
# XXIII INTERNATIONAL ROUNDTABLE ON NUCLEOSIDES, NUCLEOTIDES AND NUCLEIC ACIDS

UC San Diego

La Jolla, California

August 26 – 30, 2018

Welcome!

On behalf of the IS3NA and the XXIII IRT local organizing committee we welcome you to the University of California San Diego in La Jolla. For the next few days, a lively group of individuals from all over the world will celebrate the science of nucleosides, nucleotides and oligonucleotides. We have an intense program filled with invited and contributed lectures as well as a keynote and award presentations. As always, poster presentations play a key role in the success of the IRT meetings. All posters will therefore be displayed throughout the meeting and be featured in two early evening sessions.

In addition to our stimulating scientific program, we have planned diverse social events for you. Your participation is essential for the success of the meeting. Engage yourself in our program and enjoy the science, your colleagues, the food, and the Southern California atmosphere. We hope you will also have the opportunity to explore our campus, La Jolla, and San Diego.

We are grateful to all involved in organizing this meeting and facilitating this gathering and the associated events. We thank our colleagues for their feedback and help. We are indebted to our sponsors and exhibitors and their support, as well as to UC San Diego's Hospitality & Conference Services and our students for their dedication and assistance.

Have a wonderful and memorable time!

Professor Yitzhak Tor
Dr. Yogesh Sanghvi
Dr. Rick Hogrefe

On behalf of the IS3NA and the Local Organizing Committee

## Committees

Event Organizers
Professor Yitzhak Tor, Chair, *University of California, San Diego*
Dr. Yogesh Sanghvi, *Rasayan, Inc.*
Dr. Rick Hogrefe, *TriLink BioTechnologies*

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Professor Steve Dowdy, *University of California, San Diego*
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Professor Ulrich Muller, *University of California, San Diego*
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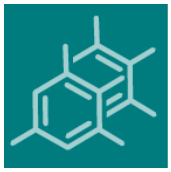






# Angels of Science

*Richard and Meghan Hogrefe*
*Robert T. Day*
*Gerald Zon*

# IRT Program at a Glance

| | Sun 8/26 | Mon 8/27 | Tue 8/28 | Wed 8/29 | Thu 8/30 |
|---|---|---|---|---|---|
| **Session Chairs** | | **N. Paul** | **S. Dowdy** | **N. Devaraj** | **D. Montesarchio** |
| **08:30 –** 08:40 | | IL1<br>S. Peyrottes | IL6<br>P. Seth | IL11<br>S. Sturla | IL15<br>S. Benner |
| 08:40 – 08:50 | | | | | |
| 08:50 – 09:00 | | | | | |
| 09:00 – 09:10 | | IL2<br>T. Koch | IL7<br>M. Manoharan | IL12<br>H. Sugiyama | IL16<br>I. Hirao |
| 09:10 – 09:20 | | | | | |
| 09:20 – 09:30 | | | | | |
| 09:30 – 09:40 | | OP1<br>O. Schmidt | OP8<br>E. Rozners | OP17<br>D. Wang | OP25<br>N. Hud |
| 09:40 – 09:50 | | | | | |
| 09:50 – 10:00 | | OP2<br>A. Ono | OP9<br>D. Perrin | OP18<br>M. Hollenstein | OP26<br>J. Chaput |
| 10:00 – 10:10 | | | | | |
| **10:10 – 10:40** | | Coffee Break | Coffee Break | Coffee Break | Coffee Break |
| **Session Chairs** | | **M. Damha** | **T. Dwyer** | **P. Herdewijn** | **R. Strömberg** |
| 10:40 – 10:50 | | AW1<br>M. Sofia | IL8<br>C. Höbartner | AW2<br>N. Sugimoto | IL17<br>W. Lee |
| 10:50 – 11:00 | | | | | |
| 11:00 – 11:10 | | | | | |
| 11:10 – 11:20 | | | OP10 | | Short oral/poster |
| 11:20 – 11:30 | | IL3<br>C. McKenna | A. Garner | IL13<br>A. Marx | presentations |
| 11:30 – 11:40 | | | OP11<br>D. O'Reilly | | |
| 11:40 – 11:50 | | | | | Next IRT<br>Closing |
| 11:50 – 12:00 | | OP3<br>S. Bogen | OP12<br>P. Kumar | OP19<br>S. Beaucage | |
| 12:00 – 12:10 | | | | | |
| **12:10 – 13:20** | | Lunch break | Lunch break<br>IS3NA Business M | Lunch break | **Lunch to go** |
| **Session Chairs** | | **S. Gryaznov** | **B. Nawrot** | **K. Seley-Radtke** | |
| 13:20 – 13:30 | | IL4<br>J. Kowalska | IL9<br>A. Bryant-Friedrich | IL14<br>Y. Mely | |
| 13:30 – 13:40 | | | | | |
| 13:40 – 13:50 | | | | | |
| 13:50 – 14:00 | | IL5<br>L. Agrofoglio | IL10<br>I. Raheem | **Chu Awards** | |
| 14:00 – 14:10 | **Registration** | | | | |
| 14:10 – 14:20 | 15:00–19:00 | | | | |
| 14:20 – 14:30 | | OP4<br>R. Veedu | OP13<br>M. Etheve-Que. | OP20<br>M. Royzen | |
| 14:30 – 14:40 | | | | | |
| 14:40 – 14:50 | | OP5<br>C. Dupouy | OP14<br>S. Vogel | OP21<br>A. Lebedev | |
| 14:50 – 15:00 | | | | | |
| **15:00 – 15:30** | | Coffee Break | Coffee Break | Coffee Break | |
| **Session Chairs** | | **B. Purse** | **A. Kore** | **F. Seela** | |
| 15:30 – 15:40 | | OP6<br>M. Bood | OP15<br>O. Yaren | OP22<br>J. Amato | |
| 15:40 – 15:50 | | | | | |
| 15:50 – 16:00 | | OP7<br>T. Wada | OP16<br>C. Ducho | OP23<br>D. Horning | |
| 16:00 – 16:10 | | | | | |
| 16:10 – 16:20 | | **Poster Session Reception** | **Poster Session Reception** | OP24<br>J. Wengel | |
| 16:20 – 16:30 | | | | | |
| **17:30** | | **Beer Garden** | | | |
| **18:00** | **Opening**<br>C. Meier<br>J-J. Vasseur | | | Transportation to Birch Aquarium | |
| **Chair** | **U. Muller** | | **18:30 Official IS3NA Event** | **Conference Banquet** | |
| | **KL**<br>G. Joyce | | | | |
| **19:00** | **Reception** | | | **Poster Awards** | |

# Program

## Sunday, 26 August

15:00 Registration Opens

18:00 Opening
Chris Meier

18:05 Jean-Louis Imbach: In Memoriam
Jean-Jacques Vasseur

18:10 Keynote Lecture
Introduction: Ulrich Mueller

**Gerald Joyce**, Salk Institute, USA
*RNA-Targeted drug discovery*

**19:00 RECEPTION**

Kindly sponsored by TriLink Biotechnologies
**Entertainment**:
Eduardo García Acosta, son jarocho band ensemble

## Monday, 27 August

**8:00 CONTINENTAL BREAKFAST**

### Session 1

**Session Chair: Natasha Paul**

8:30 Invited Lecture (**IL1**)
**Suzanne Peyrottes**, Institut des Biomolécules Max Mousseron, France
*One-Pot synthesis of nucleotides in water-medium*

9:00 Invited Lecture (**IL2**)
**Troels Koch**, Roche Innovation Center Copenhagen, Denmark
*Stereodefined LNA phosphorothioates: Design, synthesis and properties*

9:30 Oral Presentation (**OP1**)
**Olivia Schmidt,** University of Zurich, Switzerland
*Metallo base pair mediates dynamic isomerization of a duplex DNA*

9:50 Oral Presentation (**OP2**)
**Akira Ono**, Kanagawa University, Japan
*Preparations and structure elucidations of metallo-DNAs: DNA nanowire with uninterrupted one-dimensional silver ion array*

**10:10 COFFEE BREAK**

## Session 2

**Session Chair: Masad Damha**

10:40 Award Lecture (**AW1**)
**Michael Sofia**, Arbutus Biopharma Corporation, USA
*Viral hepatitis: the search for a cure*

11:20 Invited Lecture (**IL3**)
**Charles McKenna**, University of Southern California, USA
*DNA polymerases: mechanistic insights from designed substrate analogues*

11:50 Oral Presentation (**OP3**)
**Stéphane Bogen**, Merck & Company, USA
*Design and optimization of novel nucleoside prodrug clinical candidates for the treatment of HCV – Impact on metabolism and delivery to target tissue*

**12:10 LUNCH BREAK**

## Session 3

**Session Chair: Sergei Gryaznov**

13:20 Invited Lecture (**IL4**)
**Joanna Kowalska**, University of Warsaw, Poland
*Phosphate-modified nucleotides as molecular tools for probing and modulating protein activity*

13:50 Invited Lecture (**IL5**)
**Luigi Agrofoglio**, University of Orleans, France
*Overview of (E)-4-phosphono-but-2-en-1-yl acyclic nucleosides – synthesis and antiviral activity*

14:20 Oral Presentation (**OP4**)
**Rakesh Veedu**, Murdoch University, Australia
*Novel chemically modified oligonucleotides as potential molecules for precision therapy of neuromuscular diseases*

14:40 Oral Presentation (**OP5**)
**Christelle Dupouy,** University of Montpellier, France
*RNA hairpin-duplex conversion controlled by a reduction-responsive 2',2'-disulfide-bridged dinucleotide*

**15:00 COFFEE BREAK**

**Session 4**
**Session Chair: Byron Purse**

15:30 Oral Presentation (**OP6**)
**Mattias Bood**, Gothenburg University, Sweden
*Emissive quadra- and pentacyclic adenine derivatives*

15:50 Oral Presentation (**OP7**)
**Takehiko Wada**, Tohoku University, Japan
*Remarkable enhancement of RNaseH cleavage activities of RNA complexed with peptide ribonucleic acid (PRNA)–Novel backbone modification strategy for nuclease cleavage activity improvements*

**16:10 POSTER SESSION**

**17:00 BEER GARDEN**
Kindly Sponsored by TriLink Biotechnologies
**Entertainment**:
DJ Maya (https://xmayamandal.wixsite.com/citywulf)


## Tuesday, 28 August

**8:00 CONTINENTAL BREAKFAST**

**Session 5**
**Session Chair: Steven Dowdy**

8:30 Invited Lecture (**IL6**)
**Punit Seth,** Ionis Pharmaceuticals, USA
*Engineering selectivity into therapeutic oligonucleotides through chemical design*

9:00 Invited Lecture (**IL7**)
**Muthiah Manoharan**, Alnylam Pharmaceuticals, USA
*RNAi for human therapeutics: the end of the beginning*

9:30 Oral Presentation (**OP8**)
**Erik Rozners**, Binghamton University, SUNY, USA
*Amide-modified RNA: synthesis, structure and RNAi activity*

9:50 Oral Presentation (**OP9**)
**David Perrin**, University of British Columbia, Canada
*Chemically modified DNAzymes as sequence-specific ribonuclease-A mimics – From potential therapeutics to the origin of life*

**10:10 COFFEE BREAK**

## Session 6

**Session Chair: Tammy Dwyer**

10:40 Invited Lecture (**IL8**)
**Claudia Höbartner**, Universität Würzburg, Germany
*DNA catalysts and RNA aptamers for synthesis and analysis of modified RNA*

11:10 Oral Presentation (**OP10**)
**Amanda Garner**, University of Michigan, USA
*High-throughput platform assay technology for the discovery of pre-microRNA-selective small molecule probes*

11:30 Oral Presentation (**OP11**)
**Daniel O'Reilly**, McGill University, Canada
*Synergistic effects of DNA-like and RNA-like modifications in antisense oligonucleotides*

11:50 Oral Presentation (**OP12**)
**Pawan Kumar**, Alnylam Pharmaceuticals, USA
*Passenger strand carrying morpholino moiety at the 5' end improves guide strand selection and RNAi activity*

**12:10 LUNCH BREAK**

## Session 7

**Session Chair: Barbara Nawrot**

13:20 Invited Lecture (**IL9**)
**Amanda Bryant-Friedrich**, University of Toledo, USA
*Oxidative damage at a modified ribonucleic acid*

13:50 Invited Lecture (**IL10**)
**Izzat Raheem**, Merck & Company, USA
*HIV nucleoside reverse transcriptase translocation inhibitors (NRTTIs) for extended duration dosing*

14:20 Oral Presentation (**OP13**)
**Mélanie Etheve-Quelquejeu**, University of Paris Descartes, France
*Post-functionalization of RNAs for specific conjugations with peptides and proteins*

14:40 Oral Presentation (**OP14**)
**Stefan Vogel**, University of Southern Denmark, Denmark
*DNA and PNA programmed fusion of Lipid-Nanoreactors*

**15:00 COFFEE BREAK**

**Session 8**

**Session Chair: Anil Kore**

15:30 Oral Presentation (**OP15**)
**Ozlem Yaren**, Foundation for Applied Molecular Evolution, USA
*A multiplexed isothermal amplification platform to detect emerging pathogens*

15:50 Oral Presentation (**OP16**)
**Christian Ducho**, Saarland University**,** Germany
*Nucleoside antibiotics targeting bacterial peptidoglycan biosynthesis*

**16:10 POSTER SESSION (with drinks and snacks)**
**Entertainment**:
Monsieur Wulfran Trenet (https://www.reverbnation.com/wulfrantrenet/songs)

## Wednesday, 29 August

**8:00 CONTINENTAL BREAKFAST**

**Session 9**

**Session Chair: Neal Devaraj**

8:30 Invited Lecture (**IL11**)
**Shana Sturla**, ETH Zurich, Switzerland
*Finding the genomic Achilles' heel with chemistry arrows*

9:00 Invited Lecture (**IL12**)
**Hiroshi Sugiyama**, Kyoto University, Japan
*Chemical biology of nucleic acids: DNA origami and artificial genetic switch*

9:30 Oral Presentation (**OP17**)
**Dong Wang**, University of California, San Diego, USA
*Transcriptional recognition principles of naturally and synthetic modified nucleic acids: from natural biology to synthetic biology*

9:50 Oral Presentation (**OP18**)
**Marcel Hollenstein**, Institut Pasteur, France
*Expansion of the genetic code through the enzymatic construction of artificial metal base pairs*

**10:10 COFFEE BREAK**

## Session 10

**Session Chair: Piet Herdewijn**

10:40 Awards Lecture (**AW2**)
**Naoki Sugimoto**, Konan University, Japan
*Noncanonical world of nucleic acids under molecular crowding: To B or not to B*

11:20 Invited Lecture (**IL13**)
**Andreas Marx**, University of Konstanz, Germany
*Elucidating the information layer beyond the genome sequence*

11:50 Oral Presentation (**OP19**)
**Serge Beaucage**, Center for Drug Evaluation and Research, FDA, USA
*Solid-phase purification of synthetic DNA sequences*

**12:10 LUNCH BREAK**

## Session 11

**Session Chair: Katherine Seley-Radtke**

13:20 Invited Lecture (**IL14**)
**Yves Mély**, University of Strasbourg, France
*Characterization of an environment sensitive nucleoside analogue, thienoguanosine, and its application for monitoring base flipping*

13:50 **Chu Awards**

Maryam Habibian, Stanford University
Alice Ghidini, ETH
Marcella Bassetto, Cardiff University
Aurélie Lacroix, McGill University

14:20 Oral Presentation (**OP20**)
**Maksim Royzen,** University at Albany, USA
*Controlled in-cell activation of siRNA using biorthogonal chemistry*

14:40 Oral Presentation (**OP21**)
**Alexandre Lebedev**, TriLink Biotechnologies, USA
*Efficient initiation of in vitro mRNA transcription with Cap 0, Cap 1 and Cap 2 oligonucleotide primers*

**15:00 COFFEE BREAK**

**Session 12**

**Session Chair: Frank Seela**

15:30 Oral Presentation (**OP22**)
**Jussara Amato**, University of Naples Federico II, Italy
*Development and application of high throughput screening methods for the identification of conformation-selective nucleic acids ligands*

15:50 Oral Presentation (**OP23**)
**David Horning**, Salk Institute, USA
*Toward RNA life: Synthesis and replication of RNA by RNA*

16:10 Oral Presentation (**OP24**)
**Jesper Wengel**, University of Southern Denmark, Denmark
*Novel DNA-mimicking monomers for gapmer antisense oligonucleotides*

**18:00 GALA BANQUET AT BIRCH AQUARIUM**

19:30 Poster Awards

Kindly sponsored by ChemGenes


**Entertainment**: Jazz quartet
David Borgo, saxophones and flutes (davidborgo.com)
Peter Sprague, guitars (petersprague.com)
Gunnar Biggs, double bass (gunnarbiggs.com)
Andrew Munsey, drum set (music.ucsd.edu/b/Andrew+Munsey)

## Thursday, 30 August

**8:00 CONTINENTAL BREAKFAST**

**Session 13**

**Session Chair: Daniela Montesarchio**

8:30 Invited Lecture (**IL15**)
**Steven Benner**, Foundation for Applied Molecular Evolution, USA
*Darwinism from artificial genetic systems*

9:00 Invited Lecture (**IL16**)
**Ichiro Hirao**, Institute of Bioengineering and Nanotechnology, A*STAR, Singapore
*High-affinity and high-stability DNA aptamer generation using genetic alphabet expansion for SELEX (ExSELEX) and extraordinarily stable mini-hairpin technology*

9:30 Oral Presentation (**OP25**)
**Nicholas Hud**, Georgia Institute of Technology, USA
*Glycosylation of model proto-RNA nucleobases with various sugars: Implications for the prebiotic synthesis of nucleosides*

9:50 Oral Presentation (**OP26**)
**John Chaput**, University of California, Irvine, USA
*Structural basis for TNA synthesis by an evolved TNA polymerase*

**10:10 COFFEE BREAK**

## Session 14

**Session Chair: Roger Strömberg**

10:40 Invited Lecture (**IL17**)
**William Lee**, Gilead Sciences, USA
*Remdesivir (GS-5734): An antiviral nucelotide analog for the treatment of Ebola virus*

11:10 Short Oral Poster Presentations (TBA)

11:50 Closing, Next IRT, Announcements

**12:00 LUNCH TO GO**

Poster abstracts may be found online at http://irt2018.ucsd.edu/

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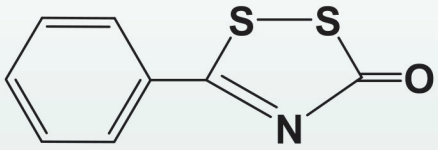
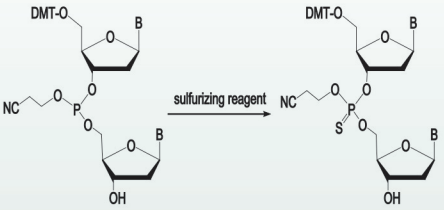
# PolyOrgSulfa (POS) – A New Sulfur Transfer Reagent for the Synthesis of Oligonucleotide Phosphorothioates

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- **Soluble and stable in anhydrous acetonitrile.**
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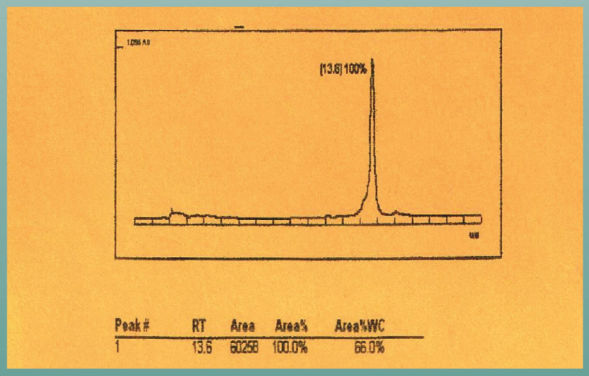
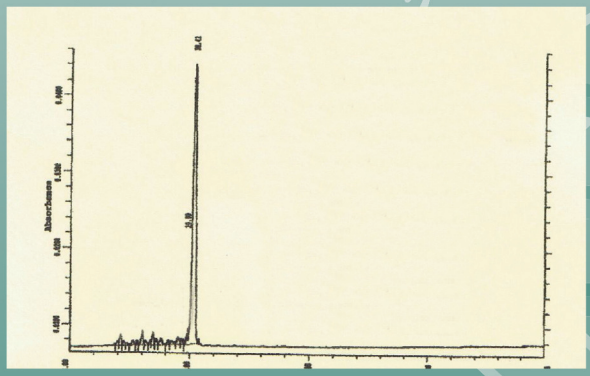
- **POS does not need aging.**
- **Odorless crystalline powder with formula weight 195.27.**
- **0.10 M solution of POS in 1% pyridine in acetonitrile is recommended.**
- **Cat. #: 16-1517**

**Anion Exchange HPLC of 31 mer (2 $\mu$m) synthesized on CPG using POS. Oligo has 26% A; 34% G; 21% T and 19% C.**




**Anion Exchange HPLC of 25 mer (200 $\mu$m) DNA phosphorothioate, synthesized on CPG using POS. Oligo has 30% A; 40% G; 26% T and 4% C.**



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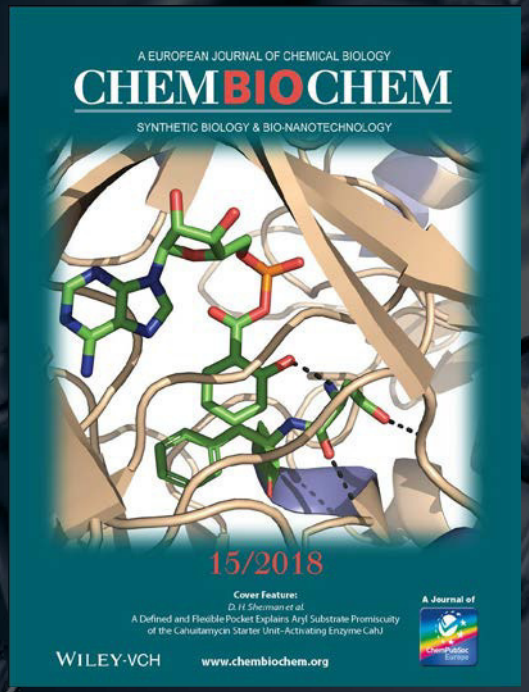


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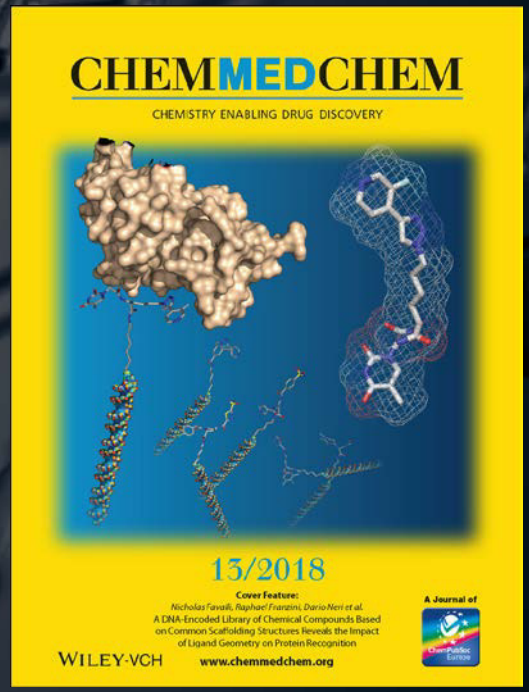


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| P047 | Olga | Perzanowska |
| P048 | Riccardo | Petrelli |
| P049 | Magdalena | Petrová |
| P050 | Karolina | Piecyk |
| P051 | Sylvie | Pochet |
| P052 | Peter | Podbevsek |
| P053 | Thazha | Prakash |
| P054 | Marcella | Bassetto |
| P055 | Sean | Purcell |
| P056 | Tomasz | Ratajczak |
| P057 | Ulrike | Reisacher |
| P058 | Marino | Resendiz |
| P059 | Joanna | Romanowska |
| P060 | Sarka | Rosenbergova |
| P061 | Petja | Rosenqvist |
| P062 | Vincent | Roy |
| P063 | Alexandra | Ruthenbeck |
| P064 | Yoshio | Saito |
| P065 | Kasper Petersen | Beck |
| P066 | George | Samaan |
| P067 | Emma Sofia | Sandell |
| P068 | Yogesh | Sanghvi |
| P069 | Shigeki | Sasaki |
| P070 | Frank | Seela |
| P071 | Anna-Lena Johanna | Segler |
| P072 | Da | Shi |
| P073 | Dongwon | Shin |
| P074 | Ondrej | Simak |

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| P075 | Michael | Smietana |
| P076 | Marcelina | Bednarczyk |
| P077 | Beata Jagoda | Starek |
| P078 | Roger | Stromberg |
| P079 | Manal | Swairjo |
| P080 | Tadao | Takada |
| P081 | Yosuke | Taniguchi |
| P082 | Kazuma | Terasawa |
| P083 | Cameron | Thorpe |
| P084 | Dilip | Tosh |
| P085 | Marc | Turner |
| P086 | Julien | Viel |
| P087 | Lucas | Bethge |
| P088 | Tomoya | Wada |
| P089 | Carston | Wagner |
| P090 | Sarah | Walsh |
| P091 | Zofia | Warminska |
| P092 | Marcin | Warminski |
| P093 | Zhiwei | Wen |
| P094 | Christopher | Wilds |
| P095 | Matthias | Winkler |
| P096 | Stanislaw | Wnuk |
| P097 | Błażej | Wojtczak |
| P098 | Sutanwi | Bhuiya |
| P099 | Junzhou | Wu |
| P100 | Madalee | Wulf |
| P101 | Chao | Yang |
| P102 | Mary | Yates |
| P103 | Eylon | Yavin |
| P104 | Ji-Seong | Yoon |
| P105 | Austin | Yu |
| P106 | Yiying | Zheng |
| P107 | Alessandra | Zimmermann |
| P108 | Ivan | Zlatev |
| P109 | Annabelle | Biscans |
| P110 | Masayuki | Endo |
| P111 | Hyuk-Jun | Jung |
| P112 | Kyeong Eun | Jung |
| P113 | Priyanka | Mangla |
| P114 | Frank | Seela |
| P115 | Nagendra | Sharma |

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| P117 | Takeshi | Yamada |
| P118 | Nicolas | Biteau |
| P119 | Verena | Böttner |
| P120 | Nikita | Brodyagin |
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| P122 | Glenn | Burley |
| P123 | Annika | Carstens |
| P124 | Brian | Cawrse |
| P125 | Gurudas | Chakraborty |
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| P130 | William | Copp |
| P131 | Roberto | Corradini |
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| P133 | Mathias Bogetoft | Danielsen |
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| P156 | Naohiro | Horie |
| P157 | Thomas | Horn |
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| P159 | Johanna | Huchting |

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| P161 | Masahito | Inagaki |
| P162 | Adolfo | Iribarren |
| P163 | Satish | Jadhav |
| P164 | Sunit Kumar | Jana |
| P165 | Tomas | Jandusik |
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| P167 | Haraldur Yngvi | Juliusson |
| P168 | Damian | Kaniowski |
| P169 | Alexander | Karpeisky |
| P170 | Hiromu | Kashida |
| P171 | Renata | Kasprzyk |
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| P175 | Kenji | Kikuta |
| P176 | Hong-Rae | Kim |
| P177 | Yuki | Kishimoto |
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| P180 | Anilkumar | Kore |
| P181 | Heidi | Korhonen |
| P182 | Ivana | Kosiova-Markusova |
| P183 | Ondrej | Kostov |
| P184 | Venubabu | Kotikam |
| P185 | Mateusz | Kozarski |
| P186 | Venkata | Krishnamurthy |
| P187 | Heike | Kropp |
| P188 | Surender | Kumar |
| P189 | Aurélie | Lacroix |
| P190 | Thomas | Lavergne |
| P191 | Hwankyu | Lee |
| P192 | Grazyna | Leszczynska |
| P193 | Yao | Li |
| P194 | Jen-Yu | Liao |
| P195 | Yuning | Liu |
| P196 | Chenguang | Lou |
| P197 | Paul | Ludford |
| P198 | Suzanne | Peyrottes |

# RNA-targeted drug discovery

Gerald F. Joyce*

*The Salk Institute, La Jolla, CA, USA*
*and Genomics Institute of the Novartis Research Foundation, La Jolla, CA, USA*

The vast majority of validated targets for drug discovery are proteins. There also have been substantial efforts to target RNAs based on either their primary structure or local secondary structure, but there have been only sparse efforts to target tertiary structured RNAs using low molecular weight compounds. Proof of principle for the specific recognition of tertiary structured RNAs comes from aptamers and riboswitches, although these are RNAs that have evolved to bind the ligand rather than ligands that were optimized to bind the RNA.

In order to discover low molecular weight compounds that target disease-relevant tertiary structured RNAs, we have turned to ultra-high-throughput screening technology and explored libraries of millions of compounds. Screens and counterscreens are performed at the level of the RNA, the corresponding RNP, and the relevant cellular phenotype. The result has been a surprisingly rich harvest of hits that have the potential to be optimized to therapeutic candidates. Many disease-related pathways include a critical RNA component that harbors a region of well-defined tertiary structure, where alteration of that structure disrupts the RNA's function. Although still in its infancy, RNA-targeted drug discovery has the opportunity to expand the range of therapeutic opportunities, especially where no suitable protein target is available.

*Corresponding author: E-mail: gjoyce@salk.edu, gjoyce@gnf.org

# Viral Hepatitis: The Search for a Cure

Michael J. Sofia

*Arbutus Biopharma, Inc., 701 Veterans Circle, Warminster, Pa 18974, USA*

Chronic viral hepatitis caused by the hepatitis B (HBV) and C (HCV) viruses afflicts more than 500 million individuals worldwide, leads to liver cirrhosis and liver cancer and results in more than 1.3 million deaths annually. The development of direct-acting antivirals (DAA) against HCV led to a revolution in the treatment of HCV where now virtually all patients diagnosed with HCV infection can be cured with safe and short duration oral therapies. The nucleotide prodrug, sofosbuvir, was a revolution in HCV therapy and the first interferon-free DAA therapy approved for curing HCV. It has become the backbone of the most effective combination curative regimens and the most successful drug launch in history. However, for HBV patients a cure has been elusive and many are relegated to lifelong nucleoside maintenance therapies. It is believed that in order to achieve an HBV cure, a combination therapy that inhibits the ability of the virus to replicate itself, eliminates the production of viral S-antigen and simultaneously reawakens the host's exhausted immune system must be developed. It is with this objective that we have undertaken an HBV cure strategy. This talk will discuss historical accomplishments and current efforts to discover therapies that will contribute to the eventual elimination of the global burden of chronic viral hepatitis

E-mail: msofia@arbutusbio.com

Noncanonical world of nucleic acids under molecular crowding:

# *To B or not to B*

Naoki Sugimoto*[a,b]

[a]*FIBER (Frontier Institute for Biomolecular Engineering Research)*
[b]*FIRST (Graduate School of Frontiers of Innovative Research in Science and Technology), Konan University, Kobe, Japan*

Noncanonical structures of nucleic acids such as triplex and quadruplex are stabilized under conditions that mimic the crowded cellular conditions and have been detected in cells. These noncanonical structures have important functions.[1-8] In this presentation, we discuss how the structures, topologies, and functions of nucleic acids differ under various conditions such as highly crowded environments to affect gene expressions such as replication, transcription, and translation. The strong effect of stall on gene expression by the noncanonical structures of nucleic acids may be one of the major causes of intractable diseases such as cancers.

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*Corresponding author: E-mail: sugimoto@konan-u.ac.jp

# Diverse approaches for the design cytosolic 5'-nucleotidase inhibitors

Suzanne Peyrottes,[a]* Laurent Chaloin,[b] Lars P. Jordheim,[c] Nushin Aghajari,[d] C. Dumontet,[c] Christian Périgaud[a]

[a] *Institut des Biomolécules Max Mousseron (IBMM), UMR 5247 CNRS, Université de Montpellier, ENSCM, Campus Triolet, cc 1705, Place E. Bataillon, 34095 Montpellier, France*
[b] *Institut de Recherche en Infectiologie de Montpellier (IRIM), UMR9004 CNRS-Université de Montpellier, 34293 Montpellier, France*
[c] *University of Lyon 1, Centre de Recherche en Cancérologie de Lyon, INSERM U1052, CNRS UMR 5286, Centre Léon Bérard, 69008 Lyon, France*
[d] *Biocrystallography and Structural Biology of Therapeutic Targets, Molecular Microbiology and Structural Biochemistry (MMSB) UMR5086 CNRS-University of Lyon1, Lyon, France*

5'-nucleotidases (EC 3.1.3.5) catalyze the hydrolysis of nucleoside 5'-monophosphates (*i.e*. nucleotides) to the corresponding parent nucleosides and inorganic phosphate, and accordingly play a key role in the metabolism of nucleotides and in the regulation of their intracellular pools for cytosolic proteins. Among them, cN-II (cytosolic 5'-nucleotidase II) is an attractive target for cancer treatments, and the combined use of cN-II inhibitors with chemotherapies which involve cytotoxic nucleosides may be of high interest. Indeed, increased activity or a high expression level of cN-II in tumor cells has been associated with resistance to cytotoxic nucleoside analogues and nucleobases [1,2]. During the last decade, we have designed, identified and studied competitive and non-competitive inhibitors of cN-II using diverse approaches (virtual screening, substrate analogue-based approach, fragment-based drug discovery) [3-5]. A selection of our results will be presented.

This work was supported by grants from the ANR (Agence Nationale de la Recherche) & INCa (Institut National du Cancer).

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*Corresponding author: E-mail: Suzanne.peyrottes@umontpellier.fr

# Stereodefined LNA Phosphorothioates: Design, synthesis and properties

Troels Koch

*Roche Innovation Center Copenhagen A/S, Fremtidsvej 3, 2970 Hørsholm, Denmark*

Locked nucleic acid (LNA) has over the last fifteen years been intensively used in RNA therapeutics. The key advantage of LNA is high affinity, and it has been confirmed that the higher affinity of LNA translates into higher potency for RNA targeting. The native internucleoside phosphates are chemically modified to phosphorothioates (PS) for nearly all LNA oligonucleotides used in RNA therapeutics. This backbone modification is crucial as it provides improved nucleolytic stability together with improving other drug like properties such as bioavailability and cellular uptake. These are predominantly driven by increased protein binding provided by PS. Classically, the introduction of PS internucleoside linkages is not stereo controlled, and therefore two stereochemical configurations Rp and Sp are created at each coupling. Consequently, antisense PS oligonucleotides consist of a large number of diastereoisomers (*i.e.* $2^n$, n = PS internucleoside linkages).

However, diastereoisomers exhibit different chemical and biological properties. This means that each diastereoisomer will exhibits its unique set of properties, and that each diastereoisomer will be different compared to the properties of the parent diastereoisomeric mixture. In some cases, the differences are large and in other cases the differences are smaller. We have shown that even a few changes in the PS configurations can give rise to dramatic changes in the global structure and electrostatics, changes that are also manifested in a differentiated set of drug properties.

This presentation will illustrate the property diversity of stereodefined LNA phosphorothioates. A focus point will be the chemistry and synthesis of LNA amidites for stereo controlled LNA synthesis and also how the development of stereo controlled LNA oligonucleotides has progressed. It will also be shown that identifying the best diatereoisomers from a large random mixture is not trivial. Several identification tactics will be shown and also how quantum mechanical modelling can be used to guide the best use of stereo defined LNA.

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E-mail: troels.koch@roche.com

# DNA polymerases: mechanistic insights from designed substrate analogues

Charles E. McKenna*[a]

[a] *Department of Chemistry, University of Southern California, Los Angeles, CA, USA*

High fidelity in DNA replication is essential to maintain the genome and avoid mutations which may lead to human diseases such as cancer. The molecular interactions and processes underlying the catalytic efficiency and base-specific selectivity of DNA polymerases have therefore long been a focus of intense study. Base excision repair (BER) is a complex process that maintains the integrity of the genome in the face of DNA mutations, including oncogenic mutations. BER has also been linked to the resistance of certain mutagenic drugs in cancer cells. DNA pol β plays an important role in BER, both as an AP site lyase and as a polymerase that adds a single nucleotide to the 3'-end of a gapped DNA site, specified by the complementary strand base. Lyase inhibition at lower nM $IC_{50}$ values has been achieved with active-site inspired compounds using pamoic acid as a structural point of departure, as will be demonstrated.

Deoxynucleoside 5'-triphosphate bisphosphonate analogues, in which the α,β- or β,γ-bridging oxygen of the triphosphate moiety ($dNp_{\alpha}Op_{\beta}Op_{\gamma}$) is replaced by a hydrolysis-resistant group such as an imido (NH) or substituted methylene group (CXY), are well known probes of the ground and transition states in pol β-catalyzed DNA repair and have been recently been found useful in studies of mutant polymerases.[1,2] Emerging results underline the advantage of having available a comprehensive 'tool-kit' of the probes encompassing all four DNA bases, to elucidate more fully base-pairing dependence of leaving group-dependent effects on dNTP incorporation catalyzed by pol β. To encompass certain key individual β,γ-pCHYp diastereomers (e.g. N = A: Y = Me; N = C: Y = F, Cl, Br) previously missing from the tool-kit, two novel chiral bisphosphonate synthons have been devised, and successfully applied to their synthesis. Using the expanded tool-kit, X-ray crystallographic ternary complex structures of analogues bound with DNA into the enzyme active site were compared with the corresponding $k_{cat}$ values derived from presteady-state kinetics.[3,4] The new results provide a unique basis to examine the correlation of ground state structural interactions with molecular mechanisms underlying pol β catalytic efficiency and fidelity.

This work was supported by a grant from the NIH (U19CA177547).

***References:*** [1] Alnajjar, K. S. et al., *Biochemistry*. **2017**, *56*, 5449. [2] Alnajjar, K. S. et al., *Biochemistry*. **2017**, *56*, 2096. [3] Oertell, K. et al. *Biochemistry.* **2018**, *57*, 3925. [4] Batra, V. K. et al., *Biochemistry* **2018**, *57*, 3934.

*Corresponding author: E-mail: mckenna@usc.edu

# Phosphate-modified nucleotides as molecular tools for probing and modulating protein activity

Joanna Kowalska[a]*, Jacek Jemielity[b]*

[a] *Division of Biophysics, Institute of Experimental Physics, Faculty of Physics, University of Wasaw, Poland*
[b]*Centre of New Technologies, University of Wasaw, Poland*

Phosphate and oligophosphate mono- and diesters are key structural elements of nucleotides and nucleic acids. Enzymes controlling phosphate transfer or phosphoester and phosphoanhydride cleavage within DNA and RNA participate in gene expression, cellular signaling, and metabolism. Consequently, phosphate ester and anhydride analogs have been invaluable tools in deciphering the mechanisms of biophosphate-related enzymatic transformations. Phosphate-modified nucleotides give also rise to molecular probes useful for the discovery of inhibitory molecules with therapeutic potential.[1]

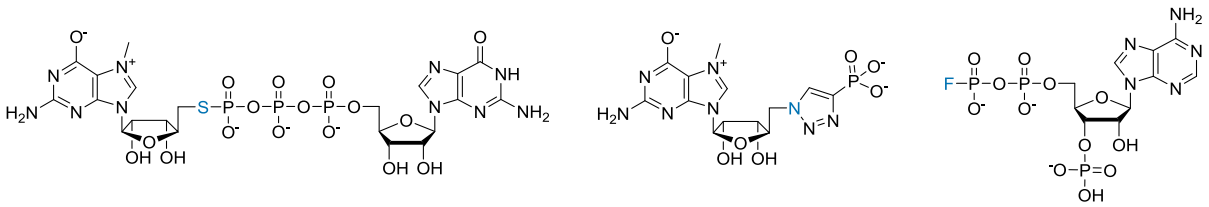


***Figure.*** *Structures of example phosphate-modified nucleotide analogs.*

We aim to use phosphate modifications to study proteins involved in mRNA translation initiation and degradation and to modulate their activity for therapeutic benefits.[2] Recently, we have also employed phosphate-modified analogs as tools to study sulfate-transferring enzymes to understand the molecular basis for sulfate-phosphate discrimination. The most important and surprising results of those studies will be reported during the talk.

This work was supported by the National Science Centre, Poland (2015/18/E/ST5/00555).

***References:*** [1] Joachimiak L., Blazewska K,M. *J. Med. Chem.*,doi: 10.1021/ acs.jmedchem.8b00249
[2] As examples see: a) Baranowski et al. *Org. Biomol. Chem.*, 2016, 14, 4595-4604; b) Wojtczak et al. *J. Am. Chem. Soc.*, 2018, 140 (18), pp 5987–5999

*Corresponding author: E-mail: jkowalska@fuw.edu.pl, j.jemielity@cent.uw.edu.pl

# Overview of (*E*)-4-phosphono-but-2-en-1-yl acyclic nucleosides - synthesis and antiviral activity

Agrofoglio Luigi A.

*Université d'Orléans, CNRS, ICOA UMR 7311, Orléans, France*

Nucleosides and their analogs form an important class of anti-infective drugs. Among them, acyclic nucleoside phosphonates (ANPs) are nowadays one of the key drugs in the treatment of DNA virus and retrovirus infections. Over the last decade, our group has described straightforward convergent syntheses a new family of ANPs in various prodrug series based on a *trans*-but-2-enyl phosphonate scaffold.

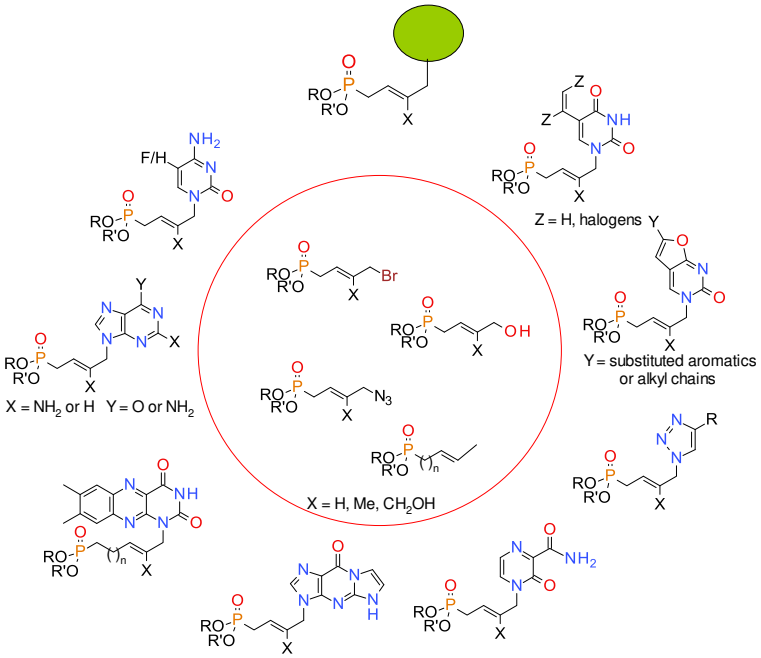



***Figure.*** *Some* (*E*)-4-phosphono-but-2-en-1-yl acyclic nucleosides

Key steps involve challenging ultrasonic-assisted olefin cross metathesis, Pd(0)-catalyzed coupling reactions, and further modifications under microwaves, giving rise to a small library of title compounds. These unique ANPs were evaluated against a wide spectrum of DNA/RNA viruses. Among them, most active compounds showed sub-micromolar/nanomolar activity. Their syntheses and some biological data will be presented.

*Corresponding author: E-mail: luigi.agrofoglio@univ-orleans.fr
http://www.icoa.fr/en/agrofoglio

# Engineering selectivity into therapeutic oligonucleotides through chemical design

Punit P. Seth

*Department of Medicinal Chemistry, Ionis Pharmaceuticals, 2855 Gazelle Court, Carlsbad, CA 92010*

The field of nucleic acid based therapeutics has seen rapid growth in the last decade. Two Antisense oligonucleotides (ASOs), Kynamro and Spinraza, were approved by the FDA recently and two additional ASOs, Inotersen and Volanosersen, completed successful phase 3 trials in 2017. In parallel to these developments, significant progress has been made in understanding the pathways by which ASOs distribute to cells and tissues and on using medicinal chemistry strategies to modulate these processes to further improve ASO potency in the clinic. Furthermore, medicinal chemistry strategies have also been used to enhance ASO specificity for applications such as allele selective gene silencing for the treatment of autosomal dominant disorders. In this talk, we will discuss recent advances in ASO medicinal chemistry which have enabled the design of more potent and specific drugs.

Corresponding author: E-mail: pseth@ionisph.com.

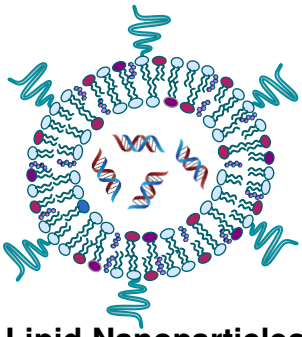
# RNAi for Human Therapeutics: *The End of the Beginning*

Muthiah Manoharan

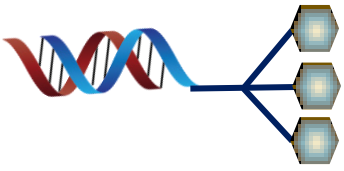
*Alnylam Pharmaceuticals, Cambridge, MA 02142 USA; mmanoharan@alnylam.com*

Synthetic small interfering RNAs (siRNAs) are potent inhibitors of gene expression through the RNA interference (RNAi) mechanism, first characterized in *C. elegans* and active in most eukaryotes. The therapeutic potential of RNAi can be fully realized when these agents are efficiently delivered into the cells of specific organs or tissues where the particular disease process originates. At Alnylam Pharmaceuticals, we have developed a three-pronged approach to enable efficient delivery of potent siRNAs into liver hepatocytes after either intravenous or subcutaneous injection. These methods include chemical modification of siRNAs, lipid nanoparticle formulation of siRNAs and multivalent *N*-acetylgalactosamine conjugation of siRNAs, resulting in localization of the siRNA to liver. With these approaches, the potential of siRNA in treatment of "undruggable" diseases that was envisioned after it was realized that this process is active in human cells is being realized as several siRNA-based drugs are in various stages of clinical testing and successful clinical results are emerging from these trials. The strategies and results will be discussed. To improve the specificity, metabolic stability, and potency of chemically modified siRNAs we have introduced chemical modifications such as *(S)*-glycol nucleic acid (*S*)-GNA, 5'-(*E*)-vinyl phosphonate (5'-(*E*)-VP) at the first nucleotide of the guide strand, and 5'-morpholino (5'-M) at the first nucleotide of the passenger strand. The application of these modifications in improving the pharmacological properties of siRNAs will also be discussed.

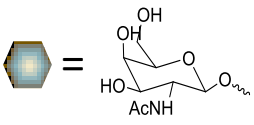
siRNA



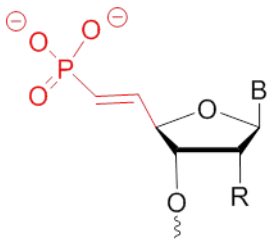
Lipid Nanoparticles



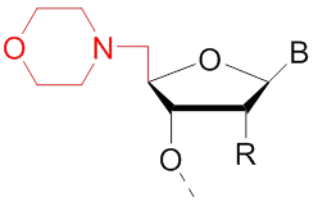
GalNAc-siRNA Conjugates




GalNAc




5'-(*E*)-VP




5'-M


(*S*)-GNA

# DNA catalysts and RNA aptamers for synthesis and analysis of modified RNA

Claudia Höbartner

*Institute of Organic Chemistry, Julius Maximilians University Würzburg, Am Hubland, 97074 Würzburg, Germany*

Natural nucleotide modifications diversify the chemical composition of DNA and RNA and have the potential to alter the structure and function of nucleic acids. In the laboratory, new functions for RNA can be evolved from initially random RNA libraries, using modified and unmodifed nucleotides to generate aptamers and ribozymes. Analogous functional DNA molecules have interesting synthetic applications but have not yet been found in nature. We explore the catalytic potential of DNA and RNA and use nucleic acids as catalysts for the synthesis of modified RNA. In addition, we are involved in engineering of fluorogenic RNA aptamers and we develop chemical labeling reactions that help in the detection and analysis of natural RNA modifications.
This presentation will discuss recent insights into the structural basis of DNA-catalyzed RNA ligation and present our ongoing efforts to enhance the catalytic abilities of DNA enzymes for site-specific labeling of RNA and for the detection of natural RNA modifications [1-3].

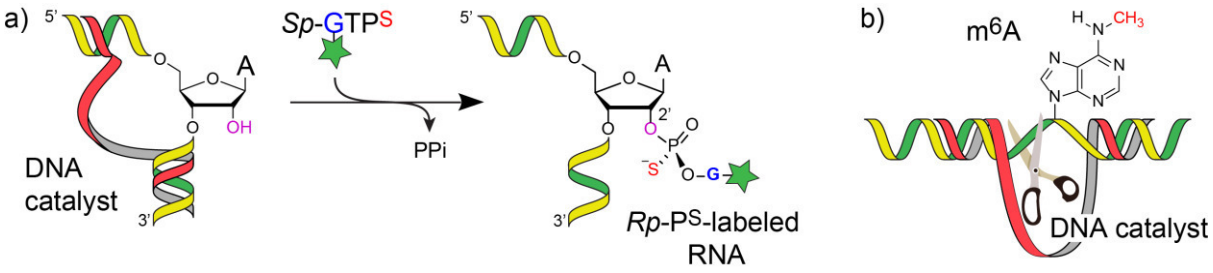



***Figure.*** *DNA catalysts for RNA labeling (a) and detection of RNA methylation (b).*

This work was supported by the DFG (Priority program "Chemical biology of native nucleic acid modifications", SPP 1784) and the European research council (ERC consolidator grant).

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*Corresponding author: E-mail: claudia.hoebartner@uni-wuerzburg.de

# Oxidative damage at a modified ribonucleic acid

Amanda Bryant-Friedrich, Immaculate Sappy, Saad Alqarni, Matthew Starr and Raziya Shaik

*Department of Medicinal and Biological Chemistry, The University Toledo, 2801 W. Bancroft St. Toledo, OH, USA.*

Pseudouridine (Ψ), the 5-ribosyl isomer of uridine (U) is the most abundant nucleic acid modification found in all domains of life and all types of RNA. Studies have shown that, urinary levels of pseudouridine are higher in Alzheimer's disease (AD) patients and that RNA oxidation is a major component in the pathogenesis of Alzheimer's disease (AD) and other neurodegenerative diseases. These studies point to a correlation between higher urinary levels of pseudouridine and oxidative stress in AD. A C5'-radical precursor for pseudouridine has been synthesized to investigate the premise that pseudouridine may play a role in the protection of RNA from oxidative damage. The synthesis of this modified nucleoside is

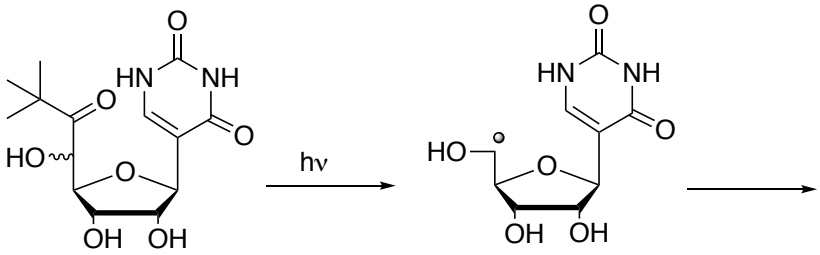


***Figure.*** *Photochemical generation of the pseudouridinyl-5'-radical*

accomplished through the construction of the pseudouridine nucleoside followed by introduction of a photolabile pivaloyl moiety. Upon photoactivation under various conditions, we will probe the products which may be obtained from this single oxidative event and determine if the modified base can limit the impact of this damage. Through the identification of products from this oxidative event, we will generate information that will allow us to determine the effect of oxidative damage on RNA structure and function. Hence, this will provide key information about the role of this nucleoside in RNA related processes and disease etiology.

This work was supported by grants from the National Science Foundation.

*Corresponding author: E-mail: Amanda.bryant-friedrich@utoledo.edu

# HIV Nucleoside Reverse Transcriptase Translocation Inhibitors (NRTTIs) for Extended Duration Dosing

*Izzat Raheem*
*Discovery Chemistry, Merck, West Point, PA*

Despite the prevalence of highly efficacious and diverse therapeutic options for the treatment of HIV, real-world effectiveness remains suboptimal. This is driven in part by poor patient adherence, often resulting in treatment failure or discontinuation. Extended duration dosing (ExDD) options with >Q6-mo dosing intervals have the potential to be transformative in the HIV space, not only addressing issues of adherence, but also providing patients with a valuable improvement in treatment convenience. Given the extremely low daily input rates and doses required to enable ExDD formulations, recent discovery efforts in our laboratories have focused on compounds in the nucleoside reverse transcriptase translocation inhibitor (NRTTI) class, MK-8591 (EFdA) in particular.

Herein, we describe our on-going efforts to advance ExDD bioerodible and non-erodible implantable formulations of our NRTTIs. We present a overview of the NRTTI mechanism, our discovery efforts to identify stucturally novel back-ups to MK-8591, as well as a summary of clinical progress of MK-8591 to date. Framed in the context of MK-8591 implants for HIV prevention, we will present implant design and optimization strategies pertinent to the discovery space, including modulation of compound physicochemical properties, polymer/formulation selection, and implant drug loading. Key data from on-going *in vitro* and *in vivo* studies will also be presented.

# Finding the Genomic Achilles' heel with Chemistry Arrows

Shana J. Sturla*

[a]*Department of Health Sciences and Technology, ETH Zurich, Zurich, Switzerland*

Carcinogenesis can be associated with an ordered progression of acquired mutations, in some cases with significant portions corresponding to point mutations in critical cell growth regulating genes. Chemical alkylating agents from endogenous metabolic processes and chemical exposures can result in the alkylation and oxidation of DNA and formation of highly pro-mutagenic types of DNA damage, such as O6-alkyl-guanine and 8-oxo-guanine. Additionally, inducing DNA damage is amongst the most commonly used strategies for treating cancer, whereby replication and transcription are stalled. However, problems related with adverse toxicities and drug resistance are common and indicate a need to better understand the basis of toxic selectivity of DNA damage, how the distribution of damage on a genomic scale relates with the acquisition of point mutations, and to create new strategies for stratifying responsive cancer patients. The talk will concern studies aiming to understand how chemically induced damage to DNA gives rise to mutations and cytotoxicity with an overarching aim of identifying modified nucleobases for diagnostic purposes in the prevention and treatment of cancer.

This work was supported by grants from the Swiss National Science Foundation and the European Research Council.

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*Corresponding author: E-mail: sturlas@ethz.ch

# Chemical biology of nucleic acids: DNA origami and artificial genetic switch

Hiroshi Sugiyama,[a,b]

[a] *Department of Chemistry, Graduate School of Science, Kyoto University, Sakyo-Ku, Japan*
[b] *Institute for Integrated Cell-Material Sciences (iCeMS) , Kyoto University, Sakyo-Ku, Japan*

The DNA origami method developed for the preparation of fully addressable two-dimensional (2-D) structures has been utilized for the selective positioning of the functional molecules and nanoparticles. We designed "DNA frame" using the DNA origami method to investigate enzymatic action and DNA structural change.[1] To observe the behaviors and reactions of DNA methyltransferase, DNA recombinase, Cas9, MOC1, and DNA repair enzymes, the substrate dsDNAs were incorporated into the cavity of the DNA frame, and the enzymes that bound to the target dsDNA were observed using HS-AFM.[1,2] We recently developed DNA nanocages and investigated the effect of confined space on the property of G-quadruplex and found that mechanical and thermodynamic stabilities of the G-quadruplex inside the nanocage are significantly increased.[3] Also a strategy for lipid-bilayer-assisted self-assembly of various DNA origami tiles into 2-D lattices was developed.[4] We have been undertaking original research on the molecular recognition of DNA by antitumor antibiotics, and the analysis of atom-specific chemical reaction on DNA. By reconstituting such knowledge, various functionalized sequence-specific DNA binding pyrrole-imidazole polyamides (PIPs) were synthesized as an artificial genetic switch, which can switch on and switch off the gene expression on demand. We recently developed alkylating PIP that could switches off cancer related KRAS gene[5] and RUNX 1-3 controling genes.[6] To switch on the gene expression we need to consider Epigenetics. We developed a SAHA-PIP containing sequence-specific pyrrole-imidazole polyamides (PIPs) and HDAC inhibiting SAHA. Evaluation of the effect of SAHA-PIPs on genome-wide gene expression in human dermal fibroblasts (HDFs) divulged that each SAHA-PIP could differentially activate the therapeutically important genes.[7] Conjugation of DNA binding domain of 'I' with HAT activating CTB remarkably activated identical cluster of genes as SAHA-PIP 'I' to substantiate the role of PIP in sequence-specific gene regulation.[8] Recently we introduced Bromodomain inhibitor to PIP to activate gene expression in sequence-specific manner.[9] In this talk recent progress of DNA origami technology and regulation of the gene expression using designed PIPs will be discussed.

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*Corresponding author: E-mail: hs@kuchem.kyoto-u.ac.jp

# Elucidating the information layer beyond the genome sequence

Andreas Marx

*Department of Chemistry and Konstanz Research School Chemical Biology, University of Konstanz, D-78457 Konstanz, Germany*

DNA is the storage of genetic information in Nature. Transmission of the genetic information from the parental DNA strand to the offspring is crucial for the survival of any living species. The entire DNA synthesis in DNA replication is catalyzed by DNA polymerases and depends on their ability to select the canonical nucleotide from a pool of structurally similar building blocks. Besides the crucial biological role of DNA polymerases, these enzymes are the workhorses in numerous important molecular biological core technologies such as the ubiquitous polymerase chain reaction (PCR), cDNA cloning, genome sequencing, and nucleic acids based diagnostics that require the use of highly modified substrates.

I will report recent results of structural and functional investigations on how DNA polymerases process unnatural and natural substrates and on how to engineer these 'nanomachines' to go beyond the scope nature provided.

Furthermore, I will report on studies on the conditional metabolism of poly(ADP-ribose), a complex nucleic acid-like posttranslational protein modification that are formed by poly(ADP-ribose) polymerases (PARPs). By new synthetic substrates of PARPs we were able to monitor poly(ADP-ribose) formation in real-time in the context of live cells.

This work was supported by grants from the European Research Council and Deutsche Forschungsgemeinschaft.

*Corresponding author: E-mail: andreas.marx@uni.kn

**Environmentally-sensitive fluorescent nucleoside analogues for surveying dynamic protein/nucleic acid interactions and interconversions of nucleic acid structures.**

Marianna Sholokh[1], Vasyl Kilin[1], Rajhans Sharma[1], Natalia Grytsyk[1], Christian Boudier[1], Dmytro Dziuba[2], Nicolas P.F. Barthes[2], Benoît Y. Michel[2], D. Shin[3], O. Mauffret[4], Alain Burger[2], Yitzhak Tor[3], and Yves Mély[1]

[1]Laboratoire de Bioimagerie et Pathologies, UMR 7021 CNRS, Université de Strasbourg, Faculté de Pharmacie, 74 route du Rhin, 67401 Illkirch, France
[2]Institut de Chimie de Nice, UMR 7272 CNRS, Université de Nice Sophia Antipolis, Parc Valrose, 06108 Nice, France
[3]Department of Chemistry and Biochemistry, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA, 92093-0358, USA
4 LBPA, Ecole normale supérieure Paris-Saclay, UMR 8113 CNRS, 94235 Cachan, France.

FRET-based techniques are highly useful for monitoring protein/nucleic acid interactions as well as conformational transitions in nucleic acids. However, these techniques require two fluorophores that are typically large and can alter the DNA/RNA structure and protein binding. Additionally, events that do not alter the donor/acceptor distance and/or angular relationship are frequently left undetected. Fluorescent nucleobases that can substitute natural nucleobases with minimal structural modifications, such as the recently developed 2-thienyl-3-hydroxychromone (3HCnt)[1,2] and thienoguanosine (thG)[3], offer a promising alternative to FRET based techniques. The photophysical properties of 3HCnt and thG have been extensively characterized. Both probes possess respectable fluorescence quantum yield and are highly responsive to environmental changes when incorporated into nucleic acids[1,4,5]. Both probes were used to monitor the recognition of hemimethylated CpG sites and further flipping of methylated cytosines (mC) by the Set and Ring Associated (SRA) domain of UHRF1, which play a key role in the replication of DNA methylation patterns[6]. Both probes were also used to survey the conversion of the (-)DNA copy of the HIV-1 primer binding site (-)PBS stem-loop into (+)/(-)PBS duplex[7]. When incorporated into the (-)PBS loop, the two probes were found to be highly sensitive to the individual steps both in the absence and the presence of a nucleic acid chaperone, providing the first complete mechanistic description of this critical process in HIV-1 replication. The combination of the two distinct probes appears to be instrumental for characterizing structural transitions of nucleic acids under various stimuli.

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7 . M. Sholokh, R. Sharma, N. Grytsyk, L. Zaghzi, V. Postupalenko, D. Dziuba, N. Barthes, B. Michel, C. Boudier, O. Zaporozhets, Y. Tor, A. Burger, & Y. Mely. Chemistry, 2018, in press.

# Darwinism from Artificial Genetic Systems

Steven A. Benner*[a,b] Shuichi Hoshika,[a] Zunyi Yang,[a] Elisa Biondi,[a] Liqin Zhang,[b] and Millie M. Georgiadis[c]

[a] *Foundation for Applied Molecular Evolution, 13709 Progress Blvd. Box 7, Alachua, FL 32615*
[b] *Firebird Biomolecular Sciences LLC, 13709 Progress Blvd. Box 17, Alachua, FL 32615*
[c] *Indiana University School of Medicine, Indianapolis IN 46202*

By dragging scientists across uncharted terrain where they are forced to answer unscripted questions, "Grand Challenge" synthesis can drive discovery and paradigm change in ways that hypothesis-directed research cannot. Here, our grand challenge seeks to reproduce the Darwinism displayed by terran biology, but on a molecular platform different from standard DNA; access to Darwinism is believed by many to distinguish the living state from the non-living state. DNA is supposed to be able to support Darwinism by two of its structural features, (a) its ability to fit into a Schrödingerian "aperiodic crystal", lattice, and (b) its "polyelectrolyte" backbone.

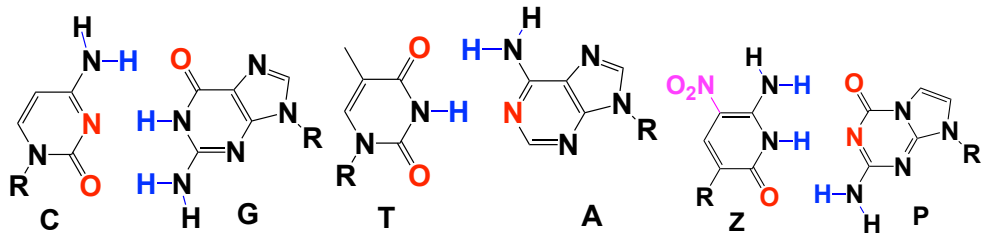


***Figure.*** *A six-letter polyelectrolyte-Schrödinger Darwinian system.*

This talk will discuss recent work that obtained Darwinian behavior from a DNA-like biopolymer built from six letter genetic alphabet. In the process of obtaining this system, two new molecular recognition systems were discovered, together with much detailed information about natural terran systems interact with natural DNA. Together with a deeper understanding of the role played in aperiodic crystal formation by the polyelectrolyte backbone and the intervening scaffolding, these results define how we search for Darwinism, and therefore life, on Mars, Europa, Enceladus, and other watery lagoons in our Solar System.

This work was supported by grants from the Templeton World Charity Foundation, NASA, and the NIH.

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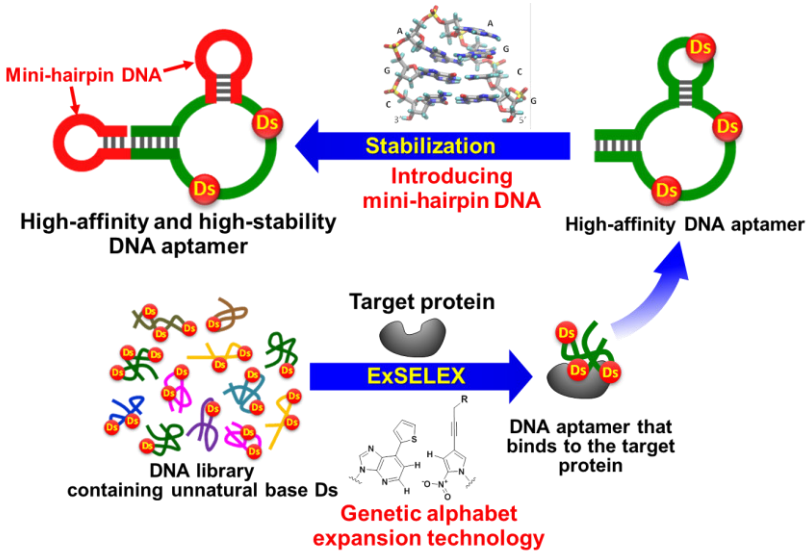
*Corresponding author: E-mail: sbenner(at)ffame.org

# High-affinity and high-stability DNA aptamer generation using genetic alphabet expansion for SELEX (ExSELEX) and extraordinarily stable mini-hairpin technology

Ichiro Hirao*[a]

[a]*Institute of Bioengineering and Nanotechnology, 31 Biopolis Way, The Nanos, #07-01, Singapore 138669, Singapore*

DNA aptamers that bind to target molecules are expected to be an antibody alternative. Initially, DNA aptamers are generated by an evolutionary engineering method (SELEX, Systematic Evolution of Ligands by EXponential enrichment). Based on the sequences obtained by SELEX, DNA aptamers and their variants are chemically synthesized for large-scale preparation and modifications. To increase aptamer abilities, such as affinity to targets and stability against nucleases, many modified methods have been reported. Recently, we developed a new method (ExSELEX) to generate high-affinity DNA aptamers by introducing hydrophobic unnatural bases as a fifth base [1]. In ExSELEX, initial DNA libraries containing the unnatural bases (Ds bases) are chemically synthesized, and the isolated DNA libraries can be amplified by PCR using an unnatural base pair (Ds–Px) as a third base pair [2]. The hydrophobic Ds bases greatly augment the affinity of DNA aptamers to targets. Forthremore, the Ds-containing DNA aptamers can be stabilized by introducing mini-hairpin DNA sequences [3], which exhibit high stability against heat and nucleases [4]. Using this method, we generated several DNA aptamers that bind to target proteins and cancer cells toward diagnostic and therapeutic applications [3, 5, 6].

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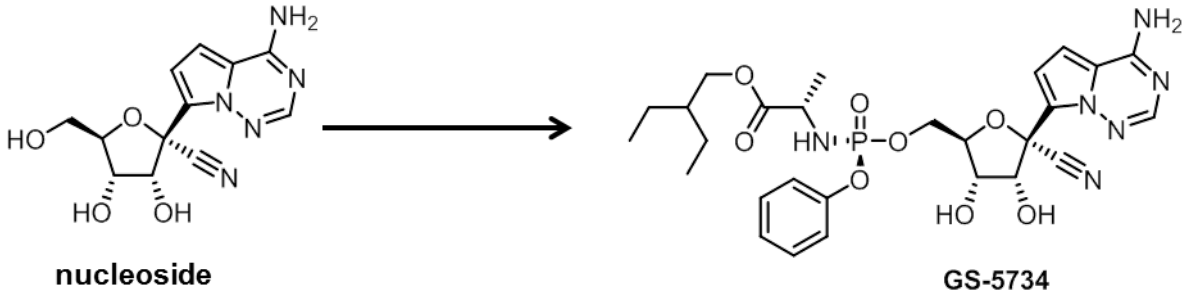
*Corresponding author: E-mail: ichiro@ibn.a-star.edu.sg

# Remdesivir (GS-5734): An Antiviral Nucelotide Analog for the Treatment of Ebola Virus

William A. Lee, Ph.D[a]

[a] *Gilead Sciences, Inc., 333 Lakeside Drive, Foster City, CA 94404*




Remdesivir is a nucleotide prodrug of a novel nucleoside analog that has shown broad spectrum in vitro activity against filoviruses, corona viruses, paramyxoviruses and flaviviruses.

Remdesivir has demonstrated potent in vivo efficacy against multiple strains of the Ebola virus in the rhesus monkey infection model. The data in rhesus, the manufacturing challenges and the limited exposure in patients exposed to the Ebola viruses will be reviewed.

*Corresponding author: E-mail: William.lee@gilead.com

# Metallo Base Pair Mediates Dynamic Isomerization of a Duplex DNA

Olivia P. Schmidt,[a] Simon Jurt,[a] Silke Johannsen,[a] Roland K. O. Sigel,[a] Nathan W. Luedtke*[a]

[a] *Department of Chemistry, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich*

Metal-mediated base pairs expand the repertoire of helical structures and dynamics available to duplex nucleic acids, thereby tuning their biological and materials properties. In addition to the widely studied T-$Hg^{II}$-T and C-$Ag^{I}$-C base pairs, a C-$Hg^{II}$-T base pair was recently observed in a crystal structure of a short, A-form duplex DNA.[1] Using fluorescent nucleobase analogs and NMR spectroscopy we discovered that $Hg^{II}$ exhibits high affinity, stoichiometric binding of C-T mismatches in duplex DNA, with thermodynamic stabilities ($K_d$ = 10 – 153 nM) similar to the widely studied T-$Hg^{II}$-T base pairs.[2] However, metal binding caused little or no change in the thermal stability ($T_m$) of duplex DNA containing a C-T mismatch.[2]

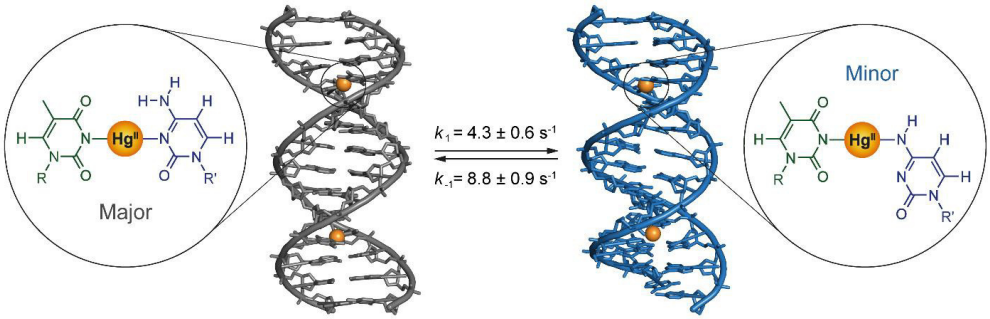



***Figure.*** *Isomerization-mediated conformational exchange of duplex DNA containing two C-$Hg^{II}$-T base pairs.*

Here we report the solution structures and dynamics of duplex DNA containing C-$Hg^{II}$-T base pairs.[3] NMR experiments revealed that C-$Hg^{II}$-T formation occurs via two distinct coordination modes to give a major species (~75 %) containing (*N3*)C-$Hg^{II}$-(*N3*)T coordination sites and a minor species (~25 %) with (*C4-NH*)C-$Hg^{II}$-(*N3*)T base pairs. The different nucleobase-metal-nucleobase connectivities resulted in two unique duplex conformations, each exhibiting characteristics of a "mixed" A/B-form duplex. The major duplex adopted a more B-form-like structure and the minor duplex had more A-form characteristics. The global interconversion of the two duplexes, was directly coupled to changes in metal ion coordination. The rate constants of their interconversion ($k_1$ = 4.3 ± 0.6 s$^{-1}$, $k_{-1}$ = 8.8 ± 0.9 s$^{-1}$) were on a similar time scale as many biochemical and material processes and can therefore serve as a low-energy barrier switch between two functional states of a duplex DNA.[3]

This work was supported by grants from the Swiss National Science Foundation.

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*Corresponding author: E-mail: nathan.luedtke@chem.uzh.ch

# Preparations and structure elucidations of metallo-DNAs: DNA nanowire with uninterrupted one-dimensional silver ion array

Akira Ono[a*], Hisao Saneyoshi[a], Jiro Kondo[b], Yoshiyuki Tanaka[c]

[a]*Department of Material & Life Chemistry, Faculty of Engineering, Kanagawa University, 3-27-1 Rokkakubashi, Kanagawa-ku, Yokohama, Kanagawa-ken 221-8686 Japan*
[b]*Department of Materials and Life Sciences, Faculty of Science and Technology, Sophia University, 7-1 Kioi-cho, Chiyoda-ku, 102-8554 Tokyo, Japan*
[c]*Graduate School of Pharmaceutical Sciences, Tohoku University, 6-3 Aza-Aoba, Aramaki, Aoba-ku, Sendai, Miyagi 980-8578, Japan*
[C]*Faculty of Phamaceutical Scienes, Tokushima Bunri University, Yamashiro-cho, Tokushima 770-8514, Japan*

Synthetic oligonucleotides containing artificial bases have been used to form metal-mediated base pairs (metallo-base pairs), in which the hydrogen bonds in Watson–Crick (W–C)-type base pairs, as found in natural DNA, are replaced by metal–base bonds[1]. Alternative methods for generating metal-mediated base pairs in DNA duplexes using only naturally occurring pyrimidine bases have also been reported[2]. Thymine–thymine (T–T) and cytosine–cytosine (C–C) pairs selectively capture Hg(II) and Ag(I) ions, respectively, and the metallo-base pairs, T-Hg(II)-T and C-Ag(I)-C, are formed in DNA duplexes. Recently, we found the unexpected potential of natural bases, that a metal-DNA nanowire with uninterrupted one-dimensional silver ion array was formed form a short oligonucleotide consisting of the natural bases and silver ions (Fig. 1)[3].

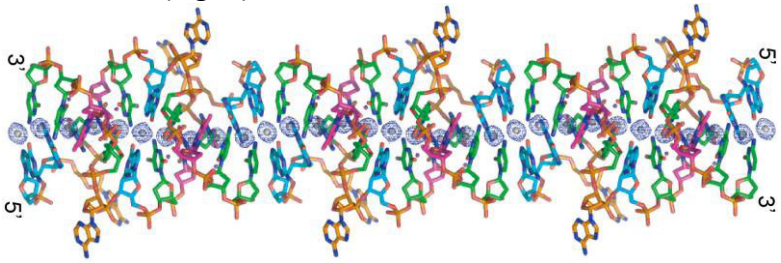


Figure 1.

In thsi presentation, solution and crystal structures of metallo-DNAs consisting of natral and modified pyrimidine bases are reported.

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(MEXT)

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*Corresponding author: E-mail: akiraono@kanagawa-u.ac.jp

# Design and optimization of novel nucleoside prodrug clinical candidates for the treatment of HCV – Impact on metabolism and delivery to target tissue

Stéphane Bogen,*[,a] Carmen Alvarez,[a] Ashok Arasappan,[a] Frank Bennett,[a] Kevin Chen,[a] Ping Chen,[a] David Cole,[a] Qun Dang,[a] Sara Esposite,[a] Vinay Girijavallabhan,[a] Ying Huang,[a] Yuhua Huang,[a] Qingmei Hong,[a] Angela Kerekes,[a] Zhidan Liu,[a] Latha Nair,[a] Weidong Pan,[a] Dmitri Pissarnitski,[a] Haiyan Pu,[a] Randall Rossman,[a] Quang Truong,[a] Vishal Verma,[a] Bancha Vibulbhan,[a] Jun Wang,[a] Zhiqiang Zhao,[a] Paul Bulger,[a] William Morris,[a] Lei Ba,[a] Eric Ferrari,[a] Anita Howe,[a] Hsueh-Cheng Huang,[a] David Olsen,[a] Li-Kang Zhang,[a] Nicole Buist,[a] Paul Walsh,[a] Erika Walsh,[a] Christine Brzostowski,[a] Karen Dingley,[a] James Lamca,[a] Randy Miller,[a] Deborah Nicoll-Griffith,[a] Theresa Nguyen,[a] Diane Rindgen,[a] Jianzhong Wen,[a] Reshma Kuvelkar,[a] Payal Sheth,[a] Jane Wen,[a] William Windsor,[a] Christopher Brynczka,[a] Carrie Markgraf,[a] Andrew Stamford,[a] Ian W. Davies,[a] F. George Njoroge,[a] David Dukhan,[b] Francois R. Alexandre,[b] Cyril Dousson,[b] Gilles Gosselin,[b] Christophe Parsy,[b] Claire Rouvière,[b] Guillaume Brandt,[b] Stéphanie Bot,[b] Julien Milhau,[b] Rachid Rahali,[b] Thierry Convard[b]

[a] *Merck & Co., Inc., USA*
[b] *Idenix an MSD Company, Montpellier, France*

Hepatitis C virus (HCV) infection remains a global health challenge. MRL has an anti-HCV pipeline covering multiple MOA (NS3, NS5A, NS5B NI). Among all known DAAs, NIs (nucleoside inhibitors) have displayed the broadest HCV genotypic coverage and the highest barrier to resistance. The complexity is that the active metabolite (nucleoside triphosphates or NTP) is formed intracellularly and its delivery is critically dependent on prodrug design and host metabolism. Formation of active metabolites in non-hepatic tissues could result in potential safety issues. This has been a challenge that resulted in multiples clinical failures. The presentation will describe the design and optimization of Merck clinical candidate nucleoside analogs and the impact on prodrug metabolism and NTP formation in the liver.

*Corresponding author: Stephane.bogen@merck.com

# Novel chemically-modified oligonucleotides as potential molecules for precision therapy of neuromuscular diseases.

Bao Tri Le,[a,b] Susan Fletcher,[a,b] Stephen D. Wilton,[a,b] Piet Herdewijn,[c] Poul Nielsen,[d] Hiroyuki Asanuma,[e] Kamal Rahimizadeh,[a,b] Madhuri Chakravarthy,[a,b] Peter R. Dodd,[f] Rakesh N. Veedu*[a,b,d]

[a] *Centre for Comparative Genomics, Murdoch University Perth, Australia*
[b] *Perron Institute for Neurological and Translational Science, Perth, Australia*
[c] *Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium.*
[d] *Department Physics, Chemistry & Pharmacy, University of Southern Denmark, Odense, Denmark*
[e] *Department of Biomolecular Engineering, Nagoya University, Nagoya, Japan*
[f] *School of Chemistry & Molecular Biosciences, University of Queensland, Brisbane, Australia*

Oligonucleotides-based technologies such as antisense oligonucleotides (AOs), DNAzymes and aptamers attracted significant attention in recent years. We have recently investigated the potential of novel chemically-modified AOs containing LNA, HNA, CeNA, morpholino, 2'-OMe and other chemistries to modulate RNA splicing in *mdx* mouse myotubes towards improving the therapy against Duchenne muscular dystrophy. Our results showed that all AOs induced *Dmd* exon-23 skipping [1-4]. In another study, we investigated the potential of DNAzymes to inhibit integrin alpha-4 gene transcript (*ITGA-4*) towards developing a therapeutic molecule for tackling inflammation in multiple sclerosis. Our results demonstrated that RNV143A, an arm-loop-arm type DNAzyme modified with a 3'-inverted dT efficiently inhibited *ITGA-4* RNA [5]. Recently, we have also developed a novel aptamer [6] against targeting amyloid-beta peptides. Our aptamer RNV95 efficiently detected low-molecular weight amyloid beta peptide aggregates in brain tissue samples of pathologically confirmed Alzheimer disease patients, and offer a great promise towards the diagnosis and therapy of Alzheimer disease.

This work was supported by grants from the Department of Health, Western Australia, Multiple Sclerosis Research Australia, McCusker Charitable Foundation and Perron Institute for Neurological and Translational Science.

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*Corresponding author: E-mail: r.veedu@murdoch.edu.au

# RNA hairpin-duplex conversion controlled by a reduction-responsive 2',2'-disulfide-bridged dinucleotide

Christelle Dupouy,* Florian Gauthier, Frédéric Beltran, Annabelle Biscans, Françoise Debart, Jean-Jacques Vasseur.

*IBMM, UMR 5247, CNRS, UM, ENSCM, Université de Montpellier, Campus Triolet, Place Eugène Bataillon, 34095 Montpellier, France*

Structured RNAs including RNA hairpins play critical roles in biology. Indeed, RNA hairpins are the most common secondary structures found in nucleic acids, and especially in transfer RNA (tRNA).[1] Their remarkable structure consisting of a double-stranded RNA (stem) and a loop with unpaired nucleotides has attracted great interest for diverse applications. However, their low enzymatic and thermal stabilities have limited their studies. To circumvent these drawbacks, several chemical modifications have been introduced into RNA hairpins.[2] Among these modifications, the functional thiol group plays a central role in the study of DNA and RNA folding into secondary and tertiary structures. Although several examples of disulfide (S-S) bridge introduced into the stem of an RNA hairpin have been described, no example of insertion of a S-S bridge into the loop part has been reported to date.

In the presented work, we describe the synthesis and the impact of a S-S bridge between 2'-*O*-positions of two adjacent nucleotides in an RNA duplex and in the loop of RNA hairpins. To form the dimethylene S-S bond within RNA, a thiol disulfide exchange was performed on solid support between the 2'-OH-positions of two adjacent 2'-*O*-acetylthiomethyl nucleotides.[3,4] We demonstrated that the S-S bridge was more tolerated in the center of the loop of RNA hairpins than in an RNA duplex, leading to their thermal and enzymatic stabilizations. Then, the influence of the S-S bridge was studied at the HIV-1 Dimerization Initiation Site (DIS) as an RNA sequence model. We showed that the S–S bridge locked the hairpin form, whereas the extended duplex form was generated after the reduction of the disulfide bond with glutathione (GSH). Furthermore, an application as an RNA molecular beacon was demonstrated, confirming these results (Figure 1). This anticipates a promising potential as a tool to study the folding of an RNA hairpin and as a probe for GSH detection.

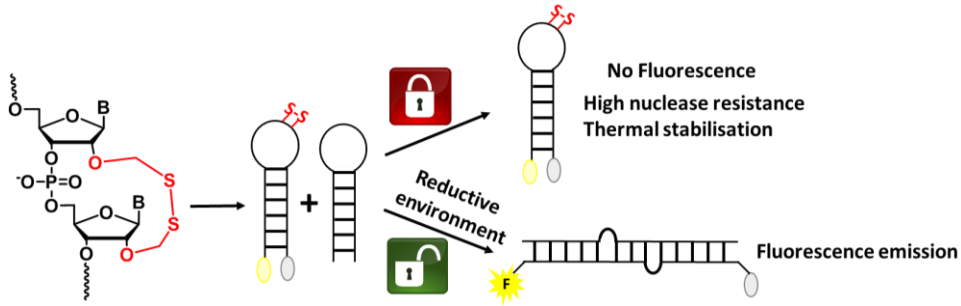


***Figure 1.*** *Application of the locked RNA hairpin by a S-S bridge in the loop as an RNA molecular beacon.*

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*Corresponding author: christelle.dupouy@umontpellier.fr

# Emissive Quadra- and Pentacyclic Adenine Derivatives

Mattias Bood,[a] Anders F. Füchtbauer,[b] Moa S. Wranne,[b] Rachel Fisher,[c] Steven W. Magennis,[c] Anita C. Jones,[d] Afaf El-Sagheer,[e] Tom Brown,[e] Anders Dahlén,[f] L. Marcus Wilhelmsson *[b], Morten Grötli *[a]

[a] *Department of Chemistry and Molecular Biology, University of Gothenburg, SE-41296 Gothenburg, Sweden.* [b] *Department of Chemistry and Chemical Engineering, Chemistry and Biochemistry, Chalmers University of Technology, SE-41296 Gothenburg, Sweden.* [c] *WestCHEM, School of Chemistry, University of Glasgow, Glasgow, G12 8QQ, UK.* [d] *School of Chemistry, University of Edinburgh, The King's Buildings, Edinburgh EH9 3JJ, UK.* [e] *Chemistry Research Laboratory, Department of Chemistry, University of Oxford, Oxford OX1 3TA, United Kingdom* [f] *AstraZeneca R&D, Gothenburg, iMED, CVRM, Pepparedsleden 1, SE-431 83 Mölndal.*

Fluorescent nucleobase analogs (FBAs) comprise an important class of probe molecules. We have developed some of the most promising, non-perturbing FBAs thus far with respect to quantum yield and brightness,[1] and recently we have focused our efforts on adenine analogs (Fig. 1).[2-3] One of these adenine analogues, qAN1, has been further developed and photophysically characterized for its use as a Förster Resonance Energy Transfer (FRET) donor in pair with $qA_{nitro}$ as an acceptor. This FRET pair has also been used in a detailed structure investigation on the DNA conformational changes upon binding of netropsin.[2]

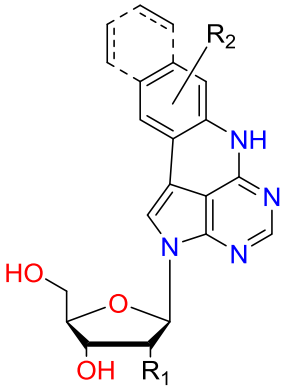


***Fig. 1.*** *Quadra- and pentacyclic adenine analogs.*

For our most recent FBA, pentacyclic adenine (pA), an unprecedented 2-photon excitation cross section for a FBA was found.[3] Furthermore, like qAN1, pA forms stable, selective base-pairs with thymine and retains the overall helical geometry of the oligonucleotide and, hence, constitutes an excellent adenine analog.

Acknowledgment: This work was supported by the Swedish Foundation for Strategic Research (SSF, grants No. ID14-0036 and IRC15-0065).

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*Corresponding authors: E-mail: marcus.wilhelmsson@chalmers.se, grotli@chem.gu.se

# Remarkable Enhancement of RNaseH Cleavage Activities of RNA Complexed with Peptide Ribonucleic Acid (PRNA) - *Novel Backbone Modification Strategy for Nuclease Cleavage Activity Improvements* -

Takehiko Wada,*[a] Masahito Inagaki,[a] Daisuke Unabara,[a] Ryohei Uematsu,[a] Hiroka Sugai,[a] Yasuyuki Araki,[a] Masaki Nishijima,[a] Satoru Ishibashi,[b] Takanori Yokota.[b]

[a] *Institute of Multidisciplinary Research for Advanced Material (IMRAM), Tohoku University, Sendai 980-8577, Japan*
[b] *Department of Neurology and Neurological Science, Tokyo Medical and Dental University, Tokyo 113-8510, Japan*

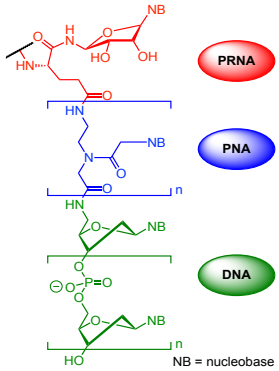


**Fig.1**PRNA-PNA-DNA Chimera

We have recently proposed a new strategy and a practical tool for cancer cell selective oligonucleotide therapeutics artificial nucleic acids, named Peptide Ribonucleic Acids (PRNAs) with active *on-off* control of functional RNA activities corresponding the cancer cell specific intracellular condition. The PRNAs can be actively *off* to *on* switching the complexation behavior with target mRNA, miRNA, and siRNA induced by lowered pH of the cancer's cytoplasm. This strategy utilizes a new category of artificial nucleic acid that carries a ribonucleoside unit tethered to a peptide backbone as a recognition and stimulus-sensitive module. In this artificial nucleic acid called PRNA, the 5'-amino-pyrimidine ribonucleoside unit, which is in the *syn* conformation in normal cellular cytoplasm condition induced by intramolecular cyclic borate ester formation with incorporated phenylborate moiety, functions as a built-in switching moiety of *syn* to *anti* nucleobase orientation switching function triggered by cancer cellular lowered pH. The results are promising, validating that the original PRNAs with *anti*-oriented nucleobases form stable complexes with the target RNA under the cancer's low cytoplasm pH (pH=ca.6.2), which are readily dissociated under normal cellular cytoplasm pH (pH = ca. 7.2). This means that the PRNA strategy can be used as a powerful tool for *on-off* switching the RNA complexation behavior, which is potentially applicable to the cancer cell selective oligonucleotide therapeutics of the next generation.[1]

Meanwhile, RNase H activities of antisense molecules would be one of the most crucial factors for practical antisense strategy. Thus, in this study, we have been designed PRNA-DNA chimeras, in which both PRNA and DNA domains work as recognition sites for the complexation with target RNAs and PRNA moieties work as recognition control/switching devices, while DNA-RNA hybrids formed in the DNA domains of the chimera should be substrates of RNase H and then target RNAs cleaved by the enzyme. (Fig. 1).

For improve a cleavage efficiency, we focused on binding mechanism of DNA/RNA duplex to RNaseH's basic binding channel and proposed chimeric neutral amide backbone of PRNA connected with negatively charged DNA's phosphate-sugar backbone (Fig. 2). In the design, the cleavage site of the target RNA should be restricted to the position of the junction site of the chimera. Fortunately, efficient and very enhanced cleavage of target RNAs compared with those with DNAs was observed for PRNA-DNA chimera/RNA complex by RNaseH. Regulation of protein synthesis by PRNA-DNA chimera was also evaluated by *in vitro* cell-free protein synthesis system, and the effective regulation was observed.

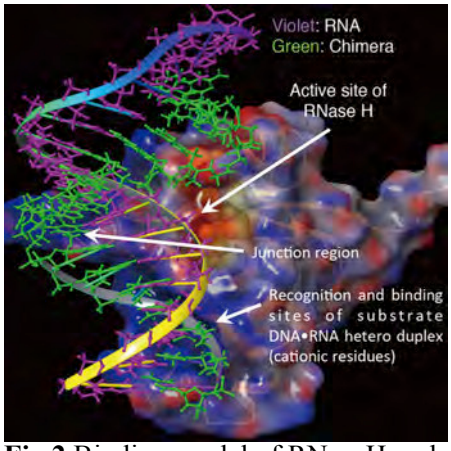


**Fig.2** Binding model of RNaseH and chimera-RNA duplex.

*Corresponding author: E-mail: hiko@tohoku.ac.jp

# Amide-modified RNA: synthesis, structure and RNAi activity

Eriks Rozners*,[a] Travis Hardcastle,[b] Irina Novosjolova,[a] Venubabu Kotikam,[a] Samwel K. Cheruiyot,[a] Daniel Mutisya,[a] Scott D. Kennedy,[c] Martin Egli,[d] Melissa L. Kelley,[b] Anja van Brabant Smith[b]

[a] *Department of Chemistry, Binghamton University, NY 13902, USA*
[b] *Dharmacon, Lafayette, CO 80026, USA*
[c] *Department of Biochemistry and Biophysics, University of Rochester, NY 14642, USA*
[d] *Department of Biochemistry, Vanderbilt University, Nashville, TN 37232, USA*

Discovery of RNA interference (RNAi) has reinvigorated interest in chemical modifications of RNA for in vivo applications. Our current work is focused on the development of novel nonionic analogues of RNA that have the phosphodiesters replaced by amide linkages (AM1 in Figure). We hypothesize that the reduced negative charge and hydrophobic nature of such modifications will not only increase the enzymatic stability, but also have the potential to optimize potency, cellular uptake, and reduce off-target effects of small interfering RNAs (siRNAs). This presentation will discuss synthesis, structure and RNAi activity of amide-modified RNA. Structural studies [1,2] show that amides are excellent mimics of the phosphodiester inter-nucleoside linkages in RNA. The local conformational changes caused by the amide linkages where accommodated easily by small adjustments in RNA structure. RNAi activity assays [2,3] show that amides are well tolerated at internal positions in both strands of siRNAs. Surprisingly, amide modifications in the middle of the guide strand and at the 5´-end of the passenger strand increased the RNAi activity compared to unmodified siRNA [3]. Most remarkably, replacement of a single phosphate linkage with an amide almost completely abolished the undesired off-target activity of the passenger strand. A crystal structure of a short amide-modified DNA-RNA hybrid in complex with RNase H shows that amide can remodel RNA-protein interactions by acting as H-bond donor, which is not possible for phosphate [3]. Our results suggest that amides are excellent mimics of phosphate backbone in RNA and may have potential to optimize biological and pharmacological properties of siRNAs. These findings are unexpected and raise the possibility that RNAi may tolerate even more substantial modifications than tried so far.

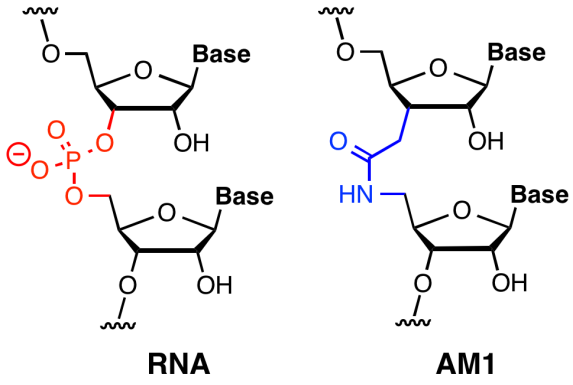


***Figure.*** *Native and amide-modified RNA.*

This work was supported by the US National Institutes of Health (R01 GM071461).

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*Corresponding author: E-mail: erozners@binghamton.edu

# Chemically Modified DNAzymes as Sequence-specific Ribonuclease-A Mimics - From Potential Therapeutics to the Origin of Life

Yajun Wang, [a] Erkai Liu, [a] Curtis Lam, [a] David M. Perrin [a] *
[a] *Chem. Dept, U. British Columbia, 2036 Main Mall, UBC Vancouver, CANADA V6T-1Z1*

For ~40 years, the discovery of a $Mg^{2+}$-independent catalyst capable of cleaving a specific mRNA target has represented a formidable intellectual challenge with implications for unmet needs in anti-mRNA therapy.[1] Yet this enduring challenge continues to demand new paradigms for sequence-specific mRNA cleavage as a powerful approach to treating diseases. While both ribozymes and DNAzymes offered great promise towards this end, few therapeutic DNAzymes (or ribozyme), have emerged. While intracellular free $Mg^{2+}$ is often too low to support catalysis by *unmodified* nucleic acid catalysts, their fundamental promise remains and presents a heightened impetus to develop modified DNAzymes to overcome this limitation. Over the past two decades, we have worked to interface chemically modified deoxynucleoside triphosphates (dNTPs) with combinatorial selection to seek DNAzymes with protein-like functionalities for catalysis in in $Mg^{2+}$-free regimes (Fig 1).[2]

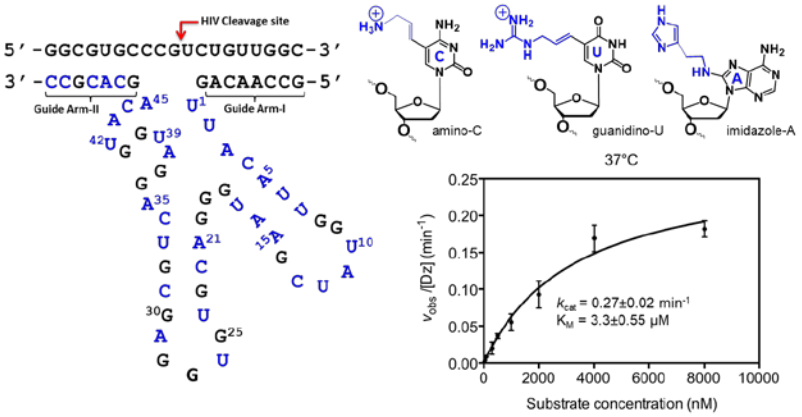


***Figure 1.*** *Densely modified DNAzymes with protein functionalities that mimic the active site of RNaseA for $Mg^{2+}$-free cleavage of specific mRNA targets:*

These exceptional catalysts display classic enzymatic properties of Michaelis-Menten kinetics in the absence of $Mg^{2+}$. In honor of the late Stanley Miller (UCSD), whose pioneering work on the origin of life included the possibility of a highly-decorated RNA world,[3] this work represents a chemist's approach to biomimicry for testing hypotheses of the origin of life in an RNA-world that must of co-opted synthetic modifications in the presence of CHO. Finally, this work underscores the use of modified dNTPS for the selection of modified aptamers.[4]

***References:*** [1] a) R. Breslow, et al, *J. Am. Chem. Soc.* **1978**, *100*, 3227-9; b) H. Lonnberg, *Org. Biomol. Chem.* **2011**, *9*, 1687-703. [2] a) D. M. Perrin, *et al. Nucl. Nucl.* **1999**, *18*, 377-91; b) L. Lermer, *et al. J. Am. Chem. Soc.* **2002**, *124*, 9960-1; c) R. Ting, *et al.*, *Nucleic Acids Res.* **2004**, *32*, 6660-72; d) M. Hollenstein *et al. ChemBioChem* **2009**, *10*, 1988-92; e) J. M. Thomas *et al. J. Am. Chem. Soc.* **2009**, *131*, 5648-58; f) Y. J. Wang, *Chem. Sci.* **2018**, *9*, 1813-21.[3] M. P. Robertson, S. L. Miller, *Science* **1995**, *268*, 702-5. [4] Y. J. Wang, et al., *Org. Biomol. Chem.* **2017**, *15*, 610-18.

*Corresponding author: E-mail: dperrin@chem.ubc.ca

# High-Throughput Platform Assay Technology for the Discovery of pre-microRNA-Selective Small Molecule Probes

Amanda L. Garner,*[ab] Daniel A. Lorenz,[b] Jorge Sandoval,[b] Ashootosh Tripathi,[a] and Tanpreet Kaur[a]

[a] *Department of Medicinal Chemistry, University of Michigan, 1600 Huron Parkway, NCRC B520, Ann Arbor, Michigan, USA*
[b] *Program in Chemical Biology, University of Michigan, 219 Washtenaw Avenue, Ann Arbor, Michigan, USA*

MicroRNAs (miRNA) are an emerging class of small RNAs that play critical roles in human development and disease. These micromanagers function at the level of post-transcriptional gene regulation, and alteration of miRNA expression levels have been implicated in many human diseases. As such, the targeting of miRNAs is considered attractive as a novel therapeutic strategy. A major bottleneck toward this goal, however, has been the identification of small molecule probes that are specific for select RNAs and methods that will facilitate such discovery efforts. Using pre-microRNAs as proof-of-concept, we have developed an innovative approach, **cat**alytic **E**nzyme-**L**inked **C**lick **C**hemistry **A**ssay or **cat-ELCCA**, for assaying RNA-small molecule and RNA-protein interactions. Using cat-ELCCA, we are working toward the discovery of new chemical space for RNA to illuminate the druggability of this under-explored nucleic acid and provide the basis for the development of next-generation, RNA-targeted small molecule therapeutics for the management of human health. Our progress toward the goals of selectively targeting Dicer-mediated pre-miRNA processing and miRNA–miRNA-binding protein interactions with small molecules and natural products will be discussed.

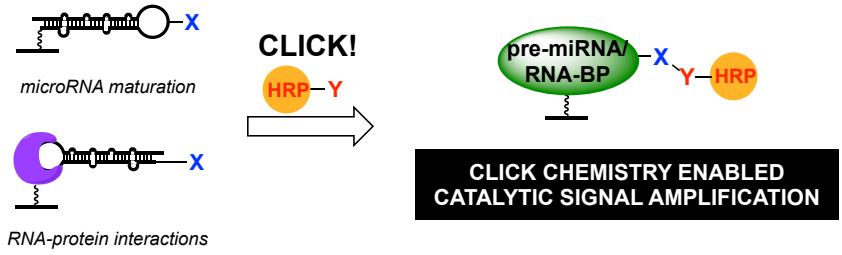


***Figure.*** *Cat-ELCCA for miRNA maturation and RNA-binding protein (RNA-BP) interactions.*

This work was supported by grants from the NIH (R01 GM118329), the Dr. Ralph and Marian Falk Medical Research Trust, and the University of Michigan Center for the Discovery of New Medicines.

***References:*** [1] Lorenz, D.A., *Bioconj. Chem.* **2015***, 26*, 19. [2] Lorenz, D.A., et al. *Chem. Commun.* **2016**, *52*, 8267. [3] Lorenz, D.A. *SLAS Disc.* **2018**, *23*, 47. [4] Lorenz, D.A., et al. *ACS Med. Chem. Lett.* **2018**, accepted. [5] Garner, A.L. *Chem. Commun.* **2018**, accepted.

*Corresponding author: E-mail: algarner@umich.edu

# Synergistic effects of DNA-like and RNA-like modifications in antisense oligonucleotides

Daniel O'Reilly [a], Xiulong Shen [b], Audrius Kilikevicius [b], Xia Xin [c], John Rossi [c], David R. Corey [b], Masad J. Damha*[a]

[a] *Department of Chemistry, McGill University, 801 Sherbrooke Street West, Canada*
[b] *Department of Pharmacology, UT Southwestern Medical Center at Dallas, 6001 Forest Park Dallas TX 75390, USA*
[c] *Beckman Research institute, City of Hope, 1500 East Duarte Road, Duarte, CA 91010*

Oligonucleotide drugs are at the forefront of modern medicine.[1] There are now 4 approved drugs with many more in clinical trials.[2] The latest of these drugs are highly modified with multiple different modifications in the same oligonucleotide.[3] Yet there is limited understanding of the inter-play between different modifications in the same oligonucleotide and the effect of that inter-play on the global structure and properties.[4]

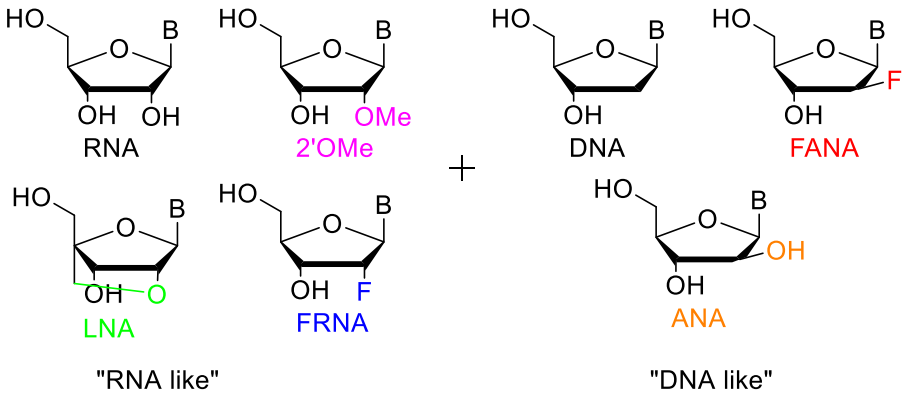


***Figure.*** *Examples of antisense oligonucleotide modifications studied for this work*

In this study we develop novel motifs for combining DNA-like modifications and RNA-like modifications. We study their structure, properties using a variety of techniques and biological functions in two different systems: Freidreich's Ataxia (upregulation) and as anti-HIV oligonucleotides (downregulation). [5]

This work was supported by grants from NSERC Canada.

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*Corresponding author: masad.damha@mcgill.ca

# Passenger strand carrying morpholino moiety at the 5′ end improves guide strand selection and RNAi activity

Rubina G. Parmar, [a] Pawan Kumar, [a] Christopher R. Brown, [a] Jennifer Willoughby, [a] Don Foster, [a] Ramesh Indrakanti, [a] Sally Schofield, [a] Vasant Jadhav, [a] Klaus Charisse, [a] Jay Nair, [a] Kallanthottathil G. Rajeev, [a] Martin A. Maier, [a] Martin Egli[b] and Muthiah Manoharan[a*]

[a] *Alnylam Pharmaceuticals, 300 Third street, Cambridge, MA 02142, USA*
[b] *Vanderbilt University, Nashville, TN 37232, USA*

Chemically modified small interfering RNAs (siRNAs) are medicines in the horizon. One of the strands of the short, double stranded siRNA is loaded into the RNA-induced silencing complex, and this so-called guide strand hybridizes to the target mRNA in a sequence-specific manner. For efficient and specific on-target activity, the loading of correct strand into RISC is critical. Thus, strategies that enhance preferential loading of the guide stand should lead to improved activity and reduced off-target effects. Nucleotides with well-characterized modifications such as 5′-*O*-methyl and several novel modifications were evaluated for their abilities to enhance guide strand loading. Among them, the 5′-moroholino group emerged as the best modification to control the strand selection (Figure 1). Here, we report synthesis of nucleoside phosphoramidites of these novel modifications. We will describe optimization of the synthesis of 5′-morpholino-bearing nucleosides and demonstrate that the presence of a morpholino at the 5′-end of the sense strand improves siRNA activity both in vitro and in vivo. Furthermore, the 5′-morpholino modification also improved metabolic stability of the siRNAs.

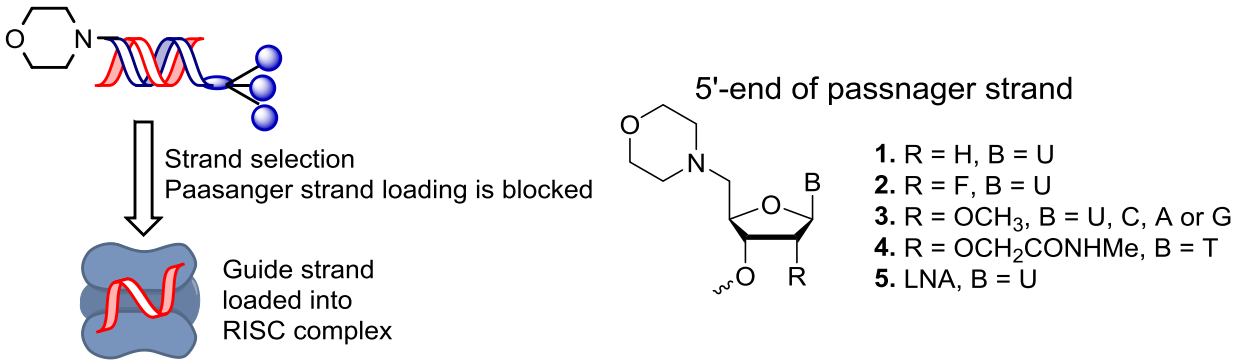



***Figure 1.*** Structure of nucleosides carrying 5′-morpholino group.

***References:*** [1] Chen, P. Y., et al. *RNA* **2008**, *234*, 2345. [2] Snead, M.N., et al. Molecular Therapy-Nucleic Acids **2013**, 2, e103 [3] Janas, M. M., Schlegel, M. K., et al. *Nature Commun.* **2018**, *9*, 723.

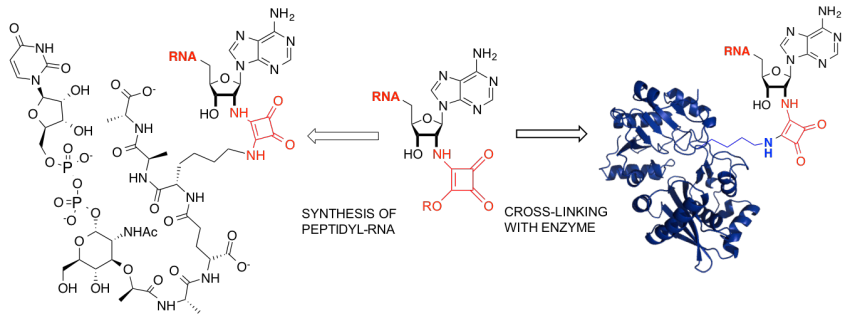
*Corresponding author: E-mail: mmanoharan@alnylam.com

# Post-functionalization of RNAs for specific conjugations with peptides and proteins

Matthieu Fonvielle[a], Nicolas Sakkas[b], Laura Iannazzo[b], Emmanuelle Braud[b], Afaf El-Sagheer[c], Tom Brown[], Michel Arthur[a] and Mélanie Etheve-Quelquejeu*[b]

[a] *Centre de Recherche des Cordeliers, Equipe 12, UMR S 1138; INSERM; Université Pierre et Marie Curie-Paris 6; Université Paris Descartes*
[b] *Laboratoire de Chimie et de Biochimie Pharmacologiques et Toxicologiques, Université Paris Descartes, UMR 8601, Paris, F-75005 France ; CNRS UMR 8601, Paris, F-75006 France*
[c] *Department of Chemistry, University of Oxford, Chemistry Research Laboratory, 12 Mansfield Road, Oxford, OX1 3TA UK*

Since few years, we develop versatile methods for the synthesis of modified aa-tRNA[1].
We report here the synthesis of electrophilic RNAs based on post-functionalization of an azido group incorporated by SPS[2]. The squaramate-RNAs specifically reacted with the primary amine of unprotected UDP-MurNAc-pentapeptide. The squaramate unit also promoted specific cross-linking of RNA to the catalytic Lys of $FemX_{Wv}$ but not to related transferases recognizing different aminoacyl-tRNAs. Thus, squaramate-RNAs provide specificity for cross-linking with defined groups in complex biomolecules due to its unique reactivity.
These modified RNAs are interesting tools to investigate in the field of RNA biology.




This project was funded by ANR (MimictRNA project). N. Sakkas was funded by a DGA-MRIS scholarship.

***References:*** [1] - a) M. Fonvielle, I. Li de la Sierra-Gallay, A. El-Sagheer, M. Lecerf, D. Patin, D. Mellal, C. Mayer, D. Blanot, N. Gale, T. Brown, H. van Tilbeurgh, M. Ethève-Quelquejeu, M. Arthur, *Angewandte Chem*., **2013**, 125, 7419-7422. b) L. Iannazzo, M. Fonvielle, E. Braud, H. Hrebabecky, E. Prochazkova, R. Nencka, C. Mathé, M. Arthur, M. Etheve-Quelquejeu, *Org. Biomol. Chem*., **2018**, 16, 1903 - 1911.
[2] - M. Fonvielle, N. Sakkas, L. Iannazzo, C. Le Fournis, D. Patin, D. Mengin-Lecreulx, A. El-Sagheer, E. Braud, S. Cardon, T. Brown, M. Arthur, M. Etheve-Quelquejeu, *Angewandte Chem*., **2016**, 55, 13553–13557.

*Corresponding author: E-mail: melanie.etheve-quelquejeu@parisdescartes.fr

# DNA and PNA programmed fusion of Lipid-Nanoreactors

*Philipp M. G. Löffler*,[a] *Oliver Ries*,[a] *Alexander Rabe*,[a] Stefan Vogel*[a]

[a]Nucleic Acid Center, Department of Physics, Chemistry and Pharmacy, University of Southern Denmark, Campusvej 55, 5230 Odense, Denmark

Chemically engineered and functionalized nanoscale compartments are used in bottom-up synthetic biology to construct compartmentalized chemical processes. Progressively more complex designs demand for spatial and temporal control over entrapped species. Here, we address this demand by a DNA-encoded design for successive fusion of multiple liposome populations. In contrast to fusion protein-dependent eukaryotic vesicle processing, this artificial fusion cascade exploits the versatile encoding-potential of DNA or PNA hybridization and is generally applicable to small and giant unilamellar vesicles.[1-3]

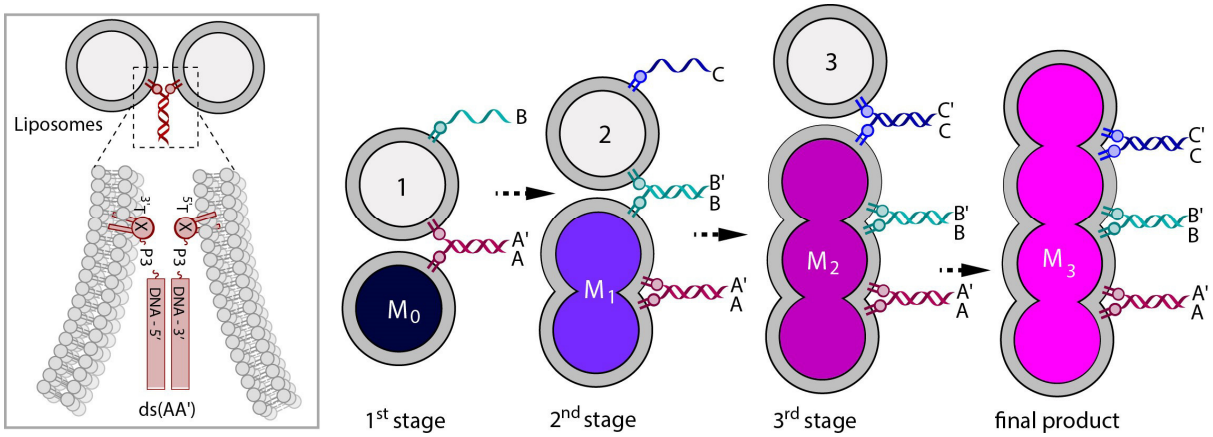



***Figure.*** *Schematic representation of a three-stage cascade of liposome fusion encoded by three sets of complementary LiNAs (lipid modified nucleic acids). Content mixing are the consequence of the zipper-like hybridization of membrane-anchored LiNAs (left insert).*

The reported liposome fusion platform is suitable for applications in the bottom-up construction and operation of nanoreactors and applicable from nanoscale (< 100 nm) to cell sized lipid particles (> 10.000 nm). In the field of synthetic biology, fusion cascades with efficient content mixing will facilitate design and construction of multi-component artificial cellular systems and protocell minimal systems. We will in addition report on research towards enzymatic and synthetic chemistry in liposomal nanoreactors.

This work was supported by grants from The VILLUM Foundation, grant no. VKR022710.

***References:*** [1] P. M. G. Löffler, O. Ries, A. Rabe, A. H. Okholm, R. P. Thomsen, J. Kjems and S. Vogel, Angew. Chem., Int. Ed., **2017**, *56*, 13228–13231. [2] Rabe A.; Löffler, P. M. G.; Vogel, S.; Chem. Comm. **2017**, *53*, 11921-11924 [3] Ries, O.; Löffler, P. M. G.; Rabe A.; Malavan, J. J.; Vogel, S.; Org. Biomol. Chem. **2017**, 15, 8936–8945.

*Corresponding author: E-mail: snv@sdu.dk

# A multiplexed isothermal amplification platform to detect emerging pathogens

Ozlem Yaren,*[a] Barry W. Alto,[b] Steven A. Benner,[a]

[a] *Foundation for Applied Molecular Evolution, Alachua, FL, USA*
[b] *Florida Medical Entomology Laboratory, University of Florida, Vero Beach, FL, USA*

Timely diagnosis of emerging infectious diseases is important to initiate patient care and take proper preventative measures for public safety in endemic regions. Current systems for clinical diagnostics are mainly based on the detection of nucleic acids by PCR. In spite of its high sensitivity, PCR-based assays require thermal cycling, and expensive instrumentation which makes their use restricted to centralized laboratories. On the other hand, development of affordable and robust point-of-care platforms for multiplexed diagnosis of infectious diseases based on isothermal nucleic acid amplification is highly desirable. Such approaches could help reduce the complexity and overall cost of the diagnostic testing, and could allow its use in low resource settings and high-risk areas.


***Figure.*** *Point-of-care diagnosis of Zika.*

Loop mediated isothermal amplification (LAMP) is a good PCR alternative due to its sensitivity and specificity. Here we present, panels of multiplexed LAMP assay where mosquito-borne (e.g. Zika, Chikungunya and Dengue), and tick-borne pathogens (causative agent of Lyme's disease, Powassan virus, etc.) can be efficiently detected within 45 minutes, without an upfront sample extraction in a range of biological samples (e.g. urine, serum, saliva and mosquito carcasses) by visualizing a fluorescence signal. Similar strategies have been applied to detect norovirus, HPV and malaria. By generating room temperature stable reagents, deployment of the assay to target areas without a chain of refrigeration has been enabled. Zika and Chikungunya detection kits have currently been tested on clinical samples in Florida Department of Health (Miami, FL), Pune (India), and Rio de Janeiro (Brazil).

This work was supported by grants Biomedical Research Program of Florida Department of Health (FDOH-7ZK15) and National Institutes of Allergy and Infectious Diseases (NIAID 1R21AI128188-01).

***References:*** [1] Yaren et al., *BMC Inf. Dis.* **2017**, *17*, 293. [2] Yaren et al., *JOVE,* **2018**, *133*, *e57051* [3] Yaren et al. *J. Virol. Methods.* **2016**, *237*, 64.

*Corresponding author: E-mail: oyaren@ffame.org

# Nucleoside antibiotics targeting bacterial peptidoglycan biosynthesis

Christian Ducho,*[a] Stefan Koppermann,[a] Kristin Leyerer,[a] Marius Wirth,[a] Patrick D. Fischer[a]

[a] *Department of Pharmacy, Pharmaceutical and Medicinal Chemistry, Saarland University, Campus C2 3, 66 123 Saarbrücken, Germany*

Several nucleoside analogues are highly valuable antiviral or anticancer drugs, but no antibacterial nucleosides are clinically established yet. At the same time, emerging resistances of bacterial strains towards established antibiotics cause an urgent need for the development of novel antibacterial agents. One approach to achieve this goal is the systematic investigation of naturally occurring antibiotics – including nucleoside derivatives – with new or previously unexploited modes of action.

Muraymycins (*e.g.* **1**-**3**, see Figure) represent a subclass of uridine-derived nucleoside antibiotics and were first isolated from *Streptomyces* sp. as a collection of 19 compounds.[1] They inhibit the bacterial membrane protein translocase I (MraY), a key enzyme in the intracellular part of peptidoglycan biosynthesis and therefore an attractive target for novel antibacterial drug candidates.[2]

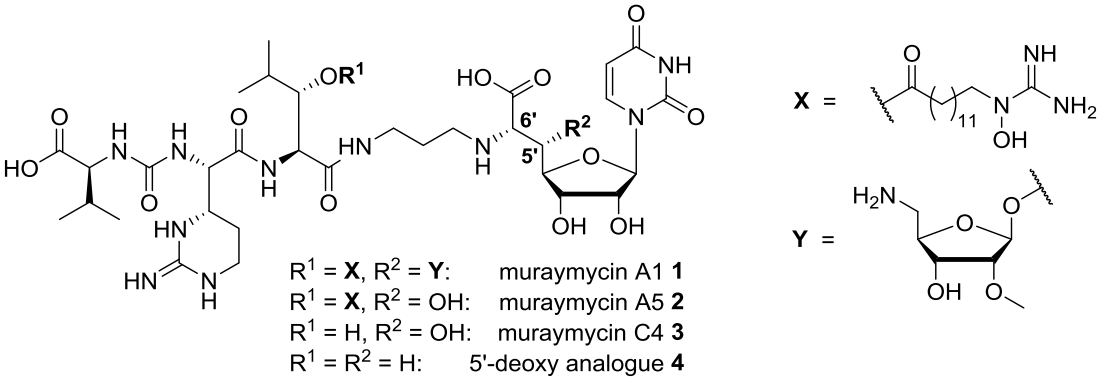



***Figure.*** *Selected naturally occurring muraymycin antibiotics* ***1***-***3*** *and synthetic analogue* ***4****.*

We have developed efficient synthetic methods for the preparation of muraymycins and their analogues, *e.g.* 5'-deoxy muraymycin C4 **4** (see Figure).[3] Using a fluorescence-based *in vitro* assay for MraY activity,[4] structure-activity relationship (SAR) data were obtained.[5] These results as well as further studies on the properties of naturally occurring muraymycins and their synthetic analogues will be presented.

This work was supported by grants from the Deutsche Forschungsgemeinschaft (DFG).

***References:*** [1] McDonald, L. A. et al., *J. Am. Chem. Soc.* **2002**, *124*, 10260. [2] Wiegmann, D. et al., *Beilstein J. Org. Chem.* **2016**, *12*, 769. [3] Spork, A. P. et al., *Chem. Eur. J.* **2014**, *20*, 15292. [4] Wohnig, S. et al., *Chem. Eur. J.* **2016**, *22*, 17813. [5] Koppermann, S. et al., *ChemMedChem* **2018**, *13*, 779.

*Corresponding author: E-mail: christian.ducho@uni-saarland.de

# Transcriptional recognition principles of naturally and synthetic modified nucleic acids: from natural biology to synthetic biology

Dong Wang *[a, b]

[a] *Division of Pharmaceutical Sciences, Skaggs School of Pharmacy & Pharmaceutical Sciences;* [b] *Department of Cellular & Molecular Medicine, School of Medicine, University of California, San Diego, La Jolla, CA 92093*

The foundation of central dogma of life relies on faithful transfer of genetic information via specific base pair recognition. Transcription is the first step of gene expression. Naturally and synthetic modified nucleic acids often changes the shape, size, and function groups of nucleobase and sugar or phosphodiester backbone. Investigation of the impacts of these DNA modifications on transcription is not only critical for us to understand how coding and decoding behaviors embedded by molecular machines are perturbed, but also provide us a platform to design novel coding and decoding behavior for synthetic biology in future.

Here we report our recent work toward understanding the transcriptional recognition of synthetic and naturally modified nucleic acids. We took a combined multidisciplinary approach including biochemistry, chemical biology, structural biology, and computational biology, to dissect the fundamental principles of nucleic acid recognition by transcriptional machinery. Finally, we will also report new efforts toward establishing a system for transcription from synthetic genomic DNA template.

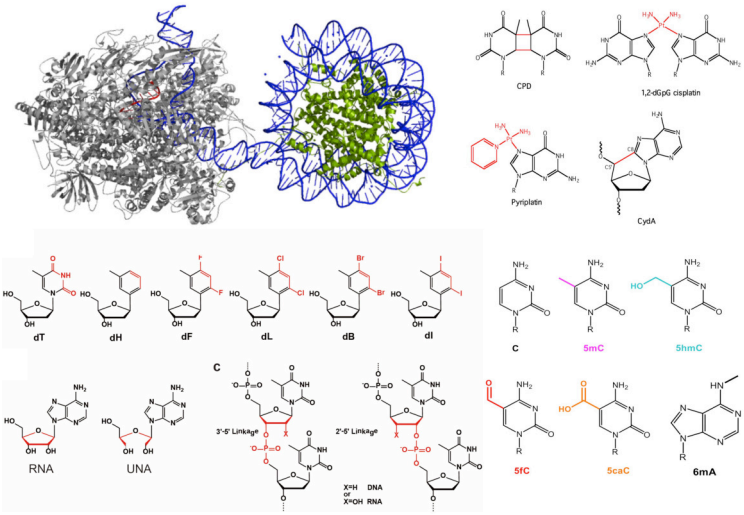



***Figure 1.*** *Structure scheme of a RNA polymerase II through nucleosome. Representative modified nucleic acids used in this work.* This work was supported by grants from GM102362.

***References:*** [1] Xu, J. et al., *Nature* **2017***, 551*, 653. [2] Wang, W. et al. *JACS* **2017***, 139*, 14436. [3] Xu, L. et al. *PNAS* **2017***, 114*, E7082. [4] Xu, L. et al. *PNAS* **2016***, 113*, 12426. [5] Wang, L. et al., *Nature* **2015***, 523*, 621. [6] Walmacq, C. et al. *PNAS* **2015***, 112*, E410. [7] Xu, L. et al. *PNAS* **2014***, 111*, E3269. [8] Xu, L. et al. *NAR* **2014***, 42*,5863. [9] Xu, L et al. *Chem. Sci.* **2014**, *5*, 567. [10] Xu, L et al. *Angew. Chem. Int. Ed.* **2013***, 52*, 12341. [11] Kellinger, M. W. et al. *JACS* **2013***, 135*, 13054. [12] Kellinger, M. W. et al. *JACS* **2012***, 134*, 8231. [13] Kellinger, M. W. et al. *Nat. Struct. Mol. Biol.* **2012***, 19*, 831.

*Corresponding author: E-mail: dongwang@ucsd.edu

# Expansion of the genetic code through the enzymatic construction of artificial metal base pairs

Marcel Hollenstein,*[a] Pascal Röthlisberger,[a] Fabienne Levi-Acobas,[a] Marie Flamme,[a] Jens Müller[b]

[a] *Laboratory for Bioorganic Chemistry of Nucleic Acids, departement of Structural Biology and Chemistry, Institut Pasteur, 28 rue du Dr Roux, 75724 Paris Cedex 15, France.*
[b] *Westfälische Wilhelms-Universität Münster, Institut für Anorganische und Analytische Chemie, Corrensstraße 28/30, 48149 Münster, Germany*

Expanding the genetic code beyond the A-T/G-C Watson-Crick canonical base pairs and 20 amino acids of natural organisms is a long standing goal in synthetic biology.[1] A reprogrammation of the genetic code can lead to the formation of nucleic acids and proteins with hitherto unknown physico-chemical and structural properties. In the field of nucleic acids, most efforts have focused on the creation of synthetic nucleotides equipped with artificial nucleobases that are capable of forming a third, unnatural base pair (UBP). Successful candidates include nucleotides bearing hydrophobic aromatic moieties and units with alternate hydrogen bond patterns.[2,3,4] Artificial metal base pairs represent a valid alternative to existing UBPs since they are fully orthogonal to the canonical systems, do not absorb near UV light or cause DNA damage, and provide DNA with metal cations which could help binding to targets or catalyze reactions. However, only few reports exist on the enzymatic formation of metal-UBPs. Here, we will present our efforts towards the enzymatic construction of artificial metal base pairs.[5] We have screened numerous nucleotide analogues bearing modified nucleobases that could serve as ligands for transition metals. We could show that some candidates mediate metal base pair formation under primer extension reactions with different polymerases. Lastly, we will also present a new strategy for the facile immobilization of DNA oligonucleotides on solid supports based on the polymerization reaction of one of our triphosphate analogues.[6]

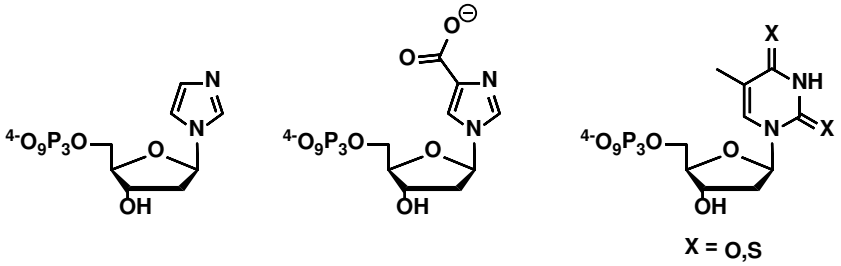



***Figure.*** *Chemical structures of some examples of nucleotides assayed for the enzymatic construction of artificial metal base pairs.*

This work was supported by Institut Pasteur.

***References:*** [1] Feldmann, A. W. et al., *Acc. Chem. Res.* **2018***, 51*, 394. [2] Zhang, Y., et al. *Nature* **2017**, *551*, 7682. [3] Kimoto, M. et al., *Nat. Biotechnol*. **2013**, *31*, 453. [4] Zhang, L. Q. et al *J. Am. Chem. Soc.* **2015**, *137*, 6734. [5] Röthlisberger, P. et al., *Org. Biomol. Chem*. **2017**, *15*, 4449. [6] Röthlisberger, P. et al., *Chem. Commun*. **2017**, *53*, 13031.

*Corresponding author: E-mail: marcel.hollenstein@pasteur.fr

# Solid-Phase Purification of Synthetic DNA Sequences

Serge L. Beaucage*[a] Andrzej Grajkowski,[a] Jacek Cieślak [a]

[a] *Laboratory of Biological Chemistry, Center for Drug Evaluation and Research, Food and Drug Administration, 10903 New Hampshire Ave., Silver Spring, MD 20993, USA*

So far, little has been done to develop high-throughput procedures for the purification of synthetic nucleic acid sequences. An efficient process for the purification of phosphorothioate and native DNA sequences is illustrated below. The process consists of functionalizing commercial aminopropylated silica gel with aminooxyalkyl entities, to enable capture of DNA sequences carrying a 5'-siloxyl ether linker with a "keto" function and/or **apurinic sites** through an oximation reaction. Shorter than full length sequences are washed away from the capture support; the full-length solid-phase-purified DNA sequence is then released from the capture support affording, after precipitation, a highly pure (ca. 98%) DNA sequence. The scalability and high-throughput features of the purification process will be presented.

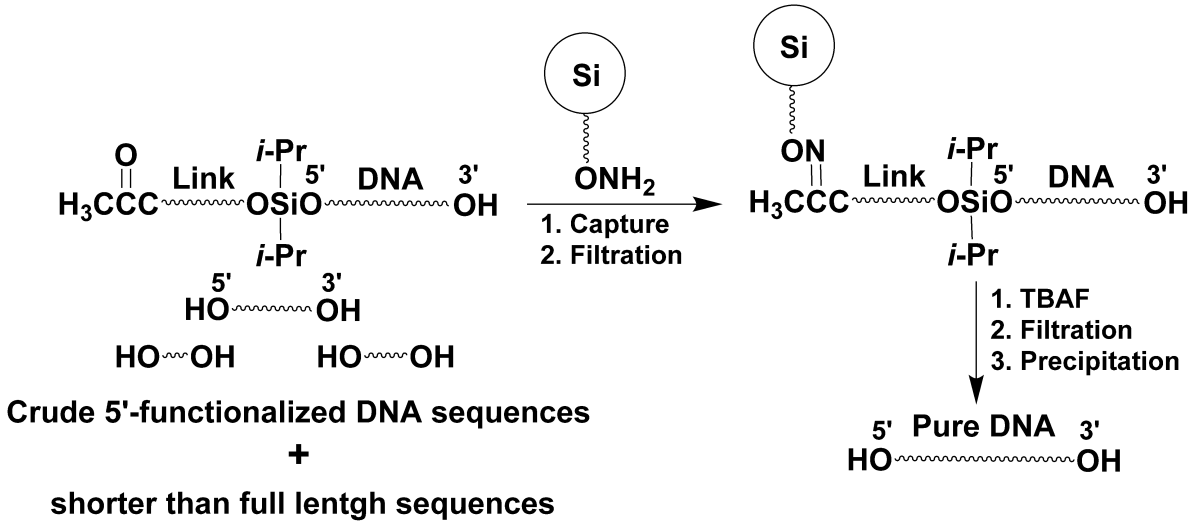



***Figure.*** *Process for the solid-phase purification of nucleic acid sequences*

This work was supported by intramural FDA funds.

***Reference:*** Grajkowski, A., Cieslak, J. and Beaucage, S. L. (**2017**). A High-Throughput Process for the Solid-Phase Purification of Synthetic DNA Sequences. In *Current Protocols in Nucleic Acid Chemistry* (M. Egli, P. Herdewijn, A. Matsuda, Y.S. Sanghvi eds.); John Wiley & Sons: Hoboken, NJ, pp.10.17.1-10.17.30 (DOI: 10.1002/cpnc.31)

*Corresponding author: E-mail: serge.beaucage@fda.hhs.gov

# Controlled in-cell activation of siRNA using bioorthogonal chemistry

Maksim Royzen,*[a] Irfan Khan,[a] Neil M. Robertson,[a] Mehmet V. Yigit*[a]

[a] *Department of Chemistry, University of Albany, 1400 Washington Ave, Albany, NY 12222, United States*

Temporal control of siRNA activation is a major challenge for RNAi-based therapeutics. The majority of the reported siRNA delivery systems rely on environmental factors, such as differences in extracellular and intracellular redox potential, ATP concentration, or pH to activate an siRNA payload. However dynamic endogenous environments are far too complex to rely on for controllable siRNA release and can result in premature siRNA activation prior to reaching the intended biological target. In addition, there are uncertainties about timing, degree and rate of the siRNA activation with spontaneous release approaches. A bioorthogonal chemistry approach to address this important challenge will be described. It is based on bond-cleaving inverse electron demand Diels-Alder reaction between *trans*-cyclooctene and tetrazine. As illustrated in the **Figure**, two major goals have been achieved: complete siRNA inactivation upon immobilization of the payload on the surface of iron oxide nanoparticles and controlled in-cell activation with the addition of a small non-toxic chemical trigger.

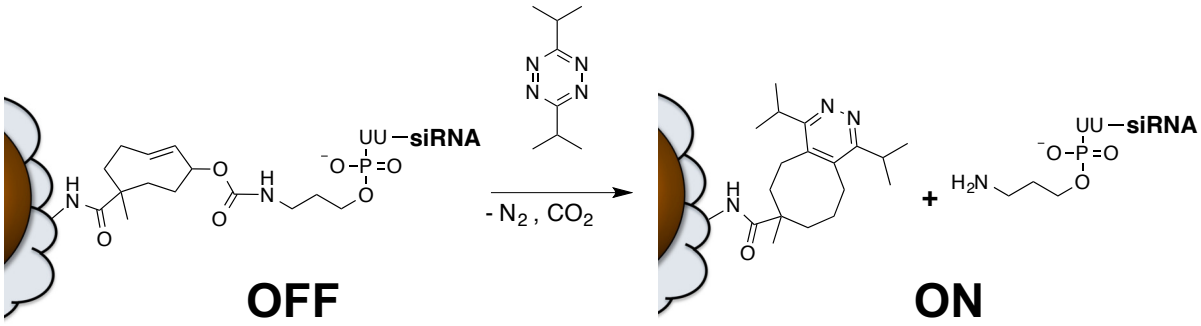



***Figure.*** *siRNA activation using bond-cleaving bioorthogonal chemistry.*

The in-cell activation approach has been demonstrated using two siRNAs against green fluorescent protein (GFP) and cyclin dependent kinase 8 (CDK8) in GFP expressing MDA- MB-231 cell line.[1,2] We anticipate that this methodology will potentially advance the clinical translation of RNAi-based therapeutics, as the described bioorthogonal chemistry can be generalized for any siRNA of choice.

This work was supported by grants from the National Science Foundation; Award# 1664577.

***References:*** [1] Khan, I. et al., *Chem. Sci.* **2017***, 8*, 5705. [2] Robertson, N. M., et al. *Nanoscale,* **2017**, *9*, 10020.

*Corresponding author: E-mail: mroyzen@albany.edu

# Efficient initiation of *in vitro* mRNA transcription with Cap 0, Cap 1 and Cap 2 oligonucleotide primers

Alexandre Lebedev[1], Dongwon Shin[1], Krist T. Azizian[1], Jordana M. Henderson[1], Christine Esau[2], Kiyoshi Tachikawa[2], Jared Davis[2], Anton P. McCaffrey[1], Richard I. Hogrefe[1] and Michael Houston[1]*

[1]*Research and Development, TriLink Biotechnologies, Maravai Life Sciences, San Diego, USA*
[2]*Arcturus Therapeutics, San Diego, USA*

We have developed a general route for *in vitro* mRNA synthesis using short oligonucleotide primers as initiators for mRNA synthesis. These primers contain all necessary elements of Cap 0, Cap 1 or Cap 2 structures. Since this approach leads directly to Cap 0, Cap1 or Cap 2 mRNAs it eliminates the need for any post-synthetic enzymatic capping to prepare fully functional capped mRNAs. The initiating capped oligonucleotide primers (ICOP), which are designed and synthesized to be fully complementary to the DNA template at the transcription initiation site, easily out-compete pppG (or any other NTPs) for initiation of synthesis of mRNA. This approach drastically reduces the synthesis of unwanted un-capped mRNAs and leads to a very high level of mRNA capping. Thus typically, with ICOP initiation, more than 95% (up to 98-99%) of mRNA are fully capped while an initiation with a traditional ARCA (Anti-Reverse Cap Analog) leads to less than 80% mRNA capping.

Since ICOPs can be synthesized with any nucleotide sequence there is a possibility to prepare mRNAs starting with any combination of purines and pyrimidines at the very 5'-end of mRNA which cannot be accomplished by using traditional *in vitro* mRNA synthesis. Also, it is possible to prepare mRNAs carrying various modification groups at 5'-end of the mRNA molecule including modified triphosphate bridge and/or modified sugars and nucleoside bases. Thus, we have synthesized mRNAs containing at 5'-end modifications: 3'-O-methylguanosine, LNA-adenosine, 2,6-diaminopurine nucleoside or N6-methyladenosine.

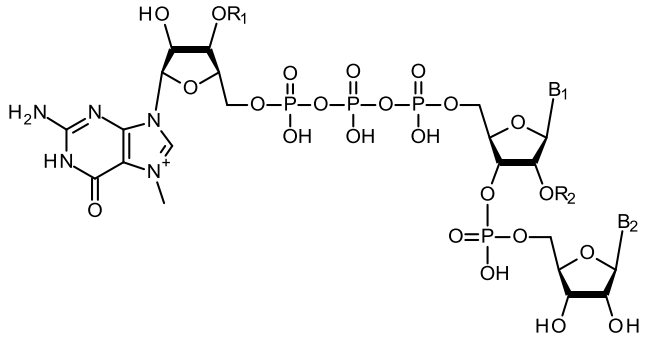


***Figure.*** *Cap 0 and Cap 1 oligonucleotide primers ($R_1$, $R_2$ = H or Me; $B_1$, $B_2$ = nucleoside base).*

In mice we have shown that *in vivo* expression from mRNA prepared with Cap 1 ($R_1$ = H, $R_2$ = Methyl, $B_1$ = adenine, $B_2$ = guanine) is much higher (> 10-fold) than expression from mRNA prepared with Cap 0 ($R_1$ = H, $R_2$ = H, $B_1$ = adenine, $B_2$ = guanine). Moreover, if Cap 1 contains N6-methyladenine in place of adenine ($R_1$ = H, $R_2$ = Methyl, $B_1$ = N6-mehyladenine, $B_2$ = guanine) its expression is increased by another 3-fold.

*Corresponding author: E-mail: MHouston@trilinkbiotech.com

# Development and application of high throughput screening methods for the identification of conformation-selective nucleic acids ligands

Jussara Amato,*[a] Chiara Platella,[b] Domenica Musumeci,[b] Ettore Novellino,[a] Bruno Pagano,[a] Antonio Randazzo,[a] Daniela Montesarchio[b]

[a] *Department of Pharmacy, University of Naples Federico II, via D. Montesano 49, 80131 Naples, Italy*
[b] *Department of Chemical Sciences, University of Naples Federico II, via Cintia 21, 80126 Naples, Italy*

The discovery of small organic molecules efficiently recognizing noncanonical DNA structures is a hot research field, particularly motivated by the need for effective anticancer agents. Indeed, structures like G-quadruplex (G4) and i-motif – whose formation in vivo has been recently demonstrated [1] – have been recognized as key elements in the regulation of cancer cell proliferation [2]. Therefore, considerable efforts are currently devoted to the discovery of novel compounds able to target them. This exceptional impulse for the search of molecules efficiently recognizing peculiar DNA structures is producing a huge number of putative ligands, for which fast and reliable screening methodologies are urgently required.
In this frame, we have recently developed a simple, cheap, and highly reproducible affinity chromatography-based method for the screening of DNA targeting compounds [3]. In this assay, the tested compounds are flowed through a resin functionalized with an oligonucleotide able to form, in proper conditions, the desired structure. Upon cation-induced control of the folding/unfolding processes of the immobilized sequence, ligands are first captured and then released depending on the used working solution. Very interestingly, this protocol allows to fully reuse the same functionalized resin batch for several experiments without loss in efficiency and reproducibility.
The proposed assay, which could be easily automated, has been successfully employed for the identification of new effective G4 ligands with biological activity [4]. Different applications of this methodology will be also discussed [5].

This work was supported by grants from the Italian Association for Cancer Research.

***References:*** [1] Biffi, G., et al. *Nat. Chem.* **2013**, *5*, 182; Zeraati, M., et al. *Nat. Chem.* **2018**, *10*, 631. [2] Brooks, T.A., et al. *FEBS J.* **2010**, *277*, 3459. [3] Musumeci, D., et al. *Anal. Chem.* **2014**, *86,* 4126. [4] Musumeci, D., et al. *Biochim. Biophys. Acta – Gen. Subj.* **2017**, 1*861,* 1341. [5] Platella, C., et al. *Anal. Chim. Acta* **2018**, doi.org/10.1016/j.aca.2018.04.071.

*Corresponding author: E-mail: jussara.amato@unina.it

# Toward RNA life: Synthesis and replication of RNA by RNA

David P Horning,[a] Katrina Tjhung,[a] Biswajit Samanta,[a] Saikat Bala,[b] John C Chaput,[b] Gerald F Joyce*[a]

[a] *Skirball Center for Chemical Biology and Proteomics, The Salk Institute, La Jolla, CA*
[b] *Department of Chemistry, University of California, Irvine, Irvine, CA*

In all extant life, genetic information is stored in nucleic acids that are replicated by polymerase proteins. In the hypothesized RNA world, prior to the evolution of genetically-encoded proteins, ancestral organisms contained RNA genes that were replicated by an RNA polymerase ribozyme. In an effort toward reconstructing RNA-based life in the laboratory, in vitro evolution was used to improve dramatically the activity and generality of an RNA polymerase ribozyme by selecting variants that can synthesize functional RNA molecules from an RNA template.[1] The improved polymerase ribozyme can synthesize a variety of complex structured RNAs, including tRNA and component fragments of itself. Furthermore, the polymerase can replicate nucleic acids, amplifying short RNA templates by more than 10,000-fold in an RNA-catalyzed form of the polymerase chain reaction (riboPCR). Thus the two prerequisites of Darwinian life — the replication of genetic information and its conversion into functional molecules — can now be accomplished with RNA in the complete absence of proteins. The polymerase can also transcribe sequences into and out of deoxyribo-,[2] threo-, or arabino- nucleic acids (DNA, TNA, and ANA), suggesting that evolutionary transitions between distinct genetic systems, including that between the RNA world to the modern DNA-based world, may have been readily accessible to RNA life.

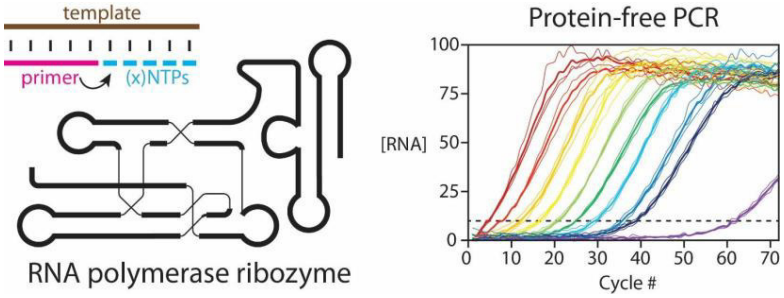



***Figure.*** *(Left) The RNA polymerase ribozyme. (Right) Quantitative riboPCR, amplifying a 20 nt template by up to 40,000 fold in the complete absence of proteins.*

This work was supported by NASA Grant NNX14AK15G and Simons Foundation Grant 287624.

***References:*** [1] Horning DP, Joyce GF (2016) Amplification of RNA by an RNA polymerase ribozyme. *Proc Natl Acad Sci USA* **113,** 9786–9791. [2] Samanta B, Joyce GF (2017) A reverse transcriptase ribozyme. *eLife* **6,** e31153.

*Corresponding author: E-mail: gjoyce@salk.edu

# Novel DNA-mimicking monomers for gapmer antisense oligonucleotides

Jesper Wengel,*[a] Mathias Bogetoft Danielsen,[a] Chenguang Lou,[a] Per Trolle Jørgensen[a]

[a] *Biomolecular Nanoscale Engineering Center, Department of Physics, Chemistry and Pharmacy, University of Southern Denmark, Campusvej 55, 5230 Odense M, Denmark*

Improved gene knock-down specificity and reduced phosphorothioate content are two important focus points for current antisense oligonucletide optimization. Based on our previous results on the RNase H compatibility of α-L-LNA and *C*-branched nucleotides in the gap region of gapmers, [1,2] we have investigated nuclease stability, RNase H activity and gene-knockdown efficiency of various gapmers. These studies have revealed the novel monomer **X**, based on a 2',3'-dideoxy-2'-fluoro-3'-*C*-hydroxymethyl-β-D-lyxofuranose scaffold, as a promising constituent of gapmer antisense oligonucleotides.

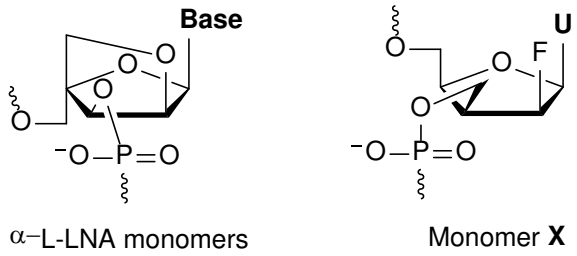



***Figure.*** *DNA-mimicking nucleotides.*

This work was supported by grants from the VILLUM FONDEN.

***References:*** [1] Sørensen, M. D., et al., *J. Am. Chem. Soc.* **2002***, 124*, 2164. [2] Vester, B., et al. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2296.

*Corresponding author: E-mail: jwe@sdu.dk

# Glycosylation of model proto-RNA nucleobases with various sugars: Implications for the prebiotic synthesis of nucleosides

David M. Fialho,[a] Gary B. Schuster,[a] Ramanarayanan Krishnamurthy[b] and Nicholas V. Hud*[a]

[a] *School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA, USA*
[b] *Department of Chemistry, The Scripps Research Institute, La Jolla, CA, USA*

The RNA World hypothesis, in most interpretations, suggests that RNA arose spontaneously on the prebiotic Earth. Such a spontaneous generation of RNA is debatable, and an ongoing goal of origins researchers. We are investigating the hypothesis that RNA evolved from an earlier "proto-RNA," a polymer that readily assembled from alternative building blocks present on the prebiotic Earth. As part of these investigations, we have found that 2,4,6-triaminopyrimidine (TAP), melamine, and barbituric acid readily form glycosides with ribose in model prebiotic reactions [1, 2].

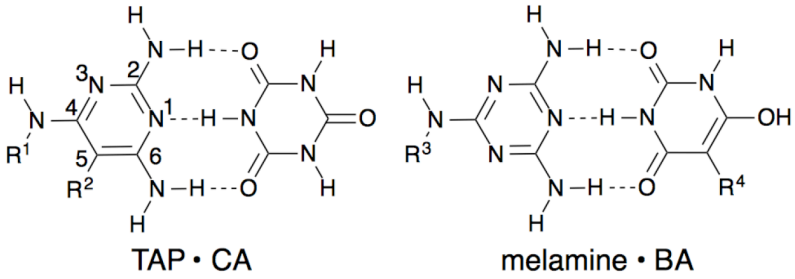



***Figure.*** *Chemical structures of two proposed proto-RNA base pairs composed of 2,4,6-triaminopyrimidine (TAP), cyanuric acid (CA), melamine, and barbituric acid (BA). $R^1$ through $R^4$ on TAP, melamine, and BA indicate positions where glycosylation has been demonstrated in model prebiotic reactions [1-3].*

We have also found that TAP is glycosylated in water by a wide variety of sugars in yields comparable to those observed with ribose [3]. Structural characterization of some of these TAP glycosides reveal constitutional and configurational similarities with extant nucleosides, suggesting that these common structural features may be prebiotic in origin. Altogether, our results suggest that prebiotic nucleoside formation would not have been restricted to ribose if proto-RNA utilized TAP and bases with similar reactivity, and that the ability to form higher-order structures may have influenced early proto-RNA monomer selection.

This work was supported by the NSF and the NASA Astrobiology Program, under the NSF Center for Chemical Evolution (CHE-1504217).

***References:*** [1] Chen, M. C., et al., *J. Am. Chem. Soc.* **2014**, *136*, 5640. [2] Cafferty, B. J., et al., *Nature Commun.* **2016**, *7*, DOI: 10.1038/ncomms11328. [3] Fialho, D. M., et al., *Org. Biomol. Chem.* **2018**, *16*, DOI: 10.1039/c7ob03017g

*Corresponding author E-mail: hud@chemistry.gatech.edu

# Structural Basis for TNA Synthesis by an Evolved TNA Polymerase

Nicholas Chim,[a] Changhua Shi,[a] Sujay P. Sau,[a] Ali Nikoomanzar,[a] and John C. Chaput*[a]

[a] *Department of Pharmaceutical Sciences, University of California, Irvine, CA., 92697 USA*

Darwinian evolution experiments carried out on xeno-nucleic acid (XNA) polymers require engineered polymerases that can faithfully and efficiently copy genetic information back and forth between DNA and XNA. However, current XNA polymerases function with inferior activity relative to their natural counterparts. Here, I discuss five X-ray crystal structures that illustrate the pathway by which α-L-threofuranosyl nucleic acid (TNA) triphosphates are selected and extended in a template-dependent manner using a laboratory evolved polymerase known as Kod-RI. Structural comparison of the apo, binary, open and closed ternary, and translocated product details an ensemble of interactions and conformational changes required to promote TNA synthesis. Close inspection of the active site in the closed ternary structure reveals a sub-optimal binding geometry that explains the slow rate of catalysis. This key piece of information provides a framework for engineering new TNA polymerase variants.

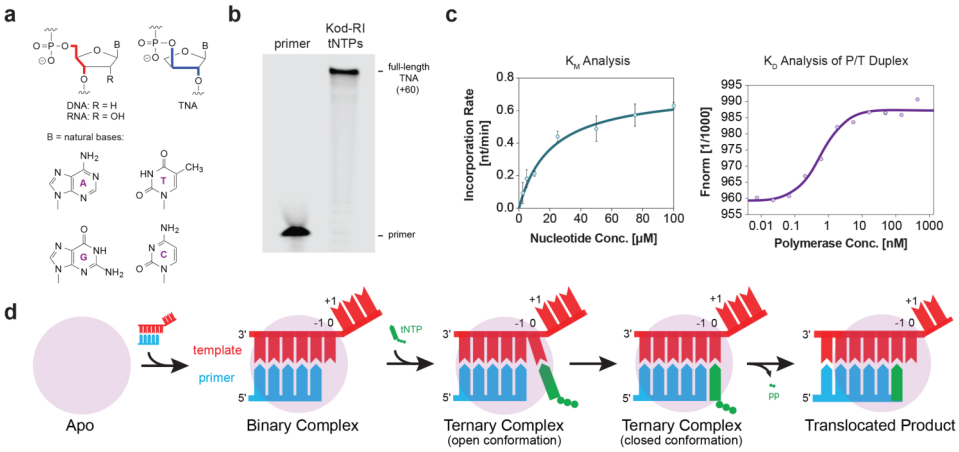



***Figure.*** *TNA synthesis by the engineered TNA polymerase Kod-RI.*

Analysis of the TNA synthesis pathway provides critical insights into the constraints of a laboratory-evolved polymerase. The combined approach of directed evolution and structure determination provides important clues that can be used to guide the development of future XNA polymerases. Such efforts open the door to a vast new world of synthetic genetics, where XNA polymers are used to create new tools for biotechnology and medicine.

This work was supported by DARPA (N66001-16-2-4061) and the NSF (1607111).

***References:*** [1] Chim, N.; Shi, C.; Sau, S. P.; Nikoomanzar, A.; Chaput, J. C. *Nat. Commun.* **2017,** *8*, 1810.

*Corresponding author: E-mail: jchaput@uci.edu

# L-DNA tetrahedron: a tumor-targeted drug delivery platform

Kyoung-Ran Kim[a] and Dae-Ro Ahn[a,b,*]

[a] *Center for Theragnosis, Korea Institute of Science and Technology (KIST), Hwarangno 14-gil 5, Seongbuk, Seoul 02792, Republic of Korea*
[b] *Division of BioMed Science and Technology, KIST School, Korea University of Science and Tehcnology (UST) Hwarangno 14-gil 5, Seongbuk, Seoul 02792, Republic of Korea*

DNA tetrahedron (Td) has been considered as one of the most practical DNA nanoconstructs due to its convenient preparation and cost-effectiveness. The previous study by Tuberfield *et al.* demonstrating cellular uptake of Td into mammalian cells has indeed provided a great opportunity for Td to play important roles in biomedical applications.[1] In this context, we recently employed the DNA nanostructure as a carrier for an efficient delivery of bioactive molecules with diagnostic and therapeutic functions.[2-4] As a continuing work in accordance with the previous efforts, we here present self-assembled tetrahedron prepared with enantiomeric L-DNA (L-Td) and report a new finding that L-Td shows tumor-specific uptake without sacrificing the biocompatibility of natural DNA nanoconstructs. They could penetrate into tumor tissue, enhance intracellular delivery of the drug, and release it at the target tissue. We also applied these useful properties of L-Td for delivery of anticancer molecules such as doxorubicin (Dox) and caspase-3 enzyme (CASP3) exclusively into tumor tissue, thereby achieving a tumor-specific therapy.[5-6]

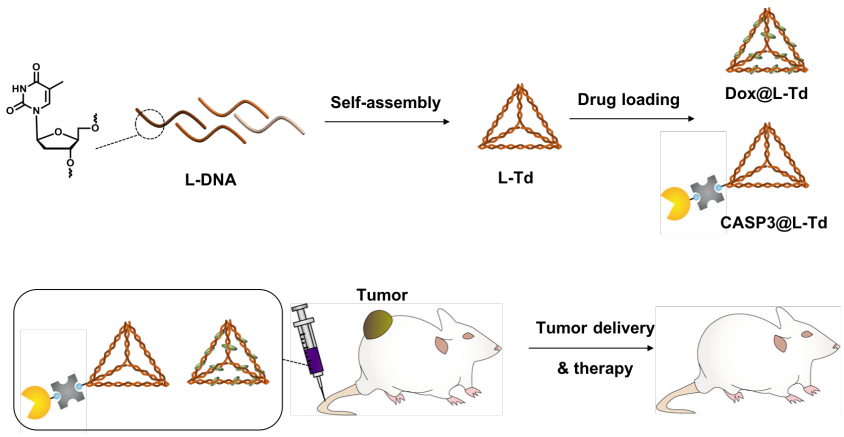



***Figure.*** *Tumor-targeted drug delivery using L-Td as a carrier*

This work was supported by grants from National Research Foundation (NRF) of Korea.

***References:*** [1] Tumberfield, A., et al., *ACS Nano.* **2011**, *5*, 5427. [2] Kim, K.-R., et al., *Chem. Commun.,* **2013**, *49,* 2010. [3] Kim, K.-R., et al., *Biomaterials,* **2013**, *34,* 5226. [4] Kim, K.-R., et al., *Chem. Sci.,* **2014**, *5,* 1533. [5] Kim, K.-R., et al., *J. Control. Release,* **2016**, *243,* 121. [6] Kim, K.-R., et al., *J. Control. Release,* **2018**, *280,* 1.

*Corresponding author: E-mail: drahn@kist.re.kr

# GalNAc conjugated LNA oligonucleotide for HBV targeting

Jacob Ravn,[a] Søren Ottesen,[a] Hassan Javanbakht,[b] Henrik Mueller,[b] Nanna Albaek*[a]

[a] *Roche Pharma Research and Early Development, Roche Innovation Center Copenhagen*
[b] *Roche Pharma Research and Early Development, Roche Innovation Center Basel*

Targeted delivery of single stranded oligonucleotides (SSO) to liver has been extensively explored in the past years and is now a well established technology platform.[1] The conjugation of an oligonucleotide to a cluster of three N-acetylgalactosamine (GalNAc)-moieties that direct specific binding to the asialoglycoprotein receptor (ASGPR) expressed specifically on the surface of hepatocytes has shown remarked increase in potency compared to unconjugated SSO. We have used this liver targeting technology platform to address the high unmet medical need of chronic hepatitis B infection (CHB) by developing liver-targeted therapeutics for CHB based on the Locked Nucleic Acid (LNA) platform.[2] We designed GalNAc-LNA-SSO to target HBV transcripts for RNase H-mediated degradation and found that the GalNAc-conjugated LNA-SSO show a remarkably higher level of potency when tested in the AAV-HBV mouse model as compared with its non-conjugated counterpart.

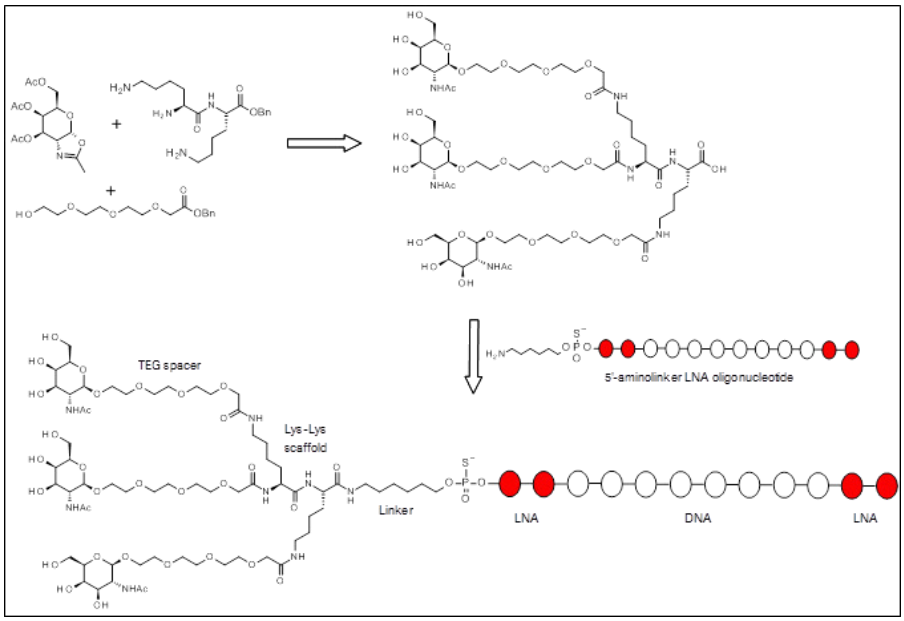



***Figure:*** *Synthesis of GalNAc conjugated LNA oligonucleotides.*

***References:*** [1] Molecular Therapy: Nucleic acids, **2017**, *6*, 116-132. [2] Molecular Therapy: Nucleic acids, **2018**, *11*, 441-454.

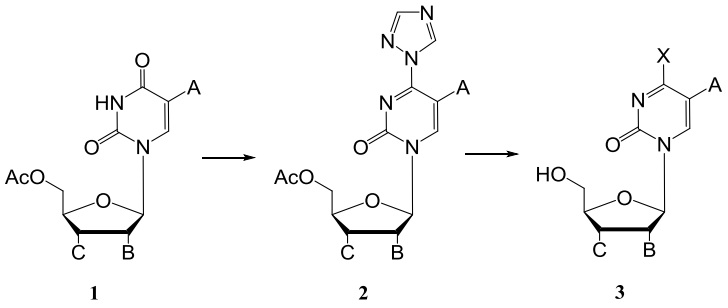
*Corresponding author: E-mail: nanna.albaek@roche.com

# NOVEL 4-MODIFIED PYRIMIDINE NUCLEOSIDES AS POTENTIAL ANTIBACTERIAL AGENTS

Liudmila A. Alexandrova[1,#] Sergey D. Negrya[1], Pavel N. Solyev[1], Andrei P. Solodinin[1], Daria P. Kolonitskaya[1], Aleksey K. Grebennikov[1], Inna L. Karpenko[1], Sergey A. Surzhikov[1], Maxim V. Jasko[1], Olga V. Efremenkova[2], and Sergey N. Kochetkov[1]

[a] Engelhardt Institute of Molecular Biology RAS, 32 Vavilov st., Moscow 119991, Russia
[b] Gause Institute of New Antibiotics, 11 Bol'shaya Pirogovskaya st., Moscow, 119021 Russia

The continued emergence of drug-resistance to existing antibacterial agents represents a severe and ongoing public health concern, which demands the discovery of new antibiotics. However the number of novel classes of antibacterial drugs launched in the clinic has been remarkably slow since the 1960s, and it is urgent to develop novel antibacterial agents to fight against drug-resistant bacterial pathogens [1]. Nucleoside analogues play an important role in medicine as antiviral agents [2], but their *in vitro* antimycobacterial activity has been reported only in the XXI century [3]. The goal of this work was the synthesis of a set of new 4-modified pyrimidine nucleoside derivatives bearing extended $N^4$-alkyl, $N^4$-oxyalkyl or $O^4$-alkyl groups (**3**), with additional modifications in the sugar moiety as potential microorganism growth inhibitors and evaluation of their stability in human blood serum, cytotoxicity and antibacterial activity towards a wide range of microorganisms.




**1, 2**: A = H, $CH_3$; B = H, OAc; C = OAc, $N_3$,
**3:** X = $NHC_nH_{2n+1}$, $NHC_nH_{2n}C_6H_5$, $HNOC_nH_{2n+1}$, $OC_nH_{2n+1}$; A = H, $CH_3$; B = H, OH; C = OH, $N_3$

Most of the compounds showed low cytotoxicity in K562, *Jurkat* and *Vero* cell cultures. The antimicrobial activity to be reported. According to the preliminary data a significant part of the synthesized compounds effectively inhibited the growth of a set of Gram-positive bacteria (including *Mycobacterium smegmatis*, drug-resistant strains of *Staphylococcus aureus, Mycrococcus luteus,* and/or *Leuconostoc mesenteroides)*.

*Acknowledgment: The study of antibacterial activity and cytotoxicity and physicochemical analysis of all compounds were supported by the Russian Science Foundation (grant No. 14-50-00060). Chemical synthesis was supported by the Russian Foundation for Basic Research (grant No. 17-04-00536).*

***References***

[1] Ventola, C.L. *Pharmacy & Therapeutics* **2015**, *40,* 277. [2] De Clercq, E. *Curr. Opin. Virol.* **2012**, 572. [3] Shmalenyuk, E.R. et al. *Russian Chem. Rev.* **2013**, *82*, 896.

[#] Corresponding author e-mail: ala2004_07@mail.ru

# Development of DNA based sensors using Linear Dichroism Spectroscopy.

Aysha Ali,[a] Haydn Little,[b] Timothy Dafforn,[b] James H.R. Tucker*[a]

[a] *School of Chemistry, University of Birmingham, Birmingham, England*
[b] *School of Biosciences, University of Birmingham, Birmingham, England*

Current established methods for biomolecular detection have long relied on the use of antibody based detection systems. These assay systems are predominantly heterogeneous and can be complicated, time consuming and costly. Here we present a novel system that combines oligonucleotide-conjugated M13 bacteriophage (M13) probes (fig. 1) and linear dichroism (LD) spectroscopy as a new diagnostic platform.[1] This approach allows the user to choose specific sequences that are required for the diagnostic target, be it a small molecule, a protein or a complementary sequence of DNA.[2]

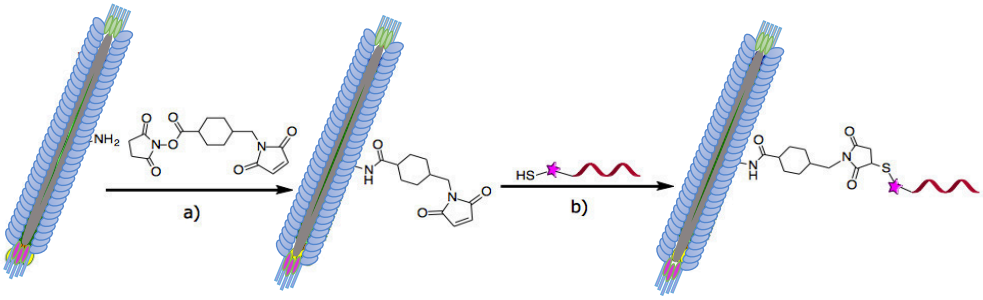



***Figure 1.*** *The bio-conjugation of an oligonucleotide to M13 in a two step reaction involving a) The NHS-ester group on the crosslinker reacting with a $NH_2$ group on the P8 protein of the M13 and b) a thiol group on the oligonucleotide reacting with the maleamide group on the same crosslinker.*

Recently we reported a PCR-based method using this new approach for the detection of the ampicillin resistance gene pBR322 (ampR).[3] Here we present a nucleic acid based assay approach for the detection of potato virus DNA targets with picomolar sensitivity.

This work is supported by grants from EPSRC.

***References:*** [1] Norden, B. et al., *Linear Dichroism and Circular Dichroism*, *RSC.* **2010.** [2] Little, H. A., PhD Thesis, *University of Birmingham*, **2016**. [3] Carr-Smith, J. et al., *ACS Synth Biol.* **2015**, *4*, 1316-1325.

*Corresponding author: E-mail: J.Tucker@bham.ac.uk

# Novel DNA aptamer based transmembrane molecules for biosensing with liposomes

Francia Allabush,*[ab] James H. R. Tucker,[a] Paula M. Mendes[b]

[a] *School of Chemistry, University of Birmingham, UK, B15 2TT*
[b] *School of Chemical Engineering, University of Birmingham, UK, B15 2TT*

DNA-lipid conjugates are comprised of either steroidal or long hydrocarbon units which anchor the molecule into liposomes by partial insertion into the lipid bilayer. These platforms are often utilized in the detection of ultra-low concentrations of nucleic acids.[1-3] Here, we report an unprecedented example of a DNA-lipid conjugate containing two cholesterol units, designed to anchor the system across the whole bilayer, providing more stabilization to the supramolecular structure (Figure 1).
We describe the design and synthesis of the bischolesterol molecule, with click chemistry and phosphoramidite coupling techniques employed to connect it to aptameric sequences of DNA in solution and on solid supports *via* automated synthesis. Cy3 and Cy5 fluorophores are also attached as signaling components for the system. The resulting DNA-bischolesterol and DNA-bischolesterol-dye conjugates were purified by reversed phased HPLC and characterized by mass spectrometry. The constructs afforded were shown to bind to target molecules as well as complementary sequences, highlighting their potential for use in biosensing with liposomes.

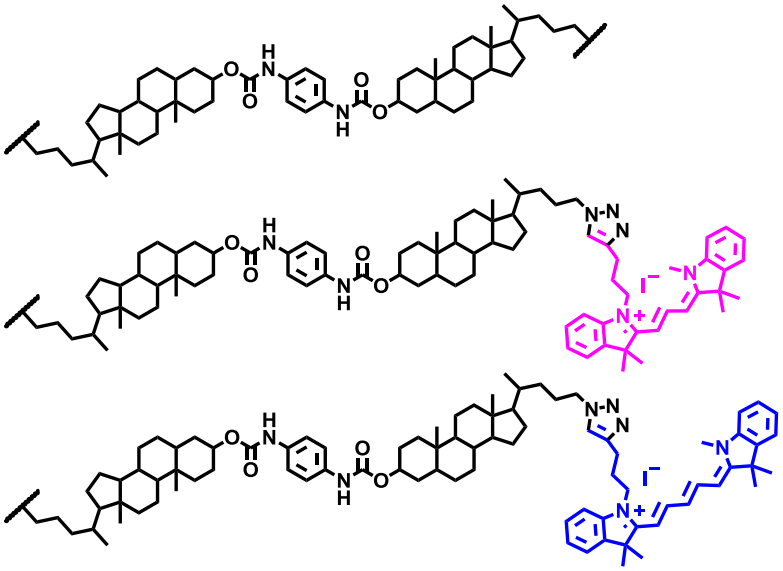


***Figure 1*** *Chemical structure of bischolesterol and bischolesterol-dye tags*

This work is supported by grants from the EPSRC, ERC, and the University of Birmingham.

***References:*** [1] Patwa, A., *et al*. *Chem. Soc. Rev.* **2011**, *40*, 5844. [2] Raouane, M., *et al*. *Bioconjugate Chem.*, **2012**, *23*, 1091. [3] Gissot, A., *et al*. *NewJ. Chem*., **2014**, *38*, 5129.

*E-mail: fxa499@bham.ac.uk

# Discovery of CC-2850, a 2'-Bromo,2'-Fluoro Ribonucleotide Prodrug with Potent Pan-genotypic Activity against Hepatitis C Virus Replication in Culture

Franck Amblard,[a] Seema Mengshetti,[a] Longhu Zhou,[a] Ozkan Sari,[a] Coralie De Schutter,[a] Hongwang Zhang,[a] Jong Hyun Cho,[a] Sijia Tao,[a] Reuben Ovadia,[a] Mahesh Kasthruri,[a] Olivia Ollinger Russell,[a] Tamara McBrayer,[b] Steven J. Coats,[b] Raymond F. Schinazi[a*a]

[a] *Center for AIDS Research, Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine, Atlanta, USA.*
[b] *Cocrystal Pharma Inc., 1860 Montreal Road, Tucker, GA 30084, USA.*

**Introduction:** Hepatitis C virus (HCV) presents a global health problem with approximately 71 million individuals infected worldwide with 80% of those progressing to chronic HCV infection. Of those chronically infected individuals, approximately 30% will develop liver cirrhosis and 10% will go on to develop hepatocellular carcinoma. Today, the most widely used drug regimens, Epclusa and Vosevi, contain the nucleoside analog Sofosbuvir (SOF). In general, pan-genotypic nucleoside HCV inhibitors display a high genetic barrier to drug resistance and are the preferred direct acting agents in the pursuit to achieve 100% sustained virologic response. Compared with previous treatments, SOF-based regimens provide a durable cure rate along with fewer side effects and they can be used successfully in fibrotic patients. However, there is still an unmet need to develop novel pan-genotypic and more efficacious nucleoside analogs which could lead to new short to ultra-short combination therapies with improved safety profiles and higher barrier to resistance. Herein, we report, the discovery of CC-2850, a β-D-2'-Br,2'-F-uridine phosphoramidate nucleotide, as a pan-genotypic, non-toxic anti-HCV agent.

**Results**: CC-2850 demonstrated specificity for HCV and showed excellent pan-genotypic activity with anti-HCV replicon activity similar to SOF. Levels of active 5'-triphosphate metabolites from CC-2850 were higher than that from SOF in huh7 cells. Its 5'-triphosphate was also a specific inhibitor of HCV NS5B polymerase and was poorly incorporated by cellular mitochondrial RNA polymerase. CC-2850 displayed low mitochondrial toxicity (MtDNA, nuclear DNA) and low effects on lactic acid levels and bone marrow toxicities similar to SOF. There was no apparent cytotoxicity to human lymphocytes, CEM, Vero, Huh-7, cardiomyocyte and HK-2 cells up to 100 µM. Mini AMES test were negative against 5 bacterial strains tested. CC-2850 was stable in dog and human plasma for up to 2 h, rapidly metabolized in human liver microsomes and showed moderate to low metabolism in human intestinal microsomes. CC-2850 and SOF show similar egress profiles in PHH. Preliminary pharmacokinetics studies in male beagles showed high levels of nucleoside 5'-triphosphate formation in liver tissue along with a high hepatic extraction ratio.

**Conclusion:** This novel nucleotide analog has a desirable profile including pan genotypic activity with no apparent cytotoxicity and an excellent preclinical profile suggesting further development towards the clinic.

*Corresponding author: E-mail: rschina@emory.edu

# $^{19}$F NMR-probes for the detection of metal-mediated base pairs

Asmo Aro-Heinilä,*[a] Tuomas Lönnberg,[a] and Pasi Virta[a]

[a] *Department of Chemistry, University of Turku, Vatselankatu 2, 20540 Turku, Finland*

Short non-coding RNAs and other structurally similar viral RNAs are important targets for the drug development. Non-coding RNAs play important roles in cellular homeostasis and are involved in many human diseases including cancer.[1] One promising approach for the improved targeting of those structures is metallo-oligonucleotides, where one or more nucleosides are replaced with metallated nucleobase analogues. In those oligonucleotides, metal-ions increase stability of the double-helical construct.[2] In our study the understanding of these metal-mediated interactions is expanded by $^{19}$F NMR using a dual-functional $^{19}$F-modified nucleotide that is capable for the metal-mediated base pairing. Detailed information may be gained by monitoring of chemical shift of fluorine, which is sensitive to electronic and environmental changes of the probe.

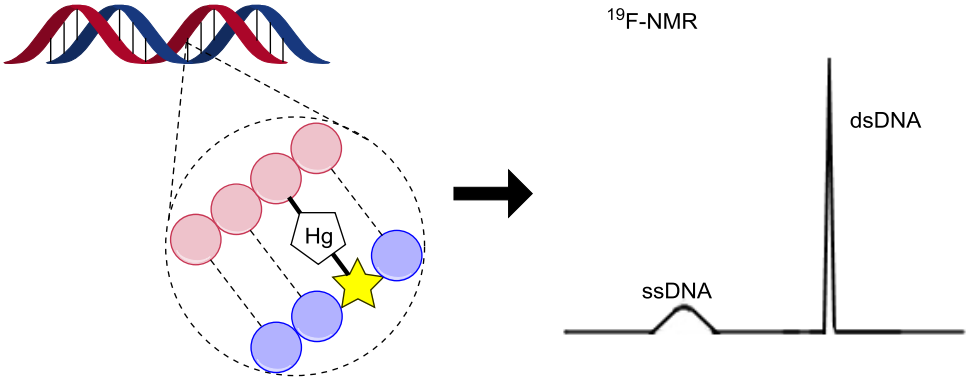



***Figure.*** *Principle of the dual functional $^{19}$F NMR/metallonucleoside analogue.*

The dual functional $^{19}$F NMR probe was incorporated into an ON and successfully mercurated. Titrations of the modified ON with complementary strands (where only opposite nucleobase varies) caused electronic and environmental changes of the modified nucleotide and consequently fluorine signal shifts. The melting temperature measurements showed that hybridization efficiency with appropriate complementary strands increased due the incorporation of mercury to the modified nucleoside.

This work was supported by Graduate School of University of Turku (UTU GS).

***References:*** [1] Sharp, P. A., Cell **2009**, 136, 577. [2] Ukale, D. et al. *Chem. Eur. J.* **2016**, *22*, 7917.

*Corresponding author: E-mail: ajmaro@utu.fi

# Selective DNA recognition by Imidazole/Pyrrole-based cyclic Oligopeptides

Sefan Asamitsu,[a] Toshikazu Bando,[a] Hiroshi Sugiyama*[ab]

[a] *Department of of Chemistry, Graduate School of Science Kyoto University, Kitashirakawa-Oiwakecho, Sakyo, Kyoto, 606-8502, Japan*
[b] *Institute for Integrated Cell-Material Science (WPI-iCeMS) Kyoto University, Sakyo, Kyoto 606-8501, Japan*

Controls of gene expressions by synthetic DNA-binding molecules have been extensively studied toward potential drugs or chemical biological tools from the past three decades.[1] Particularly, the selective targeting for certain genes is the issue to be addressed. Here we report synthetic Imidazole/Pyrrole-based cyclic oligopeptides that are capable for topology- or sequence-selectively recognizing DNAs and suppress gene expressions. Imidazole-based Cyclic Oligopeptide (**ICO**) was shown to bind to a high order DNA structrure, named G-quadruplex (G4) with a negligible binding affinity to a duplex DNA.[2] Furthermore, hybrid molecules in which a hairpin Pyrrole Imidazole Polyamide (hPIP) was attached to **ICO**, exhibited a high selectivity to a particular G4 among many types of G4s.[3] Conversely, Pyrrole-based Cyclic Oligopeptide (**PCO**) was found to both a duplex and G4 DNA. Interestingly, some **PCO** displayed the preference to a certain sequence over the others upon the binding to a duplex DNA and attenuated a specific promoter activity.

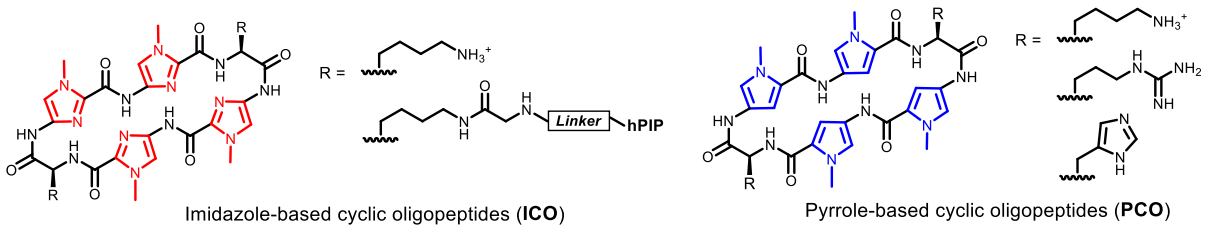



***Figure.*** *Imidazole/pyrrole-based cyclic oligopeptides.*

Collectively, a series of Imidazole/Pyrrole-based oligopeptides showed distinct and exceptional binding properties, and have the potential to be a versatile toolbox in the field of chemical biology.

This work was supported by JSPS KAKENHI (grant number 16H06356 to H.S. and 17J01932 to S.A.).

***References:*** [1] Dervan, P. B.; Edelson, B. S. *Curr. Opin. Struct. Biol.* **2003**, *13*, 284. [2] Asamitsu, S.; Li, Y.; Bando, T.; Sugiyama, H. *ChemBioChem* **2016**, 17, 1317. [3] Asamitsu, S.; Obata, S.; Phan, A. T.; Hashiya, K.; Bando, T.; Sugiyama, H. *Chem.–Eur. J.* **2018**, *24*, 4428.

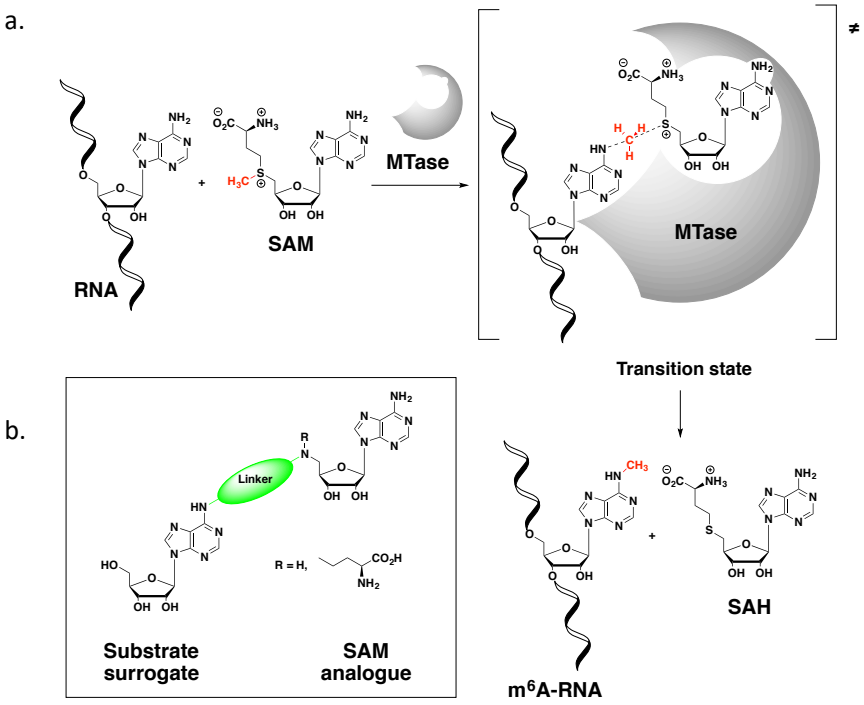
*Hiroshi Sugiyama: E-mail: hs@kuchem.kyoto-u.ac.jp

# Synthesis of SAM-adenosine conjugates for the study of m$^6$A-RNA methyltransferases.

Colette Atdjian,[a] Laura Iannazzo,[a] Emmanuelle Braud,[a] Mélanie Éthève-Quelquejeu*[a]

[a] *Laboratoire de Chimie et de Biochimie Pharmacologiques et Toxicologiques, Team "Chemistry of RNAs, nucleosides, peptides and heterocycles" Université Paris Descartes, CNRS UMR 8601, 45 rue des saints-pères,75006, Paris, France.*

RNA methyltransferases (RNMTs) catalyze the methylation of RNA using S-adenosyl-L-methionine (SAM) as methyl-donor (Scheme a). Methylation at the N-6 position of adenosine is the most abundant modification found in nearly all classes of RNAs[1] and contributes to the regulation of many biological processes in the three domains of life.[2] However, this family of enzymes remains relatively unexplored by the medicinal chemistry community and new molecules are needed for their studies.[3] Since RNMTs are suitable for bi-substrate binding, we will present the synthesis of SAM-adenosine conjugates as bi-substrate analogues (Scheme b) for RNMTs responsible for methylation of the N6-position of adenosine. Six compounds were synthesized by connecting an analogue of SAM to an adenosine unit chosen to mimic the RNA substrate, via alkyl and urea linkers.




**Scheme** : a. General mechanism of RNAs methylation catalysed by SAM dependent MTases. b. Bi-substrates general structure.

**References :**[1] Y. Niu, X. Zhao, Y. Wu, M. Li, X. Wang, Y. Yang, *Genomics Proteomics Bioinformatics* **2013**, *11*, 8-17 ; [2] J. Zhang, Y. G. Zheng, *ACS Chem. Biol.*, **2016**, 11, 583-597 ; [3] M. Schapira, *ACS Chem. Biol.*, **2016**, 11, 575-582.

*Corresponding author: E-mail: melanie.etheve-quelquejeu@parisdescartes.fr

# Synthesis and metal ion binding properties of duplexes containing thymine analogs with 1,2-diamine groups

Takahiro Atsugi,[1] Hisao Saneyoshi, [1] Akira Ono[1*]

[1]*Department of Materials and Life Chemistry, Kanagawa University, Yokohama, Japan,*

Recently, duplexes containing metal-mediated base pairs (metallo-basepairs), in which Watson-Crick base pairs are replaced by metal-base coordination complexes, have been identified [1]. In addition, extensive studies on metal-mediated pairs of natural and artificial bases have expanded the design possibilities of functional DNA molecules [2] (Fig. 1a). In this report, we describe a synthesis of oligonucleotides containing a thymine analogue having 1,2-diamino group (**X**, Fig. 1b), and metal ion binding properties of **X**-**X** pairs in duplexes.

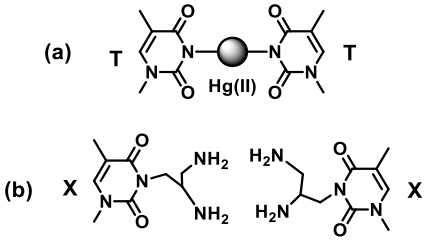



**Figure 1.** (a) metallo-base pairs consisting of thymines and a Hg(II) ion. . (b)**T**hmine analogu having 1,2-diamino group

A synthetic scheme of oligonucleotides having **X** residue is shown in Fig. 2. The synthetic oligonucleotides, **ON1** and **ON2**, consist of 2'-O-methyl strands and a 2'-deoxynucleotide (**X**).

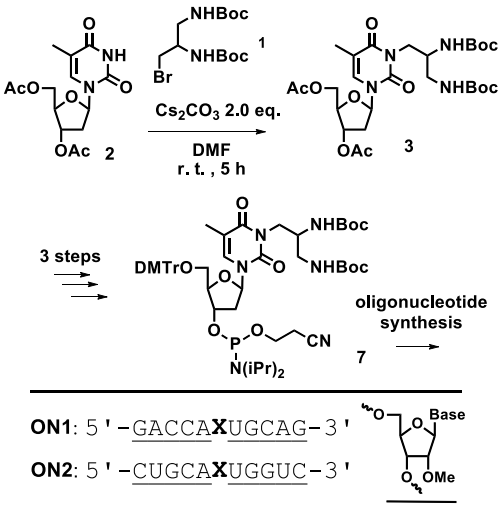



**Figure 2.** A synthtic scheme for oligonucleotides containing **X** residues.

Thermal denaturation profiles of a duplex formed from **ON1** and **ON2** in the presence and in the absence of Ni(II) ions are shown in Fig. 3 A. The duplex was stabilized in the presence of Ni(II). Thermal denaturation experiments were performed at various pH values. The duplex was stabilized in used solutions with various pH values(Fig. 3a).

In summary, oligonucleotides having 1,2-diamino side chains have been successfully synthesized. Various metal ions were incorporated between **X**-**X** pairs and formed stable metallo-base pairs. The results probe the efficiency of the new strategy in which metal ion binding chain are introduced between base pairs.

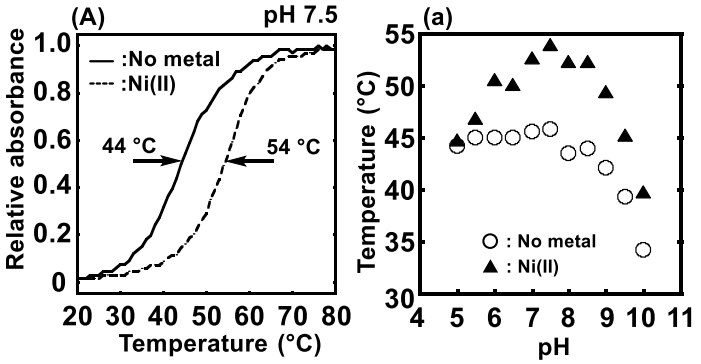



**Figure 3. A**: thermal denaturation profiles of the duplex in the presence and the absence of Ni(II) ions. **a**: plots of $T_m$ vaues at various pHs.

***References:*** [1] Y. Takezawa, J. Müller, M. Shionoya, *Chem. Lett.*, **2017**, *46*, 622-633. [2] Y. Tanaka, *et al*., *Chem. Comm*., **2015**, *51*, 17343-17360. "Feature Article"

*Corresponding author: E-mail: akiraono@kanagawa-u.ac.jp

# Structure-based mutational analysis of the twister-sister ribozyme and implications on the cleavage mechanism

Elisabeth Mairhofer,[a] Luqian Zheng,[b] Masha Teplova,[c] Dinshaw J. Patel,[c] Aiming Ren[b,*] and Ronald Micura[a,*]

[a] *Institute Organic Chemistry, Center for Molecular Biosciences Innsbruck, University of Innsbruck, 6020 Innsbruck, Austria*
[b] *Life Science Institute, Zhejiang University, Hangzhou 310058, China*
[c] *Structural Biology Program, Memorial Sloan-Kettering Cancer Center, New York 10065, NY, USA*

The twister-sister RNA motif belongs to a group of four recently discovered[1] self-cleaving ribozymes that catalyze cleavage of the intramolecular phosphodiester bond in a site-specific manner.[2] The discovery of the new ribozymes has sparked a widespread interest towards an in-depth understanding of the cleavage mechanism of these catalytic RNAs. Here, we present structure-guided mutational analyses based on our observed crystal structure of the twister-sister ribozyme.[3] Eleven conserved and spatially separated loop nucleotides are brought into close proximity at the C-A cleavage site. Comprehensive studies including cleavage assays on key base substitutions, different ribose mutations, and $Mn^{2+}$ for $Mg^{2+}$ replacements in the twister-sister construct, have revealed that the interactions between a guanine (i.e. G5) and a hydrated $Mg^{2+}$ with the non-bridging phosphate oxygens at the cleavage site are important for the cleavage activity.

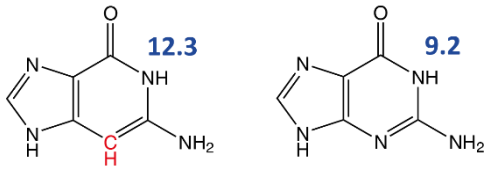



***Figure.*** *Chemical structures of 3-deaza-guanine and guanine; reported $pK_a$ values are highlighted in blue.* [4]

To gain further insight into the cleavage mechanism of the twister-sister ribozyme, in particular the involvement of G5, mutational studies with chemically modified guanosines are envisaged. To this end, we have focused on 3-deaza-guanine because of the shifted $pK_a$ value of its N1-H group compared to the native counterpart. Due to the expected altered interaction between NH-1 of G5 with the non-bridging oxygen of the scissile phosphate, a decrease in cleavage rate is expected, and should provide evidence for the mechanistic impact of G5 on phosphodiester cleavage. Here, we will delineate a synthetic path towards 3-deaza-guanosine building blocks for RNA solid-phase synthesis.

Acknowledgment: This work was supported by Austrian Science Fund FWF (P27947).

***References:*** [1] Weinberg, Z., et al., *Nature Chemical Biology* ***2011***, 11, 606-610. [2] Jimenez, R.M., et al., *Trends in Biochemical Sciences* ***2015***, 40, 648–661. [3] Zheng, L. et al., *Nature Communications* **2017**, *8*, 1180. [4] Chen, H., et al., *Biochemistry* **2017**, 56, 2985-2994.

Corresponding author: E-mail: ronald.micura@uibk.ac.at

## Mechanochemical Sensing Using DNA Templates

In this talk, I will discuss a new sensing strategy that exploits mechanochemical principles of DNA molecules. Mechanochemical coupling describes the interaction between chemical bonds in a molecule and mechanical stress experienced by the molecule. It is a key subject in the newly emerged field, mechanochemistry, which has led to a number of exotic applications in materials chemistry. However, mechanochemical principles have not been well explored in chemical sensing. Using force-based single-molecule techniques such as optical tweezers, our group has been able to measure pico-Newton force change in single DNA molecules or nanoassemblies whereupon individual analyte molecules bind specifically. Such a sensing strategy has allowed us to detect picomolar concentrations of specific targets in biological samples.

# Synthesis of oligodeoxyribonucleotides containing 2-N-heteroarylguanine residues and their properties in G-quadruplex structures

Yoshiaki Masaki, Takeshi Inde, Atsuya Maruyama, Kohji Seio*

*Department of Life Science and Technology, Tokyo Institute of Technology, 4259-J2-16 Nagatsuta-cho, Midori-ku, Yokohama, Kanagawa, JAPAN*

Genome DNA is frequently damaged by mutagenic substances. Damaged nucleobases can cause mutagenesis and subsequent harmful effects to living cells. 2-*N*-guanine adducts are known as one of the damaged nucleobases, some of which can accumulate over longer period of time than others[1]). Therefore, it is important to understand the properties of 2-N-guanine adducts.

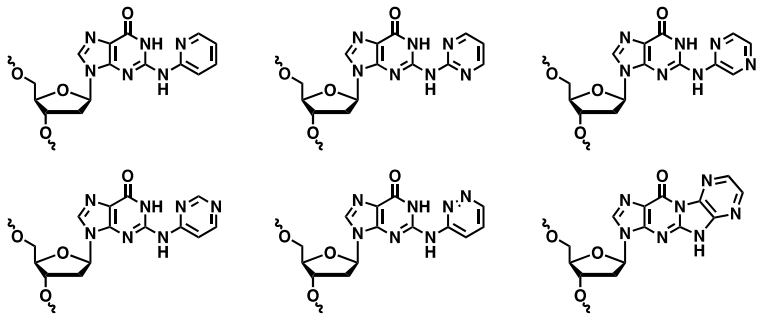


***Figure.*** *2-N-heteroarylguanine derivatives used in this study.*

We have been studied deoxyguanosine derivatives having heteroaryl group on the 2-amino group of guanine nucleobase ($G^{HA}$)[2]. The hydrogen bonding energy and stability of the two conformations, i.e., the open- and closed-type conformations, are key to determining the stability of duplex. In this study, we evaluated the effect of heteroaryl modifications on the stability of G-quadruplex structures.

This work was supported by a Grant-in-Aid for Scientific Research from the ministry of Education, Culture, Sports, Science and Technology, Japan 17H05230, 26288075, 17H04886. The authors thank Analysis Division, Technical Department, Tokyo Institute of Technology, for ESI-TFO and MALDI-TOF Mass analysis.

***References:*** [1] Turesky, J. et al. *Chem. Res. Toxicol.* **1996**, *9*, 397–408. [2] Inde, T., et al. *Org. Biomol. Chem.* **2017**, *15*, 8371-8383.

*Corresponding author: E-mail: kseio@bio.titech.ac.jp

# Phosphobetaine nucleophilic activators as tools in the synthesis of P-modified phosphates and polyphosphates of nucleosides and their analogues

Magdalena Materna, [a] Jacek Stawiński, [a] Michał Sobkowski *[a]

[a] *Department of Nucleoside and Nucleotide Chemistry, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Z. Noskowskiego 12/14, Poznań, Poland*

There are several phosphorylating agents designed for conversion of nucleosides into various nucleotide analogues. These methods often serve for limited purpose, neglect stereochemical aspects and suffer with moderate yields. During our studies on phosphorylation that involved *N*-oxides in *H*-phosphonate systems, we reported formation of oxyonium phosphobetaines[1] that possess unique structural features and chemical properties. Therefore in our present research we are focused on exploiting phosphobetaines towards their nucleophilic activator aspects that allow to modify phosphate moiety in different directions, including bridging and non-bridging positions and incorporation of heteroatoms such as sulfur and selenium as source of chirality. We work on adjusting these methods for the preparation of nucleotide analogues from ribo-, deoxyribo-, and dideoxyribo-nucleosides in a one-pot protocol.

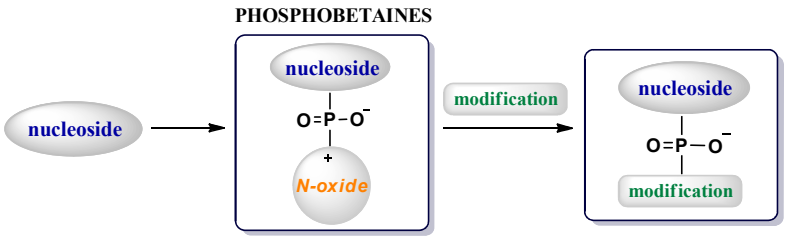



***Figure.*** *The concept of phosphobetaine strategy for nucleotide analogues.*

Our results indicate that phosphobetaines are efficient candidates to elaborate novel synthetic approaches for P-modified natural compound analogues. Experiments also suggest usefulness of this solution in stereospecific phosphorylation reactions.

This work was supported by grant from the National Science Centre of Poland (PRELUDIUM 12, Project No. DEC-2016/23/N/ST5/03024).

***References:*** [1] Materna, M., et al., *RSC Adv.* **2016**, *6*, 14448.

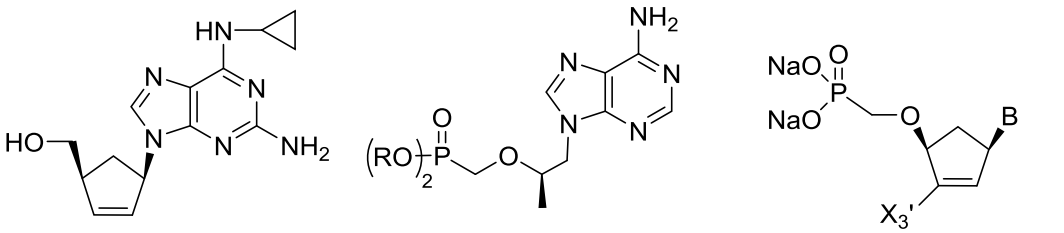
*Corresponding author: E-mail: msob@ibch.poznan.pl

# 3’-Halo-5’-norcarbocyclic nucleoside phosphonates as potent anti-HIV agents

Nadège Hamon, Malika Kaci, Jean-Pierre Uttaro, Christian Périgaud, Christophe Mathé*

*Institut des Biomolécules Max Mousseron (IBMM), UMR 5247, Université de Montpellier, CNRS, ENSCM, cc 1705, Site Triolet, Place Eugène Bataillon, 34095, Montpellier, France*

More than three decades after the discovery of the Human Immunodeficiency Virus (HIV) as the etiologic agent of AIDS, there is still no vaccine available for the prevention of AIDS and drugs are the only arsenal to treat HIV infections. However, current treatments do not allow the eradication of the virus but contain its replication at undetectable level with the obligation for the infected individuals to stay on treatment for life. This is a crucial issue because all existing anti-HIV drugs have long-term side effects and may be associated with the rapid emergence of resistant viral strains in case of faulty observance to treatment or suboptimal treatment. These concerns still promote the research for novel molecular-based anti-HIV drugs. Among these later, nucleoside and nucleotide analogues are an important class of anti-HIV drugs as illustrated with the clinical use of Abacavir, a carbocyclic nucleoside analogue, and Tenofovir disoproxil fumarate (TDF), the corresponding prodrug of (*R*)-9-(2-phosphonylmethoxypropyl)adenine (*R*-PMPA, Tenofovir), an acyclonucleoside phosphonate (Figure).




***Figure***

As a part of our research on 5’-norcarbocyclic nucleoside phosphonates as potential anti-viral agents [1], we describe here the synthesis and the antiviral evaluation of their 3’-halo (iodo and fluoro) derivatives bearing purine bases [2].

***References:*** [1] Uttaro, J. P. et al. *Tetrahedron* **2013***, 69*, 2131. [2] Hamon, N., et al. *Eur. J. Med. Chem.* **2018**, *150*, 642.

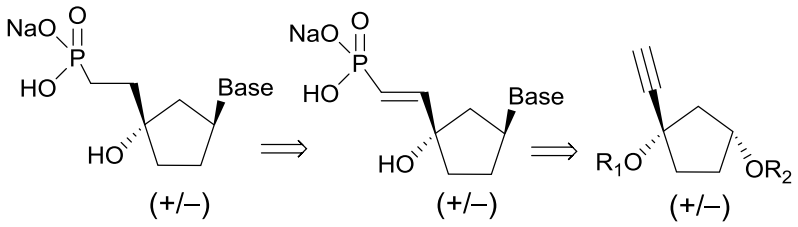
*Corresponding author: E-mail: christophe.mathe@umontpellier.fr

# Rapid synthesis of carbonucleoside phophonate analogues as potential antiviral agents via an hydrophosphonylation reaction of ethynyl carbocyclic precursors

Bemba Sidi Mohamed, Christian Périgaud, Suzanne Peyrottes, Jean-Pierre Uttaro, Christophe Mathé*

*Institut des Biomolécules Max Mousseron (IBMM), UMR 5247, Université de Montpellier, CNRS, ENSCM, cc 1705, Site Triolet, Place Eugène Bataillon, 34095, Montpellier, France*

Nucleoside analogues have been used in the treatments of viral diseases for several decades. In the search of new antiviral nucleoside analogues, a variety of structural modifications of natural nucleosides can be undertaken. Among those, the replacement of the endocyclic oxygen of the furanose ring by a methylene group gave rise to carbocyclic nucleoside analogues. Besides these changes on the sugar moiety, other modifications concern the linkage with the phosphate moiety. Thus, nucleoside phosphonates were elaborated as metabolically stable nucleotide analogues incorporating a P-C bond instead of an hydrolysable P-O bond. However, the rapid access to new carbonucleoside analogues as well as carbonucleotides combining the carbocyclic skeleton and the phosphonate linkage is rather difficult requiring multi-step and elaborate syntheses. In the present work, we describe the rapid synthesis of carbonucleoside phosphonates (Figure) as potential antiviral agents via an hydrophosphonylation reaction of suitable ethynyl carbocyclic precursor using racemic (+/-)-4-*O*-protected-2-cyclopentanone [1].




Base = adenine and guanine

***Figure***

***References:*** [1] Sidi Mohamed, B. et al., *New J. Chem.* **2018***, 42*, 974.

*Corresponding author: E-mail: christophe.mathe@umontpellier.fr

# Evaluation of Threofuranosyl Nucleic Acid (TNA) on the Gene Silencing Activity of siRNA

Shigeo Matsuda,*[a] Saikat Bala,[b] Jen-Yu Liao,[b] Christopher Theile,[a] Lauren Blair,[a] Adam Castoreno,[a] Anna Bisbe,[a] MaryBeth Kim,[a] Dale Guenther,[a] Atsushi Mikami,[a] Mark Schlegel,[a] Ivan Zlatev,[a] Jayaprakash K. Nair,[a] Klaus Charisse,[a] Kallanthottathil G. Rajeev,[a] Martin Egli,[c] John Chaput,[b] Martin Maier[a] and Muthiah Manoharan[a]

[a] *Alnylam Pharmaceuticals, 300 Third Street, Cambridge, MA 02142, USA*
[b] *Department of Pharmaceutical Sciences, University of California., Irvine, 2216 Natural Sciences I, Bldg. 517, Irvine, CA 92697, USA*
[c] *Department of Biochemistry, Vanderbilt University, School of Medicine, Nashville, TN 37232, USA*

TNA, α-(L)-threofuranosyl nucleic acid with a (3'-2') phosphodiester backbone, is known as one of nucleic acid alternatives [1]. Even though TNA has been well-studied in terms of its base pairing properties and structural features [2], little is known about its potential application in the context of short interfering RNAs (siRNAs). For this work, the TNA phosphoramidite building blocks (as shown in the Figure below) were prepared and incorporated into siRNA using optimized synthesis conditions [3]. The effects of TNA incorporation on the oligonucleotide metabolic stability, duplex thermal stability and *in vitro* gene silencing activity of siRNAs were evaluated.

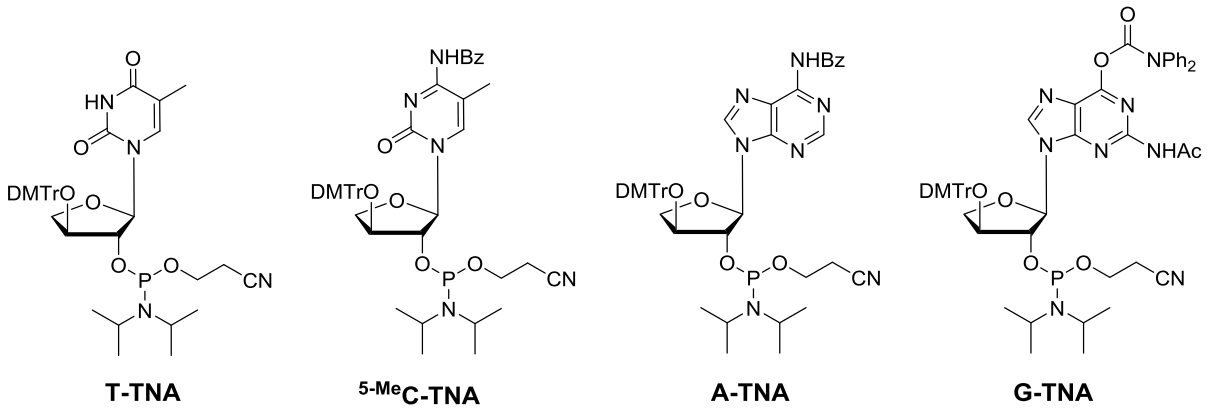



***Figure.*** *TNA phosphoramidites used in this study.*

***References:*** [1] Schoning, K. U. et al., *Science* **2000***, 290*, 1347. [2] Wilds, C. J., et al. *J. Am. Chem. Soc.* **2002**, *124*, 13716. [3] Sau, S. P., et al. *J. Org. Chem.* **2016**, *81*, 2302.

*Corresponding author: E-mail: smatsuda@alnylam.com

# Development of Isoquinoline Ligand Binding to r(CUG) Repeats

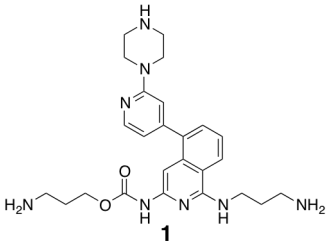
Jun Matsumoto, Jinxing Li, Asako Murata, Chikara Dohno, Kazuhiko Nakatani*

*The Institute of Scientific and Industrial Research (ISIR), Osaka University, 8-1 Mihogaoka, Ibaraki, Osaka 567-0047, Japan*

Development of small molecules that can recognize specific RNA secondary and tertiary structure has become a current hot topic for developing tools to modulate gene expression and therapeutic drugs. Expanded CUG trinucleotide repeats, known as a toxic RNA cause the genetic neurological disorder, Myotonic Dystrophy type 1 (DM1).[1] The expanded $r(CUG)_n$ repeats form a typical hairpin structure containing U-U mismatch base pairs and sequester the Muscleblind-like 1 protein (MBNL1) leading to decreasing MBNL1 level in nuclear and splicing mis-regulation. To suppress the toxic events caused by the repeats, several research groups had developed the small molecular binder to $r(CUG)_n$ repeats which could release the sequestered MBNL1 protein by competitive binding.[1-5] Here, we successfully developed novel isoquinoline ligands recognizing U-U mismatch base pair in $r(CUG)_n$ repeats. (Figure1)

We selected 1,3-diaminoisoquinoline as an uracil recognition unit by using Glide Docking on Maestro. Isoquinoline was attached with cationic functional group to gain water solubility and ionic interaction. Further to increase staking and electrostatic interactions with neighboring base or phosphate backbone, a series of 5-substituted isoquinoline ligands were synthesized.

We investigated the binding ability and sequence selectivity of the synthetic ligands to the RNA repeat sequence by SPR assay using $r(CNG)_9$ repeats. Ligand **1** showed favorable SPR response for $r(CUG)_9$ repeat and $K_D$ value was calculated to be 1.6 uM. In contrast, at least 20-fold weaker response was observed in the cases of the non-targeting sequences such as $r(CCG)_9$, $r(CAG)_9$, $r(CUG)_9$, and dsRNA. CSI-TOF MS and ITC measurements indicated that ligand **1** bound to U-U mismatch base pair in 1:1 binding stoichiometry, and the complexation occurred by enthalpy driven. Competitive binding assay with MBNL1 and r(CUG)n repeat showed inhibition activity of ligand **1** in concentration dependent manner. We will report molecular design, binding assay and inhibition of CUG-MBNL1 interaction using our ligand.




**1**

Fig1 molecular structure of ligand **1**

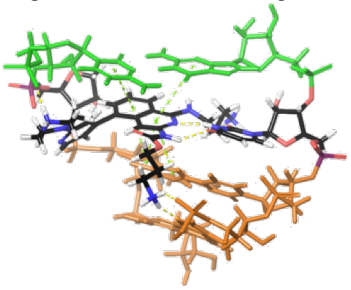


Fig2 Glide Docking of ligand into U-U mismatch base pair.

This work was supported by JSPS KAKENHI Grant-in-Aid for Specially Promoted Research (26000007) for K.N. and Grant-in-Aid for JSPS Research Fellow (201702038) for J.M.

***References:*** [1] J. F. Arambula, et Al, *PNAS,* **2009**, *106*, 16068-16073. [2] C. H. Wong, et. al., *JACS,* **2014**, *136*, 6355-6361. [3] L. A. Coonrod, et. al., *ACS Chem. Biol*., **2013**, *8*, 2528-2537. [4] J. L. Childs-Disney, et. al., *ACS, Chem. Biol.,* **2012**, *7*, 856-862. [5] J. Li, et. al., *Chem. Eur. J.,* **2016**, *22*, 14881-14889.

*Corresponding author: E-mail: nakatani@sanken.osaka-u.ac.jp

# Synthesis and Polymerase Activity of Fluorescent Cytosine TNA Triphosphate Analogues

Hui Mei, Changhua Shi, Yajun Wang, Randi M. Jimenez, Eric Yik, John C. Chaput*

*Departments of Pharmaceutical Sciences, Chemistry, Molecular Biology and Biochemistry, University of California, Irvine, CA 92697-3958.*

Threose nucleic acid (TNA) is an artificial genetic polymer that is capable of undergoing Darwinian evolution to produce aptamers with affinity to specific targets (Figure 1a).[1] This property, coupled with a backbone structure that is resistant to nuclease digestion, makes TNA an attractive artificial biopolymer for diagnostic and therapeutic applications.[2] Expanding the chemical diversity of TNA beyond the natural nucleobases would enable the development of functional TNA molecules with improved physicochemical properties. Here, we report the synthesis and polymerase activity of two fluorescent cytosine TNA triphosphate analogues 1,3-diaza-2-oxo-phenothiazine (tC$^{f}$TP, **2**) and phenyl-pyrrolocytosine (tC$^{p}$TP, **3**) (Figure 1b), which maintain Watson-Crick base pairing with guanine.[3] The two cytosine analogues have contrasting fluorescent properties, with 1,3-diaza-2-oxo-phenothiazine being insensitive to its environment and pyrrolocytosine being highly sensitive to its environment.

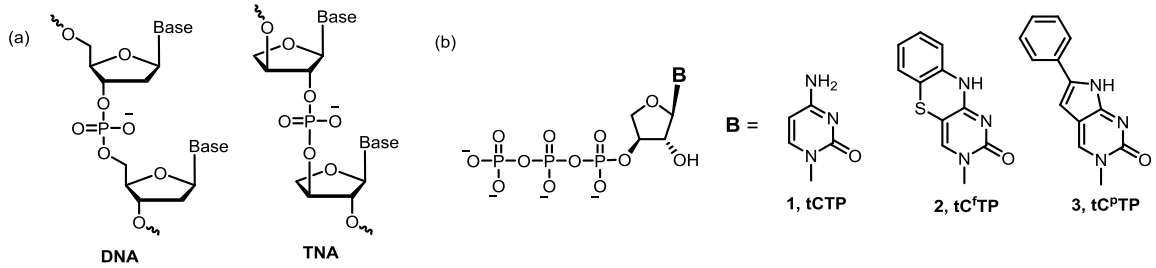



***Figure 1.*** *Molecular structure of TNA (a) and TNA triphosphates (b).*

TNA triphosphates were synthesized following a method developed by our group.[4] We found that both modified TNA triphosphates were excellent substrates for TNA synthesis by Kod-RI TNA polymerase. The overall fidelity of TNA replication for both analogues were measured to be more than 98%, which was comparable to other XNA synthesis. Together, these results provide a platform for synthesizing TNA libraries with greater hydrophobic and unique fluorescent properties. We believe these benefits will lead to the success of in vitro evolution of TNA aptamers and catalysts with enhanced functional properties.

This work was supported by grants from the DARPA Folded Non-Natural Polymers with Biological Function Fold F(x) Program (Award N66001-16-2-4061) and the National Science Foundation (Grant 1607111).

***References:*** [1] Yu, H., et al. *Nat. Chem.* **2012**, *4*, 183. [2] Dunn, M. R., et al. *Nat. Rev. Chem.* **2017**, *1*, 0076. [3] Mei, H., et al. *Nucleic Acid Res.* **2017**, *45*, 5629. [4] Bala, S., et al. *J. Org. Chem.* **2017**, *82*, 5910.

*Corresponding author: E-mail: jchaput@uci.edu

# Oligonucleotides with partially zwitterionic and cationic backbone structures

Melissa Meng,[a] Boris Schmidtgall,[a] Arne Kuepper,[b] Tom Grossmann,[b] Christian Ducho*[a]

[a]*Department of Pharmaceutical and Medicinal Chemistry, Saarland University, Campus C2 3, 66123 Saarbrücken, Germany*
[b]*Department of Chemistry and Pharmaceutical Sci., VU University Amsterdam, De Boelelaan 1108, 1081 HZ Amsterdam, The Netherlands*

Despite the favorable properties of DNA oligonucleotides (ONs), their negatively charged phosphodiester backbone entails a defective cellular uptake and low stability towards endogenous nucleases. As a novel potential strategy to overcome these issues, we introduced the nucleosyl amino acid (NAA)-modification that allows the introduction of positively charged linkages[1] into the native anionic phosphodiester backbone.

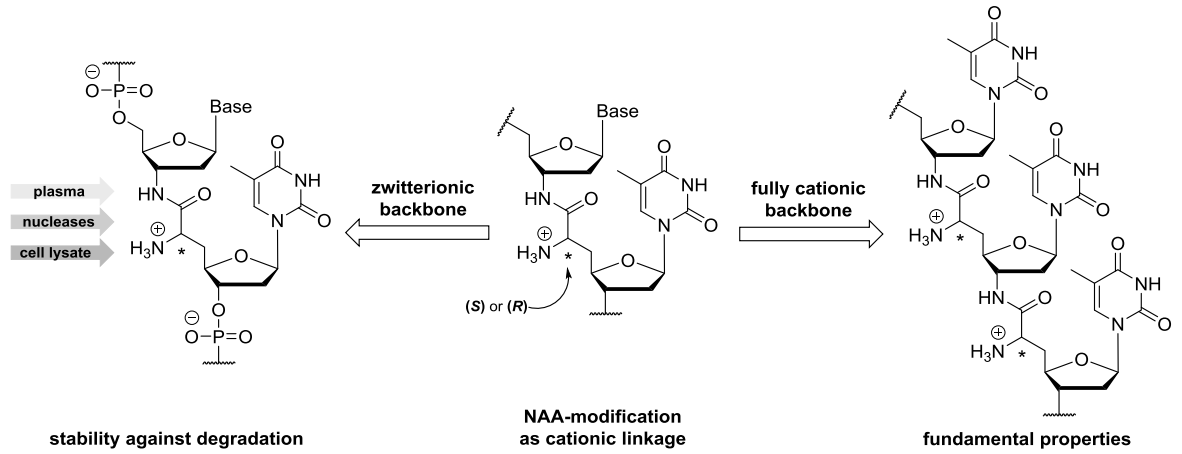



***Figure.*** *Zwitterionic and fully cationic NAA-modified ONs.*

Utilizing this backbone linkage, several partially zwitterionic[2,3] and fully cationic[4] ONs have been synthesized. These ONs are expected to evade nuclease-mediated degradation and to increase cellular uptake by reducing the overall negative charge. In preceding experiments we showed that typical chemical properties of nucleic acids, i.e. base-pairing fidelity and duplex formation, are retained in partially zwitterionic NAA-modified DNA ONs.[2,3] This is in remarkable contrast to fully cationic NAA ONs, which showed high duplex stability in complex with complementary native DNA counterstrands, but hampered base-pairing fidelity due to unspecific binding.[4] Recent research focused on further properties of NAA ONs especially regarding their biological stability. Several of these results will be presented.

This work was supported by the Deutsche Forschungsgemeinschaft, the Fonds der Chemischen Industrie and the Studienstiftung des deutschen Volkes.

***References:*** [1] Jain, M. L., et al., *Chem. Rev.* **2012**, *112,* 1284. [2] Schmidtgall, B., et al., *Chem. Commun.* **2014**, *50*, 13742. [3] Schmidtgall, B., et al., *Beilstein J. Org. Chem*. **2015**, *11*, 50. [4] Schmidtgall, B., et al., *Chem. Eur. J.* **2017**, *24*, 1544.

*Corresponding author: E-mail: christian.ducho@uni-saarland.de

# Synthesis of Chain Terminating TNA Triphosphates

Saikat Bala,[a] Jen-Yu Liao,[a] John C. Chaput*[a]

[a] *Department of Pharmaceutical Sciences, University of California, Irvine, California 92697, United States of America*

Chain terminating triphosphates are important reagents in the fields of molecular biology, pharmaceutical chemistry, and synthetic biology. [1,2,3] Nucleoside analogues that belong to the dideoxynucleoside family inhibit polymerase activity during chain elongation. The ability to site-specifically inhibit DNA synthesis has been exploited in numerous DNA sequencing techniques, including Sanger sequencing, in which truncated products are used to read the DNA sequence. [4] Members of this family of nucleoside analogues have also been used as therapeutic agents to treat cancer and viral infections. More recently, these analogues have been used to study the mechanism of polymerase-mediated DNA synthesis by trapping the enzyme in the catalytically active, closed ternary structure. X-ray crystal structures of ternary complexes reveal the coordination of the nucleoside triphosphate in the enzyme active site and the arrangement of divalent metal ions required for catalysis.

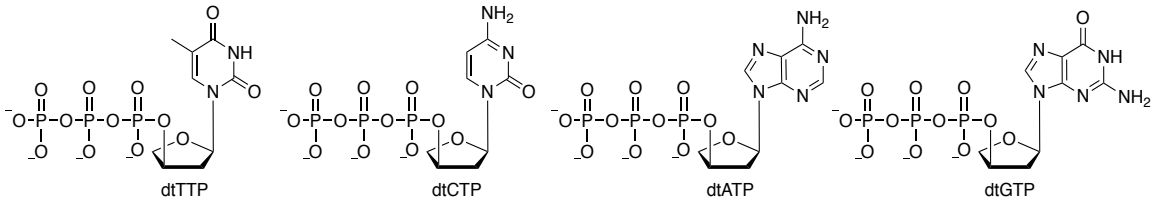



***Figure.*** *Chain terminating TNA nucleotides.*

Here, we describe the synthesis and polymerase recognition of 2′-deoxy-α-L-threofuranosyl nucleoside 3′-triphosphates (dtNTPs), which are being developed to study the mechanism of TNA synthesis by an engineered TNA polymerase.

This work was supported by DARPA (N66001-16-2-4061) and the NSF (1607111).

***References:*** [1] Chim. N. et al., *Nature. Comm.* **2017***, 8*, 1810. [2] Herdewijn. P., et al. *Nucleosides and Nucleotides* **1989**, *659*, 8(5 &6). [3] Orr. D.C, et al. *J. B.C.* **1992**, *267*, 4177. [4] Chen. C.Y.,Frontiers in Microbiology,**2014**., [5] Bala. S. et. al., JOC, **2017**, *82*, 5910.

*Corresponding author: E-mail: jchaput@uci.edu.

# Oligonucleotide Building Blocks Containing 5′-(*R*)- and (*S*)-*C*-Methyl Guanosine

Atsushi Mikami, Shigeo Matsuda, Namrata Erande, Ivan Zlatev and Muthiah Manoharan

Alnylam Pharmaceuticals, 300 Third Street, Cambridge, Massachusetts 02142, United States

Chemical modification of the nucleotide monomers that constitute siRNA strands can modulate the potency, thermodynamic stability and can enhance biostability of these nucleic acid-based drugs. They can also potentially reduce the off-target effects of siRNAs. Our laboratory has synthesized configurationally pure 5′-*C*-methyl 2′-deoxy, 2′-*O*-methyl and 2′-fluoro modified nucleosides and incorporated these monomers into siRNAs. The 5′-*C*-methyl pyrimidine nucleosides were prepared from the corresponding appropriately protected 2′-modified or 2′-deoxy nucleoside precursors [1]. The 2′-modified 5′-*C*-methyl pyrimidine-containing siRNAs had better exonuclease stability than the siRNAs with the corresponding 2′-modified pyrimidines. In the present work, we report synthesis of 5′-(*R*)- and (*S*)-*C*-methyl guanosine building blocks. It has been shown that 2′-OH of guanosine has favorable interactions with the PAZ domain of Human Ago2 [2] and we hypothesized 5′-*C*-methyl would improve the biostability. The 5′-*C*-methyl guanosine isomers have been synthesized starting from L-rhamnose [3]. In contrast to that approach, we started from guanosine, which was appropriately 3′-protected, oxidized, and methylated at the 5′ position. Pure (*R*)- and (*S*)-5′-*C*-methyl isomers were then isolated and used to make the corresponding phosphoramidites, controlled-pore glass (CPG) succinate supports, and 5′-triphosphates. Our synthesis efforts will be summarized.

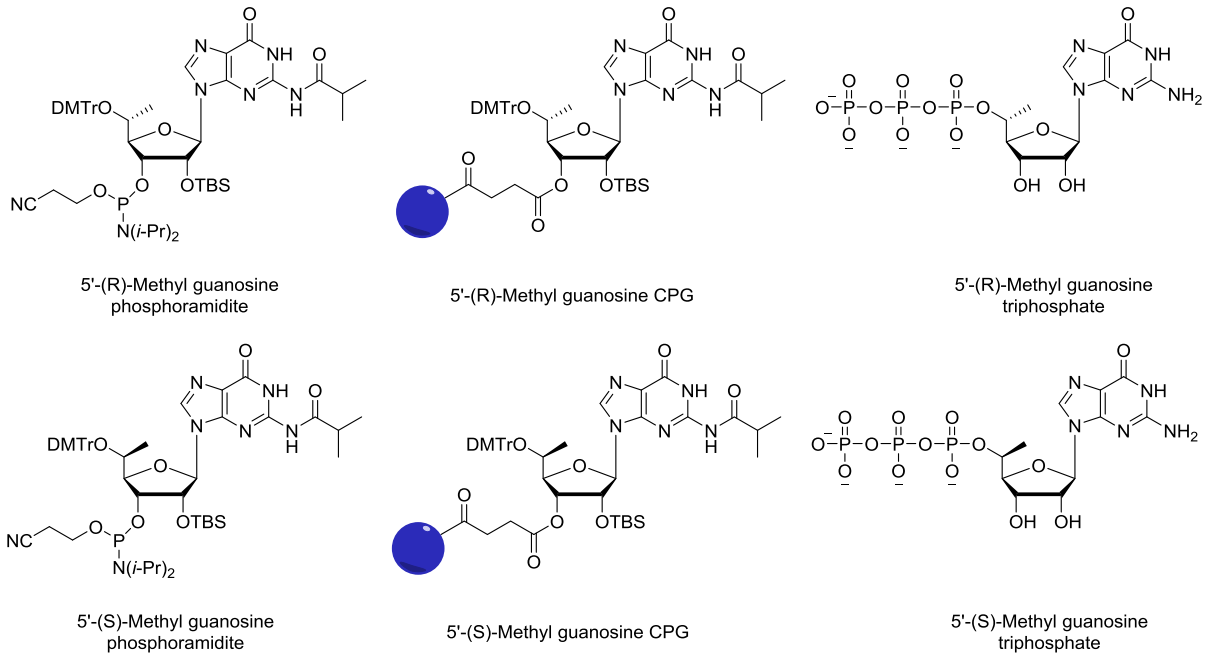



***Figure**. Various 5′-(R)- and (S)-C-methyl guanosine building blocks synthesized.*

***References:*** [1] Kel'in, A., et al., *J. Org. Chem.* **2016**, *81*, 2261−2279. [2] Kandeel, M., et al.,PLoS ONE **2014**, *9*, e94538. [3] Beigelman,L., et al., *Nucleosides Nucleotides* **1995**, *14*, 901.

# Phosphate modified PAPS analogs as tools to study sulfotransferases

A. Mlynarska-Cieslak,[a] M.R. Baranowski,[a] D. Kubacka,[a] M. Warminski,[a] J. Jemielity,[b] J. Kowalska,[*a]

[a] *University of Warsaw, Faculty of Physics, Division of Biophysics, 5 Pasteura St., 02-093 Warsaw*
[b] *University of Warsaw, Centre of New Technologies, Banacha 2c St., 02-097 Warsaw*

Sulfotransferases play an important role in many biological processes in higher Eukaryotes, such as detoxification, drug metabolism, hormone regulation, and many others. Sulfotransferases catalyze transfer of a sulfate moiety from the universal donor, 3'-phosphoadenosine-5'-phosphosulfate (PAPS)(Fig. 1), to various acceptors containing a hydroxyl or an amine group.

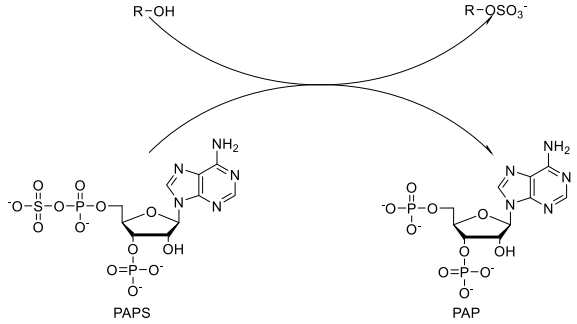



***Figure.*** *The general reaction catalyzed by sulfotransferases.*

Deregulation of sulfotransferase activity has been linked to many diseases, including Parkinson's disease, hemophilia, and breast cancer[3]. Despite the importance of sulfotransferases activity in humans and other organisms, the mechanisms of action and regulation of those enzymes have not been fully elucidated. Further studies on sulfotransferases could be significantly facilitated by novel molecular tools, such as analogues of the universal substrate (PAPS) or product (PAP). To this end, we synthesizes a series of new PAP and PAPS analogs, carrying various modifications within the phosphate moieties. The modifications, including replacing phosphate or sulfate by isomeric moieties such as fluorophosphate, thiophosphate, H-phosphonate, alkyl phosphate, and others. The preliminary biological properties of synthesized PAPS analogs were evaluated with a model sulfotransferase,[2] AtSOT18, using an HPLC-based activity assay[1] and microscale thermophoresis (MST)-based direct binding assay.

This work was supported the National Science Centre, Poland (UMO-2015/18/E/ST5/00555).

***References:*** [1] Klein M. et al., *FEBS J*. **2006,** 237(1):122-36. [2] Hirschmann F. et al., *Scientific Reports,* **2017**, 4160. [3] Chapman E. et al., *Angew. Chem. Int. Ed*. **2004**, 43, 3526 –3548.etc.

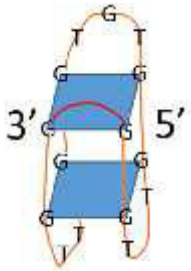
[*]Corresponding author: jkowalska@fuw.edu.pl

# Cyclic is better!
# Design, synthesis and characterization of cyclic variants of TBA

Daniela Montesarchio,[a] Claudia Riccardi,[a] Albert Meyer,[b] Jean-Jacques Vasseur,[b] Domenica Musumeci,[a,c] Irene Russo Krauss,[a,d] Luigi Paduano,[a,d] Francois Morvan[b]

[a]*Department of Chemical Sciences, University of Naples Federico II, Via Cintia 21, I-80126, Napoli, Italy*
[b]*Institut des Biomolécules Max Mousseron, University of Montpellier, CNRS, ENSCM, 34095 Montpellier Cedex 5, France*
[c]*Institute of Biostructures and Bioimages, CNR, Via Mezzocannone 16, I-80134 Napoli, Italy*
[d]*CSGI – Consorzio Interuniversitario per lo Sviluppo dei Sistemi a Grande Interfase, Via della Lastruccia 3, I-50019, Sesto Fiorentino (Fi), Italy*

The 15-mer G-rich oligonucleotide TBA (Thrombin Binding Aptamer) is the best characterized aptamer of thrombin, a "trypsin-like" serine protease involved in coagulation processes able to convert soluble fibrinogen into insoluble strands of fibrin.[1] The use of TBA has aroused a strong interest, motivated not only by its therapeutic potential, but also by its possible applications in biotechnological and bioanalytical fields.[2,3]
In this frame, we here present the design, synthesis, biophysical and biological evaluation of cyclic variants of TBA, presenting a suitable loop linking the 3'- and 5'-ends of the TBA sequence (as representatively reported in the Figure).
Upon cyclization, oligonucleotides can adopt defined molecular structures in which the sugar-phosphate backbone provides a rigid framework to hold the nucleobases. By virtue of this intrinsic preorganization, cyclic oligonucleotides typically show higher thermal stability, resistance to nucleases and improved recognition properties of the target protein, and therefore are of interest in several biological and biotechnological fields.
The new cyclic TBA analogs have been successfully synthesized using solid phase synthesis protocols exploiting different strategies so to obtain loops of different length and features. Preliminary biophysical characterization based on UV, CD, gel electrophoresis and size exclusion chromatography analysis has been performed. In addition, their resistance to nucleases has been analyzed in comparison with the unmodified TBA. The inhibitory activity towards human α-thrombin will be determined by means of DLS experiments, following the conversion of fibrinogen into fibrin promoted by thrombin.




This work was supported by grants from the Italian Association for Cancer Research.

***References:*** [1] Bock, L., et al. *Nature* **1992**, 355, 564. [2] Platella, C.; et al. *BBA - Gen. Subj*. **2017**, 1861, 1429. [3] Riccardi, C., et al. *ACS Appl. Mater. Interfaces* **2017**, 9, 35574.

*Corresponding author: E-mail: daniela.montesarchio@unina.it

# New and cheap phosphoramidites and a solid support for the synthesis of 5'- or 3'-monophosphate oligonucleotides

Mimouna Madaoui,[a] Jean-Jacques Vasseur,[a] François Morvan*[a]

[a] *IBMM, University of Montpellier, CNRS, ENSCM, Montpellier, France*

Oligonucleotides bearing a 5'-phosphate are useful in several biological applications like conjugation[1] PCR[2] or gene construction.[3] Furthermore, the small interfering RNAs (siRNAs) required the presence of a 5'-phosphate on the target-complementary strand for RNAi activity[4]

There are only two commercially available phosphoramidites **1**,[5] **2**[6] to introduce a 5'-phosphate into oligonucleotides. The same or similar chemical structures are also used to introduced 3'-phosphate starting from the solid supports **3** or **4** (Figure 1).

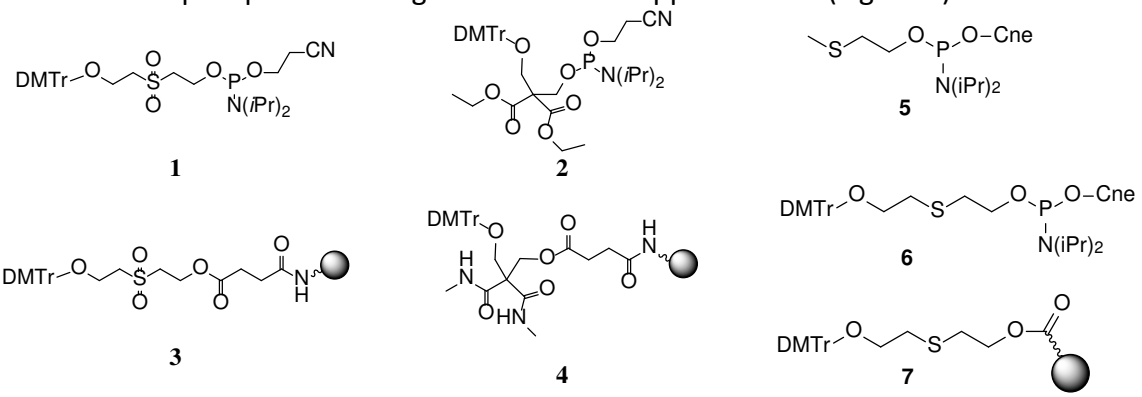



Figure 1: Reagents for the synthesis of 5'- and 3'-phosphate oligonucleotides

Since starting reagents are expensive and require several steps, we developed new phosphoramidites and a solid support using cheap reagent and a straightforward synthesis (1 or 2 steps). Methyl-thioethanol and 2,2'-thiodiethanol are about 6 and 14 times less expensive than 2,2'-sulfonyldiethanol and bis(hydroxymethyl)malonate. After solid support synthesis of oligonucleotides, a first thermolytic treatment removed the methylthioethyl or 2-ethanolthioethyl group and then a standard ammonia treatment afforded the 3'- or 5'-phosphate oligonucleotides.

This work was supported by Labex CheMISyst ANR-10-LABX-05-01.

***References:***

(1) Chu, B. C. F.; Wahl, G. M.; Orgel, L. E. *Nucleic Acids Res* **1983**, *11*, 6513-6529.
(2) Barany, F. *P Natl Acad Sci USA* **1991**, *88*, 189-193.
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(4) Manoharan, M. *Curr Opin Chem Biol* **2004**, *8*, 570-579.
(5) Horn, T.; Urdea, M. S. *Tetrahedron Lett* **1986**, *27*, 4705-4708.
(6) Guzaev, A.; Salo, H.; Azhayev, A.; Lonnberg, H. *Tetrahedron* **1995**, *51*, 9375-9384.

*Corresponding author: E-mail: francois.morvan@umontpellier.fr

# Development of fluorescent probe for RNA higher-order structure

Hirotaka Murase,[a] Fumi Nagatsugi*[a]

[a] *Institute of Multidisciplinary Research for Advanced Materials, Tohoku University*

The higher-order structures of RNA play important role in the pathway of gene expression and the sensing probes for RNA structures are highly desired. However, the basic concept for molecular design on the structural sensing is not established, because RNAs can form various higher-order structures, e.g. internal loop, AP-site, hairpin and bulge, which makes it difficult to detect them. On the other hand, these RNA structures are regarded as the fascinated targets for small molecular ligands [1][2]. In this study, a recognition molecule was designed to form multiple hydrogen bonds with the RNA base inside a hydrophobic hairpin structure. As the basic structure of the recognition molecule, we employed a 1,3-diazaphenoxazine skelton (G-clamp) to recognize a guanine base by forming four hydrogen bonds.

We synthesized the G-clamp-dimer by condensation reaction between 0.5 equivalent diamino linker and **1** prepared by previously reported method [3]. The binding affinity of the G-clamp-dimer was evaluated by fluorescence titrations using model hairpin RNAs, shRNA(-XUY-), imitated pre-miRNA-17. As a reslut, the fluorescence intensity of G-clamp-dimer was increased by addition of the shRNA(-GUG-) possessing guanines inside a hairpin in the concentration-dependent manner. In contrast, the increasing fouorecence intensity of G-clamp-dimer was not observed by addition of the shRNA(-CUA-) or single-stranded RNA (5'-AGUGA-3'). These results indicate that G-clamp-dimer selectively binds to guanine inside a hairpin loop and is a candidate for the RNA structural sensing probe. In this presentation, we will discuss the binding selectivity of G-clamp-dimer for RNA sequences and structures in detail.

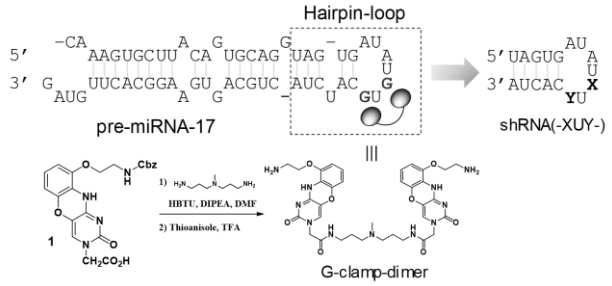


Fig. 1 Structure of target RNA and probe

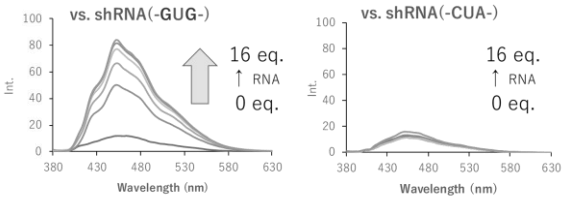


Fig. 2 Fluorescence titrations
Fluorescence emission spectra of 10 nM G-clamp-dimer in the presence of 0-160 nM sh(-XUY-) in the buffer containing 10 mM HEPES-NaOH pH 7.4 and 100 mM NaCl excited by 360 nm at 20 °C.

This work was supported by a Grant-in-Aid for Young Scientists from the Japan Society for the Promotion Sciences (JSPS).

***References:*** [1] J. F. Arambula, *et al.*, *Proc. Nat. Acad. Sci.*, **2009**, *106*, 16068. [2] Y. Sato, *et al.*, *Angew. Chem., Int. Ed.*, **2012**, *51*, 6369. [3] K. G. Rajeev, *et al.*, *Org. Lett.*, **2002**, *4*, 4395.

*Corresponding author: E-mail: nagatsugi@tagen.tohoku.ac.jp

# Development of the effective alkylating probes for G-Quadruplex

Fumi Nagatsugi*, Norihiro Sato, Madoka Hazemi Eurika,Kazumitsu Onizuka
Institute of Multidisciplinary Research for Advanced Materials, Tohoku University, 2-1-1 Katahira, Aoba-ku, Sendai 980-8587, Japan

Guanine rich single strand can form non-canonical four stranded structures known as G-quadruplexes (G4s) (Fig. 1). G4s have been found in biologically significant regions of the genoms, including telomeric DNA and promoter regions which associated with many known diseases.[1] Owing to the important regulatory role in biology, G4s have potential as therapeutic targets. For example, the small molecules stabilized G4s can interfere with the telomerase, which maintains telomere length in cancer cells, and are one of the efficient candidate for anticancer drug. Recently, covalent modifications of G4s have been suggested to be an effective strategy for stabilization of G4s.

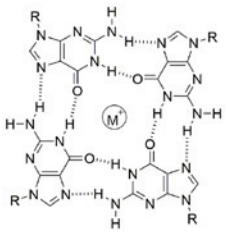

**Fig. 1** G-quadruplex structure

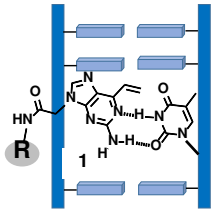


**Fig. 2** Selective chemical modification in an abasic site

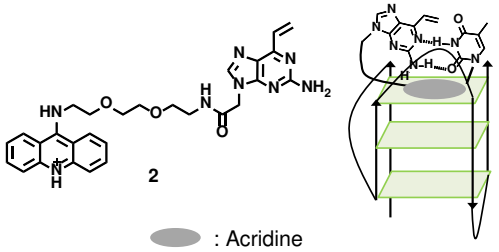


**Fig.3** Design of the alkylating probe for G4 structure

In this presentation, we report the attempts to develop the alkylating probes for G4s structure. We have previously reported that the 2-amino-6-vinylpurine (AVP) probe (**1**) exhibited efficient and selective alkylation to thymine bases at the site opposite an abasic site in DNA (Fig. 2).[1] These results suggested that AVP derivatives conjugated with DNA binding molecules might form hydrogen bonds with target nucleobases located in a hydrophobic pocket and induce the selective alkylation. Based on this concept, we designed the alkylating probe for G4s structure (Fig. 3). This probe (**2**) has a simple structure, which consists of AVP as a reactive moiety and acridine as a G4 binding site. A variety of acridine derivatives are reported as G4 ligands with high affinity and bind to guanine tetrads by means of $\pi-\pi$ interactions[2]. In our probe, acridine part was postulated to be capable of promoting the access to G4 structure and AVP might react to thymine within the loop structure of G4. We synthesized this probe and evaluated the reactivity to G4s structure in human telomere sequence. This probe showed a moderate reactivity and selectivity to the G-4 in $K^+$ buffer. Furthermore, the alkylation with this probe efficiently stabilized the G-4 structure in the $K^+$ buffer without altering the structure. In addition, the alkylation of G-4 DNA with **2** effectively caused the inhibition of the primer extension and replication. In this presentation, I will discuss these results in detail.

***References:*** [1] N. Sato, G. Tsuji, Y. Sasaki, A.Usami, T. Moki, K. Onizuka,K.Yamada, F. Nagatsugi, *Chem Commun.*, **2015**, *51*, 14885. [2] S. Sparapani, S.M. Haider, F. Doria, M. Gunaratnam, S. Neidle, *J.Am.Chem. Soc.*, **2010**, *132*,12263.

*Corresponding author: E-mail: nagatugi@tagen.tohoku.ac.jp

# Development of reduction-activated protecting groups for siRNA prodrugs

Kodai Nakamura, Akira Ono*[1], Hisao Saneyoshi*[2]

*Department of Material and Life Chemistry, Faculty of Engineering, Kanagawa University, 3-27-1 Rokkakubashi, Kanagawa-ku, Yokohama 221-8686, Japan.*

RNA interference has attracted considerable attention for life sciences and medicinal sciences, because they play an important role in high target selectivity and gene silencing activity [1]. However, small interfering RNA (siRNA) has several drawbacks such as instability in biological fluids and poor cell membrane permeability. To circumvent these problems, various chemical modifications of RNA have been reported. Specially, modifications at 2´-position such as 2´-OMe and 2´-OMOE provide benefits such as resistance to nucleases on oligonucleotides. However, these permanent protections usually decrease the activities of siRNA [2]. Therefore, it is necessary to develop biodegradable protecting group of RNA at 2´-position which are designed to be removed inside cells to liberate the active siRNA [3-1], [3-2]. These approaches above mention provide improvement of chemical and biochemical stability of RNAs in outside cells. After cellular uptake, the protecting groups were deprotected by internal triggers such as esterase and thiol to release active RNA species in diverse cell lines. We focused on the hypoxic conditions such as solid tumor cells [4]. This specific environment have been used for activities of reduction-activated prodrugs [5].

In this study, we designed bio-labile protecting group for 2´-OH group which is activated in hypoxic condition typically found in solid tumor cells (Fig. 1).

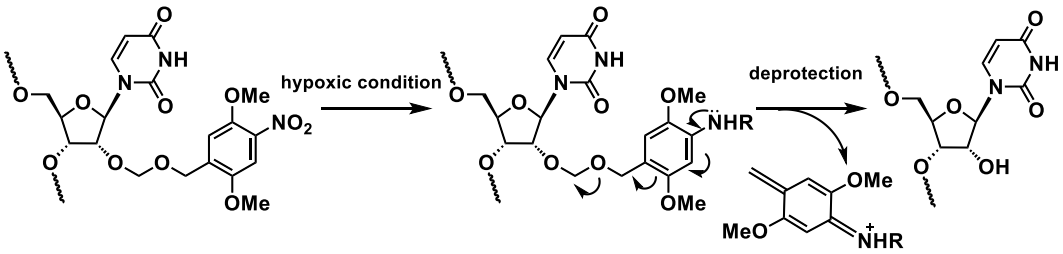


**Figure 1.** Schematic representation of the expected the behavior of pro-oligonucleotides in tumor cells.

***References:*** [1] Kurreck, J., *Angew. Chem. Int. Ed.,* **2009**, *48*, 1378-1398. [2] Czauderna F., Fechtner, M., Dames, S., Aygün, H., Klippel, A., Pronk, G., Giese, K., Kaufmann, J., *Nucleic Acids Res.*, **2003**, *31*, 2705-2716. [3-1] Parey, N., Baraguey, C., Vasseur, J-J., Debart, F., *Org. Lett.,* **2006**, *8*, 17. [3-2] Ochi, Y., Nakagawa, O., Sakaguchi, K., Wada, S., Urata, H., *Chem. Commum*., **2013**, *49*, 7620-7622. [4] Höckel, M., Vaupel, P., *Natl. Cancer. Inst*., **2001**, *91*, 266-276. [5] Wilson, W., Hay, M., *Nat. Rev. Cancer*, **2011**, *11*, 393-410.

* 1 E-mail: akiraono@kanagawa-u.ac.jp / *2 E-mail: saneyoshih@kanagawa-u.ac.jp

# *S*-Geranylated RNA is an intermediate in the SelU-catalyzed transformation of 2-thio- to 2-seleno-uridine in transfer RNA

Barbara Nawrot,*[a] Malgorzata Sierant,[a] Grazyna Leszczynska,[b] Klaudia Sadowska,[b] Patrycja Komar,[a] Ewa Radzikowska-Cieciura,[a] Elzbieta Sochacka[b]

[a]*Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, Sienkiewicza 112, 90-363 Lodz, Poland*
[b] *Institute of Organic Chemistry, Lodz University of Technology, Zeromskiego 116, 90-924 Lodz, Poland*

Using simplified anticodon-stem-loop oligonucleotide models (ASL) [1], containing 2-thiouridine, *S*-geranyl-2-thiouridine or 2-selenouridine (all deprived of any modifications otherwise present in natural bacterial $tRNA^{Lys}$), we demonstrated *in vitro*, that tRNA selenouridine synthase SelU is an enzyme with two consecutive catalytic activities. The first includes sulfur-specific geranylation of 2-thiouridine in ASL, and the resultant intermediate is a substrate for the second one, which includes substitution of a good leaving *S*-alkyl group with a Se-containing nucleophile. Finally, the formation of hypermodified bacterial 2-selenouridine is observed. We demonstrated that, at least in our model system, direct transformation of 2-thio- to 2-selenouridine RNA is not possible, even with a huge excess of the SelU enzyme. A cellular pathway, already suggested by us on the basis of chemical transformation of S2U via geS2U to Se2U [2], and now confirmed enzymatically, is presented below (Figure 1):

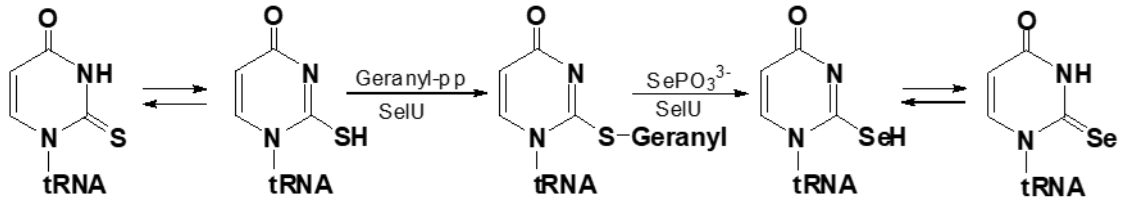


***Figure 1.*** *A proposed cellular pathway of the S2U-tRNA to Se2U-tRNA transformation going through the geS2U-tRNA intermediate (Geranyl-pp - geranyl diphosphate, $SePO_3^{3-}$-selenophosphate).*

This work was supported by grants from The National Science Centre in Poland [UMO-2014/13/B/ST5/03979] and by statutory funds of the Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences.

***References:*** [1] Sierant, M. et al., *Nucleic Acids Res.* **2016***, 44*, 10986. [2] Bartos, P. et al. *Bioorg. Chem.* **2014**, *56*, 49.

*Corresponding author: E-mail: bnawrot@cbmm.lodz.pl

# Non-hydrolysable supersubstrate of phosphatidylinositol 4-kinases

Michal Šála,[a] Hubert Hřebabecký,[a] Milan Dejmek,[a] Eva Zborníková,[a] Evzen Boura,[a] Radim Nencka*[a]

[a] *Institute of Organic Chemistry and Biochemistry of the CAS, Flemingovo náměstí 542/2, 166 10 Praha 6, Czech Republic*

Phosphorylation is essential biotransformation involved in regulation of various cellular processes. Chemically stable analogues of compounds involved in this process, such as modified substrates, products and phosphorylation agents, have recently emerged as promising tools for deciphering diverse signaling pathways, which they are implicated in. Our group has recently been focused on understanding of roles of human phosphatidylinositol 4-kinases (PI4Ks) in cell biology of various pathological processes, such as viral replication or cancer progression.[1,2] PI4Ks are enzymes responsible for transfer of a phosphate group from ATP to phosphatidylinositol, a common constituent of the plasma membranes in cells. Product of this reaction, phosphatidylinositol 4-phosphate (PI4P), plays numerous roles in cell signaling and is essentially implicated in process of membrane budding.[3]

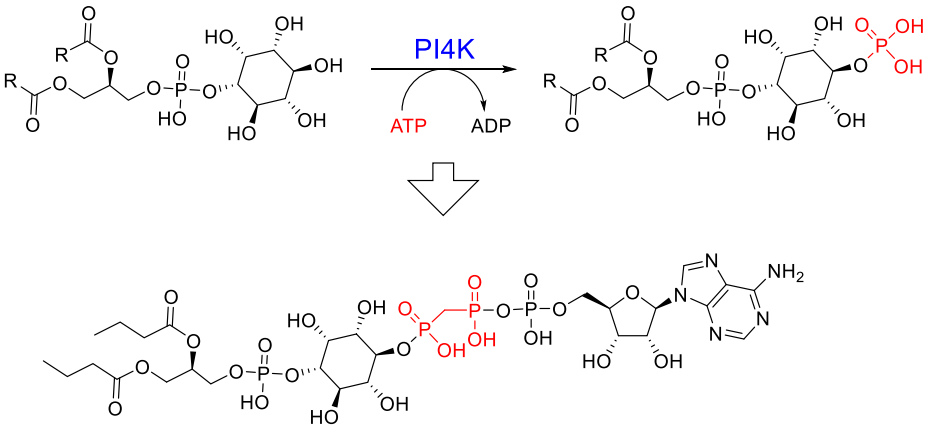


***Figure.*** *Design of non-hydrolysable supersubstrate of PI4Ks.*

Here we report the design and synthesis of novel supersubstrate complex of ADP and PI4P containing non-hydrolysable bisphosphonate motive. This compound will be used as a tool compound for our crystallization experiments in order to identify the phosphatidylinositol binding site of PI4Ks.

The work was supported from European Regional Development Fund; OP RDE; Project: "Chemical biology for drugging undruggable targets (ChemBioDrug)" (No. CZ.02.1.01/0.0/0.0/16_019/0000729).

***References:*** [1] Mejdrová, I., et al., *J. Med. Chem.* **2015***, 58*, 3767. [2] Humpolíčková, J., et al. *J. Med. Chem.* **2017**, *60*, 119. [3] Boura, E., Nencka, R. *Exp. Cell Res.* **2015**, *337*, 136.

*Corresponding author: E-mail: nencka@uochb.cas.cz

# Condensing the information in DNA by introducing additional bases

Poul Nielsen,*[a] Mick Hornum,[a] Charlotte Reslow-Jacobsen,[a] Kasper Petersen Beck,[a] Pawan Kumar,[a] Pawan K. Sharma,[b] Michael Petersen[a]

[a] *Department of Physics, Chemistry and Pharmacy, University of Southern Denmark, 5230 Odense M, Denmark.*
[b] *Department of Chemistry, Kurukshetra University, Kurukshetra-136 119, India.*

The double-headed nucleotides shown below exposes both their nucleobases for pairing in the DNA duplex and hereby functions as condensed dinucleotide mimics. With for instance $U_T$ both nucleobases are forming Watson-Crick base pairs with complementary adenines. In a duplex with $U_T$ and $U_A$ in each complementary sequence, the duplex is extended with an additional T-A base-pair.[1,2]

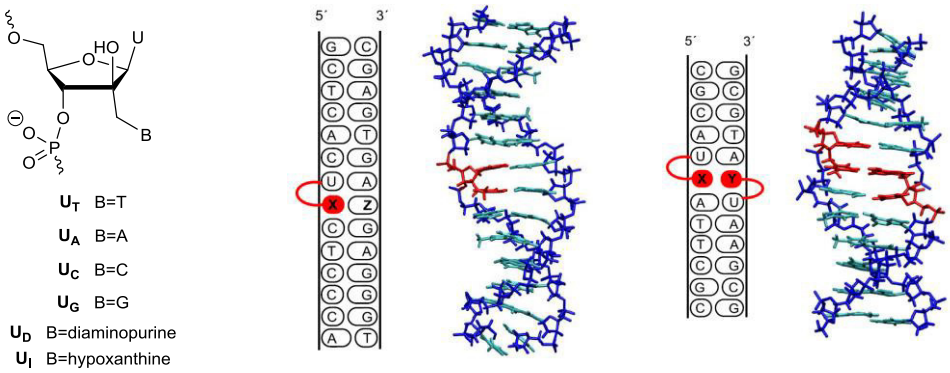


**Figure**. Double-headed nucleotides. Models of the two general motifs and representative snapshots from MD-simulations.

We hereby present the full series with all four nucleobases attached, i.e. $U_T$, $U_A$, $U_C$ and $U_G$, as well as two analogues with other purine bases $U_D$ and $U_I$. Overall, $U_T$, $U_A$, $U_C$ and $U_G$ all work as condensed dinucleotides obeying the Watson-Crick base-pairing roles for the additional bases. However, whereas mis-match discrimination was very efficient for both $U_C$ and $U_G$, neither T nor A in $U_T$ and $U_A$ discriminates G as efficiently as in a natural DNA duplex.[2] We have now improved this by replacing A with D using $U_D$. Also $U_I$ containing hypoxanthine, a known wobble base in RNA, has been studied and found to show more uniform recognition. Overall, the presented double-headed nucleotides are building blocks that concentrate the molecular information of the nucleobases on smaller entities. In principle it takes only one phosphate for every second nucleobase to store the same information as in normal DNA.

This work was supported by grants from The Danish Council for Independent Research, Natural Sciences and by the Villum Foundation.

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*Corresponding author: E-mail: pouln@sdu.dk

# Synthesis, evaluation of antiviral activity and cytotoxicity of novel 2'-C-Methyl-7-Deaza-Adenosine Analogues

B. Raayudu[a], B. Shiva Krishna[a], M. Laxman[a], J. Rajani[a], Sk. Sahabuddin[a], **Shyamapada Banerjee[a]**, Yogesh Sanghvi[a,b], Luděk Eyer[c,d] and Daniel Růžek[c,d]

[a] Sapala Organics Pvt. Ltd. Plot Nos. 146B & 147 IDA Mallapur, Phase-II, Hyderabad-500076, Telangana, India

[b] Rasayan Inc. 2802 Crystal Ridge Road, Encinitas, CA 92024-6615, USA

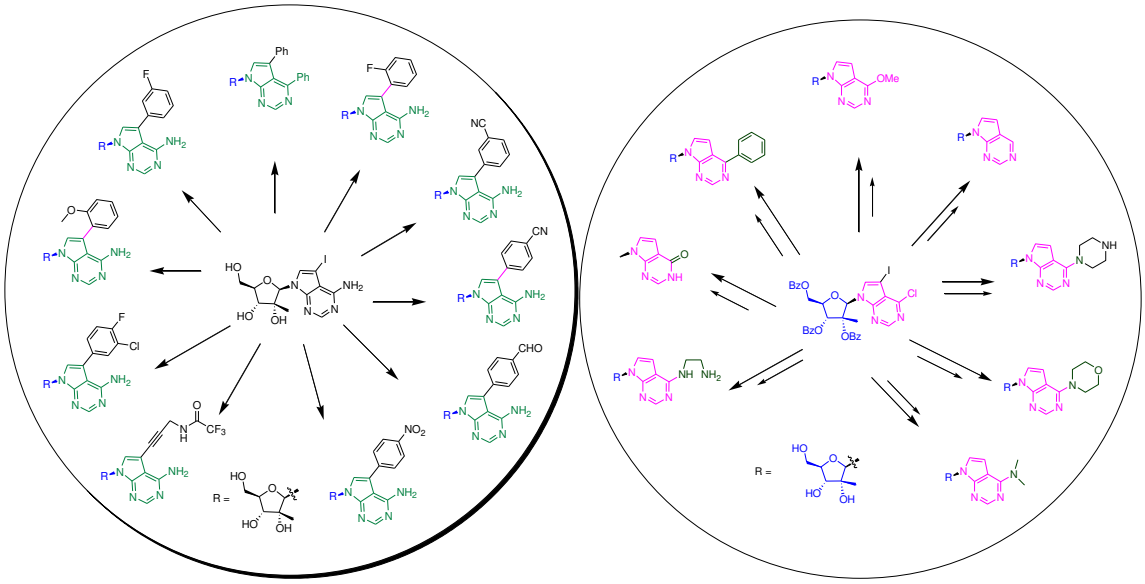
[c] Department of Virology, Veterinary Research Institute, Hudcova 70, CZ-62100 Brno, Czech Republic

[d] Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, Branisovska 31, CZ-37005 Ceske Budejovice, Czech Republic

## Abstract:

2'-C-Methyl-6-chloro-7-iodo-7-deazaadenosine was synthesised via glycosylation of (3R,4R,5R)-5-((benzoyloxy)methyl)-3-methyltetrahydrofuran-2,3,4-tribenzoate with 4-chloro-5-iodo-7H-pyrrolo[2,3-d]pyrimidine. 2'-C-Methyl-6-chloro-7-iodo-7-deazaadenosine was then converted into various structurally interesting [1,2,3] 2'-C-methyl-7-deazaadenosine, and their 7-substituted analogues.

Antiviral activity[4] and cytotoxicity of a set of about 15 nucleoside analogues were evaluated using tick-borne encephalitis virus (strain Hypr, a representative of West-Europe TBEV strain) and porcine kidney stable (PS) cells or human hepatoma (Huh-7) cells. No inhibition/reduction of TBEV growth was observed after 3 days incubation, however, some of the compound tested were found to be highly cytotoxic for both cell lines at concentration of 50 μM. The details of synthesis and outcome of antiviral activity will be presented as poster.



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# Comprehensive structure activity study of lipid conjugated antisense oligonucleotides in muscle

Thazha Prakash*, Jinghua Yu, Mehran Nikan, Richard Lee, Adam Mullick, Steve Yeh, Audrey Low, Alfred Chappell, Michael Oestergaard, Hans Gaus, Eric Swayze, Punit Seth

*Ionis Pharmaceuticals, 2855 Gazelle Ct., Carlsbad, CA 92010, USA*

Antisense oligonucleotide (ASO) based drug development is evolving as an effective therapeutic modality [1]. There are several antisense drugs for treating muscle related diseases advancing in the clinic [1]. Therapeutic efficiency of a drug depends on its availability at the target site. Hence, improving the distribution of ASO in the muscle will be beneficial for improving therapeutic efficiency of antisense drugs for targets expressed in muscle. We hypothesized that improving plasma protein binding will increase uptake of ASOs in muscle. To test this hypothesis, we investigated the effect of conjugating hydrophobic ligands, capable of interacting with plasma proteins, on productive uptake of ASO into muscle. An extensive SAR to identify optimal hydrophobic ligand for improving potency of ASO in muscle was carried out. We designed and synthesized structurally diverse saturated, unsaturated and phospholipid conjugated ASOs with a range of hydrophobicity. Plasma protein binding of lipid ASO conjugates were examined. Sub-cellular distribution of lipid ASO conjugates in heart was determined using cell sorting experiments. Activity of lipid conjugated ASOs targeting Malat-1, CD-36 and DMPK mRNA were evaluated in mice. Our results suggest that conjugation of hydrophobic ligands improved uptake and potency of ASOs in muscle.

***References:*** [1] Crooke, et al. *Cell Metab.* **2018**, 27, 714-739.

*Corresponding author: E-mail: tprakash@ionisph.com

# Evaluating the Rate and Substrate Specificity of Laboratory Evolved XNA Polymerases

Ali Nikoomanzar,[a] Matthew R. Dunn,[a] and John C. Chaput*[a]

[a] *Department of Pharmaceutical Sciences, University of California, Irvine, CA., 92697 USA*

Engineered polymerases that can copy genetic information between DNA and xeno-nucleic acids (XNA) hold tremendous value as reagents in future biotechnology applications. However, current XNA polymerases function with inferior activity relative to their natural counterparts, indicating that current polymerase engineering efforts would benefit from new benchmarking assays. Here described is a highly parallel, low-cost method for measuring the average rate and substrate specificity of XNA polymerases in a standard qPCR instrument. This approach, termed polymerase kinetic profiling (PKPro), involves monitoring XNA synthesis on a self-priming template using high-resolution melting (HRM) fluorescent dyes. PKPro analysis reveals that XNA polymerases function with rates of ~1–80 nt/min and exhibit substrate specificities of ~0.1–5-fold for xNTP versus dNTP. Because PKPro is performed in 96-well format, this technique could also be used to rapidly screen polymerase reaction conditions or engineered polymerases variants.

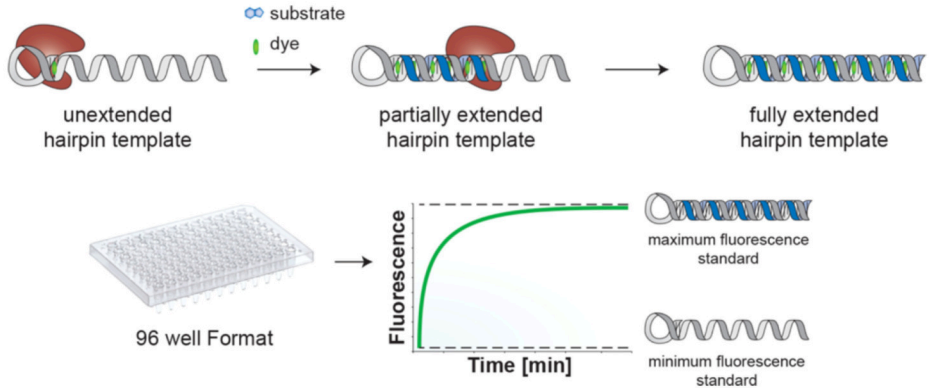


***Figure.*** *Polymerase Kinetic Profiling (PKPro).*

In summary, the field of polymerase engineering would benefit from highly parallel methods that provide quantitative information about the rate of XNA synthesis. Such assays are not meant to replace traditional kinetic assays that are necessary for detailed characterization of a final polymerase design but rather to increase throughput by reducing the time required to evaluate existing enzymes and benchmark the evolutionary process used to obtain new XNA polymerases.

This work was supported by DARPA (N66001-16-2-4061) and the NSF (1607111).

***References:*** [1] Nikoomanzar, A.; Dunn, M.R.; Chaput, J. C. *Anal. Chem.* **2017,** *89*, 12622-12625.

*Corresponding author: E-mail: jchaput@uci.edu

# Studies on the selectivity of nucleoside antibiotics

Giuliana Niro, Christian Ducho*

*Department of Pharmaceutical and Medicinal Chemistry, Saarland University, Campus C2 3, 66123 Saarbrücken, Germany*

Due to increasing resistances against established antibiotics, the need for novel drugs which address unexplored bacterial targets is urgent. Nucleoside antibiotics (e.g., muraymycins and mureidomycins) represent such a new class, which inhibits peptidoglycan formation blocking an early intracellular step, i.e., the reaction catalyzed by the bacterial membrane protein MraY.[1-3]
So far, the reason for different antimicrobial activities and selectivities of the nucleoside antibiotics is poorly understood. Muraymycins are mainly active against Gram-positive bacteria but also against Gram-negative *E. coli* with increased membrane permeability, while there is a loss of activity against wild-type *E. coli*. Mureidomycins on the other hand are mainly active against Gram-negative *P. aeruginosa* but inactive against most Gram-positive pathogens. However, Ichikawa and Matsuda reported structurally modified muraymycin analogues which gained antimicrobial activity against Gram-negative *P. aeruginosa* (see figure).[4]

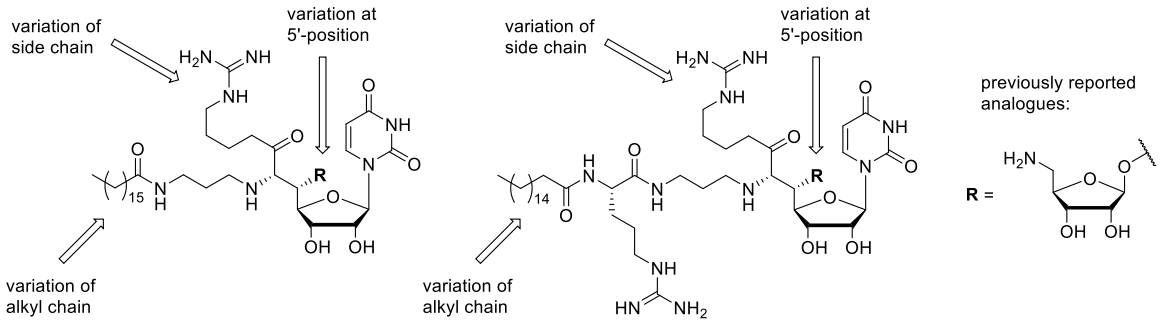


***Figure.*** *Structure of muraymycin analogues with activity against P. aeruginosa (as reported by Ichikawa and Matsuda [4] and envisioned variations*.

Our main goal is a more detailed understanding of the activity of such analogues against *P. aeruginosa*. Therefore, we synthesize further analogues for structure-activity relationship studies with variations at the 5'-position, of the guanidine side chain as well as of the alkyl chain, to gain more insights into the differences in selectivity and activity. Current results from these studies will be presented.

This work was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie. G.N. thanks the Fonds der Chemischen Industrie for a PhD fellowship.

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*Corresponding author: E-mail: christian.ducho@uni-saarland.de

# Fluorescently labelled m$^7$GpppG analogs as probes for fluorescence polarization studies – structure optimization and application

Anna Nowicka,[a] Marcin Warmiński,[a] Renata Kasprzyk,[b] Błażej Wojtczak,[b] Dorota Kubacka,[a] Joanna Kowalska,*[a] Jacek Jemielity *[b]

[a] *Faculty of Physics, University of Warsaw, Pasteura 5 St, 02-093 Warsaw, Poland*
[b] *Centre of New Technologies, University of Warsaw, Banacha 2c St, 02-097 Warsaw, Poland*

Fluorescence polarization (FP) is a method widely used for studying molecular interactions and enzyme activities.[1] This technique represents an excellent tool to search ligands of the tested protein and can be used in high-throughput approaches.
FP method can be successfully applied to study cap-dependent processes such as pre-mRNA splicing, translation and mRNA degradation. Cap (m$^7$GpppN$_n$) is a unique structure occurring at the 5' end of mRNA which protects mRNA from degradation and stimulates gene expression processes *via* interactions with cap-binding proteins. The abnormal activities of these proteins is related to cancer transformation, spinal muscular atrophy and intellectual disabilities.[2,3,4] Search for the ligand which regulates the activity of cap-binding proteins may lead to the discovery of new drug candidates.

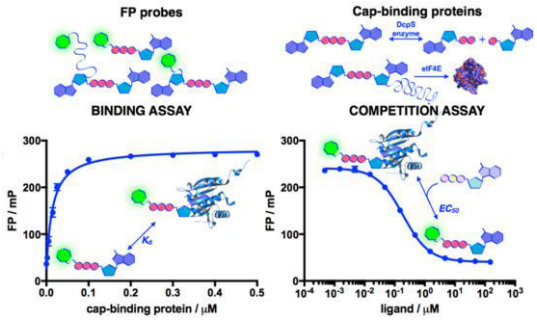


***Figure.*** *Overview of the project – synthesis of the fluorescently labelled cap structures and development of fluorescence polarization method against DcpS and eIF4E.*

Here, we present the development of fluorescence polarization method for searching potent ligands of DcpS and eIF4E. For this purpose fluorescently-labelled probes based on the cap structure were synthetized. Probes differ in site of fluorophore incorporation (ribose, base, phosphate) and length of the linker. Probes with optimal properties were selected and used for development of fluorescence polarization method and applied in high-throughput screening.

This work was supported by grants from The National Science Centre (UMO-2016/21/N/ST4/03750)

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*Corresponding author: E-mail: jkowalska@fuw.edu.pl, j.jemielity@cent.uw.edu.pl

# Development of cell-permeable oligonucleotides bearing GSH-activated protecting groups

Takayuki Ohta, Akira Ono*1, Hisao Saneyoshi*2

*Department of Material and Life Chemistry, Faculty of Engineering, Kanagawa University, 3-27-1 Rokkakubashi, Kanagawa-ku, Yokohama 221-8686, Japan.*

Synthetic oligonucleotides are important biological tools that can be used to treat various human diseases. However, their applications as therapeutic agents have been limited by their poor cell membrane permeability and poor stability towards enzymatic degradation. One possible strategy to improve cellular uptake is the pro-oligonucleotide approach in which the negatively charged phosphodiester moieties are protected by biodegradable protecting groups [1][2].

In this study, we focused on the use of glutathione (GSH) to activate pro-oligonucleotides. GSH exists in higher concentration in cells (10 mM) against in extracellular mediums (10 μM) [3]. Because of this GSH concentration gradient, pro-oligonucleotides should be stable in the extracellular medium and, after cellular uptake, the protecting groups would be deprotected by the abundant intracellular GSH.

We designed and synthesized a series of GSH-labile protecting groups for the protection of phosphodiester moieties in oligonucleotides (Figure 1). The GSH-labile protecting groups were incorporated into phosphoramidite monomer units which were applied to solid phase DNA synthesis.

Deprotection of the protecting groups by GSH was tested. In the presence of GSH (10 mM), the protecting groups were deprotected. On the other hand, pro-oligonucleotides were stable in a buffer containing GSH (10 μM). Cell membrane permeabilities of synthesized oligonucleotides were tested. Efficacies of cellular uptake were related to the number and location of the protecting groups.

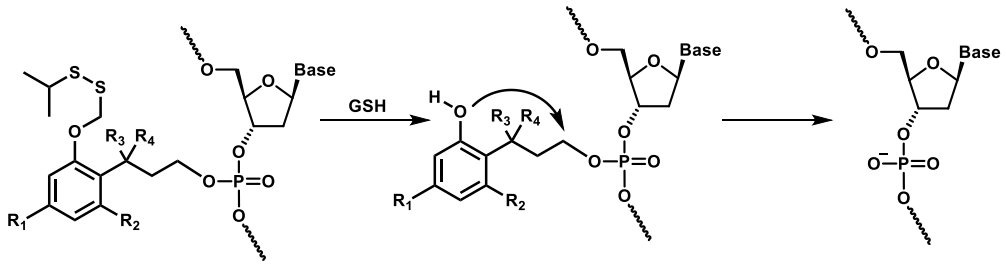


***Figure 1.*** *Schematic representation for deprotection pathway of the protecting groups*

***References:***[1] J. C. Bologna, E. Vives, J. L. Imbach and F. Morvan, *Antisense Nucleic Acid Drug Dev.*, **2002**, *12*, 33-41.[2] B. R. Meade, K. Gogoi, A. S. Hamil, C. Palm-Apergi, A. v. d. Berg, J. C. Hagopian, A. D. Springer, A. Eguchi, A. D. Kacsinta, C. F. Dowdy, A. Presente, P. Lonn, M. Kaulich, N. Yoshioka, E. Gros, X.-S. Cui and S. F. Dowdy, *Nat. Biotechnol.*, **2014**, *32*, 1256-1261.[3] G. Saito, J. A. Swanson, K. D. Lee, *Adv. Drug Delivery Rev.*, **2003**, *55*, 199-215.

*1 E-mail: akiraono@kanagawa-u.ac.jp / *2 E-mail: saneyoshih@kanagawa-u.ac.jp

# Synthesis and properties of fluorescent nucleic acids containing pyrene-based D-π-A dyes

Takumi Okuda,[a] Yuuya Kasahara,[b] Satoshi Obika*[a, b]

[a]*Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan.*
[b]*National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN), 7-6-8 Saito-Asagi, Ibaraki, Osaka 567-0085, Japan.*

Fluorescent oligonucleotide is a powerful tool to investigate the structure and function of nucleic acids. Base-modified fluorescent nucleosides that could change their fluorescent property in response to a local environment within a DNA duplex have been developed and introduced into oligonucleotides as reporter molecules.[1] Pyrene is one of the most utilized fluorophore for sequence-specific detection of nucleic acids due to its unique fluorescent properties[2], such as solvatochromism, ability to form excimers and acting as a π-π-stacking moiety. However, it is difficult to use pyrene-modified fluorescent nucleosides for molecule imaging in biological samples. Pyrene shows short absorption and emission wavelengths and low quantum yield in water. Herein, we focused on an electron donor-π-acceptor (D-π-A) system to improve the fluorescent properties of pyrene derivatives.[3] We designed pyrene derivatives bearing piperidine and three different acceptors, i.e. uracil ($^{PP}$U), 5-ethynyluracil ($^{PPA}$U) and *N*-substituted carboxamide ($^{PPAA}$U) (Figure 1).

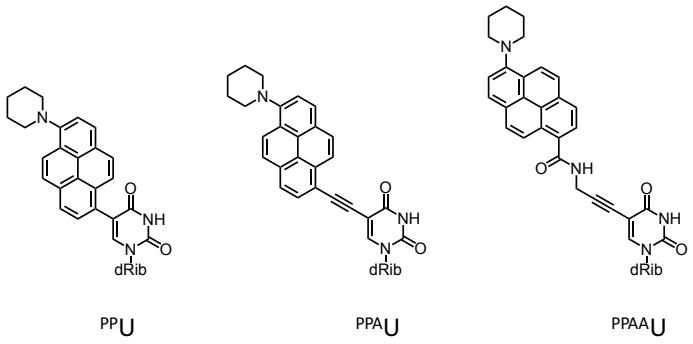


***Figure 1.*** *Fluorescent nucleic acids containing pyrene-based D-π-A dyes.*

We have succeeded to synthesize three pyrene-modified fluorescent nucleosides and evaluated the fluorescent properties. Interestingly, emission intensity of fluorescent nucleosides, $^{PP}$U and $^{PPA}$U, utilizing the pyrimidine base as electron-withdrawing group was drastically decreased in water (pH = 7.4). On the other hand, $^{PPAA}$U containing the *N*-substituted carboxamide as electron acceptor showed high quantum yield and long emission wavelengths.

In conclusion, we successfully synthesized novel pyrene-modified fluorescent nucleosides, $^{PP}$U, $^{PPA}$U and $^{PPAA}$U. Among them, $^{PPAA}$U showed improved fluorescent properties.

***References:***

[1] Tor, Y., et al. *Chem. Rev.* **2010***, 111*, 2579. [2] Venyaminova, A. G., et al. *Molecules* **2017**, *22*, 2108. [3] Konishi, G., et al. *RSC Adv.* **2014**, *4*, 36480.

*Corresponding author: E-mail: obika@phs.osaka-u.ac.jp

# Synthesis of cleavage-resistant, fluorescentlly labeled cap analogue as a molecular probe for screening potential inhibitors of human Decapping Scavenger

Teodor Olejko,[ab] Sylwia Walczak,[ab] Anna Nowicka,[bc] Joanna Kowalska,[c] Jacek Jemielity*[b]

[a] *College of Inter-Faculty Individual Studies in Mathematics and Natural Sciences, University of Warsaw, Warsaw, Poland*
[b] *Centre of New Technologies, University of Warsaw, Warsaw, Poland*
[c] *Division of Biophysics, Institute of Experimental Physics, Faculty of Physics, University of Warsaw, Warsaw, Poland*

The 5’ mRNA cap protects mRNA from 5’ → 3’-exonuclease degradation and has an important role in gene expression processes including translation initiation. The cap interacts with several proteins involved in mRNA metabolism which have often been linked to disease development. Therefore, synthetic cap analogues are useful in wide range of applications, including modulating activity of key cap-dependent enzymes. One of them is Decapping Scavenger (DcpS) – a pyrophosphatase cleaving the triphosphate chain within the cap to release $m^7$GMP and a 5’-diphosphate from the rest of the molecule. DcpS degrades cap structure released from mRNA 3’ → 5’ decay. DcpS is also a molecular target in Spinal Muscular Atrophy (SMA) treatment, thus its inhibitors are potential therapeutic agents. Fluorescent molecular probes that bind DcpS with high affinity can be used in the discovery of tightly binding inhibitors of DcpS as potential therapeutics for SMA. Here, we designed and synthesized a fluorescently labelled mRNA cap analogue with resistance and high binding affinity to DcpS (Figure). The key modification to achieve both features was phosphorothiolate group neighbouring 7-methylguanosine moiety. Carboxyfluoresceine dye was attached to the base of second nucleoside via diamine linker using NHS chemistry. The preliminary spectroscopic and biochemical characteristics of the probe and its applications for discovery of DcpS inhibitors by fluorescence polarization method also will be presented.

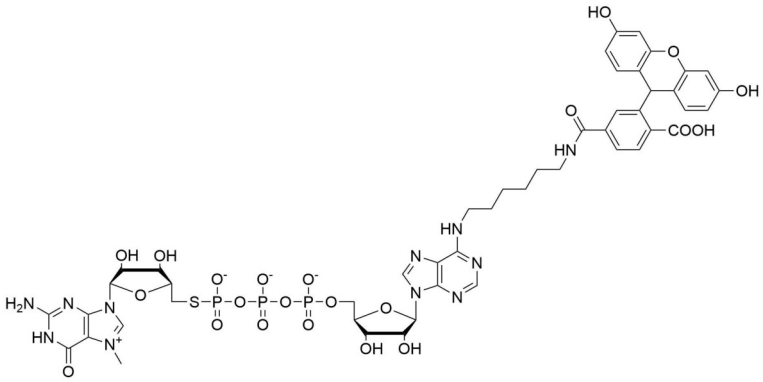


***Figure.*** *Designed structure of the probe $m^7$GspppA-HDA-FAM*

***References:*** [1] Wojtczak, B. A. et al., J. Am. Chem. Soc. **2018***, 140*, *5987-5999*.

*Corresponding author: E-mail: Jacek.Jemielity@fuw.edu.pl

# Spontaneous pseudorotaxane formation targeting nucleic acids

Kazumitsu Onizuka,*[a] Jumpei Matsuyama,[a] Takuya Miyashita,[a] Yuuya Kawasaki,[b] Kazunobu Igawa,[b] Katsuhiko Tomooka,[b] Fumi Nagatsugi*[a]

[a] *Institute of Multidisciplinary Research for Advanced Materials, Tohoku University, 2-1-1 Katahira, Aoba-ku, Sendai, Miyagi 980-8577, Japan*
[b] *Institute for Materials Chemistry and Engineering and Department of Material and Molecular Sciences, Kyushu University, Kasuga, Fukuoka 816-8580, Japan*

Rotaxane is a molecular architecture consisting of a dumbbell shaped molecule which is threaded through a molecular ring. In the nucleic acid chemistry, unique mechanically interlocked molecular architectures such as catenane and rotaxane have been constructed by taking advantage of base pairing. A variety of methods to construct interlocked molecular architectures have been developed for DNA/RNA nanotechnology and topological labeling.[1] However, these methods either require a toxic chemical reagent or an enzyme to form topological structures; thus, it is difficult to construct them inside the cell.

Currently, we have developed novel methods to form topological DNA/RNA architectures that neither require a toxic chemical reagent nor an enzyme (Figure 1). Here we report a method to spontaneously form a pseudorotaxane architecture using only a pair of reactive oligo deoxynucleotides (ODNs), which we designed and synthesized, and then performed the pseudorotaxane formation reaction with both DNA and RNA oligonucleotides. The reaction proceeded smoothly without any extra reagents at 37 $^{o}$C and pH 7.2, leading to the formation of a stable complex on a denaturing polyacrylamide gel.[2] The yield with optimized pseudorotaxane-forming ODNs (prfODNs) reached 85% in 5 min for RNA target. In addition, we developed fluorescent off-on prfODN using fluorogenic Cu-free click chemistry with 3-azidocoumarin derivative[3] and DACN (diazacyclononyne)[4]. This pseudorotaxane formation method would be new tools for RNA studies and DNA/RNA nanotechnology.

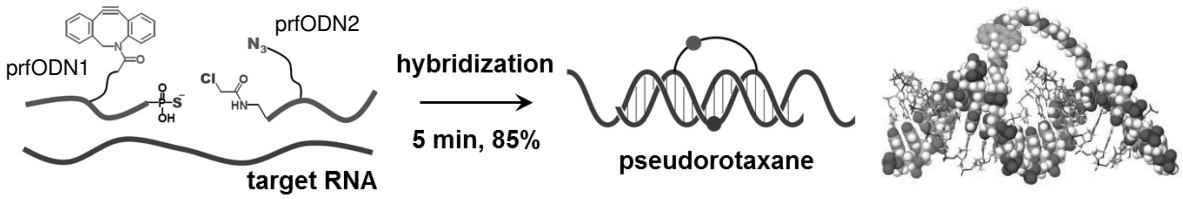


***Figure 1*** *Pseudorotaxane formation reaction*

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*Corresponding author: E-mail: onizuka@tohoku.ac.jp, nagatugi@tagen.tohoku.ac.jp

# α-Tocopherol conjugation to single stranded antisense oligonucleotides improves activity in muscle in non-human primates

Michael E. Østergaard,* Hans Gaus, Alfred Chappell, Michaela Jackson, Jinghua Yu, Thazha P. Prakash, Frank Rigo, Punit P. Seth

*Ionis Pharmaceuticals, 2855 Gazelle Ct., Carlsbad, CA 92010, USA*

Phosphorothioate-modified antisense oligonucleotides (ASOs) distribute broadly in animals after systemic injection but accumulate preferentially in the liver and kidney. As a result, higher doses are required to reduce gene expression in extra-hepatic tissues such as skeletal muscle and heart. These tissues also present additional challenges to ASO delivery as the polyanionic nucleic acid macromolecule has to be transported across the continuous endothelium to access the muscle cells. Lipid-conjugated ASOs associate with plasma proteins such as albumin as well as lipoproteins which can facilitate distribution across the continuous endothelium by transcytotic processes. We investigated the effect of conjugating hydrophobic moieties such as lipids, α-tocopherol and cholesterol to single stranded ASOs to determine if this can enhance ASO distribution and activity in skeletal muscle. ASO conjugates were evaluated for plasma protein binding, tissue distribution and potency in mice. The α-tocopherol and cholesterol conjugated ASOs showed enhanced association with lipoproteins in plasma. In contrast, the palmitate conjugated ASOs showed enhanced association with albumin. Both α-tocopherol and cholesterol conjugated ASOs showed improved potency for reducing gene expression in skeletal muscle and heart in mice but, the cholesterol conjugated ASO also showed some toxicity at higher doses. To determine if ASOs conjugated to hydrophobic moieties show enhanced potency in larger species, we evaluated an α-tocopherol conjugated ASO targeting dystrophia myotonica-protein kinase mRNA in non-human primates. The α-tocopherol conjugated ASO increased drug accumulation and improved activity in skeletal muscle and heart in non-human primates.

*Corresponding author: E-mail: moesterg@ionisph.com

# Chemical synthesis of both anomers of 2'-deoxy-C-nucleosides and evaluation of antiviral activity

P. Srishylam[a], S. Rajendra Prasad[a], D. Sudhakar Reddy[a], Shyamapada Banerjee[a], Paidi Yella Reddy[a], Santosh Penta[c], Yogesh S. Sanghvi[a,b]

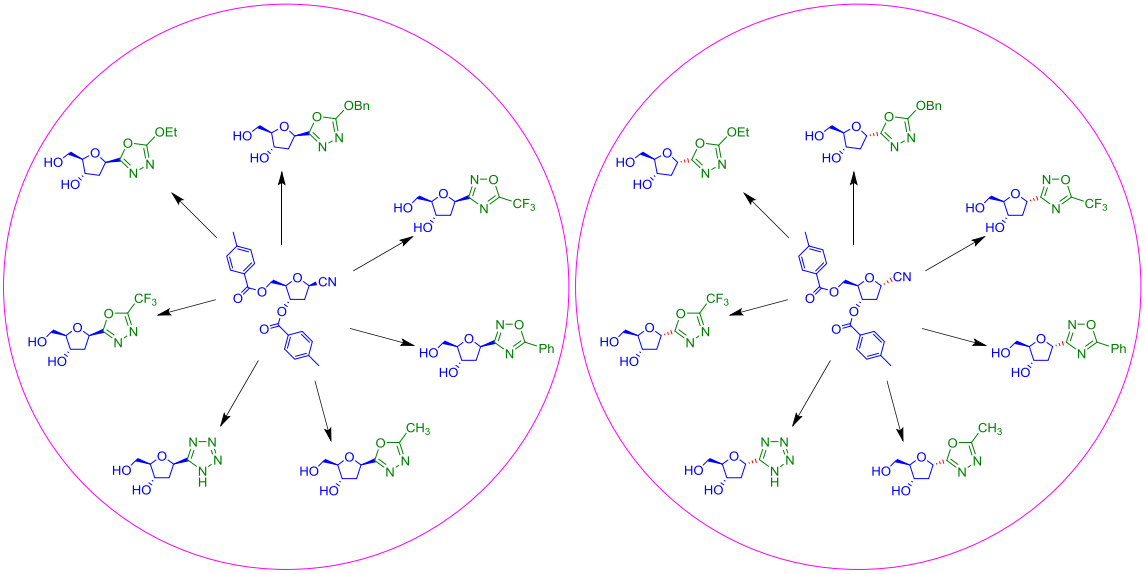
[a] Sapala Organics Pvt. Ltd, Plot Nos. 146B & 147 IDA Mallapur, Phase-II, Hyderabad-500076, Telangana. India

[b] Rasayan Inc. 2802 Crystal Ridge Road Encinitas, CA 92024-6615, USA. [C] NIT, Raipur, India

## Abstract:

Majority of the therapeutic nucleosides[1] as drugs on the market are N-nucleosides. Often C-nucleoside are difficult to synthesize [2,3] and yet to be commercialized as drugs. Unlike natural and synthetic N-nucleosides, C-nucleosides are stable to enzymatic and acid-catalyzed hydrolysis of the glycosidic bond. These properties of C-nucleosides offer a distinct advantage over the N-nucleosides. C-Nucleosides have also attracted the interest of researchers interested in hydrogen-bond interactions alternative to those produced in the classical Watson–Crick model.

Synthesis of a common building block by C-C bond formation at C1 and further integration of the heterocycle was central theme to our approach. One of the most important types of C-glycosyl intermediates is the glycosyl cyanide, which is usually obtained as mixture of cyanide anomers from 1-chloro carbohydrates by reaction with trimethyl silyl cyanide in the presence of a Lewis acid as catalyst. These two anomers of glycosyl cyanides were transformed into various new C-nucleosides. The synthesis and antiviral activity of these novel C-nucleosides will be presented as poster.



## Reference:

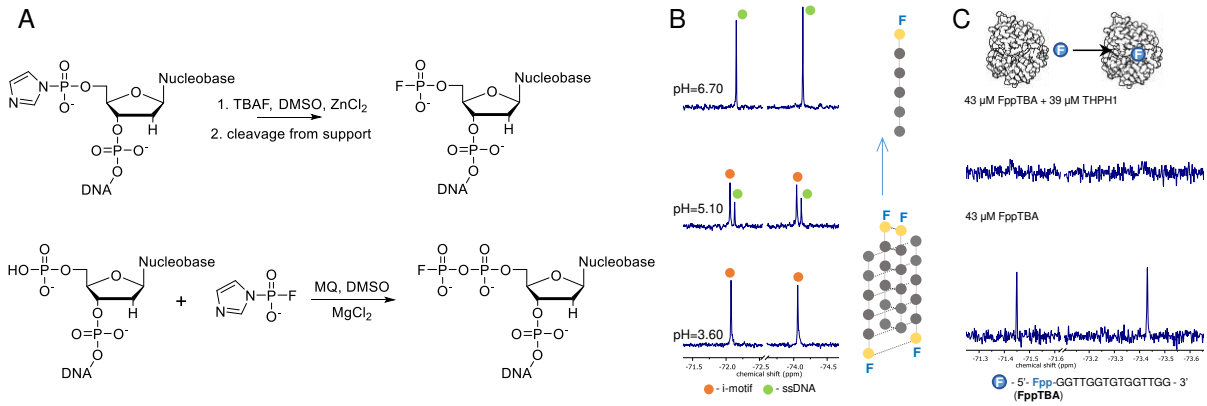
1. Jan Štambaský , Michal Hocek and Pavel Kočovský, *Chem. Rev.*, **2009**, *109*, 6729–6764
2. Erik De Clercq, *J. Med. Chem.*, **2016**, *59 (6)*, 2301–2311
3. Qinpei Wu, Claire Simons, *Synthesis*, **2004**, *10*, 1533–1553

# Oligonucleotide 5'-fluoro(di)phosphates enable monitoring of duplex, G-quadruplex and i-motif DNA formation by $^{19}$F NMR

Marek R. Baranowski,[a] Marcin Warminski,[a] Jacek Jemielity,[b] Joanna Kowalska*[a]

[a] *Faculty of Physics, University of Warsaw, Poland*
[b] *Centre of New Technologies, University of Warsaw, Poland*

Labelling of nucleic acids with fluorine is commonly used to study DNA hybridization, tertiary structure formation, or protein-nucleic acid interactions by $^{19}$F NMR. Absence of fluorine atoms in natural deoxy- and ribonucleic acids make this biopolymers as an ideal molecular probes to study structural changes in combination with nuclear magnetic resonance phenomenon. $^{19}$F NMR is almost as sensitive as $^{1}$H NMR but chemical shift dispersion is 50-fold wider making fluorine spectra much simpler to analyze. Moreover, $^{19}$F NMR chemical shift is exceptionally sensitive to the local environment changes of fluorine atom allowing to monitor even minor structural and conformational transformations. Here, combining known chemical methods, we synthesized oligodeoxyribonucleotides (ODNs) 5'-labelled with fluorophosphate or fluorodiphosphate moiety (FpODN or FppODN, A) [1, 2]. The usefulness of fluoro(di)phosphate oligonucleotide analogs in $^{19}$F NMR studies was verified by $^{19}$F NMR monitoring of hybridization with complementary unlabeled oligonucleotide, as well as formation of mismatched duplexes, G-quadraplexes, and i-motifs (B). The fluoro(di)phosphate moiety at the 5' end of oligo generally did not interfere with secondary and tertiary structure formation and provided sensitivity sufficient for NMR studies at micromolar concentrations. Distinct chemical shifts for single stranded and double stranded oligonucleotides allow to differentiate these two forms in $^{19}$F NMR spectra. Finally, we found that FpODN aptamers allow observation of protein-nucleic acid interactions using $^{19}$F NMR (C). Altogether, our results designate 5'-fluoro(di)phosphate moiety as a synthetically available, universal, and non-interfering $^{19}$F label for ODNs.




This work was supported by the Ministry of Science and Higher Education (DI2013 014943) and National Centre for Research and Development (LIDER 003/L-5/2013) in Poland.

***References:*** [1] Baranowski et al. *J. Org. Chem*., **2015**, 80, 3982–399. [2] Thillier et al. *RNA*. **2012**, 18, 856-868.

*Corresponding author: E-mail: jkowalska@fuw.edu.pl

# Thymidine nucleotides with alkyne-linked long flexible tethers for efficient postsynthetic DNA functionalization through CuAAC.

Alessandro Panattoni,[a] Michal Hocek*[a,b]

[a]*Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Gilead & IOCB Research Center, Flemingovo namesti 2, CZ-16610 Prague 6, Czech Republic;* [b]*Department of Organic Chemistry, Faculty of Science, Charles University in Prague, Hlavova 8, Prague-2 12843, Czech Republic;*

The copper-catalyzed alkyne-azide 1,3-dipolar cycloaddition (CuAAC) is an ideal tool for the postsynthetic modification of nucleic acids, and is, indeed, widely employed for DNA labeling, cross-linking, and biomolecular ligation.[1,2] Thymidine derivatives containing terminal alkynyl groups, in particular, 5-ethynyl-dU (**1**, ***Figure 1***) and 5-(1,7-octadiynyl)-dU (**2**, ***Figure 1***), have been extensively employed for this purpose.[1,2] However, the steric hindrance caused by the proximity of the nucleobase to the acetylene moiety considerably affects the CuAAC, and longer and more flexible linkers between the nucleobase and the terminal alkyne are known to favor the click proceeding.[3]

Here, we present the synthesis of two alternative building blocks, useful for postsynthetic functionalization of DNA through the CuAAC. Thymidine nucleoside derivatives **3** and **4** (***Figure 1***), substituted at the α position either with an *O*-propargyl-diethylene glycol side chain (**3**) or with a 10-undecynyl side chain (**4**), were synthesized and converted to the respective 5'-*O*-triphosphates (**3a** and **4a**, ***Figure 1***) and their 3'-*O*-phosphoramidites (**3b** and **4b**, ***Figure 1***). Oligonucleotides bearing these base modifications were highly reactive toward the CuAAC.

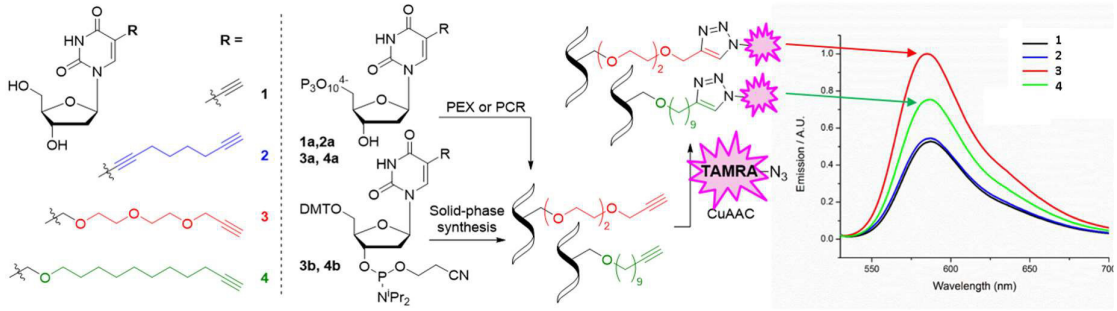



***Figure 1***

This work was supported by by Marie Sklodowska-Curie Innovative Training Network (ITN) Click Gene (H2020-MSCA-ITN-2014-642023).

***References:*** [1] A. H. El-Sagheer, T. Brown, *Chem. Soc. Rev.*, **2010**, 39, 1388-1405. [2] P. M. E. Gramlich, C. T. Wirges, A. Manetto, T. Carell, *Angew. Chem. Int. Ed.*, **2008**, 47, 8350-8358 [3] P. M. E. Gramlich, S. Warncke, J. Gierlich, T. Carell, *Angew. Chem. Int. Ed.*, **2008**, 47, 3442-3444.

*Corresponding author: E-mail: michal.hocek@uochb.cas.cz

# A Chimeric Nucleobase - Phenylazo Derivative as an Intrinsic Nucleobase Quencher

Gyeongsu Park,[a] Christie Ettles,[a] Robert H.E. Hudson*[a]

[a] *Department of Chemistry, Western University, London, Ontario, Canada*

Molecular beacons are important bioanalytical probes which are most often constructed from a single-stranded oligonucleotide which has been labelled at opposite termini with a fluorophore and a quencher. When the fluorophore and quencher are in close proximity, no fluorescence is observed due to fluorescence resonance energy transfer (FRET). DABCYL (4-dimethylaminoazobenzene-4'-carboxylic acid) has been widely used as a quencher in molecular beacons; however, it is unable to form a base-pair and is conventionally placed as an overhanging residue. This produces a derivative wherein the chromophore has substantial mobility and limits the types of other conjugates that can be prepared. In order to overcome these limitations, we have embarked on the synthesis of peptide nucleic acid (PNA) analogue possessing DMPAU (5-[(4-dimethylaminophenyl)diazenyl]uracil) as the nucleobase. DMPAU has DABCYL-like properties due to the installation of an azo moiety at the 5-position of the uracil base. This base is designed to have the ability to form a complementary base pair with adenine by canonical hydrogen bonding and also to quench the fluorescence emission in a molecular beacon construct. DMPAU PNA analogue possesses the same UV-Vis absorbance range as DABCYL and provides a reasonable quenching effect toward blue fluorophores.

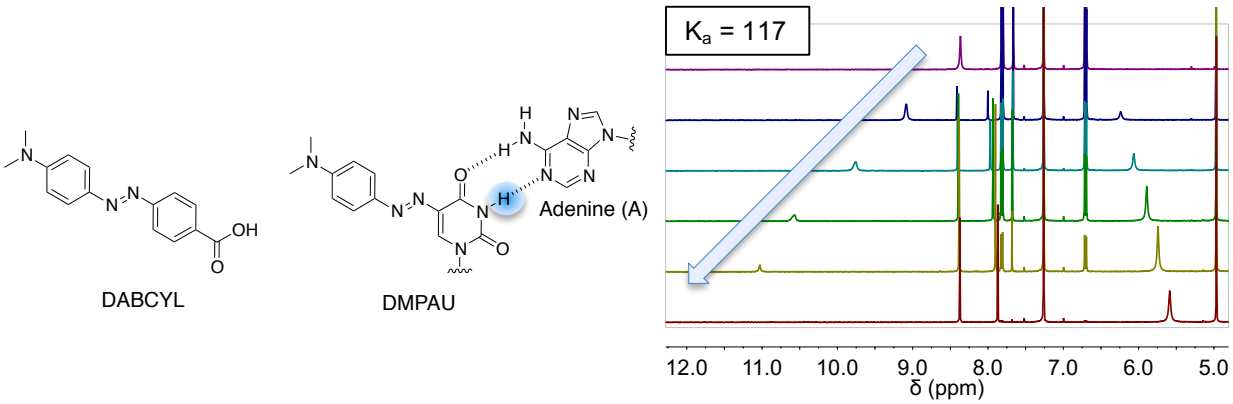



***Figure.*** *Structure of DABCYL and DMPAU with complementary nucleobase, adenine, and $^1$H NMR binding study.*

This work was supported by grants from NSERC Canada.

***References:*** [1] Kramer, F. et al., *Nat. Biotechnol.* **1996***, 14*, 303. [2] Moustafa, M. Design and Syntheses of Novel Quenchers for Fluorescent Hybridization Probes. thesis, The University of Western Ontario, 2011. [3] Hudson, R. H. E. et al. *Acta Crystallogr. E.* **2014**, *70,* 556.

*Corresponding author: E-mail: rhhudson@uwo.ca

# Synthesis and Application of Highly Emissive Deoxyguanosine and Thymidine Analogues

Soyoung Park[*a] and Hiroshi Sugiyama[*a,b]

[a] *Department of Chemistry, Graduate School of Science, Kyoto University*
[b] *Institute for Integrated Cell-Material Science (iCeMS), Kyoto University*

The development of fluorescent nucleoside analogues has engaged the interest of many scientists because of the nonemissive nature of natural nucleic acid bases. Fluorescent nucleosides have provided powerful and essential tools and contributed to advance for understanding biological phenomenon.

Tor and coworkers have developed isomorphic fluorescent RNA nucleosides derived from thieno[3,4-d]-pyrimidine, which have very significant photophysical features including visible light emission and a high quantum yield. Inspired by their study, we have focused on the potential of isomorphic nucleoside analogues based on a thieno[3,4-*d*]-pyrimidine core and exploited the emissive DNA nucleoside analogues, **$^{th}$dG**. We demonstrated that **$^{th}$dG** enabled the visual detection of Z-DNA. We also synthesized an emissive 2'-O-methylated guanosine analogue, 2'-OMe-thG, and developed a visible-light nanothermometer. Thieno[3,4-d]-pyrimidine T-mimic deoxynucleoside (**$^{th}$dT**) and its triphosphate **$^{th}$dTTP** were synthesized and have been investigated as fluorescent thymidine surrogates. Very recently, we developed a Watson–Crick base-pairable FRET pair that consists of the highly emissive DNA analogues **$^{th}$dG** as the energy donor and tC as the energy acceptor. Herein, we describe synthesis, photophysical characterization and application of thieno[3,4-*d*]-pyrimidine-based deoxyribonucleosides.

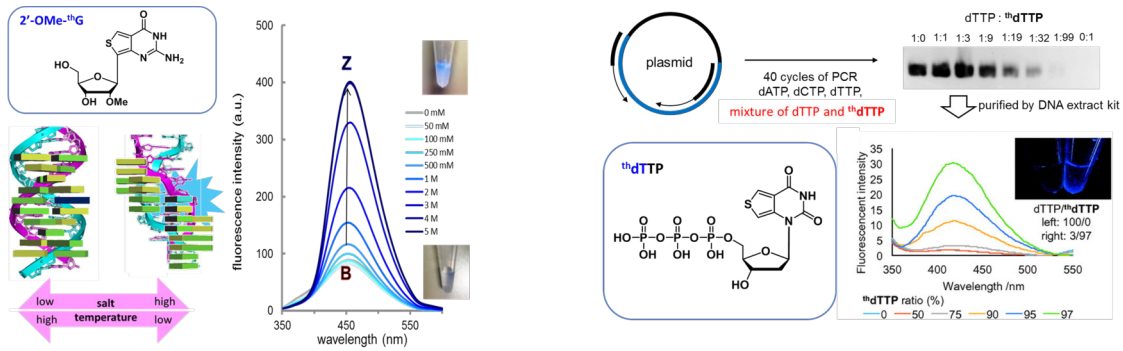



***Figure.*** *Observation of the conformational changes from B- to Z- DNA and PCR amplification for long fluorescent DNA*

***References:*** [1] J. H. Han, S. Yamamoto, S. Park, H. Sugiyama, *Chem. Eur. J.*, **2017**, 23, 7606–7613. [2] I. Okamura, S. Park, R. Hiraga, S. Yamamoto, H. Sugiyama, *Chem. Lett.*, **2017**, *46*, 245–248. [3] S. Yamamoto, S. Park, H. Sugiyama, *RSC Adv.*, ***2015***, *5*, 104601–104605. [4] H. Otomo, S. Park, S. Yamamoto, H. Sugiyama, *RSC Adv.* **2014**, 4, 31341–31344. [5] S. Park, H. Otomo, L. Zheng, H. Sugiyama, *Chem. Commun.* **2014**, *50*, 1573–1575.

[*]Corresponding author: oleesy@kuchem.kyoto-u.ac.jp, hs@kuchem.kyoto-u.ac.jp

# Gold nanoparticle-nucleotide conjugates as molecular probes for monitoring activity of cap-binding proteins

O. Perzanowska[a], M. Majewski[a], J. Kowalska[a], J. Jemielity[b*]

[a] *Division of Biophysics, Institute of Experimental Physics, Faculty of Physics, University of Warsaw, Pasteura 5, 02-093 Warsaw, Poland*
[b] *Centre of New Technologies, University of Warsaw, S. Banacha 2c, 02-097 Warsaw, Poland*

$m^7G$ cap is a nucleotide structure present at the 5' end of eukaryotic mRNA. I takes part in many important cellular processes, such as mRNA maturation, transport, initiation of translation and degradation.[1] Cap partakes in these processes through multiple interactions with cap-binding proteins, thereby regulating their activity. Abnormal activity of cap-biding proteins can lead to, or be an indicator of, disease development.

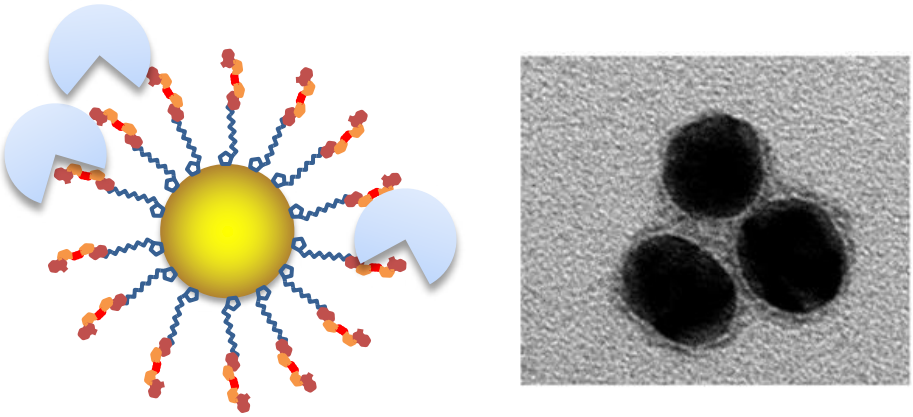


***Figure 1.*** *Gold nanoparticle-cap analog conjugates, bound by eIF-4E protein.*

Here, we aimed to develop cap-decorated gold nanoparticles as molecular tools useful in studying activity of cap-binding proteins. We synthesized gold nanoparticle-cap analog conjugates taking advantage of lipoic-acid functionalization of cap structure (Fig. 1). Taking advantage of electrical and optical properties of gold nanoparticles[2], such as sensitivity to composition of monolayer encasing them, or ability to quench fluorescence of nearby chromophores [3] we characterized the physicochemical and biological properties of gold nanoparticles.. This allowed us to develop sensitive methods for detecting cap-binding proteins and studying their interactions with the aid of our conjugates

***References:*** [1] A. K. Banerjee et al., *Microbiological Rev*., 1980, 175-205. [2] R. G. Rayavarapu et al., *J. Biomed. Imaging*, 2007, 1, 5-5 [3] E. Dulkeith et al., *Phys. Rev. Let*., 2002, 89, 203002

* jemielity@cent.uw.edu.pl

# Antinociceptive effects of a new series of $N^6$,5'-disubstituted adenosine derivatives

Riccardo Petrelli,[a] Mirko Scortichini,[a] Giorgio Fioravanti,[a] Livio Luongo,[b] Lavecchia A,[c] Sabatino Maione,[b] Karl-Norbert Klotz,[d] and Loredana Cappellacci*[a]

[a] *School of Pharmacy, Medicinal Chemistry Unit, University of Camerino, via S. Agostino 1, 62032 Camerino (MC), Italy*
[b] *Department of Experimental Medicine, Section of Pharmacology "L. Donatelli", University of Campania "L. Vanvitelli", via S. Maria di Costantinopoli 16, 80138 Naples, Italy*
[c] *Department of Pharmacy, "Drug Discovery" Laboratory, University of Naples "Federico II, via D. Montesano 49, 80131 Naples, Italy*
[d] *Institut für Pharmakologie and Toxikologie, Universität Würzburg, Versbacher Straβe 9, D-97078 Würzburg, Germany*

Adenosine receptors (ARs) belong to the family of G-protein coupled receptors (GPCRs). Four human ARs subtypes have been characterized, named $A_1$, $A_{2A}$, $A_{2B}$ and $A_3$ ARs, that recognize adenosine and represent potential drug targets for a wide range of diseases. ARs have distinct distribution, signal transduction pathways, physiological effects, pharmacological properties and therapeutic application. [1]
The $A_1$AR is the best-characterized adenosine receptor subtype. Selective $A_1$ adenosine receptor ($A_1$AR) agonists have antinociceptive, antiarrhythmic, neuro- and cardioprotective effects and reduce lipolysis in adipose tissue and intraocular pressure in glaucoma.
Our previous work discovered that combining the appropriate 5'- and $N^6$-substitution in adenosine derivatives, highly selective human (h) $A_1$AR agonists [2] or highly potent dual h$A_1$AR agonists and h$A_3$AR antagonists [3] can be obtained. The latter may be useful in the treatment of epilepsy and glaucoma. The substitution of the OH in 5' of the sugar moiety of $N^6$-substituted adenosine derivatives with a chlorine atom, brought to one of the most potent and selective h$A_1$AR agonist (i.e. 5'-chloro-5'-deoxy-$N^6$-(±)-*endo*-norbornyl-adenosine), while the replacement with a 5'-*C*-ethyl-tetrazolyl moiety maintained the high $A_1$ potency, but restored also the $A_3$AR affinity, leading to very potent dual h$A_1$AR agonists and $A_3$AR antagonists. [4]
In order to explore new heterocycles in 5'-position of adenosine derivatives, a series of 5'-deoxy-5'-(3,5-dimethyl)-pyrazolyl-$N^6$-substituted adenosine derivatives have been synthesized and tested in binding assays at all AR subtypes. Moreover, a molecular modeling and antinociceptive activities will be discussed.

This work was supported by grants from the University of Camerino (Grant FAR 2014/15 FPI000044), and by the Italian MIUR funds (Grant 20094BJ9R7 and Grant 200928EEX4_004).

***References:*** [1] Jacobson, K. A. et al. *Neuropharmacology* **2016**, *104*, 31. [2] Franchetti, P. et al. *J. Med. Chem.* **2009**, *52*, 2393. [3] Petrelli, R. et al. *J. Med. Chem.* **2015**, *58*, 2560. [4] Petrelli, R. et al. *J. Med. Chem.* **2017**, *60*, 4327.

*Corresponding author: E-mail: loredana.cappellacci@unicam.it

# Anomeric 4´-Alkoxy-α-D-2´-Deoxy-Oligonucleotides

Magdalena Petrová*, Miloš Buděšínský, Pavel Novák, Ivana Dvořáková, Šárka Rosenbergová, Ivan Rosenberg

*Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo nám. 2, 166 10 Praha 6, Czech Republic*

In contrast to natural β-D-oligodeoxynucleotides, α-D-anomeric analogues (α-D-DNA) are resistant to nucleases. Uniformly modified α-D-DNAs form stable parallel duplexes with complementary natural ssRNA and ssDNA, and triplexes with polypurine-polypyrimidine DNA duplexes. Phosphodiester 4´-alkoxy-β-D-deoxynucleotides adopt predominantly C3´-*endo* conformation, which results in increasing hybridization ability and selectivity towards natural RNA [1].

The synthesis of α-D-4´-methoxythymidine is based on the procedure described for β-D-analogue [2]. The starting α-D-thymidine was obtained by mild acid anomerization of 3´,5´-di-*O*-acetylthymidine [3], and convenient separation of 3´-TBDPS α/β anomeric mixture. A series of unmodified, partially, and fully modified α(β)-D-homooligothymidylates were prepared by phosphoramidite method on solid phase, and their properties are under investigation.

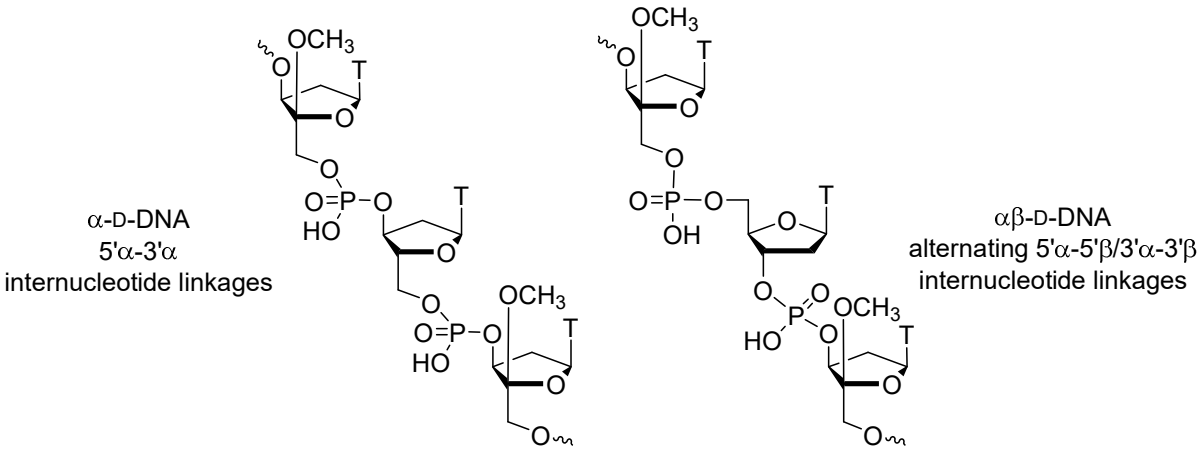



***Figure.*** *Examples of modified homooligothymidylates.*

This work was supported by the grant # 17-12703S (Czech Science Foundation).

***References:*** [1] Liboska, R. et al., *Org. Biomol. Chem.* **2011***, 9*, 8261. [2] Petrová, M. et al., *Org. Lett.* **2015***, 17*, 3426. [3] Ward, D. I., et al. *Tetrahedron Lett.* **1993**, *34*, 6779.

*Corresponding author: E-mail: petrova@uochb.cas.cz

# Isoxazole-containing mimetics of 5’ cap mRNA as inhibitors of the translation initiation process: synthesis, biological and biophysical evaluation

Karolina Piecyk,[a] Marzena Jankowska-Anyszka,[a] Maria Janowska,[a] Maciej Lukaszewicz,[b] Karol Kamel,[c] Sebastian Kmiecik,[d] Edward Darzynkiewicz,[b] Karolina Piecyk*[a]

[a] *Faculty of Chemistry, University of Warsaw, 02-093, Warsaw, Poland*
[b] *Centre of New Technologies, University of Warsaw, 02-097 Warsaw, Poland*
[c] *Institute of Bioorganic Chemistry, Polish Academy of Sciences, Z. Noskowskiego str. 12/14, 61-704 Poznan, Poland*
[d] *Biological and Chemical Research Centre, Faculty of Chemistry, University of Warsaw, 02-093, Warsaw, Poland*

Heterocyclic compounds comprise the major family of organic compounds. These are enormously essential with wide range of synthetic, pharmaceutical and industrial applications and are famous for their biological activities. One of the most interesting five membered heterocyclic rings is an isoxazole. Isoxazole derivatives exhibit numerous biological activities and they act mainly as antimicrobial, antiviral, anticancer, immunomodulatory and anti-diabetic agents. The current research deals with the synthesis of a new series of synthesis and biological evaluation of isoxazole-containing mimetics of 5’ cap mRNA and their ability to inhibit the cap-dependent translation initiation.

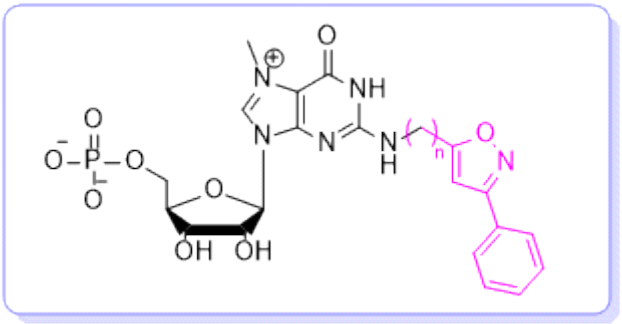



***Figure.*** *Exemplary isoxazole-containing mimetics of 5’ cap mRNA*

This work was supported by grant from the National Science Centre, Poland (DEC-2016/21/D/ST5/01654 )

***References:*** [1] Photon, X. Y. et al., *Chem. Rev.* **2011***, 123*, 1234. [2] Fluorophore, Q. Y., et al. *J. Am. Chem. Soc.* **2011**, *234*, 2345. [3] Decay, E., et al. *J. Am. Chem. Soc.* **2011**, *345*, 12345. etc.,

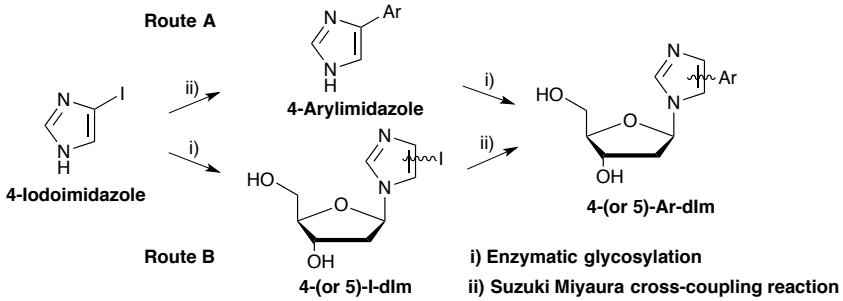
*Corresponding author: E-mail: kpiecyk@chem.uw.edu.pl

# Expedient and generic synthesis of imidazole-based nucleosides via a regiocontrolled enzymatic glycosylation step

Sophie-Vichier-Guerre, Laurence Dugué, Frédéric Bonhomme, Sylvie Pochet*

*Chemistry and Biocatalysis Unit, Institut Pateur, CNRS UMR3523, 28 rue du Dr Roux, 75724 Paris Cedex 15, France*

Nucleoside 2'-deoxyribosyltransferase (NDT, EC 2.4.2.6) catalyzes the transfer of 2-deoxyribose between purine and pyrimidine bases. Interestingly, NDT tolerates a wide range of modified nucleobases from azole derivatives to expanded-size purines.
As part of our ongoing efforts to enlarge the repertoire of imidazole-based nucleosides, we developed a convenient and versatile two-step synthesis that makes use of an enzymatic *N*-transglycosylation step. To illustrate the scope of this approach, a diverse set of 4-(hetero)aryl-1(*H*)-imidazoles featuring variable sizes and hydrogen-bonding patterns was prepared through microwave-assisted Suzuki-Miyaura cross-coupling reactions (Scheme 1 route A) [1]. Next these imidazole derivatives were converted to 2'-deoxyribonucleosides in good to high yields using NDT from *L. leichmannii* (*Ll*NDT) [2].




***Scheme 1**. Synthetic routes to imidazole-based nucleosides*

We also determined experimental conditions allowing to manage regioselectivity (N3 *vs* N1-isomers) in NDT glycosylation of imidazole derivatives, thus providing a simple access to isomers not readily accessible by chemical routes. Interestingly, a series of flexible nucleosides was easily obtained in one-step from 4 or 5-iodo-imidazole nucleoside by by Suzuki-Miyaura cross-coupling reaction with a series of (hetero)aryl-boronic acids in aqueous media (Scheme 1 route B) [3]. This chemoenzymatic approach is compatible with a one-pot two-step process affording a straightforward access to a broad array of potential anticancer and antiviral drugs, as well as new DNA building blocks for synthetic biology.

This work was supported by grants from the Institut Pasteur and CNRS.

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*Corresponding author: E-mail: sylvie.pochet@pasteur.fr

# Thrombin binding aptamer G-quadruplex stabilized by pyrene modified nucleotides

Matic Kovačič,[a] Peter Podbevšek,[a,b] Hisae Tateishi-Karimata,[c] Naoki Sugimoto,[c,d] Janez Plavec*[a,b,e]

[a]*Slovenian NMR centre, National Institute of Chemistry, Ljubljana, Slovenia*
[b]*EN-FIST Centre of Excellence, Ljubljana, Slovenia*
[c]*Frontier Institute for Biomolecular Engineering Research, Kobe, Japan*
[d]*Graduate School of Frontiers of Innovative Research in Science and Technology, Kobe, Japan*
[e]*Faculty of Chemistry and Chemical Technology, University of Ljubljana, Ljubljana, Slovenia*

Guanine-rich regions of the human genome can adopt non-canonical secondary structures. The role of these four-stranded G-quadruplex DNA in regulating gene expression has become indisputable. Ligand molecules consisting of polyaromatic moieties are especially suitable for targeting G-quadruplexes due to the large exposed surface area of four guanine bases. A predictable way of (de)stabilizing specific G-quadruplex structures through efficient base stacking of polyaromatic functional groups could become a valuable tool in our therapeutic arsenal.

We have investigated the stabilizing effect of pyrene conjugated thymine nucleotides incorporated at single positions into the thrombin binding aptamer model system. Our results show that in specific cases structural changes induced by the pyrene moiety stacking on nearby nucleobases result in stabilization of the G-quadruplex structure. The effect strongly depends on the position of the modification in the oligonucleotide sequence. We show that careful positioning of polyaromatic groups in functional oligonucleotides can be used to boost the stability of G-quadruplex structures and that this approach has potential in modulating stability of future G-quadruplex based therapeutics.

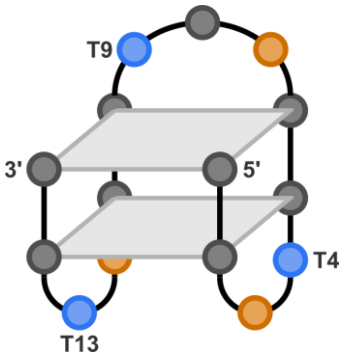



***Figure.*** *Schematic representation of the TBA G-quadruplex structure. Loop thymine positions where pyrene incorporation resulted in a stabilizing and destabilizing effect are shaded in blue and orange, respectively.*

This work was supported by the Japan Society for the Promotion of Science (JSPS) and Slovenian Ministry of Education, Science and Sport (MESS) under the Japan-Slovenia Research Cooperative Program and a grant from the Slovenian Research Agency (P1-0242).

*Corresponding author: E-mail: janez.plavec@ki.si

## GLP1 receptor for targeted delivery of antisense oligonucleotides to pancreatic ß-Cells

Thazha Prakash,[4*] Carina Ämmälä[1], William Drury III[1], Laurent Knerr[1], Ingela Ahlstedt[1], P. Stillemark-Billton[1], C. Wennberg-Huldt[1], E-M. Andersson[1], Eric Valeur[1], Rasmus Jansson-Löfmark[1], Linda Sundström[2], Johan Meuller[2], Josefine Claesson[2], Patrik Andersson[3], C. Johansson[3], Richard Lee[4], Brett Monia[4], Shalini Andersson[1], Punit Seth[4]

*[a] [1]Cardiovascular Renal and Metabolic Diseases, IMED Biotech Unit, AstraZeneca, Gothenburg, Sweden. [2]Discovery Sciences, IMED Biotech Unit, AstraZeneca, Gothenburg, Sweden.[3]Drug Safety and Metabolism, IMED Biotech Unit, AstraZeneca, Gothenburg, Sweden.[4]Ionis Pharmaceuticals, 2855 Gazelle Court, Carlsbad, California 92010, USA.*

Antisense oligonucleotide (ASO)-based drug development is evolving as an effective therapeutic approach [1]. To fully realize the potential of this technology, it is necessary to improve the potency of ASOs in extrahepatic tissues. Inability to selectively deliver antisense therapies to ß-cells is a substantial barrier to the development of innovative and safe treatments for ß-cell specific diseases. G-protein coupled receptors (GPCR) has been employed to increase cellular uptake of estrogen conjugated to GLP-1 peptide [2]. Activation of GPCRs initiates receptor internalization, endocytosis and trafficking through endosomes [4], with surface recycling controlling the rate and extent of receptor trafficking back to the cell surface. It has been suggested that the low abundance and limited ability to internalize large amount of cargo makes GPCRs inappropriate for drug delivery [3]. Here, we used a GLP1 receptor (GLP1R) peptide agonist conjugated to ASO to explore the concept of delivering ASO using GLP1R mediated internalization. We show that the GLP1 receptor can be used as a targeting approach for efficient and selective delivery of ASO to pancreatic ß-cells in cells and in animals.

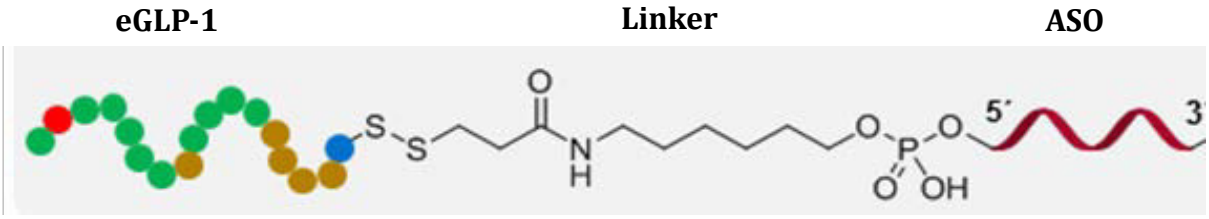



***Figure.*** *GLP-1 Conjugated Antisense Oligonucleotide.*

***References:*** [1] Crooke, et al. Cell Metab. **2018**, 27, 714-739 [2] Finan et al. *Nature Medicine* **2012,** *18*, 1847-1856 [3] Juliano et al. *J Drug Target* **2013**, *21*, 27-43. [4] Drake 2006, *Circ Res 99*, 570-582

*Corresponding author: E-mail: tprakash@ionisph.com

# *In silico* design, synthesis and evaluation of novel antiviral nucleoside analogues as potential treatments for *Arbovirus* infections

Marcella Bassetto,*[a] Juliane Nolte,[b] Benno Schreiner, [b] Joachim Bugert,[b] Andrea Brancale[a]

[a] *School of Pharmacy and Pharmaceutical Sciences, Cardiff University, King Edward VII Avenue, CF103NB, Cardiff, United Kingdom*
[b] *Institut für Mikrobiologie der Bundeswehr, Neuherbergstraße 11, 80937, Munich, Germany*

Vector-borne emerging diseases such as Chikungunya and Zika infections currently represent global concerns, due to their recent outbreaks in the Americas, Europe and the US. Chikungunya virus (CHIKV) is an *Arbovirus* associated with an acute pathology characterised by fever, rash and arthralgia, a condition which is often severe and may persist for several months or become chronic in the 10% of infected individuals.[1] Zika virus (ZIKV) is a *Flavivirus* also transmitted to humans by mosquitoes, responsible for an acute febrile illness associated with severe neurological complications, such as the Guillan-Barré syndrome in adults, and microcephaly and neurological disorders in newborns to women infected during pregnancy.[2] Currently, no therapeutic options are available to treat these two viral diseases.
Among the viral non-structural proteins responsible for virus replication, the polymerase represents a promising target for antiviral drug design, as its inhibition with nucleoside analogues is one of the most successful strategies in antiviral drug discovery for several other viruses.[3] Using computational approaches, a homology model was built for CHIKV nsP4 polymerase, using the Norwalk virus polymerase as template. The resulting structure was optimised by including the nucleic acid components of the elongation complex, the RNA template strand, the RNA growing strand and the incoming nucleoside triphosphate. In a similar fashion, a model for the active complex of ZIKV NS5 polymerase with the nucleic acid elements was built using the HCV polymerase complex structure as template. The two models were validated by docking known nucleoside inhibitors of the two viruses, in their triphosphate forms, in the NTP binding site, and used to screen *in silico* a virtual library of novel potential nucleoside inhibitors. These calculations guided the selection of new nucleoside analogues with good predicted binding into the two polymerases, and the best hits were chosen for chemical synthesis, along with their phosphoramidate prodrugs. The rational design, synthesis and biological evaluation of these novel compounds will be discussed.

This work was supported by the Sêr Cymru II programme, part-funded by Cardiff University and the European Regional Development Fund through the Welsh Government.

***References:*** [1] Burt, F.J., et al., *Lancet Infect. Dis.* **2017***, 17*, e107. [2] Esposito, D.L.A., et al. *Immunology* **2018**, *153*, 435. [3] Seley-Radtke, K.L., et al. *Antiviral Res.* **2018**, *154*, 66.

*Corresponding author: E-mail: bassettom@cardiff.ac.uk

# Nucleic Acid Mediated Precision Glycocalyx Engineering

Sean C Purcell, Nate Marroquin, Mia L Huang, Kamil Godula*

*Department of Chemistry and Biochemistry, University of California San Diego, 9500 Gilman Dr. La Jolla, CA 92093, USA*

We report a nucleic-acid mediated cell surface engineering strategy to target the activity of synthetic heparan-sulfate proteoglycan (HSPG) mimetics to pluripotent stem cells in a co-culture model, enhancing differentiation toward the neural lineage. The glycocalyx is an extensive network of cell surface biomolecules capable of regulating biological events including cellular communication and development.[1] Of particular interest are HSPGs, which can facilitate interactions between growth factors and their receptors, ultimately influencing differentiation and cell fate. One such HSPG facilitated interaction, between fibroblast growth factor 2 (FGF2) and its cell surface receptor (FGFR) is known to promote neural differentiation through activation of the Erk1/2 protein-kinase signaling cascade[2]. The Godula lab has demonstrated previously the ability to modify the surface of mouse embryonic cells deficient in HSPG biosynthesis by passively inserting synthetic nanoscale glycomimetics into the cell membrane[3]. These materials effectively rescued FGF2 mediated Erk1/2 activity and promoted neural specification[3]. Of great interest is developing materials that not only direct stem cell fate in this manner, but that can selectively target transplanted stem cells amidst the biological mileu. To this aim we have developed aptamer glycoconjugates (ApGCs), which utilize a nucleic acid targeting motif specific to pluripotent cells to direct and non-covalently anchor the glycoconjugates to the cell surface. The ability to precisely deliver and anchor HSPG mimetics to the cell surface allows for selective control over differentiation, and is primed to further elucidate the mechanisms by which proteoglycans regulate cellular fate. This strategy offers a powerful tool to elucidate the roles of HSPGs in regulating cellular differentiation and has applications in cell therapy and regenerative medicine.

This work was supported by grants from the University of California San Diego and a GAANN fellowship (UC San Diego Dept. of Chemistry).

***References:*** [1] Varki, A. et al., Essentials of Glycobiology *(3rd ed.)*, Cold Springs Harbor Press **2017**. [2] Huang ML, et al. *Glycobiology* **2016**, *26(8)*, 797. [3] Huang ML, et al. *J. Am. Chem. Soc.* **2014**, *136(30)*, 10565.

*Corresponding author: E-mail: kgodula@ucsd.edu

# Synthesis, structure and biological evaluation of 1,5-anhydroaltritol cap analogs

Tomasz Ratajczak,[a]Anna Nowicka,[b]Dorota Kubacka,[b]Joanna Kowalska,[b]Jacek Jemielity*[a]

[a]*Laboratory of Bioorganic Chemistry, Centre of New Technologies, University of Warsaw, Banacha 2C, Poland*
[b]*Division of Biophysics, Faculty of Physics, University of Warsaw, Pasteura 5 02-093 Warsaw, Poland*

**Introduction**. All eukaryotic and some viral mRNAs possess characteristic structure of cap localized at 5' end consisting of *N*-7 methylguanosine linked to RNA chain by 5'-5' triphosphate bridge. Cap interacts with several cap-binding proteins like: translation initiation factor (eIF4E), methyltransferases or decapping enzymes (DcpS, Dcp1/2) being involved in mRNA processing, its stability and translation. Due to important role of cap in a cellular processes cap analogs attractmore and moreattention aspotential therapeutic agents [1,2].
**Results.** I synthesized series of 1,5-anhydroaltritol cap analogs (Figure 1). In biological studies

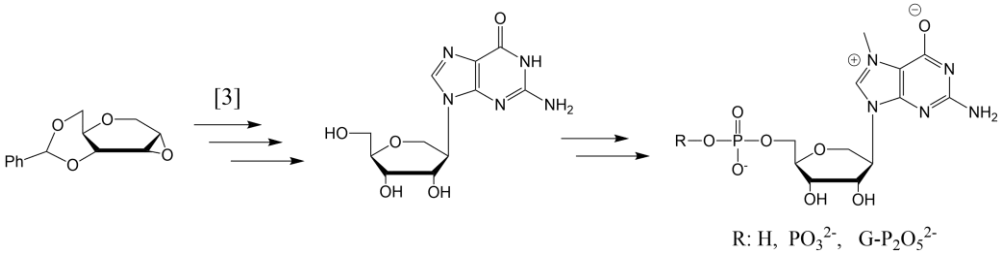



***Figure 1.*** *1,5-anhydroaltritol cap analogs.*

these compounds were fully resistant to hydrolytic activity of DcpS (Graph 1A, C). 1,5-anhydroaltritol analogs were investigated according to their affinity to translation initiation factor 4E (eIF4E) using fluorescence anisotropy method [3]. Results showed lower binding constant of all 1,5-anhydroaltritol analogs compared to unmodified cap.

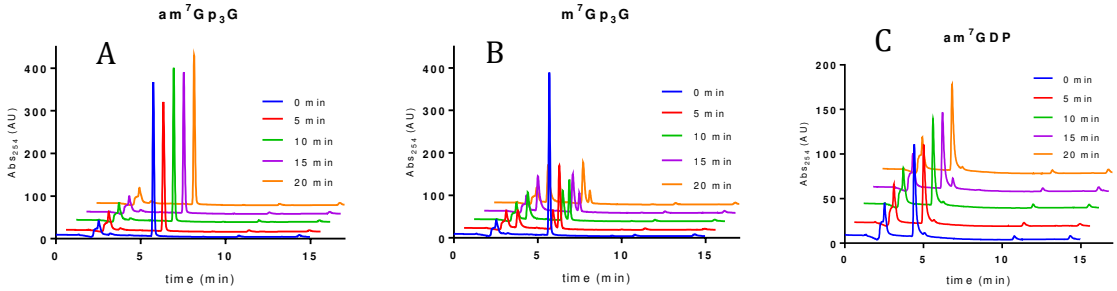



***Figure 2.*** *Enzymatic hydrolysis by DcpS enzyme. (A),(C) 1,5-anhydroaltritol cap analogs, (B) unmodified cap.*

This work was supported by grants from the National Science Centre. Grant No. 2016/20/S/ST5/00362

***References:*** [1] Kowalska, J. et al., *Bioorg. & Med. Chem. Lett.* **2009**, *19*,1921. [2] Ziemniak, M., et al. Future Med. Chem. **2013**, 5, 1141. [3] Allart et al. *Nucleosides Nucleotides Nucleic Acids* **1999**, *23*, 439.

*Corresponding author: E-mail: j.jemielity@cent.uw.edu.pl

# Enzymatic Synthesis and Bioorthogonal Labeling of Triazine-modified Oligonucleotides

Ulrike Reisacher,[a] Hans-Achim Wagenknecht*[a]

[a] *Institute of Organic Chemistry, Karlsruhe Institute of Technology (KIT), Fritz-Haber-Weg 6, 76131 Karlsruhe, Germany*

Bioorthogonal reactions have become a valuable method for postsynthetic labeling of biopolymers like proteins and nucleic acids. Besides copper-catalyzed 1,3-dipolar cycloadditions, the development of copper-free "click"-reactions such as SPAAC[1,2], iEDDA or "photoclick"[3] as rapid nontoxic alternatives has gained increasing interest over the past decade.[4]

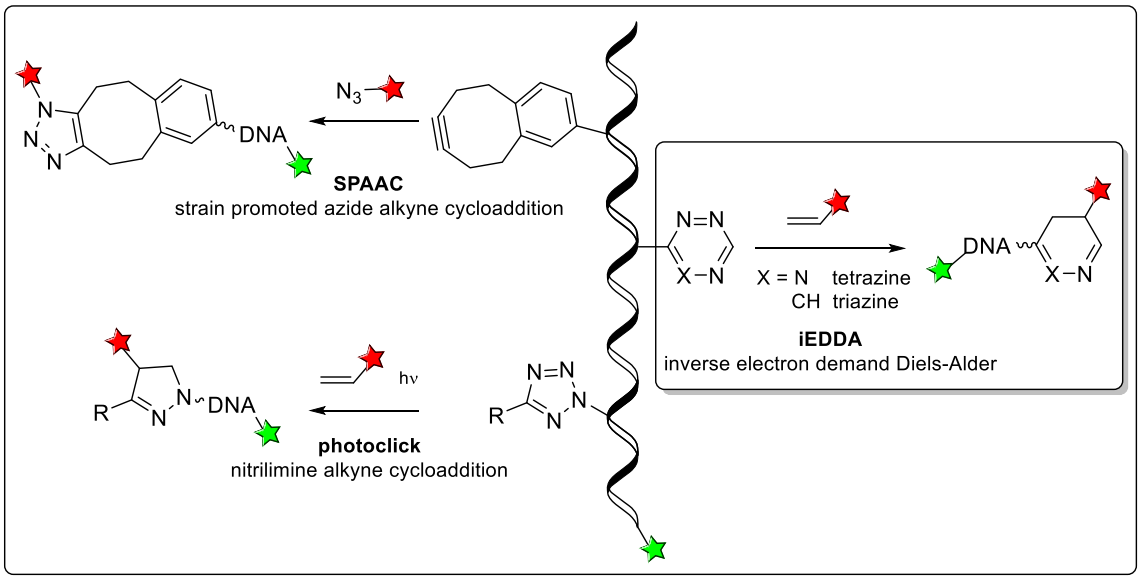



***Figure.*** *Copper-free postsynthetic bioorthogonal labeling.*

Our group developed a wide range of uridine derivatives with bioorthogonal reporters that are useful for those reactions.[5] For instance, tetrazines are reactive dienes for iEDDA, however, in previous studies they showed low labeling yields due to their instability. To overcome this problem the more stable triazines can be used as bioorthogonal reagents.[6,7] Therefore a new triazine-modified nucleoside triphosphate was synthesized and afterwards enzymatically incorporated into oligonucleotides. The high yields of postsynthetic iEDDA show the potential of this small but highly stable compound as tool for bioorthogonal labeling.

***References:*** [1] Wagenknecht, H.-A. et al, *Chem. Commun* **2014**, 50, 11218. [2] Schepers, U. et al, *Bioconjugate Chem.* **2016**, 27, 457. [3] Wagenknecht, H.-A. et al, *Angew. Chem. Int. Ed.* **2014**, 53, 14580. [4] Wagenknecht, H.-A. et al, *ChemBioChem* **2015**, 16, 1541. [5] Wagenknecht, H.-A. et al, *J. Org. Chem.* **2016**, 81, 7527. [6] Wagenknecht, H.-A. et al, *ChemBioChem* **2017**, 18, 1473. [7] Prescher, J. A. et al, *J. Am. Chem. Soc.* **2015**, 137, 8388.

*Corresponding author: wagenknecht@kit.edu

# Incorporation of thiophene and benzothiophene groups to stabilize duplexes of RNA: Insights into the nature of thermal stability

Marino J. E. Resendiz,*[a] Lamont Sharp,[a] Diego Minjares,[a] Paula G. Lewis,[a] Cesar Rios,[a] Chase Barker,[a] Haobin Wang[a]

[a] *Department of Chemistry, University of Colorado Denver, 1151 Arapahoe St. Denver, CO 80204, USA*

Modifying nucleic acids with small aromatic groups has been used for structural probing, in imaging, or in molecular recognition for biomedical purposes. Thus, it is an attractive strategy that potentially facilitates attaining control of the structure of RNA for therapeutic applications. This work describes the use of thiophene-based probes to obtain strands of RNA with unique biophysical properties via the stabilization of its secondary structure. Density functional theory was used as an aid to explain the observed changes. Thiophene and benzothiophene were incorporated via standard solid-phase chemistry from their corresponding phosphoramidites. The modifications were installed at the C2'-O-position of internal sites of RNA [CUACGGAAUCAU].

Structural and thermodynamic parameters were obtained via circular dichroism. Interestingly, modification of both strands led to stabilization of the corresponding duplexes and the nature of this result was explored using density functional theory through analysis of structural parameters, i.e., pseudorotational angles, π-π stacking, and covariance matrices; which indicated that this modification induces a conformational change around the dihedral angle between the glycosidic bond and the C2'-O-methylthiophene. Increasing the aromatic nature of the modification led to increased stability and the nature of this difference is under current study.

In conclusion, thiophene and benzothiophene units can be installed at key positions within various structural elements of RNA to induce its stability. Current research is being carried out to explore the reactivity between these probes to obtain distinct RNA motifs with new biophysical properties.

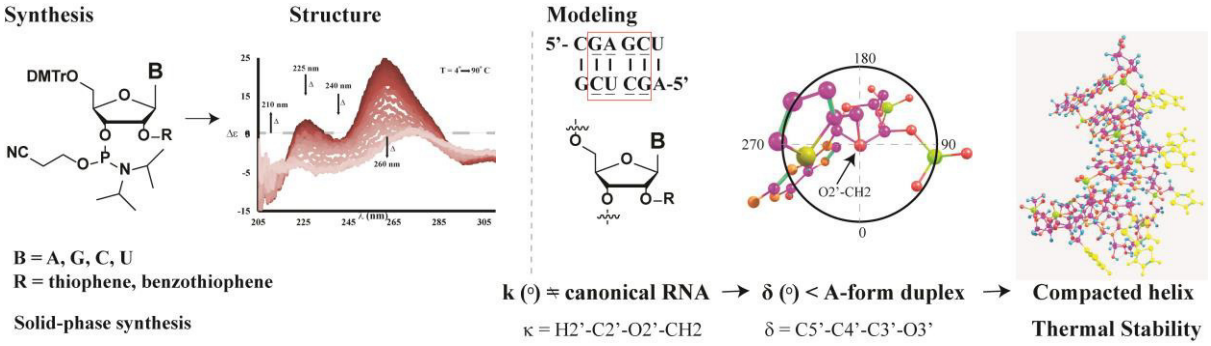



***Figure.*** *Synthesis. structure, and modeling of duplexes of RNA containing thiophene or benzothiophene groups at the C2'-O-position*

*Corresponding author: marino.resendiz@ucdenver.edu

# Nucleoside 5’-diphosphates and their analogues - synthesis, selected properties and anti-HIV activity

Marta Rachwalak[a], Tomasz Jakubowski[a], Justyna Gołębiewska[a], Jacek Stawiński[a], Joanna Romanowska*[a]

[a] *Department of Nucleoside and Nucleotide Chemistry, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Z. Noskowskiego 12/14, Poznań, Poland*

Nucleoside 5’-diphosphates with different substituents and their analogues containing sulfur and selenium modification at non-bridging positions of the phosphorus atoms have been explored as a new type of pronucleotides for the purpose of anti-HIV therapy. The synthetic protocol, based on *H*-phosphonate chemistry, has been developed.
For the preparation of these compounds, we have been exploring *H*-phosphonate chemistry. Nucleoside *H*-phosphonate monoesters (or their thio or seleno analogues) of type **1** after conversion to the silyl derivatives with trimethylsilyl chloride (TMSCl), can be oxidized by elemental iodine which lead to highly reactive pyridinium adducts of type **2**. After the subsequent reaction with appropriate alkyl or aryl phosphate derivatives (for nucleoside 5’-diphosphates and their analogues with heteroatoms at α-P position), or thio(seleno)phosphate (for β-P thio or seleno derivatives) of type **3** or **4**, we obtained quantitatively the corresponding nucleoside 5’-diphosphate analogues **5** or **6** (***Figure 1***).

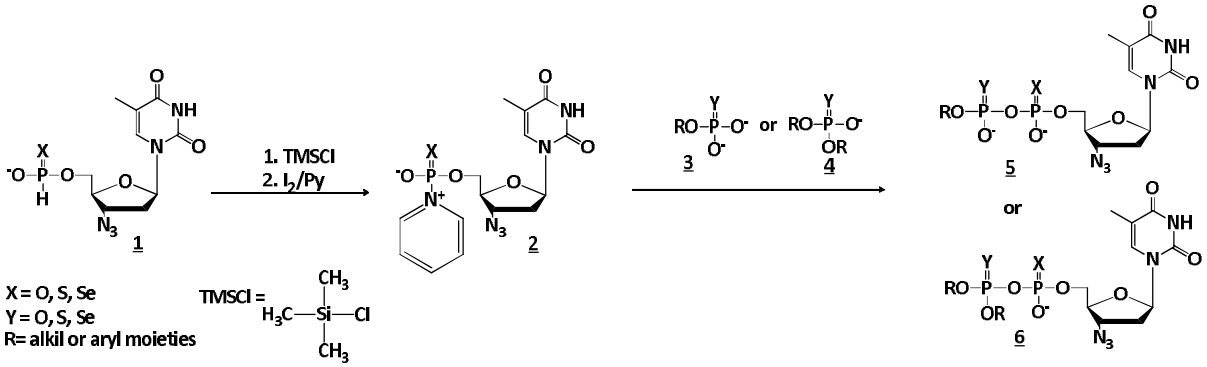



***Figure 1.*** *A general method for the synthesis of nucleoside 5’-diphosphates and their analogues containing sulfur and selenium*

It was found that some of the synthesised nucleoside 5’-diphosphates revealed high anti-HIV potency and very low cytotoxicity and they might be considered as new, attractive potential anti-HIV prodrugs. For nucleoside 5’-diphosphates and their β-P-thio(seleno) analogues, it is likely that these compounds may act as true pronucleotides.

Financial support from the Polish Ministry of Science and Higher Education (the KNOW program) is greatly acknowledged.

*Corresponding author: E-mail: joarom@ibch.poznan.pl

# Study on Substrate Specificity of *E.coli* Uracil-DNA Glycosylase: Search for Oligonucleotide Inhibitors.

Šárka Rosenbergová*[1], Ondřej Páv[1], Ivana Kóšiová-Markusová, Magdalena Petrová[1], Radek Liboska[1], Pavel Novák[1], Ivana Dvořáková[1], Anna Ligasová[2], Karel Koberna[2], and Ivan Rosenberg[1]

[1]*Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo nám. 2, 166 10 Prague 6, Czech Republic.* [2]*Institute of Molecular and Translational Medicine, Palacký University in Olomouc, Olomouc 7900, Czech Republic*

Uracil-DNA glycosylase (UNG or UDG) is a DNA repair enzyme which prevents mutagenesis *via* eliminating uracil (but neither thymidine nor some 5-substituted uracils are eliminated) from DNA by cleaving the *N*-nucleosidic bond and thus initiating the base-excision repair (BER) pathway [1].
From this point of view UNG could be considered as potential target for cancer treatment.
The specificity of UNG towards uracil nucleobase prompted us to run the structure-activity study on series of short oligonucleotides containing central (i) various 5-substituted 2'-deoxyuridine units, (ii) 2'-deoxyuridine analogs bearing 3'- or 5'-phosphonate moiety, and (iii) 2'-deoxyuridine unit with neighbor internucleotide linkage-modified nucleotide unit. The obtained results showed sensitivity of the enzyme towards modification of both deoxyuridine unit and structural changes in a close proximity of 2'-deoxyuridine unit. This study could contribute to better understanding the structural requirements of *E. coli* uracil-DNA glycosylase for oligonucleotide binders.

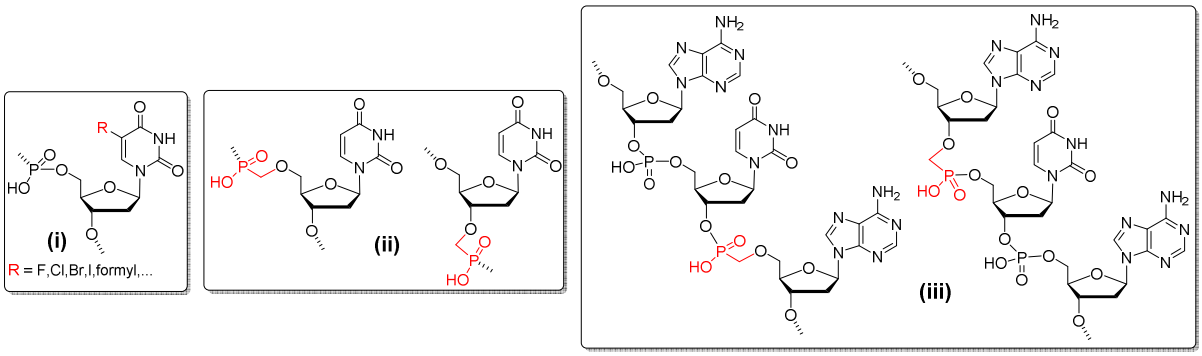



***Figure 1.*** Examples of modified 2'-deoxyuridine (**i**) and (**ii**) units and d(AUA) (**iii**) units

This work was supported by grant # 15-31604A (Ministry of Health, CR, all rights reserved).

***References:*** [1] Slupphaug, G. et al., *Nature.* **1996**, *384*, 87–92. [2]

*Corresponding author: E-mail: sarka.rosenbergova@uochb.cas.cz

# Activity of semisynthetic C-nucleosides against RNAPs

Petja Rosenqvist,*[a] Ranjit Prajapati,[b] Kaisa Palmu,[b] Pasi Virta,[a] Mikko Ora,[a] Heidi Korhonen,[a] Mikko Metsä-Ketelä,[b] Georgi Belogurov[b]

[a] *Department of Chemistry, University of Turku, FI-20014, Turku, Finland*
[b] *Department of Biochemistry, University of Turku, FI-20014, Turku, Finland*

RNA polymerases (RNAP) are one of the major targets for antivirals and antibiotics. [1] However, there are currently no approved drugs in clinical use targeting the active site of bacterial RNAP. In the present work, the aim has been to produce derivatives of natural C-nucleosides by combination of synthetic biology and chemical synthesis, and to assay the produced 5´-triphosphates for inhibitory activity against four different RNAPs; the "multi-subunit" eukaryotic and bacterial RNAPs, and the "single-subunit" mitochondrial and viral polymerases. First, the C-nucleoside 5´-triphosphates bind to the active site and then, they may get incorporated to the elongating RNA, both events having effect on normal transcription.

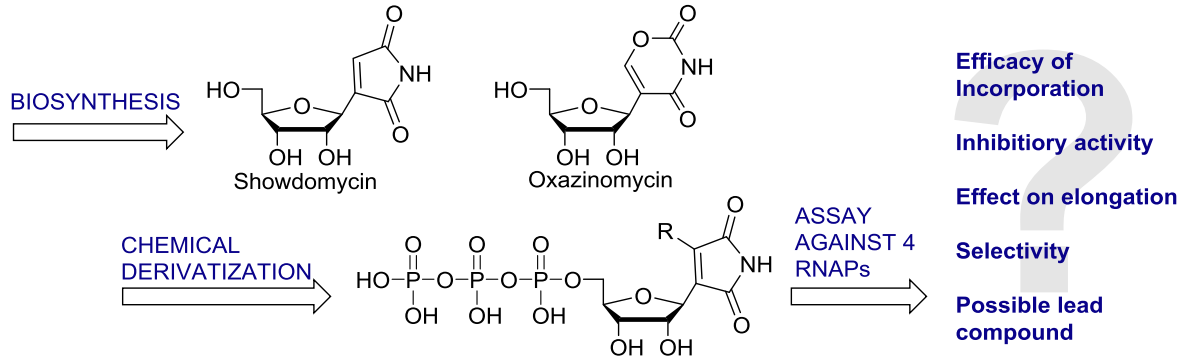



***Figure.*** *General scheme of presented study and the structures of relevant compounds.*

The C-nucleosides showdomycin and oxazinomycin are obtained by cultivating certain strains of *Streptomyces* soil bacteria. Attempts have also been made to produce C-nucleoside analogs by pathway engineering.[2] Of particular interest is the maleimide-type base moiety of showdomycin that is susceptible to Michael addition by nucleophiles such as unbound cysteine residues in enzymes, giving it the ability to form covalent bonds with its target. Reactivity of the compound can be controlled by addition of modifications to the base moiety which also affects the selectivity of the nucleotide towards a certain type of RNAP.

This work was supported by grants from the Sigrid Juselius Foundation.

***References:*** [1] Ma, C. et al., *Microbiol. Mol. Biol. Rev.* **2016***, 80*, 139. [2] Palmu, K. et al., *ACS Chem. Biol.* **2017***, 12*, 1472.

*Corresponding author: E-mail: pesaro@utu.fi

# Phosphonoamidate synthons for the synthesis of acyclic nucleotide derivatives through cross metathesis assisted by ultrasound on water

Presenter Roy Vincent,[a]* Bessières Maxime,[a] Hervin Vincent, [a] Snoeck Robert,[b] Andrei Graciela,[b] Lohier Jean-François,[c] Luigi. A. Agrofoglio[a]

[a]*Université d'Orléans et CNRS, Institut de Chimie Organique et Analytique, UMR 7311, Orléans, France*
[b]*Rega Institute for Medical Research, KU Leuven, B-3000 Leuven, Belgium*
[c]*Université de Caen et CNRS, Laboratoire de Chimie Organique et Analytique, UMR 6507, Caen, France*

Nucleosides and their analogs form an important class of anti-infective drugs against viruses, tumors, bacteria. In some cases, to circumvent the initial rate-limiting first phosphorylation step and to increase their bioavailability, there are prepared as stable phosphate prodrugs, such as phosphoramidates, pivaloyloxymethyl analogs, ….. In recent years, our team have focused on more environmentally friendly chemistry in order to : - decreasing the number of steps; - avoiding protection-deprotection steps; - promoting catalytic instead of stoichiometric reactions and - using low energy consuming materials.
Herein, we report a straightforward approach to hitherto unknown nucleoside phosphonoamidates through a convergent one-step reaction, [1] allowing us to revisit the biological activity of a large class of drug (natural or modified nucleobases, nucleosides) with increased therapeutic index. The key step of this strategy involves the cross-metathesis reaction of various olefinic phosphonoamidate synthons, under ultrasound activation on water. We have shown the undeniable contribution of this procedure compared to conventional heating or microwave activation in organic solvent. Each phosphonoamidate synthon isomers were obtained in gram scale by flash column chromatography and in good yields.[2]

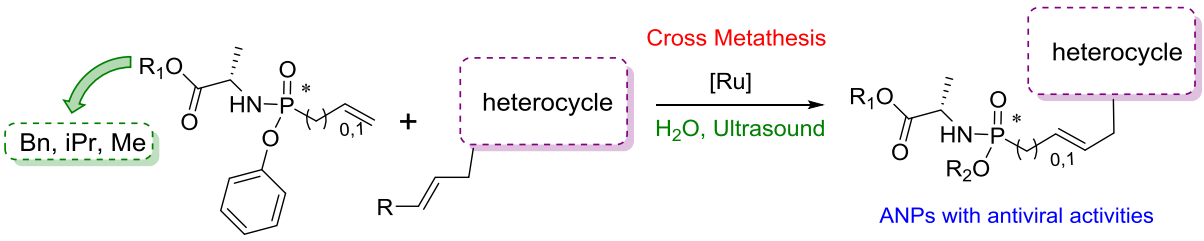



***Figure 1.****Convergent synthesis of antiviral ANPs.*

This work was supported by grants from the Labex SynOrg

***References:*** [1] Agrofoglio, L. A. Roy, V.; *et al*. PCT Int. Appl. **2010**, WO 2010146127 A1 20101223. [2] Bessières, M. , et al. *Eur. J. Med. Chem.* **2018**, *146*, 678-686.

*Corresponding author: E-mail: Vincent.roy@univ-orleans.fr

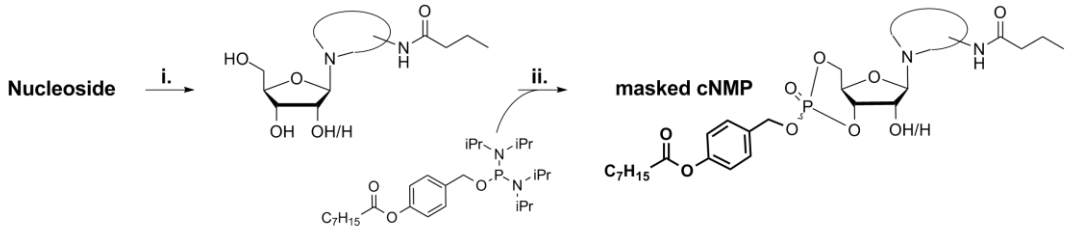
# Masked cNMPs as novel chemical tools for cell-based assays

Alexandra Ruthenbeck,[a] Viacheslav Nikolaev,[b] Björn-Ph. Diercks,[b] Chris Meier*[a]

[a] *Department of Chemistry, Organic Chemistry, University of Hamburg, Martin-Luther-King Platz 6, 20146 Hamburg, Germany*
[b] *Institute for Experimental Cardiovascular Research, University Medical Center Hamburg-Eppendorf, Martinistraße 52, 20246 Hamburg, Germany*

SFB1328

Adenine nucleotide (AN) 2nd messengers like 3',5'-cyclic adenosine monophosphate (cAMP) are central elements of intracellular signaling, but many details of the underlying processes remain elusive still. The nucleotides are net-negatively charged at physiologic pH which limits the applicability in cell-based settings. Thus, assays rely on sophisticated techniques like microinjection [1], which needs highly trained staff and elaborate instrumentation. The set-up further is not feasible for medium- to high-throughput formats, and the mechanic stress that cells are exposed to raises the probability of false-positives. Membrane-permeable, bioreversibly-masked AN 2nd messenger derivatives are potential tools to overcome these limitations. However, such derivatives are sparsely described. The few examples comprised mainly acetyloxymethyl (AM) esters, but proved insufficient regarding chemical stability, and unsatisfactory in terms of reproducibility of cell-assay results.[2-4]




**i:** (**A**, **dA** & **G**) *N*-acylation via transient TMS-PGs. **ii:** (**U** & ***N*(Bu)-(d)Nucl**) phosphordiamidite-based 3',5'-monophosphorylation

***Figure 1.*** *Synthetic approach towards AB-masked cNMPs of U, A, dA and G.*

Here, we present the straightforward synthesis of masked cNMPs (Fig.1) employing the well-established acyloxybenzyl (AB) group [5,6]. We further show hydrolysis studies on chemical stability and enzymatic activation of the AB-masked nucleotides, and discuss their favorable characteristics. In a cell-based setup, we used a FRET-based cAMP-sensor [7] to demonstrate high permeability of our masked cAMP and its prompt intracellular conversion to cAMP within minutes. Consequently, our novel AB-masked cNMPs constitute highly successful bioprecursor-tools for non-invasive studies on intracellular signaling. In contrast to existing techniques, our compounds overcome the necessity for demanding single-cell preparations and accordingly, facilitate cell-based settings significantly. They are therefore a cornerstone in advancing the possibilities of cell-studies and, more broadly, the understanding of (patho-)physiological processes.

***References:*** [1] Wolf, I. M. A. & Guse, A. H., *Front. Oncol.* **2017**, 7, 1–11. [2] Parkesh, R. et al., *Cell Calcium* **2008**, 43, 531–538. [3] Rosen, D. et al., *Biochem. Biophys. Res. Commun.* **2012**, 418, 353–358. [4] Schultz, C. et al., *J. Biol. Chem.* **1993**, 268, 6316–6322. [5] Thomson, W. et al., *J. Chem. Soc. Perkin Trans 1.* **1993**, 1239–1245. [6] Meier, C., *Antivir. Chem. Chemother.* **2017**, 25, 69–82. [7] Börner, S. et al., *Nat. Protoc.* **2011**, 6, 427–438.

*Corresponding author: E-mail: meier@chemie.uni-hamburg.de

# Development of fluorescent benzo[*g*]imidazo[4,5-*c*]quinoline nucleoside for monitoring base-pair-induced protonation with cytosine: Distinguishing cytosine *via* changes in fluorescence intensity and wavelength

Yoshio Saito,* Shogo Siraiwa, Masaki Yanagi

*Department of Chemical Biology and Applied Chemistry, College of Engineering, Nihon University, Koriyama, Fukushima 963-8642, Japan*

A novel fluorescent benzo[*g*]imidazo[4,5-*c*]quinoline nucleoside $^{\mathbf{BIQ}}\mathbf{A}$ (**1**) comprising a 3-deaza-2'-deoxyadenosine skeleton was developed and applied for monitoring $^{\mathbf{BIQ}}\mathbf{A}$–C base-pair formation in oligodeoxynucleotide (ODN) duplexes. The newly developed $^{\mathbf{BIQ}}\mathbf{A}$ showed distinct photophysical properties associated with its protonated/deprotonated forms (monomer: p$K_a$ 6.2) *via* dramatic changes in its absorption and fluorescence spectra. In ODN duplexes, the induced protonation of $^{\mathbf{BIQ}}\mathbf{A}$ was observed, even under alkalescent conditions when cytosine was the opposite base on a complementary strand; the resulting $^{\mathbf{BIQ}}\mathbf{A}$–C base pairs were stable. By monitoring the protonation of $^{\mathbf{BIQ}}\mathbf{A}$ under alkalescent and neutral conditions, we could discriminate cytosine base *via* spectral changes in absorption and fluorescence. Similarly, we found that the demonstrated 3-deaza-2'-deoxyadenosine $^{\mathbf{3z}}\mathbf{A}$ forms a stable base pair with cytosine base *via* $N^1$ protonation in ODN duplexes at acidic and neutral conditions (pH < 7.0). At lower pH values, ODNs containing $^{\mathbf{3z}}\mathbf{A}$ could clearly discriminate cytosine base through melting temperature ($T_m$) measurements. Thus, ODNs containing indicator nucleosides like $^{\mathbf{BIQ}}\mathbf{A}$ and $^{\mathbf{3z}}\mathbf{A}$ exhibit great potential as bioprobes for genetic analysis and structural studies of nucleic acids.

This work was supported by MEXT KAKENHI Grant Number 25410180.

***References:*** [1] S. Siraiwa, A. Suzuki, R. Katoh, Y. Saito, Org. Biomol. Chem. 2016, 14, 3934.

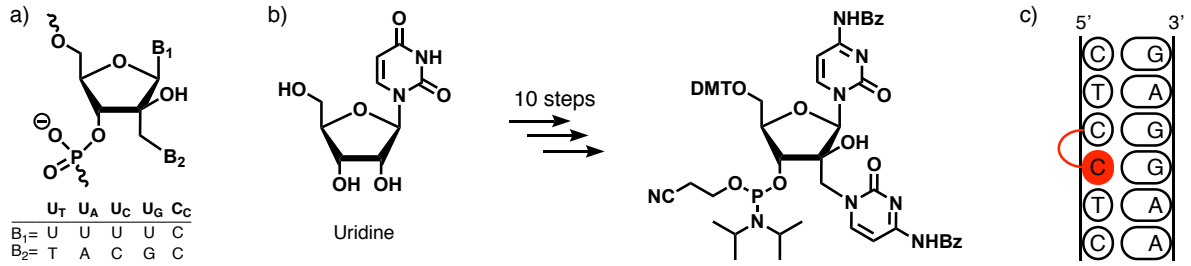
*Corresponding author: E-mail: saitoy@chem.ce.nihon-u.ac.jp

# Synthesis and recognition properties of a double-headed nucleotide with two cytosine nucleobases

Kasper Petersen Beck,[a] Mick Hornum,[a] Charlotte Reslow-Jacobsen,[a] Poul Nielsen*[a]

[a] *Department of Physics, Chemistry and Pharmacy, University of Southern Denmark, Campusvej 55, 5230 Odense M, Denmark.*

Double-headed nucleotides are nucleotides with two nucleobases. Adding the extra nucleobase in the 2'-position with a methylene linker, allows the nucleobase to be oriented towards the core of the duplex where it can participate in Watson–Crick base-pairing. In practice, these double-headed nucleotides work as condensed dinucleotides as they can make two sets of base-pairs for every phosphate. To this point the full set of canonical nucleobases have been attached and studied in the form of $U_T$, $U_A$, $U_C$ and $U_G$, and thus only with uracil in the natural position. [1,2]




| | $U_T$ | $U_A$ | $U_C$ | $U_G$ | $C_C$ |
|---|---|---|---|---|---|
| $B_1$= | U | U | U | U | C |
| $B_2$= | T | A | C | G | C |

***Figure.*** *(a) General structure of the double-headed nucleotides; (b) synthesis of $C_C$; (c) motif of $C_C$ when incorporated into DNA and placed opposite two guanines.*

Here we present the first double-headed nucleotide with two cytosine nucleobases, $C_C$. The synthesis of $C_C$ proceeds in 10 steps from uridine to the activated and fully protected nucleoside and includes the simultaneous conversion of two uracils to protected cytosines. In this study the recognition properties of $C_C$ are examined for the purpose of evaluating the mis-match discrimination of both the natural and the additional cytosine. Furthermore, the properties of the $C_C$ double-headed nucleotide as part of triplex-forming oligonucleotides (TFOs) are studied.

This work was supported by grants from The Danish Council for Independent Research and Natural Sciences.

***References:*** [1] Sharma, P. K.; Kumar, P.; Nielsen, P. *Aust. J. Chem.* **2016**, *69*, 1094-1101. [2] Hornum, M.; Sharma, P. K.; Reslow-Jacobsen, C.; Kumar, P.; Petersen, M.; Nielsen, P. *Chem. Commun.* **2017**, *53*, 9717-9720.

*Corresponding author: E-mail: pouln@sdu.dk

# Nucleoside analogue inhibitors of GTP cyclohydrolase I (GCYH-I) as a potential new class of antibiotics

George Samaan, Ryan Murphy, Naduni Paranagama, Manal A. Swairjo,* Byron W. Purse*

*Department of Chemistry and Biochemistry, San Diego State University, California, USA*

Despite a pressing global need, no new class of antibiotics has been clinically approved in more than 30 years. Our project investigates the design and synthesis of novel antifolate compounds, which will inhibit the synthesis of folate in some species of pathogenic bacteria, leading to bacterial cell death. The enzyme GTP cyclohydrolase I catalyzes the first step in folate (vitamin $B_9$) biosynthesis in bacteria and biopterin biosynthesis in humans. Recently it was discovered that many bacteria use an essential GTP cyclohydrolase I enzyme, GCYH-IB, that bears little structural homology to the human form, GCYH-IA.[1,2] Accordingly, GCYH-IB is a new potential antibiotic target in the clinically validated folate biosynthesis pathway.

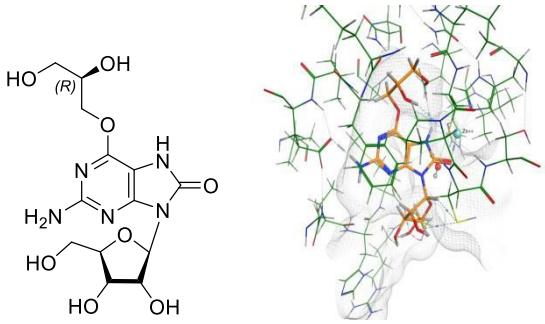



***Figure.*** *Structure of compound G3 and docking model showing its predicted mode of binding to GCYH-IB.*

8-oxo-GTP is a known substrate-analogue inhibitor of GCYH-IA, and we used *in vitro* assays to show that it also inhibits bacterial GCYH-IB, but with reduced potency. We hypothesized that by modifying 8-oxo-GTP to exploit active site features unique to GCYH-IB, we could develop a new inhibitor class with high selectivity for the bacterial enzyme. We have designed and synthesized an initial set of compounds to test structure–activity relationships in the inhibition of GCYH-IA and -IB. Two compounds, G1 and G2, showed lower inhibition of the bacterial enzyme than of the human enzyme. The third compound (*S*)-G3 showed no significant difference in the inhibition of the two enzymes. A further modification, G3, showed more inhibition of the bacterial enzyme (50%) than the human enzyme (15%), demonstrating in principle that these active site differences can be targeted.

***References:*** [1] Sankaran, B., *et al. J. Bacteriol.* **2009**, *191,* 6936. [2] Paranagama, N., *et al. Biochemical J.* **2017**, *474,* 1017. [3] Nampalli, S., *et al. Bioorganic Med. Chem. Lett.* **2000**, *10,* 1677.

*Byron W. Purse: E-mail: bpurse@sdsu.edu

# Size and Charge of DNA-Adducts Influence Activity and Fidelity of TLS Polymerase Enzymes

Emma Sandell*[1], Michael H. Räz[1], Hailey L. Gahlon[1], Kiran Patil[2], Dennis Gillingham[2],and Shana J. Sturla[1]

[1]*ETH Zürich, Department of Health Sciences and Technology, Switzerland*
[2]*University of Basel, Department of Chemistry, Switzerland*

High red meat consumption is a known human carcinogen, and is associated with carboxymethylation of DNA including the formation of the DNA adduct *O*$^6$-carboxymethylguanine O$^6$-CMG. Bulky DNA adducts, like O$^6$-CMG, can impede replicative polymerases and lead to replication fork stalling, or an alternative means for replication to proceed is by translesion synthesis (TLS). TLS requires specialized DNA polymerases that have different sized active sites capable of accommodating bulky DNA lesions, leading to preferential bypass of specific alkylated or bulky DNA adducts. However, these polymerases have high error rates and can contribute to establishing point mutations. We have previously found that various human TLS polymerases can bypass *O*$^6$-CMG. The highest base incorporation activity was observed for polymerase κ and η, and the highest fidelity for polymerase κ. However, it was not evident what structural aspects of the O$^6$-CMG adduct, such as its size and charge, influence the activity and fidelity of base incorporation by TLS polymerases. To better understand the impact of the *O*$^6$-CMG structure on TLS activity, we synthesized DNA templates with minimally altered *O*$^6$-CMG structures by coupling primary amines to a carboxylic acid group of a site-specific O$^6$-CMG modified DNA sequence. Standing-start primer extension assays, carried out with Pol κ and η showed that polymerase η preferentially incorporated dATP and dTTP and polymerase κ preferred the correct base dCTP for incorporation. Furthermore, we observed that a negative charge of the adduct group increase the error-free bypass of polymerase κ while a non-charged or positive charged adduct increase mutagenicity. Polymerase η was not influenced by charge, but by size, since bigger-sized adducts lead to more false-incorporations by polymerase η. These results demonstrate that adduct size and charge impacts base incorporation and the fidelity of the TLS polymerases.

*Corresponding author: E-mail: esandell@student.ethz.ch

***References:*** [1] Bypass of Mutagenic O$^6$-Carboxymethylguanine DNA Adducts by Human Y- and B-Family Polymerases, M. Räz *et al*., *Chemical Research in Toxicology,* **2016***, 29, 9, 1493-1503*

# Pd/PTABS: An Efficient Catalytic System for Synthesis of Base-Modified Nucleosides

Yogesh S. Sanghvi,[a] Shatrughn Bhilare,[b] Vijay Gayakhe,[b] Ajaykumar Ardhapure,[b] Siva Sankar Murthy Bandaru,[c] Carola Schulzke,[c] Anant Kapdi*[b]

[a] *Rasayan Inc., 2802 Crystal Ridge Road, Encinitas, California, 92024-6615, USA*
[b] *Dept. of Chemistry, Institute of Chemical Technology, Matunga, Mumbai 400019, India*
[c] *Institute for Biochemie, Ernst-Moritz-Arndt-Universitat, 17489 Greifswald, Germany*

Modifications of heterocyclic bases in nucleosides have been explored for their potential biological activities like antiviral, antibacterial and anticancer properties. Palladium catalyzed cross-coupling reactions plays a vibrant role to form C-C, C-N and C-O bonds in these modifications.[1] Highly water soluble and versatile catalytic system consisting of palladium acetate and PTABS (7-phospha-1,3,5-triaza-admantane butane sultonate) ligand have been employed for Suzuki-Miyaura, Heck, Sonogashira, amination and etherification under mild reaction conditions.[2]

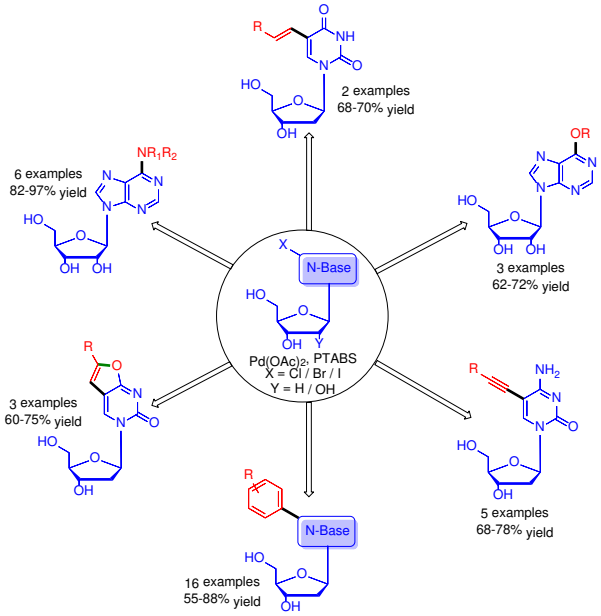



***Figure 1*** *Cross-coupling reactions.*

The synthesized PTABS ligand along with palladium acetate shows excellent reactivity towards different cross-coupling reaction of halo nucleoside. The developed catalytic system allows column-free isolation of Suzuki-Miyuara cross-coupled product of halo nucleoside with possible recyclability of catalytic system. A novel copper-free Sonogashira coupling protocol for the pyrimidine nucleosides has also been established via a one-pot synthesis of FV-100, a nucleoside-based drug in phase 3 clinical trials for herpes zoster treatment. In case of Heck reaction, we demonstrated the synthesis of antiviral drug: BVDU.[3]

This highly efficient catalytic system allows amination of 6-chloro-9-(β-D-ribofuranosyl)-9*H*-purine with different amines at ambient temperature. The validation of this strategy has been proved via synthesis of uracil based anti-diabetic drug, alogliptin which is an oral anti-diabetic.[4] Additionally, the catalytic system allows the cross-coupling of 6-chloro-9-(β-D-ribofuranosyl)-9*H*-purine with different phenols offering the corresponding ether product under mild reaction conditions.

***References:*** [1] Shaughnessy, K. H. *et al*., *Molecules* **2015**, *20*, 9419. [2] Kapdi, A. R. *et al*., *RSC Adv.* **2014**, *4*, 17567. [3] Bhilare, S. *et al*., *RSC Adv*. **2016**, *6*, 83820. [4] Bandaru, S. S. M. *et al*., *Org. Lett.* **2018**, *20*, 473.

*Corresponding author:
E-mail: ar.kapdi@ictmumbai.edu.in

# Application of the Functionality Transfer Oligonucleotide for the Site-Selective Internal Labeling of RNA

Shigeki Sasaki, *[a] Ikuya Oshiro, Takuya Matsumoto,[a] Gakuro Harada,[a] Yosuke Taniguchi,[a]
[a] *Graduate School of Pharmaceutical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan*

We have established the functionality-transfer reaction for the site-specific modification of RNA, using the functionality-transfer ODN probe (FT-ODN) incorporating 2´-deoxy-6-thioguanosine functionalized with the (*E*)-pyridinyl vinyl ketone moiety [1]-[3]. The transfer was accomplished by a sequential reaction of a Michael addition by the 4-amino group of the cytosine base followed by elimination of 6-thio-dG. The transfer reaction was accelerated in the presence of $NiCl_2$. The 4-thiothymidine unit was also functionalized with the (*E*)-pyridinyl vinyl ketone, which was efficiently and specifically transferred to the 6-amino group of the target adenosine in RNA [3].
In this study, the alkyne group was introduced to (*E*)-pyridinyl vinyl ketone transfer group. The specifically modified RNA was further subjected to the Click reaction with azide derivatives. This method is useful for the site-selective internal labeling of RNA with a variety of molecules such as fluorescent dyes and polymers.

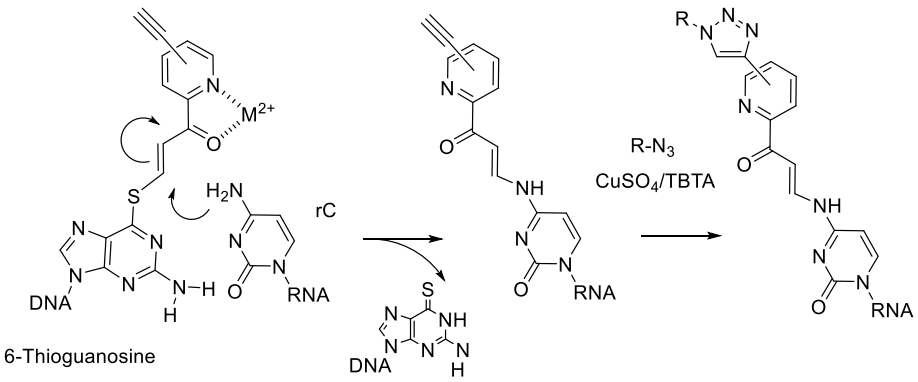



***Figure.*** *The Functionality Transfer Reactions and the Following Click Reaction.*

This work was supported by a Grant-in-Aid for Scientific Research (B) (15H04633) from the Japan Society for the Promotion of Science (JSPS).

***References:*** [1] Sasaki S, Onizuka K, Taniguchi Y. *Chem Soc Rev* **2011**, *40*, 5698.
[2] Jitsuzaki D., Onizuka K., Nishimoto A., Oshiro I., Taniguchi Y. and Sasaki S*. *Nucleic Acids Res.* **2014***, 42*, 8808.
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*Corresponding author: E-mail: sasaki@phar.kyuhsu-u.ac.jp

# 5-Aza-7-deaza-2’-deoxyguanosine and 2'-deoxycytidine form programmable silver-mediated base pairs with metal Ions in the core of the DNA double helix

Frank Seela,[a,b*] Xiurong Guo,[b] Peter Leonard,[b] Sachin A. Ingale,[b] Jiang Liu,[b] Hui Mei,[b] Martha Sieg[b]

[a]*Laboratory of Bioorganic Chemistry and Chemical Biology, Center for Nanotechnology, Heisenbergstraße 11, 48149 Münster, Germany*
[b]*Laboratorium für Organische und Bioorganische Chemie, Institut für Chemie neuer Materialien, Universität Osnabrück, Barbarastraße 7, 49069 Osnabrück, Germany*

5-Aza-7-deaza-2’-deoxyguanosine (dZ)[1] displays a similar shape as the parent 2’-deoxyguanosine (dG) from which it can be constructed by transposition of nitrogen-7 to the bridgehead position-5. It lacks the major groove binding site as the positional change moves the dG-acceptor position from nitrogen-7 to nitrogen-1. The nucleoside fits nicely in double helical DNA.

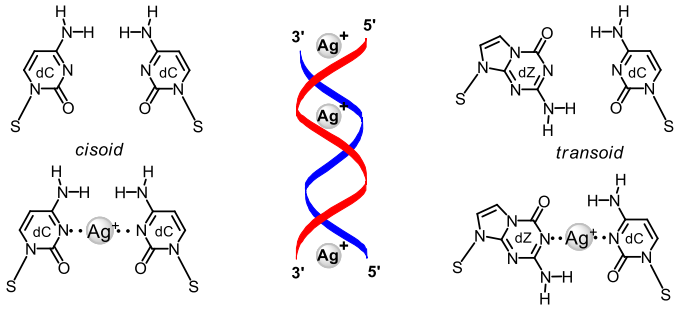


***Figure.*** *Right side: Proposed motif for the programmable metal-mediated transoid dZ-Ag$^+$-dC base pair with one silver ion. Left side: the cisoid dC-Ag$^+$-dC pair and Middle: Schematic view of a programmed DNA helix.*

Nucleoside dZ form a silver-mediated hetero base pair with dC and displays selective base recognition.[2] The proposed dZ-Ag$^+$-dC base pair motif represents a mimic of the H-bonded Watson-Crick dG-dC pair and shows relationship to the homo dC-Ag$^+$-dC pair. The metal ion pair can coexist with H-bonded canonical pairs in the same DNA duplex. It is positively charged, and slightly less than the dG-dC pair, shows sequence dependence and consumes one or two silver ions. It is appropriate for the programmable incorporation of silver ions in the DNA double helix and the selective base recognition make it suitable to be used in a silver ion mediated coding system.

***References:*** [1] Rosemeyer, H. and Seela F. *J. Org. Chem.* **1987**, *52*, 5136. [2] Xiurong Guo, Peter Leonard, Sachin A. Ingale, Jiang Liu, Hui Mei, Martha Sieg and Frank Seela *Chem. Eur. J.* **2018**, *23*, 11776.

*Corresponding author: E-mail: Frank.Seela@uni-osnabrueck.de

# Improving the chemical synthesis of spin-labeled nucleic acids

Anna-Lena Johanna Segler,[a] Haraldur Yngvi Juliusson[a] and Snorri Thor Sigurdsson*[a]

[a] *University of Iceland, Department of Chemistry, Science Institute, Dunhaga 3, 107 Reykjavik, Iceland.*

The understanding of the function and behavior of biomolecules, such as nucleic acids, is based on information about their structures and dynamics. Electron paramagnetic resonance (EPR) spectroscopy is a valuable technique to access such information. For this method a paramagnetic center is required. Nitroxides, commonly used as spin labels, are small organic molecules that possess an unpaired electron. These molecules are fairly stable because of the delocalization of the unpaired electron between the nitrogen and the oxygen, as well as electron donating and steric effects of the alkyl groups that flank the nitroxide. Nucleic acids do not have a paramagnetic center but various methods can be used to incorporate spin labels into both DNA and RNA. One approach is the phosphoramidite method, in which the spin-labeled nucleoside is converted to a phosphoramidite, the building block for automated chemical synthesis of nucleic acids. However, during the automated chemical synthesis, nitroxide spin labels are partially reduced to the corresponding amines through a disproportionation reaction under acidic conditions.[1, 2] This is mainly due to two reagents used on the synthesizer, the tetrazole activator and dichloroacetic acid used for trityl deprotection. It is possible to separate a spin-labeled oligonucleotide from one containing the reduced label by denaturing gel electrophoresis when the oligonucleotides are short but it becomes nontrivial when the strands are longer than ca. 13-15 nucleotides. We report here a protecting group strategy to circumvent the reduction of the nitroxide. Specifically, we have prepared an acylated hydroxyl amine as a protected form of the nitroxide, which is stable through oligonucleotide synthesis. The protecting group can subsequently be removed under standard conditions used for deprotecting the oligonucleotide, yielding a hydroxylamine that is oxidized to a nitroxide during the deprotection. We show how this approach was used to synthesize both DNA and RNA oligonucleotides that contain rigid nitroxide spin labels.

This work was supported by a grant from the Icelandic Research Fund (173727-051).

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*Corresponding author: E-mail: snorrisi@hi.is

# Identification of chemical modulators of RfaH with large-scale virtual ligand screening

Da Shi,[a,b] , Dmitri Svetlov[c,d], Irina Artsimovitch,*[d] Ruben Abagyan*[b]

[a] *Department of Chemistry and Biochemistry, UC San Diego, CA 92093, USA*
[b] *Skaggs School of Pharmacy and Pharmaceutical Science, UC San Diego, CA 92093, USA*
[c] *Department of Chemistry and Biochemistry, The Ohio State University, Columbus, OH 43210, USA*
[d] *Department of Microbiology and The Center for RNA Biology, The Ohio State University, Columbus, OH 43210, USA*

Transcription factors from the NusG family bind to the elongating RNA polymerase to enable synthesis of long RNAs in all domains of life. In bacteria, NusG frequently co-exists with specialized paralogs that activate expression of a small set of targets, many of which encode virulence factors. *Escherichia coli* RfaH is the exemplar of this regulatory mechanism. In contrast to NusG, which freely binds to RNA polymerase, RfaH exists in a structurally distinct autoinhibitory state in which the RNA polymerase-binding site is buried at the interface between two RfaH domains[1]. Binding to an *ops* DNA sequence triggers structural transformation wherein the domains dissociate and RfaH refolds into a NusG-like structure. Our previous research identified the key residues modulating this conformational switch[2]. In this work, we used computational and experimental approaches to identify chemicals that can bind to RfaH and modulate its function. We performed a docking screening with 2 million chemicals from ZINC database[3] against the predicted pocket on RfaH that overlaps with the binding site on RNA polymerase. We then tested ten top-ranking chemicals for the ability to inhibit RfaH function in an *in vitro* transcription assay. We found that two ligands inhibited RfaH recruitment to the transcription complex, possibly by disrupting interactions between RfaH and the β' subunit of RNA polymerase. Our findings provide chemical probes to further dissect the mechanism of RfaH and establish the feasibility of using chemicals to interfere with antitermination effects of RfaH. RfaH is required for virulence in several Gram-negative pathogens, and blocking RfaH function would have a significant therapeutic potential.

This work was supported by a grant from the National Institute of General Medical Sciences of the National Institutes of Health [R01 GM67153 to I.A.].

***References:*** [1] Belogurov, GA *et al*., *Mol Cell* **2007**, *26*, 117. [2] Shi, D. *et al*., *Nucleic Acids Res.* **2017***, 45*, 15. [3] Irwin, JJ. *et al*., *J. Chem. Inf. Model.* **2012**, *52*, 7.

*Corresponding authors: E-mails: ruben@ucsd.edu and artsimovitch.1@osu.edu

# Exploring the Capping Code: CleanCap™ Co-transcriptional Capping Allows the Syntheses of Cap 0, Cap 1, Cap 2 and Cap 1 ($^{m6}$A) Capped Messenger RNAs

Dongwon Shin,[a] Krist T. Azizian,[b] Jordana M. Henderson,[a] Richard I. Hogrefe,[a] Michael Houston,[a] Alexandre Lebedev[a] and Anton P. McCaffrey[a*]

[a] *TriLink BioTechnologies, LLC, San Diego, CA 92121, USA*
[b] *Current address: Synthetic Genomics, Inc., La Jolla, CA 92037, USA*

Messenger RNA (mRNA) therapy is a popular platform technology for expressing proteins in cells or *in vivo* because there is minimal risk of insertional mutagenesis. mRNA transfection is used to express proteins for genome editing, protein replacement, vaccines and antibody expression. To avoid an innate immune response, transfected mRNAs should mimic the 5' cap structure of non-immunogenic endogenous mRNAs.

During eukaryotic RNA capping, Cap 0 ($^{m7}$GpppN) is formed as an intermediate. Methylation of the 2'-O position of the first cap-proximal nucleotide forms Cap 1 ($^{m7}$Gppp$N_m$N). In ~50% of transcripts, the 2'-O position of the second cap-proximal nucleotide is also methylated to form Cap 2 ($^{m7}$Gppp$N_mN_m$). N6-methylation of adenosine at the first cap-proximal nucleotide ($^{m7}$Gppp$^{m6}A_m$N) is the second most frequently found modification in mRNA and occurs in conjunction with Cap 1 (and potentially Cap 2).

The immunogenic role of mRNA caps requires elucidation. Viral attenuation occurs after deleting methyltransferases that RNA viruses encode to convert Cap 0 to Cap 1. IFITs bind Cap 0 and activate antiviral translational repression. Thus, Cap 1 (and possibly Cap 2) marks endogenous mRNAs as "self" RNAs. The role of Cap 2 and Cap 1 ($^{m6}$A) is poorly understood because such capped mRNAs have not been produced synthetically at scale. In a recent study, Cap 1 ($^{m6}$A) caps may increase stability and translation while decreasing de-capping of mRNA (Mauer et al., *Nature* **2017**, *541*, 371-375).

Traditional co-transcriptional capping utilizes ARCA (Anti-Reverse Cap Analog) to produce immunogenic Cap 0 with poor capping (~70%) and low yield. Post-transcriptional enzymatic capping to produce Cap 0 or Cap 1 is hindered by highly structured 5' ends, requires further purification and is expensive. Methods to produce Cap 2 mRNAs have not been commercially available. We developed CleanCap™, a novel co-transcriptional capping method to yield Cap 0, Cap 1, Cap 2, Cap 1 ($^{m6}$A) or unnatural caps (***Figure***). Capping with CleanCap™ is reproducibly efficient (90-99%), less expensive than enzymatic capping and is done in a "one-pot" reaction without additional purification. Our studies in a THP-1 Dual monocyte cell line indicate that these various CleanCap™ mRNAs exhibit altered expression and immunogenicity. Further *in vivo* studies to characterize these mRNAs are ongoing.

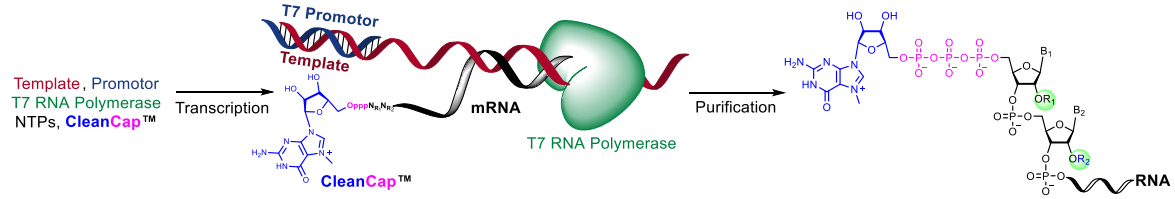


***Figure.*** Capped mRNA synthesis by *in vitro* transcription: Cap 0: $R_{1\text{-}2}$ = H; Cap 1: $R_1$ = $CH_3$, $R_2$ = H; Cap 2: $R_{1\text{-}2}$ = $CH_3$; Cap 1 ($^{m6}$A): $R_1$ = $CH_3$, $R_2$ = H, $B_1$= $^{m6}$A; $B_{1\text{-}2}$= A, C, G, U or $^{m6}$A ($B_1$)

*Corresponding author: E-mail: amccaffrey@trilinkbiotech.com

# α-L-4′-Methoxy-2′-Deoxy-Oligonucleotides

Ondřej Šimák* Miloš Buděšínský, Pavel Novák, Šárka Rosenbergová, Magdalena Petrová, Ivana Dvořáková, Ivan Rosenberg

*Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo nám. 2, 166 10 Praha 6, Czech Republic*

The L-oligonucleotides (L-DNAs), mirror compounds of natural D-configured oligonucleotides, are potential antisense compounds. In contrast to the β-L-LNA, the conformationally locked α-L-LNA in duplex with DNA adopt right-handed helical conformation and form normal Watson-Crick base pairs with all nucleobases in the anti-conformation [1]. Interesting results were achieved with (α-L-RNA)-(α-L-LNA) chimeric oligonucleotides which hybridize selectively with RNA and exhibit significant resistance towards 3'-exonuclease cleavage, and thus they were classified as a promising type of antisense molecules [2].
The synthesis of α-L-4′-methoxythymidine is based on the addition of methoxy group to the appropriately modified 4′-enolacetate [3]. The steric hindrance at 3′-position plays determining role in configuration of arising product. A series of unmodified, partially, and fully modified α-L-homooligothymidylates were prepared by phosphoramidite method on solid phase, and their properties are under investigation.

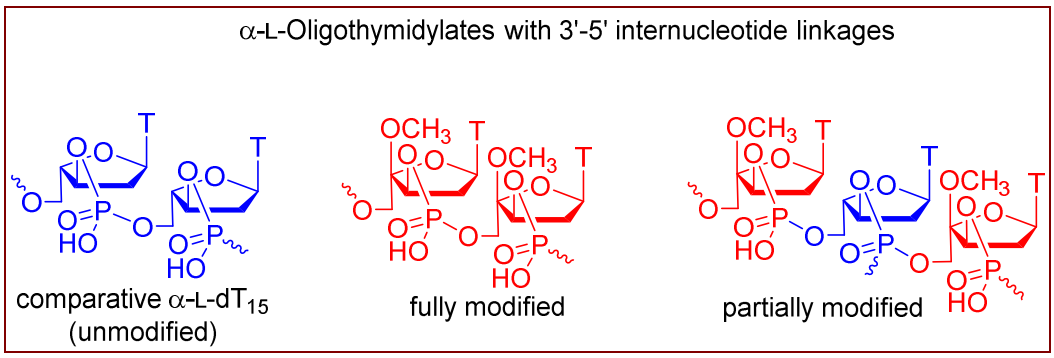


***Figure.*** *Examples of homooligothymidylates.*

This work was supported by the grant # 17-12703S (Czech Science Foundation).

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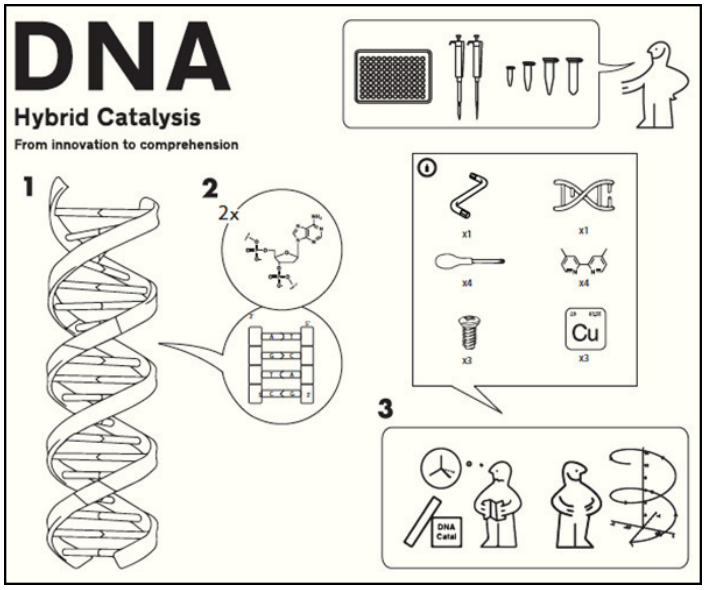
*Corresponding author: E-mail: simak@uochb.cas.cz

# INNOVATIVE DNA-BASED ASYMMETRIC CATALYSIS

Michael Smietana,*[a] Jean-Jacques Vasseur,[a] and Stellios Arseniyadis,[b]*

[a] *IBMM, Université de Montpellier, ENSCM, CNRS, Montpellier, France*
[b] *School of Biological and Chemical Sciences, Queen Mary University of London, London, UK*

DNA-based artificial metalloenzymes have recently drawn considerable attention because of their unique features that comprise a chemically stable chiral double helix associated with many programmable secondary structures. This field has been thriving in the last few years, resulting in the development of a few synthetic transformations by several groups including ours.[1-5] The concept of DNA-based asymmetric catalysis lies in embedding an achiral transition metal catalyst in a DNA double helix that provides the necessary chiral microenvironment to promote the formation of one enantiomer over another for any given reaction. The most recent efforts to unveil new reactivities have been accompanied with the willingness to understand the mechanisms by which the chirality is transferred. We'll present here our most recent results.




***References:*** [1] Duchemin, N.; Heath-Apostolopoulos, I.; Smietana, M.; Arseniyadis, S. *Org. Biomol. Chem*. **2017**, *15*, 7072. [2] Duchemin, N.; Benedetti, E.; Bethge, L.; Vonhoff, S.; Klussmann, S.; Vasseur, J.-J.; Cossy, J.; Smietana, M.; Arseniyadis, S. *Chem. Commun*. **2016**, *52*, 8604. [3] Amirbekyan, K.; Duchemin, N.; Benedetti, E.; Joseph, R.; Colon, A.; Markarian, S. A.; Bethge, L.; Vonhoff, S.; Klussmann, S.; Cossy, J.; Vasseur, J.-J.; Arseniyadis, S.; Smietana, M. *ACS Catal*. **2016**, *6*, 3096. [4] Benedetti, E.; Duchemin, N.; Bethge, L.; Vonhoff, S.; Klussmann, S.; Vasseur, J.-J.; Cossy, J.; Smietana, M.; Arseniyadis, S. *Chem. Commun*. **2015**, *51*, 6076. [5] Wang, J.; Benedetti, E.; Bethge, L.; Vonhoff, S.; Klussmann, S.; Vasseur, J.-J.; Cossy, J.; Smietana, M.; Arseniyadis, S. *Angew. Chem. Int. Ed*. **2013**, *52*, 11546.

* E-mail: michael.smietana@umontpellier.fr; s.arseniyadis@qmul.ac.uk

# Biophysical characterization of mononucleotides with C8-modified (7-methyl)guanine as potential inhibitors of cap dependent processes.

M.Bednarczyk,[a,d] A.Mamot,[b,d] R.Kasprzyk,[c,d] A.Nowicka ,[a,d] J.Kowalska,[a,d] J.Jemielity*[d]

[a] *Faculty of Physics, University of Warsaw, Poland*
[b] *Faculty of Chemistry, University of Warsaw, Poland*
[c] *College of Inter-Faculty Individual Studies in Mathematics and Sciences, University of Warsaw, Poland*
[d] *Centre of New Technologies, University of Warsaw, Poland*

Cap analogs – chemically modified derivatives of a natural nucleotide structure present at the 5' end of messenger RNA – have a great potential for use as small-molecule drugs targeting cap dependent proteins – eIF4E and DcpS. These proteins play a crucial role in gene expression and are responsible for developing some severe diseases, such as cancers, neuromuscular and viral diseases.
The eukaryotic translation initiation factor 4E, eIF4E, is a cap-binding protein playing an important role in initiation of mRNA translation. Moreover, studies have shown the implication of eIF4E in oncogenesis and its increased level in cancer cells.
Decapping scavenger enzyme, DcpS, is a HIT family pyrophosphatase that acts in the 3'-5' mRNA decay pathway and it is identified as a therapeutic target for spinal muscular atrophy (SMA).[1]

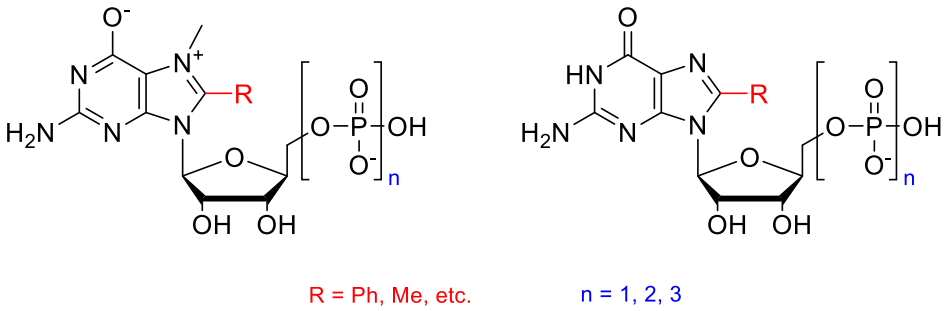



***Figure.*** *Possible modifications in mononucleotide analogs.*

Here, we present a library of mononucleotide cap analogs modified at C8 position mono-, di- or triphosphate of ($m^7$)Guo via Suzuki-Miyaura reaction to investigate the specificity of inhibition of eIF4E, DcpS and other cap binding proteins. Mononucleotides, unlike dinucleotides with more rigid structure, are more flexible and capable of conformational adjustments and therefore can bind selected proteins more tightly. The results of biophysical characterization of novel nucleotides as inhibitors of eIF4E and DcpS will be presented.

This work was supported by grants from the Foundation for Polish Science (TEAM/2016-2/13).

***References:*** [1] Ziemniak M. et al., *Fut. Med. Chem.,* **2013***, 5 (10)*, 1141-1172.

*Corresponding author: E-mail: j.jemielity@cent.uw.edu.pl

# Graphene oxide as fluorescence quencher - developing HTS method for studies on mRNA 5'-end-interacting proteins

Beata Jagoda Starek ,[a] Renata Kasprzyk,[b] Joanna Kowalska,[b] Jacek Jemielity *[c]

[a] *Division of Biophysics, Faculty of Physics, University of Warsaw Pasteura 5, 02-093, Warsaw, Poland*
[b]*College of Inter-Faculty Individual Studies in Mathematics and Natural Sciences, University of Warsaw, Zwirki I Wigury 93, 02-089 Warsaw, Poland.*
[c]*Centre of New Technologies, University of Warsaw, Banacha 2c, 02-097, Warsaw, Poland.*

Fluorescence methods are one of the best monitor progress of enzymatic reactions or protein-ligand complex formation, due to their high sensitivity and selectivity. There are numerous fluorescence techniques that can be exploited for this purpose, such as fluorescence intensity (FLINT), fluorescence polarization (FP), and fluorescence resonance energy transfer (FRET) measurements. FLINT method is the simplest approach, which does not require a complex experimental set-up. Moreover, it can be used to monitor cleavage of small-molecule probes, in contrast to FP method which requires significant changes in size of the fluorescent molecule. However, in not every case fluorescence intensity changes are observed upon the cleavage. One of the solutions to this problem is using additional components, such as selective quenchers that can differentiate the fluorescence signals of cleaved and uncleaved probes. It has been reported that graphene oxide (GO), due to its large surface and the presence of the functional groups, can be used as selective quencher in enzymatic studies.[1]

In our work, we employed the quenching properties of GO to study inhibition of Decapping Scavenger enzyme (DcpS), which is one of the proteins responsible for the degradation of mRNA 5' cap structure. DcpS activity has been identified as a molecular target in Spinal Muscular Atrophy (SMA) and its inhibitors are potential therapeutics against SMA.[2]

To obtain the fluorescent probe we performed CuAAC reaction between and azido-dye (6-FAM) and alkyne modified nucleotide ($m^7$GTP analog) [4] or GTP and butynyl *C*-phosphonate as reference compounds. We characterized fluorescent properties of the synthesized analogs and studied their interactions with DcpS and GO. We observed that graphene oxide quenched the fluorescence of 6-FAM labelled $m^7$GTP very strongly compared to non-methylated or butynyl *C*-phosphonate fluorescent analogs. Based on this phenomenon we developed a *High Throughput Screening* (HTS) assay for DcpS inhibition studies. To show the utility of our method, we applied it to characterize some of the known DcpS small-molecule inhibitors.

***References:*** [1] H. Jang, et. al., *J. of Mat. Chem.* **2014** vol. 2, pp. 2452-2460. [2] J. Singh, et. al., *ACS Chem. Biol.* **2008**, vol. 3, 711-722, 2008. [3] A. Yamamoto, et. al., *Eur J of Pharma and Biopharma*, 2009. *J. Am. Chem. Soc.* **2009**, 71(3), 484-489, [4] Wanat et. al., *Org. Lett*. **2015**, 3062–3065

*Corresponding author: E-mail: j.jemielity@cent.uw.edu.pl

# Optimizing reaction conditions for Cu(I) catalyzed conjugation to phophorothioate containing oligonucleotides

Malgorzata Honcharenko,[a] Dmytro Honcharenko,[a] Roger Strömberg*[a]

[a] *Department of Biosciences and Nutrition, Karolinska Institutet, NEO,Hälsovägen 9, Huddinge, Sweden*

The Oligonucleotide therapeutics, like other drugs, must reach disease-associated tissues to function. By conjugation to peptides, sugars etc, it is possible to enhance cellular uptake and delivery to specific tissues. The use of Cu (I) catalyzed 1, 3 – dipolar cycladdition ("click chemistry") between an azide and a terminal alkyne for conjugation to biomolecules has exploded in the last decade. This is due to efficacy, orthogonality to most reactions carried out in synthesis of biomolecules and due to being carried out under conditions where functional groups in biomolecules do not react.

Phosphorothioate internucleosidic linkages are still the by far most commonly used modifications of oligonucleotides for therapeutic use. To conjugate localization signals and uptake enhancers to oligonucleotide phosphorothioates (ON PS) is of course desirable. Today this is typically done by various methods but mostly not with "click chemistry" and usually then in moderate yields. There seems to be a general conception that Cu(I) catalyzed conjugation will not work well with ON PS. There is of course the option to use copper free click chemistry, but the disadvantage is that specific strained alkynes are needed and these add some bulk and also require additional synthesis efforts.

Despite the general conception about Cu (I) promoted click chemistry and ON PS being troublesome, there is very little reported. Here we report on a study that investigates the possibility of using Cu (I) catalyzed Huisgen cycloaddition in conjugation reactions with thiophosphate modified oligonucleotides. The study includes reactions both on solid phase and in solution, different sources for the copper ions, different conditions and reaction times as well as assessment of side product formation.

# The Structural Basis of Threonylcarbamoyl Adenosine Biosynthesis in Bacteria

Amit Luthra,[a] William Swinehart,[b] Naduni Paranagama,[a] Susan Bayooz,[a] Phuc Phan,[a] Boguslaw Stec,[a] Dirk Iwata-Reuyl,[b] Manal A. Swairjo*[a]

[a] *Department of Chemistry and Biochemistry, San Diego State University, California, USA*
[b] *Department Chemistry, Portland State University, Portland OR, USA*

N6-threonylcarbamoyladenosine ($t^6A$) is a universally conserved modified nucleoside found at position 37 in the anticodon stem-loop of many tRNAs decoding ANN codons (N is any nucleotide) in all domains of life. The $t^6A$ modification shapes the architecture of the anticodon stem-loop, a requisite step for accurate decoding of mRNA codons, and its absence results in growth defects and neurodegeneration in humans. In bacteria, $t^6A$ biosynthesis - an essential biological process and an attractive antibacterial drug target – is catalyzed by the proteins TsaB, TsaC/TsaC2, TsaD and TsaE. To elucidate the molecular mechanisms underlying $t^6A$ biosynthesis in bacteria, we characterized structurally, biochemically, and mechanistically the biosynthesis system from *Thermotoga maritima* using a combination of crystallographic, SAXS, kinetic, and mutagenesis approaches. Our results show that a heterocomplex $TsaB_2D_2$ constitutes the minimal tRNA binding platform that supports threonylcarbamoyl (TC) transfer to tRNA before inactivation and release of modified tRNA. TsaE-catalyzed ATP hydrolysis reactivates the platform, enabling multiple turnovers. Our high-resolution crystal structure of the $TsaB_2D_2E_2$ complex bound to ATP in the ATPase site, and ADP in the TC-transfer site, combined with extensive mutagenesis, reveal the communication routes that govern the order and timing of TC transfer and ATP hydrolysis in the course of synthesis.

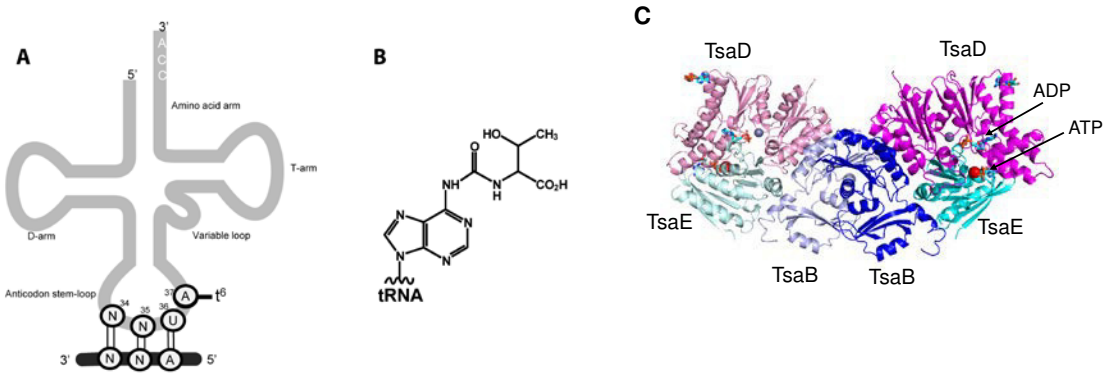



***Figure.*** *Location (A) and structure (B) of $t^6A$ in tRNA, and the 2.5-Å crystal structure of the* T. maritima *$TsaB_2D_2E_2$ complex bound to nucleotides.*

This work was supported by grant GM110588 from the NIH.

***References:*** Luthra *et al.*, *Nucl. Acids. Res*. 2018, 46, 1395.

*Corresponding author: E-mail: mswairjo@sdsu.edu

# Functional Nucleic Acid Probes Possessing Stacked Chromophores

Tadao Takada,* Shunya Ishino, Yurika Honda, Mitsunobu Nakamura, Kazushige Yamana*

*Department of Applied Chemistry, Graduate School of Engineering, University of Hyogo, 2167 Shosha, Himeji 671-2280, Japan*

Nucleic acids modified with fluorescent dyes or (photo) electrochemical probes can be exploited in various fields ranging from molecular biology to molecular electronics.[1-2] In previous work, we have demonstrated that artificial hydrophobic pockets in DNA created by spacer molecules can serve as a scaffold to control the organization of the functional dyes. We also showed that abasic sites generated through enzymatic reactions in DNA can work as binding pockets for hydrophobic molecules and efficient conjugation reactions.[3-5]

In this study, we demonstrate the construction of stacked functional dyes within a DNA duplex and their photochemical properties, which is potentially applicable for the design of fluorescent nucleic acid probes. We show the site-specific covalent modification of DNA with perylenediimide (PDI) derivatives and the photochemical properties of PDI-DNA conjugates possessing a homo- and hetero-dimer composed of the PDI derivatives (Figure 1).[6] We also report the preparation of DNA with thiazole oranges (TO) exhibiting a unique excimer-like emission.

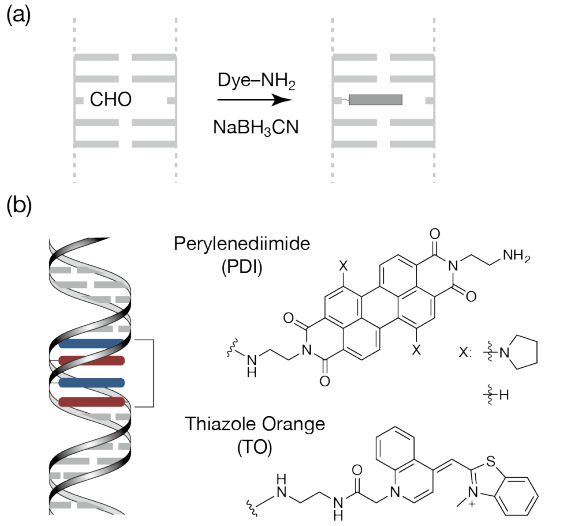



Figure 1. (a) Site-specific incorporation of functional dyes into DNA. (b) Stacked functional chromophores constructed within DNA π-stack. Chemical structures of perylenediimide derivatives (PDI) and thiazole orange (TO).

***References:*** [1] Y. N. Teo, E. T. Kool, *Chem. Rev.*, **2012**, *112*, 4221-4245. [2] D. Gorl, X. Zhang, F. Wurthner, *Angew. Chem. Int. Ed.*, **2012**, *51*, 6328-6348. [3] T. Takada, K. Yamaguchi, S. Tsukamoto, M. Nakamura, K. Yamana, *Analyst*, **2014**, *139*, 4016-4021. [4] T. Takada, M. Ido, A. Ashida, M. Nakamura, K. Yamana, *ChemBioChem*, **2016**, *17*, 2230-2233. [5] T. Takada, A. Ashida, M. Nakamura, M. Fujitsuka, T. Majima, K. Yamana, *J. Am. Chem. Soc.*, **2014**, *136*, 6814-6817. [6] T. Takada, S. Ishino, A. Takata, M. Nakamura, M. Fujitsuka, T. Majima, K. Yamana, *Chem. Eur. J.*, **2018**, *24*, 8228-8232.

*Corresponding author: E-mail: takada@eng.u-hyogo.ac.jp, yamana@eng.u-hyogo.ac.jp

# Synthesis and evaluation of modified 7-deaza-dG triphosphate derivatives for 8-oxo-dGTP mimicry

Yosuke Taniguchi,*[a] Hui Shi,[a] Yoshiharu Shigematsu,[a] Shigeki Sasaki[a]

[a] *Graduate School of Pharmaceutical Sciences, Kyushu University, 3-1-1 Maidashi Higashi-ku Fukuoka 812-8582, Japan*

The nucleotide repair enzyme, hMTH1, hydrolyzes the oxidized nucleoside triphosphates, 8-oxo-dGTP and 2-oxo-dATP, to prevent the genotoxicity due to incorporation into DNA. It is reported that hMTH1 is necessary for the survival of cancer cells, but it is not necessary for survival of normal cells. However, there has been controversy about whether hMTH1 becomes novel anticancer target or not.

Recently, we found that 8-halogenated 7-deaza-dG (8-halo-7-deaza-dG) behaved as 8-oxo-dG in duplex DNA. We, therefore, synthesized its triphosphate (8-halo-7-deaza-dGTP) derivatives and evaluated the hydrolysis properties of them using hMTH1. It was found that these triphosphates were poor substrates for hMTH1 but they exhibited strong competitive inhibition against hMTH1 activity. This inhibitory effect was caused by the slower rate of hydrolysis by possible enzyme structural changes, specifically different stacking interactions with between 8-halo-7-deaza-dGTP and 8-holo-7-deaza-dGMP. Furthermore, they were poorly incorporated by both KF(exo-) and human DNA polymerase β opposite dC or dA into the template DNA. These result indicated that these triphosphates have no critical genotoxicity during the DNA replication or repair processes. These results indicated that these nucleotide analogues will be good inhibitors and chemical probes for hMTH1 in cell.

In this presentation, we will show the synthesis and properties of 8-halo-7-deaza-dGTP and their derivatives.

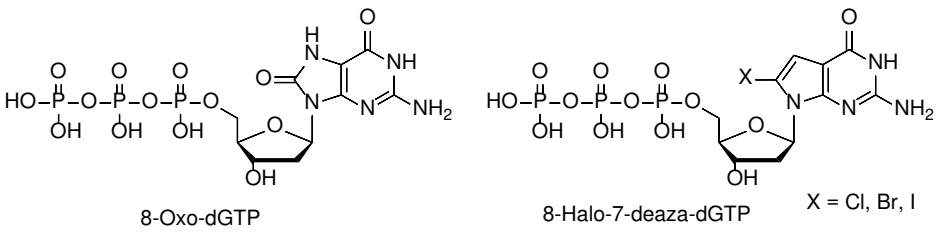



***Figure.*** *Structures of 8-oxo-dGTP and modified 7-deaza-dGTP derivatives.*

***References:*** [1] Yin, Y., Sasaki, S., Taniguchi, Y., *Bioorg. Med. Chem.* **2016***, 24*, 3856. [2] Yin, Y., Sasaki, S., Taniguchi, Y., *ChemBioChem.* **2016***, 17*, 556. [3] Yin, Y., Sasaki, S., Taniguchi, Y., *ChemBioChem.* **2015***, 16*, 1190. [4] Yin, Y., Taniguchi, Y., Sasaki, S., *Tetrahedron.* **2014***, 70*, 2040.

*Corresponding author: E-mail: taniguch@phar.kyushu-u.ac.jp

# Synthetic study of bio-reduction cleavable linker for oligonucleotides

Kazuma Terasawa, Akira Ono*1, Hisao Saneyoshi*2

*Department of Material & Life Chemistry, Faculty of Engineering, Kanagawa University*
*3-27-1 Rokkakubashi, Kanagawa-ku, Yokohama 221-8686, Japan*

In recent oligonucleotide-based drug development, delivery of oligonucleotides to the target site of action is a major challenge. Conjugations of functional molecules such as carbonhydrates vitamins and peptides with the oligonucleotides have been developed. [1] This strategy enables oligonucleotides to deliver to their target site of action. However, the conjugated oligonucleotides often reduce the biological activity. [2] To avoid the loss of activity, use of cleavable type linkers have been reported to date.[4]

Our group also reported a cleavable linker was prepared to functionalize the 5'-terminal end of oligonucleotides. [3] This linker which can modify oligonucleotide at 5'-position with functional molecules using CuAAC reaction. Resulted conjugated oligonucleotides would be cleaved in hypoxic tumor cells to release active oligonucleotides. However, this linker limited to introduction only at 5'-terminal ends. In this study, we propose a new cleavable linker structure which can link functional molecules at desired positions in oligonucleotides.

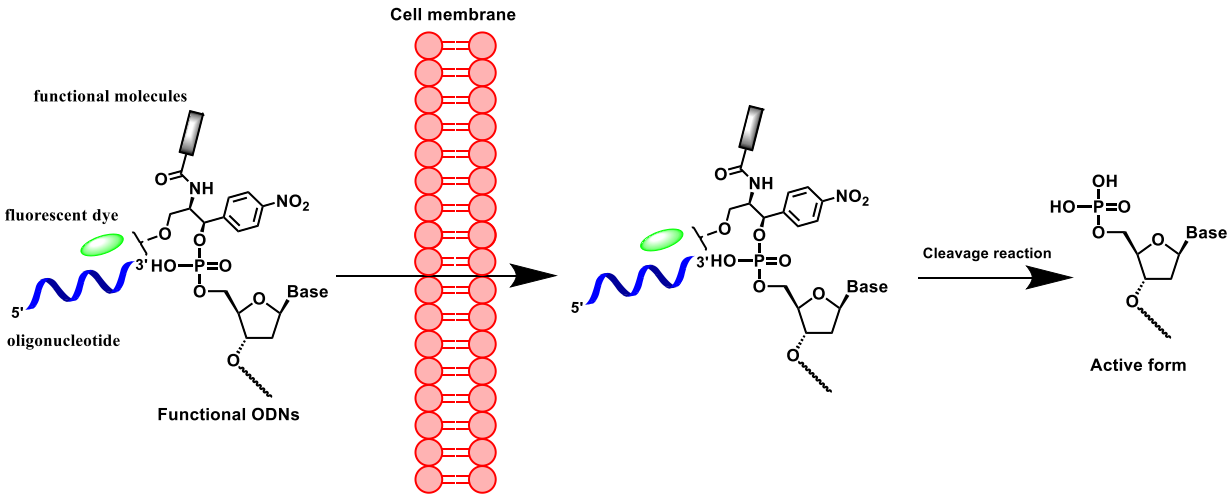



***Figure.*** *Expected action of bio-reduction cleavable linker*

***References:*** [1] J. Winkler *et al*, *Ther. Deliv.*, **2013**,*4(7),* 791-809. [2] Y. Ji *et al, Angew. Chem. Int. Ed,*. **2016**, *55*, 2152-2156. [3] H. Saneyoshi *et al, J. Org. Chem*., **2017**, *82,* 1796−1802. [4] P. T. Wong *et al, Chem Rev*., **2015**, *115,* 3388−3432.

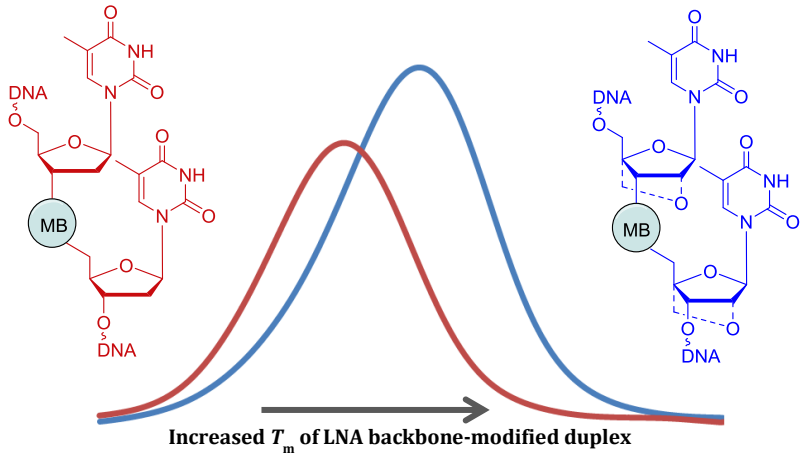
*1 E-mail: akiraono@kanagawa-u.ac.jp / *2 E-mail: saneyoshih@kanagawa-u.ac.jp

# Enhanced Binding Affinity of Backbone-Modified Locked Nucleic Acid (LNA) Oligonucleotides Towards RNA Targets

Cameron Thorpe[a], Sven Epple[a], Benjamin Woods[a], Afaf El-Sagheer[ab], Tom Brown[a]

[a] *Department of Chemistry, University of Oxford, 12 Mansfield Road, Oxford, OX1 3TA, UK, Chemistry.*
[b] *Department of Science and Mathematics, Faculty of Petroleum and Mining Engineering, Suez University, Suez 43721, Egypt*

Backbone-modified oligonucleotides can be used for antisense therapeutics, exon skipping and gene interference applications. Replacement of phosphodiesters with modified backbone analogues allows for improved enzymatic stability and increased biological half-lives. Though enzymatically stabilising, these modifications often result in disrupted duplex structures with lower thermodynamic stability. We report a novel system of modified backbones with locked nucleic acids (LNAs) to combine enzymatic and duplex stability as well as provide neutral oligonucleotide frameworks with the potential for enhanced cell delivery. LNAs are a bicyclic modified system which can enhance duplex stability by up to +10 °C per modification.[1] With the recent development and approval of several oligonucleotide drugs by the FDA, namely Eteplirsen and Nusinersen, this work offers the opportunity to develop the next generation of oligonucleotide drugs. Our approach has the potential to provide high efficacy and low toxicity for LNA-based therapeutics, delivering increased binding to RNA targets.[2]




Enhanced duplex stability of LNA-backbone oligonucleotide

This work was supported by funding from the Biotechnology and Biological Sciences research Council (BBSRC) [grant number BB/M011224/1].

***References:*** [1] Kaur, H. et al. Chemical Reviews **2007**, *107*, 4672–4697, [2] Kumar, P. et al, Chem. Commun., **2017**, *53*, 8910-891.

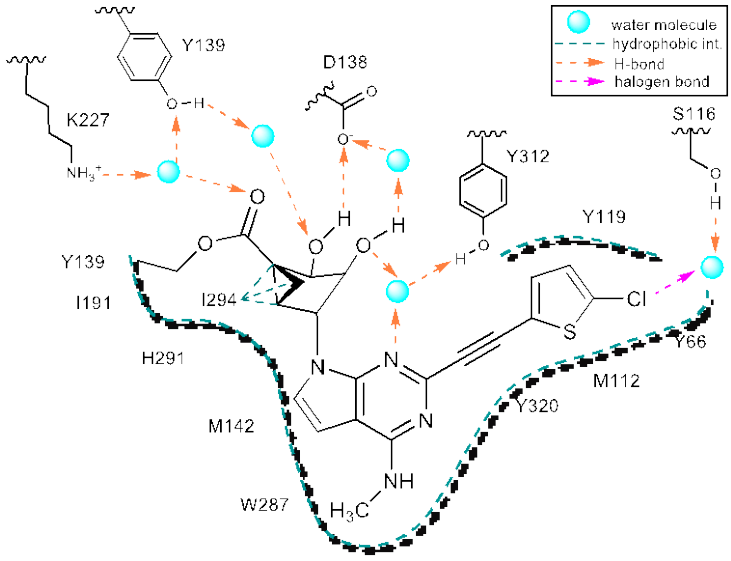
*Email : cameron.thorpe@dtp.ox.ac.uk, tom.brown@chem.ox.ac.uk

# Discovery of the First Nucleoside Scaffold for Opioid Receptor Antagonists

Dilip K. Tosh,[a] Antonella Ciancetta,[a] Philip Mannes,[a] Eugene Warnick,[a] Aaron Janowsky,[b] Amy J. Eshleman,[b] Elizabeth Gizewski,[c] Tarsis F. Brust,[d] Laura M. Bohn, [d]John A. Auchampach,[c] Zhan-Guo Gao,[a] and Kenneth A. Jacobson*[a]

[a] National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892 USA. [b] Oregon Health and Science University, Portland, OR 97239 USA. [c] Medical College of Wisconsin, Milwaukee, WI 53226 USA. [d] Departments of Molecular Medicine and Neuroscience, The Scripps Research Institute, Jupiter, Florida 33458 USA.

In the course of screening off-target effects of rigid (N)-methanocarba-adenosine 5′-methylamides $A_3$ adenosine receptor (AR) agonists, we discovered μM binding hits at the δ–opioid receptor (DOR) and translocator protein (TSPO). In an effort to increase OR and decrease AR affinity by structure activity analysis of this series, antagonist activity at κ(K)OR appeared in 5′-esters (ethyl, MRS7232; propyl, MRS7251), which retained TSPO interaction (μM). 7-Deaza modification of C2-(arylethynyl)-5′-esters but not 4′-truncation enhanced KOR affinity (MRS7299 and MRS7300, $K_i$ ~40 nM), revealed μ-OR and δ–OR binding and reduced AR affinity. Molecular docking and dynamics simulations located a putative KOR binding mode consistent with the observed affinities placing C7 in a hydrophobic region. 3-Deaza modification permitted TSPO but not OR binding, and 1-deaza was permissive to both; ribose-restored analogues were inactive at both. Thus, we have repurposed a known AR nucleoside scaffold for OR antagonism, with a detailed hypothesis for KOR recognition.




* Corresponding author: Email: kennethj@niddk.nih.gov

# Fluorescent Nucleoside Analogues towards Monitoring Retroviral Replication in Live Cells

Marc B Turner,[a] Karena M Fassett,[b] Danielle Slemons,[b] Roland Wolkowicz,[b] Byron W Purse*[a]

[a] *Department of Chemistry & Biochemistry, San Diego State University, 5500 Campanile Dr, San Diego CA, 92182, USA*
[b] *Department of Biology, San Diego State University, same address as above*

Fluorescent tags and probes for nucleic acids have been pivotal in biochemical research by providing new capabilities in visualizing cellular structures and processes. Conventional fluorophores for labeling nucleic acids in cells are effective, but can disrupt natural cellular structure or leave artifacts from sample preparation. For example, 5-ethynyl-2'-deoxyuridine (EdU) allows for coupling of a common fluorophore with an azide linker using Cu(I)-catalyzed azide-alkyne click-chemistry.[1] This method accommodates many bright fluorophores but disrupts natural cellular function with toxic conditions, thereby offering single time-point images of disrupted cells. Using tricyclic cytidine (tC) analogues, which have been tuned for a variety of fluorescent responses to base pairing and stacking, we aim to attain the advantage of dynamically imaging nucleic acids while avoiding any conjugation chemistry.

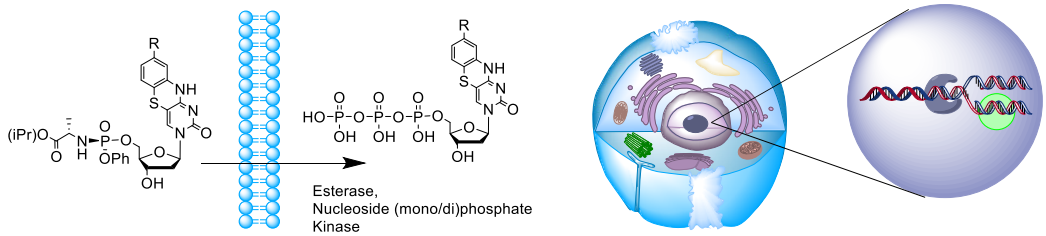



**Fig 1.** Strategy for delivering nucleoside analogues to cells. Enzymatic processing yields a triphosphate for subsequent integration into replicated DNA.

We performed *in vitro* kinetics measurements with tC-derived triphosphates and viral reverse transcriptase (RT) enzymes partly because these viral polymerases lack proofreading capabilities[2]. We measured the standard Michaelis-Menten kinetic parameters $V_{MAX}$ and $K_M$ using RT from AMV, MLV, and HIV. The three RTs exhibit different kinetics for parent tC triphosphate and 8-DEA-tC[3] triphosphates. For live cell studies, we synthesized tC-derived phosphoramidates that can traverse cell membranes and be enzymatically converted to active triphosphates intracellularly. The delivery of these molecules to 298T and SUPT1 human T-cells was studied using flow cytometry, with initial results suggesting cell delivery and fluorescence retention. In the presence of living cells and infectious retroviruses we hypothesize that fluorescently labelled DNA will be mostly the product of retroviral DNA synthesis.

This work was supported by the National Science Foundation (CHE-1709796 and CHE-1800529).

***References:*** [1] Neef, a. B.; Luedtke, N. W. *Proc. Natl. Acad. Sci.* **2011**, *108* (51), 20404–20409. [2] Xiong, Y.; Eickbush, T. H. *EMBO J.* **1990**, *9* (10), 3353–3362. [3] Burns, D. D.; et al. Purse, B. W. *J. Am. Chem. Soc.* **2017**, *139* (4), 1372–1375.

*Corresponding author: E-mail: bpurse@sdsu.edu

# Synthesis and Biological Activity of Amide-Linked RNA

Julien Viel,[a] Venubabu Kotikam,[a] Harry Haran,[a] Eriks Rozners*[a]

[a] *Department of Chemistry, Binghamton University, NY 13902, USA*

Amide linkages are promising modifications for optimization of short interfering RNAs (siRNAs) as potential therapeutic agents as well as tools for basic science (1-3). However, the realization of their full potential requires further investigation into optimum amide placement and number (1). Progress in this area is impeded by the relatively low coupling yields observed for the amide-modified RNA monomers currently in use. Preliminary results within our group indicate that coupling must be optimized in order to allow for the efficient production of siRNA strands with more than six amide-modifications.

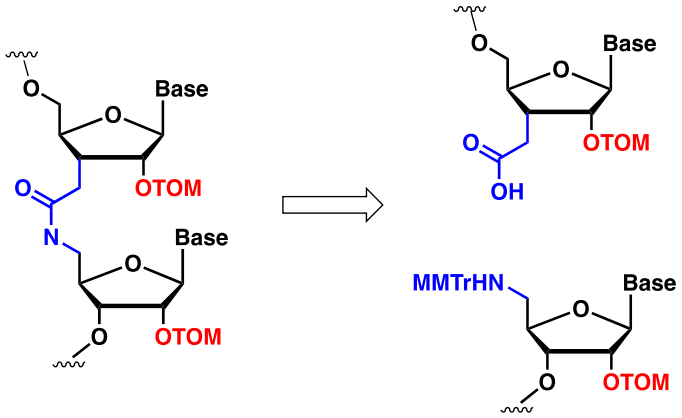



***Figure.*** *Amide-modified RNA coupling*

This presentation will discuss optimization of amide coupling efficiency by optimizing protecting groups, coupling agents and reaction time and temperature. We hypothesize that the relatively low coupling efficiency is caused by steric hindrance of the currently-used tert-butyldimethylsilyl (TBDMS) 2'-OH protecting group. Results of coupling optimization will be discussed in terms of different protection strategies aimed to decrease or move protecting group bulk away from the ribonucleoside ring. Since amide-modified RNA monomers are not susceptible to epimerization during carboxylic acid activation, we have used strong activating reagents not suitable for typical protein amino acids is possible. Coupling condition optimization results using such strong carboxylic acid activators will presented. The optimization of coupling time and temperature in concert with the strategies above will also be discussed. Such investigations are facilitated by the use of an automated microwave-capable peptide synthesizer.

Through the development of efficient coupling strategies compatible with automated solid-phase-synthesis, the investigation of the biological properties of amide-linked RNAs will be greatly facilitated, and will lead to a substantial increase in their applicability as basic science tools.

This work was supported by the US National Institutes of Health (R01 GM071461).

***References:*** [1] Mutisya, D., et al. *Nucleic Acid Res.* **2017***, 45,* 8142. [2] Mutisya, D., et al. *Nucleic Acid Res.* **2014***, 42,* 6542. [3] Tanui, P., et al. *Org. Biomol. Chem.* **2014***, 12,* 1207.

*Corresponding author: E-mail: erozners@binghamton.edu

# Refinement of the molecular design of hepatocyte-targeted siRNA conjugates for the treatment of liver-related disorders

Lucas Bethge,[a] Steffen Schubert,[a] Ute Schaeper,[a] Judith Hauptmann,[a] Adrien Weingärtner,[a] Christian Frauendorf,[a] Manuela Aleku,[a] Marie Wikström Lindholm [a*]

[a] *Silence Therapeutics GmbH, Robert-Rössle-Str. 10, 13125 Berlin, Germany*

Iron overload represents a serious complication in many blood disorders, such as β-thalassemia. Silence Therapeutics has entered non-clinical development of a novel, effective and safe treatment for this unmet medical need. Targeting a key modulator of iron homeostasis with a GalNAc-conjugated siRNA efficiently reduces serum iron levels in mice for at least six weeks after a single injection. The treatment is well tolerated and shows no signs of toxicity. On a parallel track for technology innovation we examine different GalNAc-conjugated siRNA design options. Special areas of interest are siRNA modification patterns, end stabilisation, linker chemistry, and finally number and location of GalNAc units. Taken together these design strategies enable us to fine-tune RNAi activity for GalNAc conjugates and to broaden the therapeutic options for diseases originating in the hepatocyte.

*Corresponding author: E-mail: m.lindholm@silence-therapeutics.com

# Elucidating dynamic interactions between siRNA and proteins using a pair of nucleoside chemical probes

Tomoya Wada, Mayu Yamada, Noriko Saito-Tarashima, Noriaki Minakawa*

*Graduate School of Pharmaceutical Science, Tokushima University, Shomachi 1-78-1, Tokushima 770-8505, Japan*

Understanding the dynamic interaction between siRNA and proteins is useful for designing chemically-modified siRNA drugs. In this study, we have developed logical chemical probes 7-bromo-7-deazaadenosine (7Br-7deA) and 3-bromo-3-deazaadenosine (3Br-3deA) (Figure 1) [1]. We hypothesized that the incorporation of these probes into the A position in an siRNA sequence would cause the bromo groups at each deaza position to disturb siRNA–protein interactions, thereby clarifying which groove (major or minor) is critical for these interactions. Using siRNAs incorporating these probes, we analyzed the interaction between siRNA- and RNAi-related proteins. The results showed that (i) 3Br-3deA(s) at the 5'-end of the sense strand enhanced the RNAi activity of these modified siRNAs, and (ii) the direction of RISC assembly was determined by the interaction between Argonaute2, which is the main component of RISC, and siRNA in the minor groove near the 5'-end of the sense strand. In addition, we also analyzed the interaction between siRNA and pattern recognition receptors that induce an innate immune response. Interaction with TLR3, which is known to recognize double-stranded RNA, was evaluated by reporter gene assay, revealing that steric hindrance on the minor groove side at the 5'-end of the sense strand suppressed innate immune response. However, evaluation by whole blood assay [2] using human blood revealed that the steric hindrance at the same position enhanced the innate immune response. Therefore, we are now developing photo-cross-linking probes with a diazirine group at each deaza position in order to analyze these conflicting results in more detail. In this presentation, the synthesis of these probes and findings related to them will also be reported.

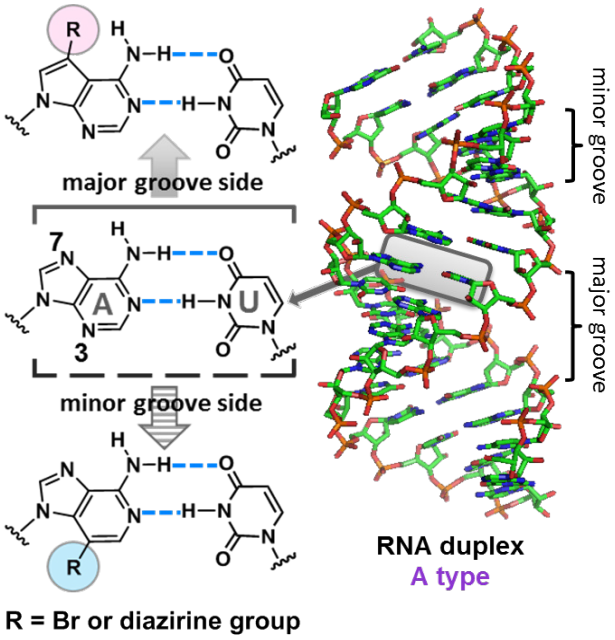



***Figure 1.*** *Schematic of chemical probe concept*

***References:*** [1] Noriko Saito-Tarashima, Hirotaka Kira, Tomoya Wada, et al., *Org. Biomol. Chem.* **2016**, *14*, 11096. [2] Christoph Coch, et al., *PLos ONE*. **2013**, *8*, e71057.

*Corresponding author: E-mail: minakawa@tokushima-u.ac.jp

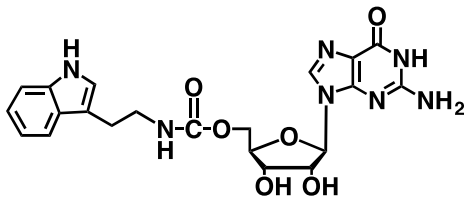
# HINT1: A Potential Non-Opioid Target for Pain

Carston R. Wagner[a*], Rachit Shah[a], George Wilcox[b], Alex Strom[a] and Mary Lunzer[a]

[a]Department of Medicinal Chemistry, University of Minnesota, Minneapolis, MN, USA
[b]Department of Neuroscience, University of Minnesota, Minneapolis, MN, USA

Over 100 million Americans suffer a significant decrease in their quality of life and productivity due to chronic pain. Consequently, there is an increasing demand for the development of new approaches for the treatment of pain.

Through the use of medicinal chemistry, biochemistry and neuropharmacology studies, our team has recently demonstrated that Histidine Triad Nucleotide Binding Protein 1 (HINT1) is a key mediator of the cross-regulation of the μ-opioid receptor (MOR) and the glutamatergic N-Methyl-D-Aspartate Receptor (NMDAR).[1] The ability of MOR activation to reduce pain is highly dependent on its interaction with NMDAR. We have found that HINT1 directs the association of MOR with NMDAR. Upon binding with morphine and activation of analgesia, Zn is mobilized and binds to the HINT1 active site, resulting in Protein Kinase C gamma (PKCγ) recruitment. NMDAR phosphorylation by PKCγ results in activation of the channel and Calmodulin-Dependent Kinase II (CaMKII) suppression of MOR. Consistent with these observations, HINT1 knock-out mice exhibited enhanced analgesia and resistance to tolerance with no apparent adverse side-effects.




***TpGc***

Through kinetic, mutagenic and x-ray crystallography studies, our group has delineated key features of the catalytic mechanism and potential biochemical role of human HINT1.[2] These studies have enabled the rational design of inhibitors of hHINT1. We have demonstrated that the dosing of mice with one of these inhibitors, 5'-tryptamine guanosine carbamate (TpGc), can significantly enhance morphine analgesia, reverse morphine tolerance and reduce neuropathic pain. In addition, we have also recently demonstrated that TpGc can abolish chemotherapeutic induced neuropathic pain, as well as block the antagonist effect of the mu-opioid receptor on NMDAR activation in the spinal cord. Taken together, our results demonstrate the unique regulatory role of HINT1 on MOR and NMDAR, as well as reveal it to be a potential new non-opioid target for the development of pain therapeutics.

This work was funded by a grant from the Wallin Foundation.

***References:*** [1] Garzon , J, et. al, *Neuropharmacology*, **2015**, *89*, 412, [2] Maize, K. M., et.al, *Molecular Pharmaceutics*, **2017**, *14*, 3987.

*Corresponding author: E-mail: wagne003@umn.edu

# Fluorogenic thiazole orange TOTFO probes stabilize parallel DNA triplexes at pH 7 and above

Sarah Walsh,[a,b] Afaf El Sagheer,[a,c] Tom Brown*[a]

[a] *Department of Chemistry, University of Oxford, 12 Mansfield Road, Oxford OX1 3TA, UK.*
[b] *ATDBio Ltd., Robert Robinson Ave, Oxford, OX4 4GA, UK.*
[c] *Chemistry Branch, Department of Science and Mathematics, Suez University, Suez 43721, Egypt.*

Triplex-forming oligonucleotides (TFOs) selectively bind to the major groove of double stranded DNA (dsDNA) through Hoogsteen hydrogen bonding. Instability of DNA triplexes at neutral pH and above severely limits their applications. Here, we demonstrate that the introduction of a thiazole orange (TO) intercalator onto a thymine nucleobase in TFOs resolves this problem. The stabilising effects are additive; multiple TO units produce nanomolar duplex binding and triplex stability can surpass that of the underlying duplex. In one example, a TFO containing three TO units increased the triplex melting temperature at pH 7 by a remarkable 45 °C relative to the unmodified triplex. Importantly, this approach allows the development of probes that can be used *in vivo* for target sequences outside the conventional polypyrimidine targets, which are currently necessary for stable triplex formation. This represents a significant step in the development of triplex based therapeutic and diagnostic tools. By overcoming the instability of triplexes across a broad range of pH and sequence contexts, these very simple 'TOTFO' probes could expand triplex applications into many areas including triplex nanotechnology,[1] gene inhibition and regulation,[2] DNA repair and recombination,[3] inhibition of enzymatic binding[4] and site-specific DNA damage.[5]

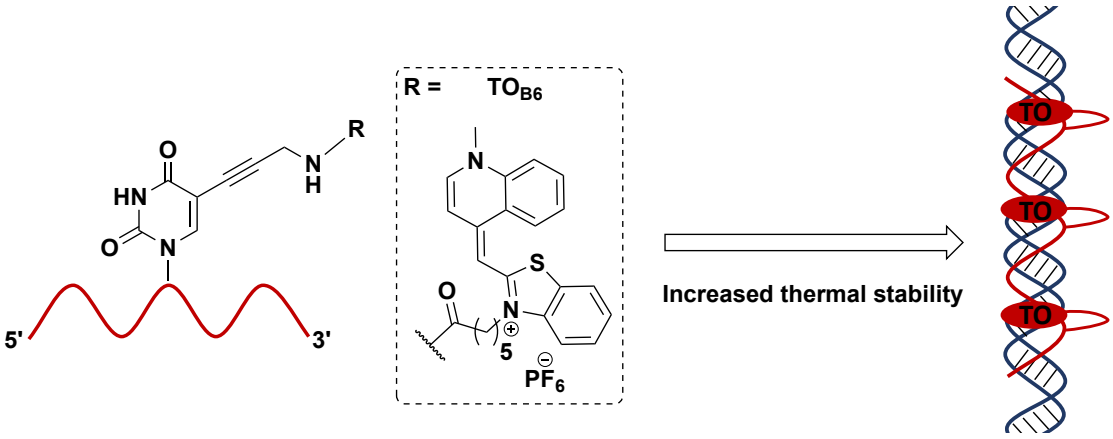



***Figure:*** *Multiple additions of thiazole orange incorporated via propargylamino dT linker into triplexes will enhance the triplex stability and fluorescence intensity.*

This work has received funding from the European Union's Horizon 2020 programme under the Marie Sklodowska-Curie Grant no. 642023.

***References:*** [1] Y. Hu, A. Cecconello, A. Idili, F. Ricci, I. Willner, *Angew. Chemie - Int. Ed.* **2017**, *56*, 15210–15233. [2] A. R. Morgan, R. D. Wells, *J. Mol. Biol.* **1968**, *37*, 63–80. [3] A. Mukherjee, K. M. Vasquez, *Biochimie* **2011**, *93*, 1197–1208. [4] L. Maher, B. Wold, P. Dervan, *Science* **1989**, *245*, 725–730. [5] V. Malnuit, M. Duca, R. Benhida, *Org. Biomol. Chem.* **2011**, *9*, 326–336.

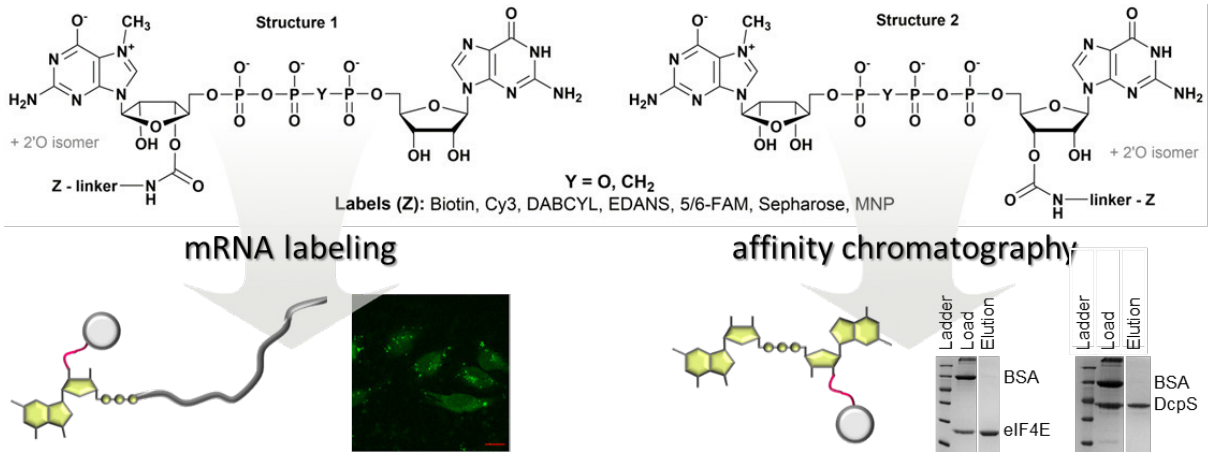
*Corresponding author: E-mail: tom.brown@chem.ox.ac.uk

# 5' cap analogs and their conjugates – tools for site-specific sequence-independent labeling of mRNA and isolation of cap-binding proteins

Zofia Warminska[a,b] Pawel J. Sikorski[a] Marcin Warminski[c] Joanna Kowalska[c] Jacek Jemielity*[a]

[a] *Centre of New Technologies, University of Warsaw, 02-097 Warsaw, Poland*
[b] *College of Interfaculty Individual Studies of Mathematics and Natural Sciences, University of Warsaw, 02-097 Warsaw, Poland*
[c] *Division of Biophysics, Institute of Experimental Physics, Faculty of Physics, University of Warsaw, 02-093 Warsaw, Poland*

The 7-methylguanosine cap structure is a unique feature present at the 5’ ends of messenger RNAs (mRNAs), which is bound by numerous cap-binding proteins. Along with growing interest in the mechanisms of mRNA decay and localization, there is an increasing need for tools enabling its fluorescent labeling or affinity tagging.
To address that demand, we developed a new class of 5’ cap analogs, functionalized with amine or carboxyl groups attached to 2’/3’-OH of ribose moiety through linkers of different length [1]. Selection of the attachment positions was dictated by the specificity of recognition by major cap-binding proteins regulating various processes, such as translation initiation, mRNA degradation, etc. Analogs modified within 7-methylguanosine [Structure 1] enabled the site-specific labeling of RNA at the 5’ end without disrupting basic function of mRNA in protein biosynthesis [2]. We also demonstrated the utility of fluorescently labeled RNAs in Dcp2-mediated decapping assays, RNA decay assays, and RNA visualization in cells. Analogs modified within the second nucleoside [Structure 2] were conjugated with Sepharose, magnetic nanoparticles or biotin and used as affinity resins for purifying of cap-binding proteins such as Initiation Factor 4E and Decapping Scavenger. Experiments with standard mixtures showed that such resins are selective for cap-binding proteins and enable their efficient purification.




This work was supported by the Polish National Center of Research and Development (STRATEGMED1/235773/19/NCBR/2016) and by the National Science Centre, Poland (UMO-2013/09/B/ST5/01341).

***References:*** [1] Warminski, M. et al., *EurJOC.* **2015***, 28*, 6153. [2] Warminski, M. et al., *Bioconjugate Chem.* **2017***, 28*, 1978.

*Corresponding author: E-mail: j.jemielity@cent.uw.edu.pl

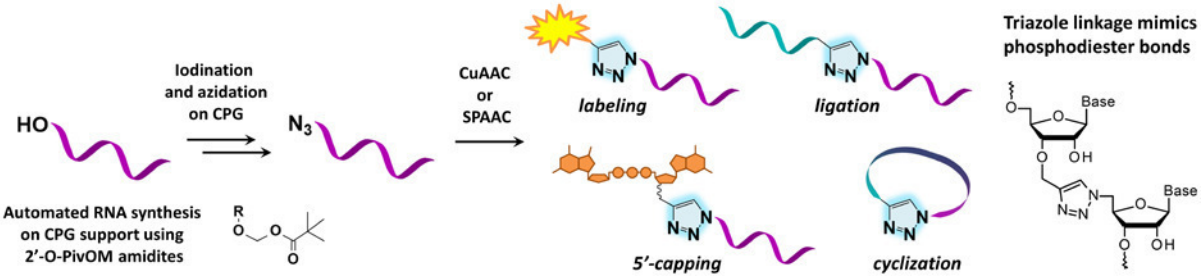
# RNA 5’-azides: solid-phase synthesis and reactivity in azide-alkyne cycloadditions

Marcin Warminski,[a] Joanna Kowalska, Jacek Jemielity*[b]

[a] *Division of Biophysics, Institute of Experimental Physics, Faculty of Physics, University of Warsaw, Pasteura 5, 02-093 Warsaw, Poland;*
[b] *Centre of New Technologies, University of Warsaw, Banacha 2c, 02-097 Warsaw, Poland;*

Azide-alkyne cycloadditions (AACs) have proven their wide utility for conjugation of various molecules, including (oligo)nucleotides.[1] One of the limitations of AACs in nucleic acid chemistry is azide functionalization of oligos, which is generally not compatible with phosphoramidite method and requires modification of the monomers or solid supports.[2] A simple and straightforward protocol for iodination of 5’-hydroxyl and substitution with azide was described for functionalization of DNA, but it failed to produce modified RNAs, since the iodination reagents interfere with 2’-O-silyl protection (e.g. TBDMS, TOM).[3] To eliminate that problem, we performed the solid-phase synthesis of RNA using commercially available 2’-O-PivOM amidites,[4] followed by 5’-iodination and substitution with $NaN_3$.[5]

Using this procedure we synthesized 13 oligoribonucleotides of different sequence and length (2–21 nt), 4 of them bearing additional 3’-O-propargyl modification. Reactivity of such modified RNA fragments in Cu(I)-catalyzed (CuAAC) or strain-promoted (SPAAC) cycloaddition was exemplified by fluorescent labeling, conjugation with mRNA 5’ end analogs (5’ cap) and non-templated ligation, including cyclization of bisfunctionalized oligos.




When using RP HPLC purified compounds, we observed complete conversion of 5’-azides into the desired product in less than one hour, without significant degradation of oligo. We were able to efficiently ligate different pairs of RNA fragments forming a 27-nt analog of sarcin-ricin loop, as well as cyclize di-, tri-, and even 9-nucleotide chains, without using a splint. Interestingly, triazole linkage formed in that reactions was identified earlier as a good mimic of phosphodiester bond in DNA replication and transcription.[1] CuAAC cyclization is then an atractive alternative to other chemical cyclization methods, which are generally limited to very short oligos (up to 4-nt).[6] Finally, we verified the biological activity of 5’-capped RNA and established that our tirazole-linked oligonucleotide cap analog binds to translation initiation factor 4E (eIF4E) ~5-times stronger than dinucleotide cap ($m^7$GpppG), which makes such compounds good candidates for studies on translation initiation process.

This work was supported by National Science Centre Poland (NCN 2017/24/T/NZ1/00345).

***References:*** [1] El-Sagheer, A. H., et al., *Chem. Soc. Rev.* **2010**, *39*, 1388. [2] Coppola, C., et al., *Eur. J. Org. Chem.* **2011**, *2011*, 1155. [3] Kool, E. T., et al. *Curr. Prot. in Nucl. Acid Chem.* **2001**. [4] Lavergne, T., et al., *Chem. - Eur. J.* **2008**, *14*, 9135. [5] Warminski, M., et al., *Org. Lett.* **2017**, *19*, 3624. [6] Micura, R., *Chem. – Eur. J.* **1999**, *5*, 2077.

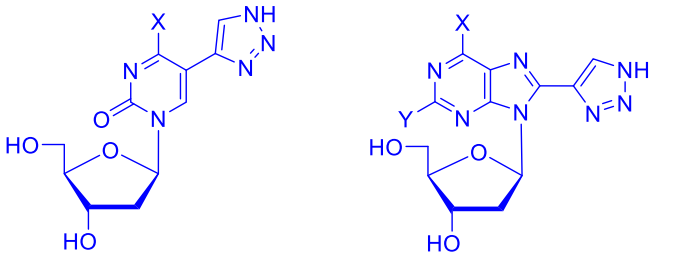
*Corresponding author: E-mail: j.jemielity@cent.uw.edu.pl

# Synthesis and fluorescent properties of 5-pyrimidine and 8-purine nucleosides with 1,2,3-triazol-4-yl substitution

Zhiwei Wen, Paloma Tuttle, Anna Vasilyeva, Laura Gonzalez, Antonija Tangar, Jaroslava Miksovska, and Stanislaw F. Wnuk*

*Department of Chemistry and Biochemistry, Florida International University, Miami, Florida 33199, USA*

The fluorescent *N*-unsubstituted 1,2,3-triazol-4-yl analogues of the four natural bases of DNA (*i.e.* 5-TrzdU **1**, 5-TrzdC **2**, 8-TrzdA **3** and 8-TrzdG **4**, Figure) have been synthesized by metal-catalyzed reactions between the 5-ethynylpyrimidine or 8-ethynylpurine nucleosides with trimethylsilyl azide ($TMSN_3$). CuI-catalyzed cycloaddition (DMF/$H_2O$, 90 °C, 5 h) gave 1,2,3-triazoles as sole products however in low to moderate yield (10% for 5-TrzdC **2** to 50% for 5-TrzdU **1**). Combination of $CuSO_4$/sodium ascorbate gave triazoles in improved yields (38% for 5-TrzdC **2** to 52% for 5-TrzdU **1**). Interestingly, $Ag_2CO_3$-catalyzed cycloaddition (DMF, 2 eq. $H_2O$, 80 °C, 1 h) of 8-ethynylpurine nucleosides with $TMSN_3$ produced 8-triazolylpurines as sole products (8-TrzdG **4**, 55%). Analogous cycloadditions of 5-ethynylpyrimidine nucleosides produced mixture of 5-triazolylpyrimidine nucleosides (5-TrzdC **2**, 7%) and the corresponding 5-(1-azidovinyl)pyrimidine byproducts (e.g., 5-(1-azidovinyl)-2'-deoxycytidine, 48%). The novel *N*-unsubstituted 1,2,3-triazol-4-yl nucleosides analogues showed excellent fluorescent properties in MeOH. The 8-purine analogue 8-TrzdA **3** exhibits the highest quantum yield of 44% while the 8-TrzdG **4** had quantum yield of 9%. The 5-pyrimidine analogues 5-TrzdU **1** and 5-TrzdC **2** showed a large Stokes shift of ~110 nm with the maximum emission approximately at 408 nm and quantum yield of 2%. The 8-TrzdA was converted to 5'-triphosphates using Yoshikawa protocol followed by coupling with pyrophosphate. The application of these fluorescent nucleosides and nucleotides with the minimalistic modification at heterocyclic bases to cell imaging and DNA modifications will be discussed.




**5-TrzdU (1)**: X = OH
**5-TrzdC (2)**: X = $NH_2$

**8-TrzdA (3)**: X = $NH_2$, Y = H
**8-TrzdG (4)**: X = OH, Y = $NH_2$

***Figure.*** *Unsubstituted 5-(1,2,3-triazol-4-yl) pyrimidine and purine nucleosides*

This work was supported by NIGMS (1SC1CA138176 to SFW). ZW is recipient of the FIU Dissertation Year Fellowship.

*Corresponding author: E-mail: wnuk@fiu.edu

# Repair of $O^4$-alkylated-2'-deoxyuridine by $O^6$-alkylguanine DNA alkyltransferases is enhanced by fluorine at the C5 position

Christopher J. Wilds,*[a] Laura Sacre,[a] Derek K. O'Flaherty,[a] Philippe Archambault,[a] Francis McManus,[a] William Copp,[a] Gilles H. Peslherbe,[a] Heidi M. Muchall[a]

[a] *Department of Chemistry and Biochemistry and Centre for Research in Molecular Modeling (CERMM), Concordia University, 7141 Sherbrooke St. West, Montréal, Québec, Canada*

The introduction of alkyl lesions at the $O^6$- and $O^4$-positions of 2'-deoxyguanosine and thymidine, respectively, can be disruptive for DNA polymerase activity. [1] $O^6$-Alkylguanine DNA alkyltransferases (AGTs) can remove these alkyl adducts from DNA by a direct repair mechanism involving an active site cysteine. [2] However, repair proficiency towards the alkylated nucleobases varies amongst AGTs. For example, the *E. coli* AGT variant OGT displays greater repair proficiency towards alkyl groups at the $O^4$-position of thymidine relative to human AGT. In the present study, the influence of replacing the C5-methyl group of thymidine with fluorine on AGT mediated removal of $O^4$-alkyl groups was explored. [3,4] A series of oligonucleotides containing $O^4$-alkylated 5-fluoro-2'-deoxyuridine residues (**Figure 1**) were synthesized. UV thermal denaturation studies revealed that these modifications destabilized duplex DNA by ~10°C. In general, the AGT variants demonstrated improved proficiency at removing the various $O^4$-alkyl groups from 5-fluoro-2'-deoxyuridine, relative to their thymidine and 2'-deoxyuridine counterparts. Computational assessment of the $O^4$-alkylated nucleobases revealed that the C5-fluorine modification aids in the cleavage of the $O^4$-Cα bond of 5-fluoro-uracil relative to thymine and uracil through a cascade effect, which may be a contributing factor to the enhanced repair of the adducts observed by the AGTs.

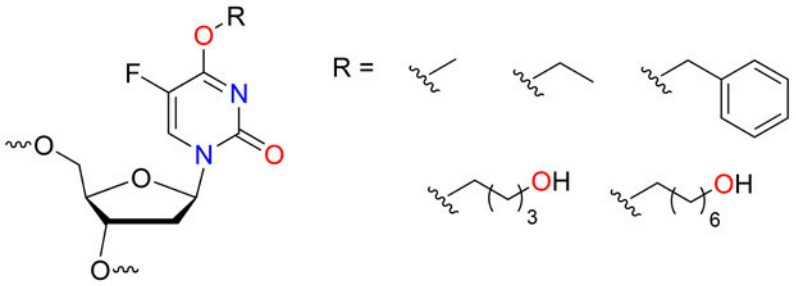



***Figure 1.*** *Structures of $O^4$-alkylated 5-fluoro-2'-deoxyuridine adducts.*

This research was supported by grants from the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Canada Foundation for Innovation (CFI). Computational resources were provided by Compute Canada.

***References:*** [1] Shrivastav, N. et al., *Carcinogenesis* **2010**, *31*, 59. [2] Pegg, A. E., *Chem. Res. Toxicol.* **2011**, *24*, 618. [3] Sacre, L. et al., *Eur. J. Org. Chem.* **2017**, 3003. [4] Sacre, L. et al., *ChemBioChem* **2018**, *19*, 575.

*Corresponding author: E-mail: chris.wilds@concordia.ca

# Synthesis and antiviral evaluation of fluorescent T-1105 and T-1106 analogues

Matthias Winkler, [a] Evelien Vanderlinden, [b] Lieve Naesens, [b] Chris Meier*[a]

[a] *Department of Organic Chemistry, Martin-Luther-King-Platz 6, 20146 Hamburg, Germany*
[b] *Rega Institute for Medical Research, Herestraat 49, 3000 Leuven, Belgium*

There is a great need for the development of drugs that have therapeutic efficacy in treating diseases caused by highly pathogenic RNA viruses. Recently, the fluorinated pyrazinecarboxamide T-705 (favipiravir), and the defluoro analogues T-1105 and T-1106 have demonstrated their potency in treating viral infections in against several RNA viruses.
Here, we report on the chemical synthesis of fluorescent T-1105 and T-1106 analogues via Sonogashira cross coupling. Starting from the brominated precursors, different alkyne based push-pull fluorophores were introduced at the 5-/6-position of the pyrazine heterocycle. The photophysical properties of the target compounds were evaluated in different solvents to study their solvatochromic properties.
In addition, these new analogues were tested against several RNA and DNA viruses. With their unique mechanism of action and broad range of antiviral activity, these pyrazinecarboxamide derivatives are promising drug candidates and can therefore serve as a promising starting point for the development of new antivirals. Furthermore they can serve as chemical probes for mechanistic studies of T-1105/T-1106, which mechanism of action is still debated.

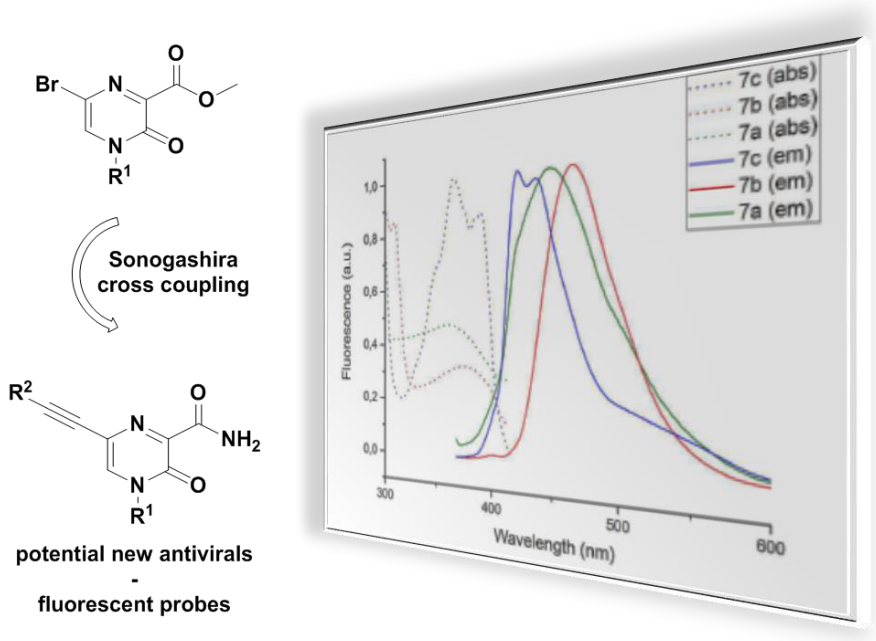



***Figure.*** *Schematic overview.*

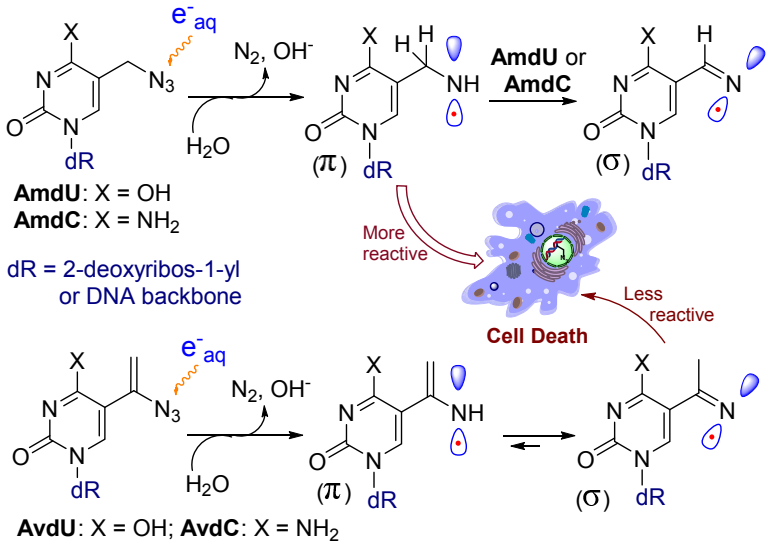
*Corresponding author: chris.meier@chemie.uni-hamburg.de

# Radiation damage to cells augmented by electron-induced aminyl radicals in C5 azido-modified pyrimidine nucleosides incorporated into DNA-fragments

Zhiwei Wen,[a] Amitava Adhikary,[b] Michael D. Sevilla,[b] and Stanislaw F. Wnuk*[a]

[a] *Department of Chemistry and Biochemistry, Florida International University, Miami, FL 33199, USA;* [b] *Department of Chemistry, Oakland University, Rochester, MI, 48309, USA*

Two classes of C5 azido-modified pyrimidine nucleosides have been investigated as potential radiosensitizers: (a) C5-azidomethyl modified 2'-deoxyuridine (AmdU) and 2'-deoxycytidine (AmdC), and (b) 5-(1-azidovinyl) modified 2'-deoxyuridine (AvdU) and 2'-deoxycytidine (AvdC). The latter class was prepared by the regioselective Ag-catalyzed hydroazidation of 5-ethynyl substrates with $TMSN_3$. The AmdU and AmdC were converted to their 5'-triphosphates using Yoshikawa protocol followed by coupling with pyrophosphate. Phosphates AmdUTP and AmdCTP were incorporated into DNA-fragments during DNA-replication and base excision repair (BER) by human or *Escherichia coli* DNA-polymerase-catalyzed reactions. Radiation-mediated prehydrated electrons which are formed in homogeneous aqueous glassy (7.5 M LiCl) systems in the absence of oxygen at 77 K, led to site-specific formation of novel and neutral π-aminyl radicals (RNH•) from AmdU, AmdC, AvdU, and AvdC. ESR studies and DFT calculations showed that RNH• generated from these azido nucleosides undergo facile conversion to thermodynamically more stable σ-iminyl radicals, R=N•. In AmdU and AmdC, RNH• underwent a bimolecular conversion to R=N• involving an α-azidoalkyl radical as intermediate; however, AvdU, and AvdC, RNH• was found to tautomerize to R=N•. Our work provides the first evidence of formation of RNH• and its facile conversion to R=N• in azido-modified nucleoside under a reductive environment. These aminyl and iminyl radicals generate DNA damage via oxidative pathways. Furthermore, we show that these azido-nucleosides can be successfully applied as radiosensitizers in EMT6 tumor cells under hypoxic and normoxic conditions.




This work was supported by NIH grants (R01CA045424 to MDS, 1SC1CA138176 to SFW)

*Corresponding author: E-mail: wnuk@fiu.edu

# Dinucleotide cap analogs modified with fluorescent molecular rotors

Blazej A. Wojtczak,[a] Anna Nowicka,[b] Pawel J. Sikorski,[a] Joanna Kowalska,[b] Jacek Jemielity*[a]

[a] *Centre of New Technologies, University of Warsaw, Banacha 2c St, 02-097 Warsaw, Poland*
[b] *Division of Biophysics, Institute of Experimental Physics, Faculty of Physics, University of Warsaw, Pasteura 5 St, 02-093 Warsaw, Poland*

The cap structure containing monomethylated (MMG) or trimethylated (TMG) form of guanine is present at the 5 'end of mRNA (MMG) and snRNAs (TMG). It plays a number of functions in the cell, the most important are splicing, initiation of translation, and nuclear export/import.[1] Being selectively recognized by specific proteins e.g.: CBC, eIF4E, and snurportin 1 exploration of cap-dependent processes in the cell are difficult, mainly due to overlapping of biological processes. The later can be recognized and distinguished using synthetic non-hydrolyzable cap analogs conjugated to fluorescent molecular rotors that are useful reporters of their microenvironment for visualizing capped RNA in cells. Because noncovalent binding to active sites of protein is associated with increased fluorescence emission, molecular rotors are excellent protein probes, indicating not only the presence of protein (similar to antibody-conjugated fluorescent markers) but they also respond to environmental changes and can, therefore, be used to probe protein structure.[2]

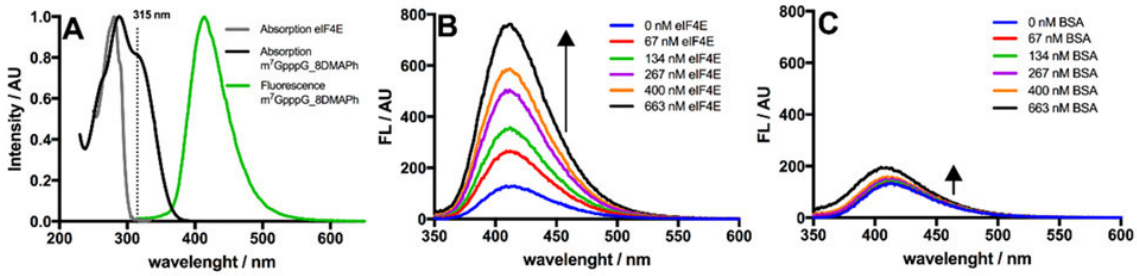



***Figure.*** *A) Absorption and emission spectra of eIF4E (grey), m$^7$GpppG-8DMAPh (black) and emission (green); B) shows the emission spectra of m$^7$GpppG-8DMAPh in the presence of increasing concentration of eIF4E and C) small changes of fluorescence emission in the presence of increasing concentration of BSA.*

In this work, we present the synthesis and biological properties of a series of dinucleotide cap analogs modified with fluorescent molecular rotors as tools to study cap-dependent biological processes.

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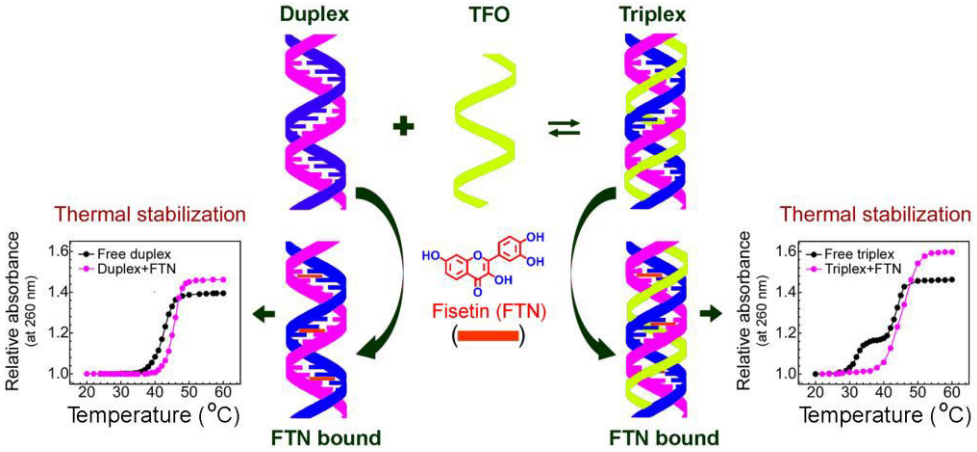
*Corresponding author: E-mail: j.jemielity@cent.uw.edu.pl

# EXPLORATION ON THE COMPARATIVE BINDING ASPECTS OF THE BIO-FLAVONOID FISETIN WITH TRIPLE AND DOUBLE HELICAL FORMS OF RNA

Sutanwi Bhuiya, Lucy Haque, Rapti Goswami, Suman Das*

*Physical Chemistry Section, Department of Chemistry, Jadavpur University, Raja S. C. Mullick Road, Jadavpur, Kolkata 700032, India*

RNA duplex can accommodate single stranded 'triplex forming oligonucleotide' (TFO) to form triplex. Low thermodynamic stability of triplex due to presence of Hoogsten base pairing limits their applications in antisense and antigene strategies, gene regulation etc. One means of increasing the stabilization of triplex is through the association with small molecules. Here, we have prepared poly(U).poly(A)*poly(U) triplex [where .(dot) represents the Watson-Crick base pairing and *(asterisk) denotes the Hoogsteen base pairing, herein after U.A*U] by mixing 2:1 molar ratio of poly(U) and poly(A) and studied the interaction of naturally occurring flavonoid Fisetin (FTN) with the prepared triplex (U.A*U) and the corresponding duplex (A.U). Different spectroscopic, viscometric and theoretical studies revealed that FTN intercalates to both triplex and duplex RNA with comparatively higher




affinity towards triplex ($5.9 \times 10^6$ $M^{-1}$) than duplex ($1.0 \times 10^5$ $M^{-1}$). FTN causes higher thermal stabilization of the RNA triplex (~14 °C) in comparison to RNA duplex (~4 °C). Fluorimetric studies ascertain that U.A*U and A.U primarily bind the photo-produced tautomer of FTN in the excited state. Steady-state and time-resolved anisotropy measurements illustrate considerable modulations of the spectroscopic properties of the tautomeric FTN within the RNA environment. Theoretical study explains the experimental absorption and emission (dual fluorescence) behavior of FTN along with the ESIPT process.

This work was supported by University Grants Commission (UGC), New Delhi, India [F. No. 43-243/2014 (SR), MRP-MAJORCHEM-2013-37991].

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*Corresponding author: E-mail: sumandas10@yahoo.com

# Global Mapping of 8-oxoguanine in Genomic DNA by Click-code-seq

Junzhou Wu, Maureen McKeague, Shana J. Sturla*

*Department of Health Sciences and Technology, ETH Zürich, Schmelzbergstrasse 9, 8092 Zürich, Switzerland*

The capacity to sequence DNA damage at single nucleotide resolution on a genome-wide scale is anticipated to allow scientists to better decipher the complex causal link between chemical exposures and mutation outcomes in the genome. Despite advances in high-throughput sequencing technology, the low abundance and inability to specifically amplify DNA adducts, such as the key oxidation product 8-oxoguanine, has prohibited single nucleotide mapping within whole genomes. We leveraged the specificity of the base excision repair enzyme, formamidopyrimidine-DNA glycosylase, to recognize and remove 8-oxoguanine from DNA, generating a free 3'-hydroxyl handle at the damage site [1]. We coupled the specificity of the repair enzyme with the efficiency of a one-pot click-DNA ligation reaction to insert a readable oligonucleotide code sequence [2]. The biocompatible code enabled high-throughput, base resolution sequencing of 8-oxoguanine sites. We applied the new method, termed click-code-seq to map 8-oxoguanine sites in a eukaryotic (yeast) genome. The thousands of 8-oxoguanine sites were distributed in genomic features with patterns suggesting their relationship to chromatin assembly and transcription. Click-code-seq overcomes significant hurdles in DNA damage sequencing and provides a new approach for generating comprehensive, sequence-specific information about 8-oxoguanine patterns in whole genomes that may be adaptable to other types of DNA damage. The results are expected to initiate the advancement of a critical understanding of the impact of genome-wide damage distribution on normal and pathological biological processes.

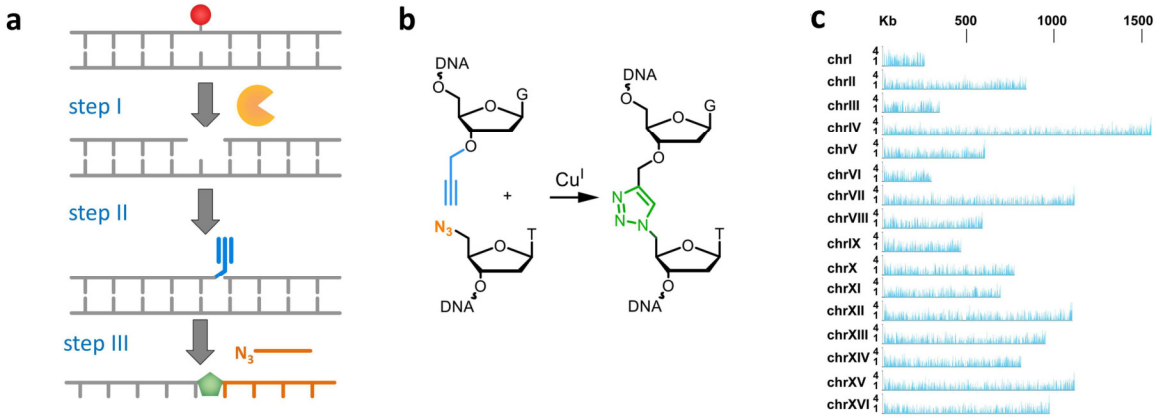


***Figure.*** *a) The three step scheme for labelling 8-oxoG. b) The click ligation reaction to produce a ligated DNA. c) The click-code-seq map of 8-oxoG in genomic DNA from BY4741 cells.*

This work was supported by Swiss National Science Foundation (grant 156280 to S.J.S).

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*Corresponding author: E-mail: sturlas@ethz.ch

# Protein-mediated enrichment and characterization of 5’ end of RNA polymerase III transcripts

Madalee Wulf, Jingmin Jin, Bo Yan, Nan Dai, George Tzertzinis, Laurence Ettwiller, Ira Schildkraut, Larry McReynolds, Ivan R. Corrêa Jr.*

*New England Biolabs, 240 County Road. Ipswich, MA, USA*

RNA polymerase III (Pol III) is a specialized nuclear enzyme that produces small non-coding RNAs, including transfer RNAs, the 5S ribosomal RNA, and the U6 spliceosomal RNA. It is generally accepted that the majority of Pol III transcripts have either triphosphate or monophosphate 5' ends. However, some RNA pol III transcripts are post-transcriptionally modified to generate unusual cap-like structures at their 5’ ends (*x*ppp5’N, wherein *x* has a non-nucleotide structure such as a methyl group). The detection of 5’ terminal nucleotides is typically achieved through a laborious and time-consuming protocol, which includes pre-incubation of cells with radioactive $^{32}$P-orthophosphate, fractionation of RNA, digestion to ribonucleotides with a non-specific nuclease, thin-layer chromatography (TLC), and comparative analysis of autoradiograms using known ribonucleotide markers. Other approaches involve the treatment of fractionated RNA with 5’ specific enzymes, such as RNA 5’ polyphosphatase and 5´ phosphate-dependent exonuclease. Here, we present a platform for the rapid and unambiguous identification of 5’ ends of cellular RNA by mass spectrometry (MS). In this platform, a target RNA is first hybridized to an RNA probe that is complementary to the 5' end of the target. This probe:RNA duplex is then enriched through binding to the viral protein p19. The p19-bound RNA is partially digested with single-stranded specific ribonuclease to produce a blunted probe:RNA duplex. Finally, the duplex is analyzed by MS, either directly or after digestion to single nucleosides. We demonstrate the potential of this approach by detecting the 5’ terminal nucleotides of Pol III transcripts 5S, 7SK and 7SL.

*Corresponding author: E-mail: correa@neb.com

# Construction and Functional Modification of siRNA Nanoparticles

Chao Yang, Dejun Ma, Zhen Xi*

*State Key Laboratory of Elemento-Organic Chemistry and National Engineering Research Center of Pesticide, College of Chemistry, Nankai University, Tianjin, China, 300071,*

Synthetic small interfering RNAs (siRNAs) have been considered a promising class of therapeutics for treatment of various undruggable diseases including cancer, and numerous efforts have been made to enhance RNAi efficiency during the past decades[1-3]. Nevertheless, achieving highly potent RNAi toward clinical translation remains many challenges, such as stability, targeting delivery and cellular uptake of siRNA[4]. The RNA nanotechnology is an attractive candidate for enhancing RNAi efficiency [5]. Recently, we have designed well-dispersed, size-controllable Archimedean Solid-like RNA nanoparticles (ASRN) by engineering parameters such as sequence length and nucleotide type[6]. Based on canonical Watson−Crick base pairing, we successfully constructed self-assembled siRNA nanoparticles through annealing between sense and antisense strands of RNA trimer.

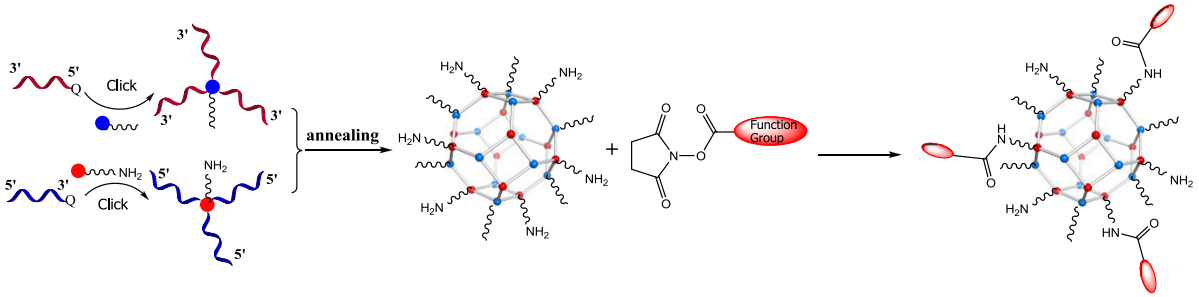


***Figure.*** *Construction and Functional Modification of siRNA Nanoparticles.*

The postsynthetic conjugation strategy was employed to introduce some functional groups including targeting molecules and reporter molecules to the free amino group of the siRNA nanoparticles. Following that, we explored gene-silencing efficiency of the Modified siRNA Nanoparticles in vitro and in animal models.

This work was supported by the National Natural Science Foundation of China (21332004), the Ministry of Science and Technology of the People's Republic of China (2017YFD0200501) and Wan Xiang Scholarship

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*Corresponding author: E-mail: zhenxi@nankai.edu.cn

# Synthesis of Flexible Antiviral Agents Against Emerging and Reemerging Infectious Diseases

Mary K. Yates,[a] Wahiba Aoudai,[b] Etienne Decroly,[b] Bruno Canard,[b] Donald Smee,[c] Arissa Falat,[a] Marcella Bassetto,[d] Andrea Brancale,[d] Katherine L. Seley-Radtke*[a]

[a] *Department of Chemistry and Biochemistry, UMBC, Baltimore MD, USA*
[b] *CNRS, Aix Marseille University, Marseille France*
[c] *Department of Animal, Dairy, and Veterinary Sciences, Utah State University, Logan UT, USA*
[d] *School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff United Kingdom*

Flaviviruses, such as Dengue (DENV) and Zika (ZIKV) viruses, represent a severe health burden due to their ease of transmission, propensity to become epidemics, and the development of unexpected pathologies. While there are currently no FDA approved treatments or vaccines for these viral diseases, nucleoside analogues have long served as the cornerstone for antiviral therapeutics due to their ability to inhibit viral DNA or RNA replication. The Seley-Radtke lab has developed various types of flexible nucleoside analogues, called "fleximers", that have demonstrated the ability to overcome point mutations within the binding site of biologically significant enzymes.

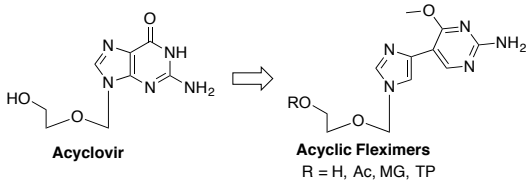


***Figure.*** *Structure of Acyclovir and the designed acyclic Fleximers including the parent, acetate protected prodrug, McGuigan prodrug, and triphosphate analogues.*

Preliminary studies have demonstrated that several acyclic flexible analogues of the FDA-approved drug Acyclovir have activity against numerous RNA viruses in vitro, including DENV and ZIKV, demonstrating their broad-spectrum potential. Furthermore, studies to determine the mechanism of action of the compounds found that the fleximers inhibit DENV and ZIKV methyltransferases (MTases) but not human MTases, which makes these compounds promising antiviral therapeutics. Computational analysis found that, due to their flexibility, these analogues retain interactions necessary for recognition in the MTase binding site. The results of these studies are reported herein.

This work was supported by the National Institute of Health with funding for the Chemistry-Biology Interface Program – T32GM066706 (KSR and MKY)

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*Corresponding author: E-mail: kseley@umbc.edu

# The detection of RNA biomarkers in human cancer tissues by FIT-PNA probes – a route for fluorescence guided cytoreductive surgery

Eylon Yavin,[a,*] Dina Hashoul,[a] Aviram Nissan,[b] Maria Falchenko,[a] Ganeshkumar Moorthy,[a], Odelia Shimshon,[a], Vera Paviov,[b] Rachel Shapira[b]

[a] *School of Pharmacy, Faculty of Medicine ,The Hebrew University of Jerusalem, Hadassah Ein-Kerem, Jerusalem, 91120, Israel.*
[b] *Department of General and Oncological Surgery, The Chaim Sheba Medical Center, Tel Hashomer, Israel.*

FIT-PNAs (forced-intercalation-Peptide Nucleic Acids) are nucleic acid based probes that turn their fluorescence upon hybridization to a complementary RNA/DNA sequence. [1] We have recently developed FIT-PNAs with a new surrogate base (BisQ) that brightly fluoresces at the red-region (613nm) and shown that a fluorescent readout at single nucleotide polymorphism (SNP) resolution in living cells is detected after 30 minutes by simple incubation of the FIT-PNA in cell culture [2]. We have also targeted an oncogenic long non-coding RNA (lncRNA) termed CCAT1 (colon-cancer associated transcript 1) that has been found to be highly expressed in a variety of tumors [3, 4]. Herein we have designed BisQ FIT-PNAs for CCAT1 as well as a cancer associated mRNA - KRT20. Fresh human tissues taken from peritoneal post-surgery patients were tested with CCAT1 and KRT20 FIT-PNAs. Following simple spraying (in PBS) of the FIT-PNA probe directly on the tissue examined resulted in substantial fluorescence at regions suspected as cancerous ones. No signal was observed when the FIT-PNAs were sprayed on normal (non-cancerous) fresh human tissues taken from bariatric surgeries. We envision that such PNA-FIT probes may be useful for non-invasive RNA biomarker detection in-vivo by defining cancer margins during cytoreductive surgery.

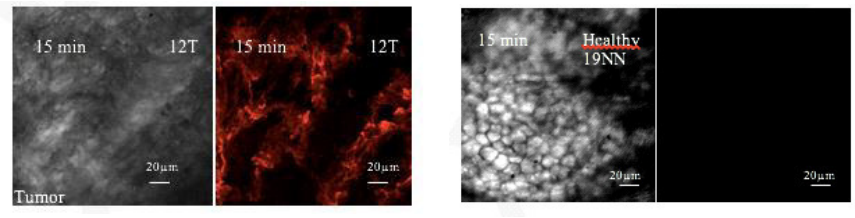


***Figure.*** *Fresh human peritoneal cancer tissue (right) and normal tissue (left) from bariatric surgeries sprayed with the FIT-PNA probe (1 μM in PBS). Images taken after 15 minutes.*

This work was supported by grants from the Israel Science Foundation (grant No. 480/13) and the Israel ministry of industry and trade (Kamin, grant No. 55330).

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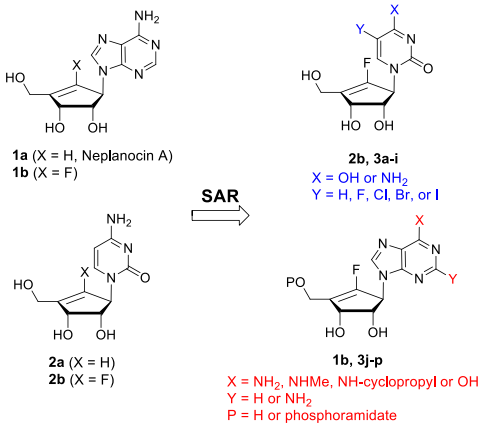
*Corresponding author: E-mail: eylony@ekmd.huji.ac.il

# Structure-Activity Relationships of Fluorocyclopentenyl-purines and -pyrimidines as Antitumor Agents

Ji-seong Yoon, Dnyandev B Jarhad, Gyudong Kim, and Lak Shin Jeong*

*College of Pharmacy, Seoul National University, Seoul 08826, South Korea*

Based on the potent anticancer activity of 6'-fluorocyclopentenyl-cytosine **2b**[1] in phase IIa clinical trials for the treatment of gemcitabine-resistant pancreatic cancer,[2] we carried out a systematic structure-activity relationship study of 6'-fluorocyclopentenyl-pyrimidines **3a-i** and -purines **3j-o** to discover novel anticancer agents. We also synthesized the phosphoramidate prodrug **3p** of adenine derivative **1b** to determine if the anticancer activity depends on the inhibition of DNA and/or RNA polymerase in cancer cells and/or on the inhibition of *S*-adenosylhomocysteine (SAH) hydrolase. All the synthesized pyrimidine nucleosides exhibited much less potent anticancer activity in vitro than the cytosine derivative **2b**, acting as RNA and/or DNA polymerase inhibitor, indicating that they could not be efficiently converted to their triphosphates for anticancer activity. Among all the synthesized purine nucleosides, adenine derivative **1b** and $N^6$-methyladenine derivative **3k** and **3n** showed potent anticancer activity, showing equipotent inhibitory activity as the positive control, neplanocin A (**1a**) or Ara-C. However, the phosphoramidate prodrug **3p** showed less anticancer activity than **1b**, indicating that it does not seem to act as RNA and/or DNA polymerase inhibitor as **2b**. This result also demonstrates that the anticancer activity of **1b** largely depends on the inhibition of SAH hydrolase. The deamination of $N^6$-amino group, addition of the bulky alkyl group at the $N^6$-amino group, or introduction of the amino group at the C2 position almost abolished the anticancer activity.

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*Corresponding author: E-mail: lakjeong@snu.ac.kr

# An RNA three-way junction involved in Zika virus replication

Austin Yu,[a] Thomas Hermann*[a]

[a] *Department of Chemistry and Biochemistry, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093, USA*

The Zika virus (ZIKV) is a member of the genus Flavivirus which includes Dengue virus (DENV), West Nile virus, and Yellow Fever virus.[1] ZIKV is an RNA virus and estimated to have emerged in 1920 in East Africa.[1,2] ZIKV is a pathogen transmitted through mosquitoes (*Aedes aegypti* and *Aedes albopictus*) and sexual transmission.[1,2,3] Increasing temperatures throughout the United States have also increased the potential zones of habitation for ZIKV-carrying mosquitoes.[4] The symptoms of a Zika infection include rash, fever, and Guillain-Barré syndrome.[2,3,5] Additionally, ZIKV infection is frequently misdiagnosed as DENV infection, especially in areas heavily afflicted by DENV.[2] Increased zones of habitation within the United States have caused rising concerns about the spread of ZIKV since currently there is no vaccine or FDA-approved antiviral cure. We believe that non-coding RNA elements in the ZIKV, like other viruses, can be allosterically modulated. By monitoring conformational changes in the RNA three-way junction of the ZIKV stem loop A (SLA) using fluorescence and functional assays, we hope to discover small molecules which selectively bind and capture the ZIKV SLA in a non-native conformation. If the conformational changes are great enough, the ZIKV SLA will be unable to effectively bind with its viral RNA-dependent RNA polymerase, thereby inhibiting the ZIKV replication process. We hope to characterize the structure of the ZIKV SLA and develop selectively binding molecules which may contort the replication promoter to inhibit viral replication.

This work was supported by grants from the US Department of Education and UC San Diego Academic Senate.

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*Corresponding author: E-mail: tch@ucsd.edu

# Synthesis of β,γ-$CH_2$-, β,γ-CHF-, and β,γ-$CF_2$-8-Oxo-dGTP Analogues

Yiying Zheng,[a] Boris A. Kashemirov,[a] Charles E. McKenna*[a]

[a]*Department of Chemistry, University of Southern California, Los Angeles, CA 90089, USA*

8-Oxo-2'-deoxyguanosine (8-oxo-dG), a major oxidative DNA lesion, is believed to contribute significantly to aging and cancer.[1,2] This mutagenicity occurs due to the conformational shift of the N9-C1' glycosidic bonds from *anti* to *syn*. 8-Oxo-dG (*anti*) forms a Watson-Crick base pair with 2'-deoxycytosine (dC), while 8-oxo-dG (*syn*) forms a Hoogsteen base pair with 2'-deoxyadenosine (dA). The *anti*-conformation enables error-free bypass of the lesion by replicative polymerases, whereas the *syn*-conformation is responsible for the mutagenic effect.[3] DNA polymerases often prefer the misincorporation of dA to the accurate incorporation of dC. This leads to propagation of G to T transversions, which are commonly observed in mutations linked to human cancers. To study the mechanism of insertion of 8-oxo-dGTP, we have elaborated a small set of corresponding nucleotides with variable bisphosphonate leaving groups replacing pyrophosphate, by analogy with our recently elaborated[4,5] 'CXY-dNTP toolkit'.

Three novel β,γ-methylene ($CH_2$)-, β,γ-monofluoro (CHF)-, and β,γ-difluoro ($CF_2$)-8-oxo-dGTP CXY bisphosphonate analogues were synthesized. They were prepared by two methods: a) conjugation of the morpholidate of 8-oxo-dGMP in anhydrous dimethyl sulfoxide with the tributylammonium salt of the appropriate methylenebisphosphonic acid; or b) coupling of the tributylammonium salt of the appropriate methylenebisphosphonic acid with activated 5'-*N*-methyl phosphorylimidazolide 8-oxo-dG. The compounds were characterized by LC-MS, HRMS and NMR.

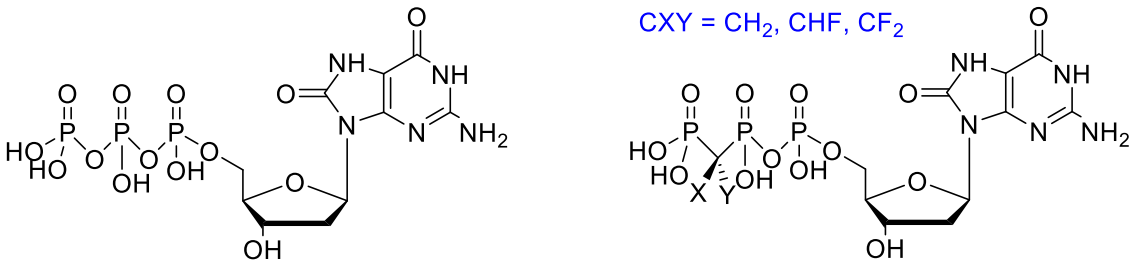


***Figure.*** *8-Oxo-dGTP (left) and β,γ-$CH_2$-, β,γ-CHF-, and β,γ-$CF_2$-8-oxo-dGTP analogues (right).*

This work was supported by NIH (U19CA177547) and a Dornsife Graduate School Fellowship.

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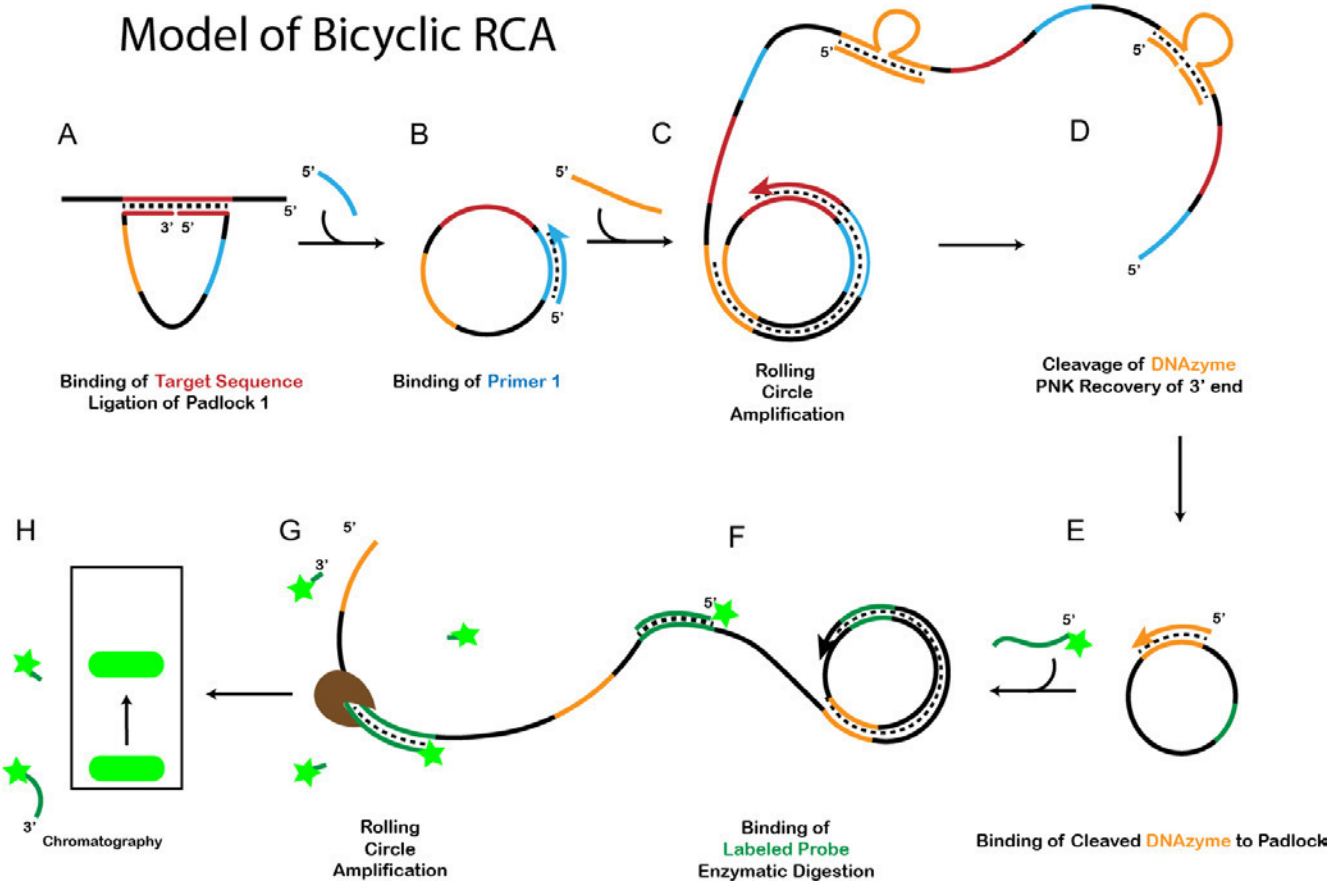
*Corresponding author: E-mail: mckenna@usc.edu

# Isothermal DNA Detection Utilizing Bicyclic Amplification of Padlock Probes

Alessandra Zimmermann[a], Ian White*[b], Jason Kahn*[a]

[a] *Department of Biochemistry, University of Maryland, College Park, USA*
[b] *Department of Bioengineering and Nucleotides, University of Maryland, College Park, USA*

Recent developments in catalytic nucleic acids and modified DNA bases have enabled novel ways of detecting and amplifying oligonucleotides for diagnostic purposes. To enable isothermal analysis with simple, multiplexed detection we leverage these developments in rolling circle amplification (RCA) of oligonucleotide padlock probes for a one-pot, two-stage amplification and detection of single stranded DNA or RNA oligonucleotides. In this novel platform, recognition of the target sequence occurs via target-templated circularization of a primary padlock oligonucleotide (A), followed by rolling circle extension (B,C). The primary amplicon is designed to incorporate a known self-cleaving DNAzyme sequence (D), allowing *in situ* generation of a primer for initiating RCA of a secondary padlock (E). This secondary amplicon, in turn, allows selective degradation of a fluorescently-labeled probe-DNA (F, G) which can subsequently liberated can ultimately be detected through separation by paper chromatography from the undigested probe and paper-based surface-enhanced Raman spectroscopy (SERS) for a sensitive, specific, multiplexable readout (H). This two-stage RCA is designed to minimize false positive outputs as well as increase the amplification potential of the reaction to allow for a lower detection limit.




*Corresponding author: E-mail: jdkahn@umd.edu

# Bis-RNAi™ Conjugates for Simultaneous Silencing of Two Transcripts

Ivan Zlatev,[a] Christopher S. Theile,[a] Adam Castoreno,[a] Anna Bisbe,[a] Stephanie Williams,[a] Tuyen Nguyen,[a] Mark K. Schlegel,[a] Scott Waldron,[a] June Qin,[a] Shannon Fishman,[a] Nathan Taneja,[a] Ryan Malone,[a] Scott Lentini,[a] Jayaprakash Nair,[a] Kristin Fong,[a] Mangala Soundar,[a] Abigail Liebow,[a] Klaus Charisse,[a] Kallanthottathil G. Rajeev,[a] Kevin Fitzgerald,[a] Muthiah Manoharan,[a] Vasant Jadhav[a] and Martin A. Maier*[a]

[a]*Alnylam Pharmaceuticals, Research Department, 300 Third Street, Cambridge, MA 02142, U.S.A.*

siRNAs covalently conjugated to synthetic multivalent *N*-acetylgalactosamine (GalNAc) ligands represent a promising new class of RNAi therapeutics with demonstrated human proof-of-concept across multiple clinical programs. Simultaneous silencing of two different transcripts could result in enhanced therapeutic benefit e.g. in the case of an antiviral therapy the ability to simultaneously hit two different target sites may reduce viral resistance.

Here we present the design and evaluation of siRNA conjugates capable of silencing two different transcripts as a single chemical entity, termed bis-RNAi™. This approach ensures that cells receive both siRNAs simultaneously and with the same efficiency thereby maximizing the therapeutic benefit of dual target silencing. Multiple designs were evaluated and critical design parameters, such as placement of the ligand and the nature of the linker between the siRNAs, were identified. Through optimization of those features robust *in vivo* activity, comparable to a cocktail of individual siRNA conjugates, was achieved.

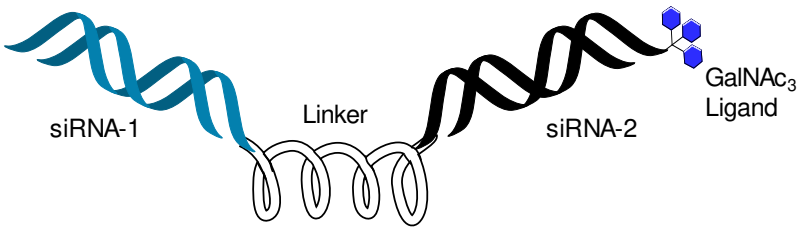



***Figure.*** *Schematic representation of a Bis-RNAi™ molecule combining two different siRNAs*

***References:*** [1] Chang, C.I., et al. *Mol. Cells*, **2009**, *27*, 689. [2] Peng, W., et al. *Nucleic Acid Ther.* **2013**, *23*, 281. [3] Wu, Y.Y., et al. *J. Mol. Histol.* **2013**, *44*, 433.

*Corresponding author: E-mail: mmaier@alnylam.com

# Use of lipid conjugates for extra-hepatic siRNA delivery *in vivo*

Annabelle Biscans,[a,b,*] Andrew H. Coles,[a,b] Dimas Echeverria,[a,b] Anastasia Khvorova.[a,b]

[a]*RNA Therapeutics Institute, University of Massachusetts Medical School, MA*
[b]*Department of Molecular Medicine, University of Massachusetts Medical School, MA*

Oligonucleotide therapeutics (i.e. antisense, small interfering RNA (siRNA), aptamers) are emerging as a new class of drugs alongside that of small molecules and biologics. However, their delivery into diseased cells and tissues remains a challenge.
One promising approach to deliver siRNAs into selected tissues is their conjugation with chemical entities. The use of N-Acetylgalactosamine (GalNAc) as a conjugate, which efficiently delivers siRNAs to hepatocytes, has shown encouraging clinical data for treatment of liver associated disorders.[1-2] Although highly promising, the utility of this technology is limited to liver delivery only.
Chemical engineering of lipid composition of lipid nanoparticles (LNPs) allowed changes in siRNA distribution.[3] Based on this knowledge, to expand siRNA distribution, our lab has explored the use of lipophilic conjugates.[4-5] We have developed a panel of chemical moieties with differences in their structure, length and degree of saturation to evaluate the impact of the lipophilic conjugates on siRNA biodistribution. All oligonuleotides were synthesized from functionalized solid supports, purified by High-Performance Liquid Chromatography (HPLC), and characterized by Liquid Chromatography-Mass Spectrometry (LC-MS). The effect of the lipophilic conjugates on siRNA pharmacokinetics and tissue distribution have been evaluated *in vivo* using a combination of fluorescent microscopy (n=3 per conjugate) and a Peptide Nucleic Acid (PNA) hybridization assay (quantification of oligonucleotides in blood or tissues, n=3 per conjugate). We demonstrated that the chemical composition of the conjugates has a major impact on siRNA pharmacokinetics and tissue distribution profiles. Diverse novel conjugated-siRNA accumulated in several tissues beyond liver (e.g. kidney, lung, heart, spleen and adrenal glands).
Reaching extra-hepatic tissue delivery paves the path towards expanding RNAi clinical utility beyond the liver and opens the door to the treatment of many more diseases.

This work was supported by National Institutes of Health grants [RO1GM10880304, S10 OD020012]

***References:*** [1] Huang, H. et al., *Mol. Ther. Nucleic Acids* **2017***, 6*, 116. [2] Rajeev, K. G., et al. *ChemBioChem* **2015**, *16*, 903. [3] Akinc, A., et al. *Nat. Biotechnol.* **2008**, *326*, 561. [4] Nikan, M., et al. *Bioconjugate Chem.* **2017**, *28*, 1758. [5] Nikan, M., et al. *Mol. Ther. Nucleic Acids* **2016**, *5*, e344.

*Corresponding author: E-mail: annabelle.biscans@umassmed.edu

# Physical property of G-quadruplex in a confined DNA origami nanocage

Masayuki Endo,*[a] Prakash Shrestha,[b] Sagun Jonchhe,[b] Tomoko Emura,[a] Kumi Hidaka,[a] Hiroshi Sugiyama,[a] Hanbin Mao[b]

[a] *Department of Chemistry, Graduate School of Science, Kyoto University, Kyoto, Japan*
[b] *Department of Chemistry and Biochemistry, Kent State University, Kent, OH*

Physical properties of biomolecules change depending on the environment. For investigating the properties of biomolecules in the confined environment, precise design of a nanoscale space to place the molecules is critical. Various DNA nanostructures and nanosystems have been created by programming the sequences of DNA strands and the functionalization.

We investigated the effect of confined space on the property of individual biomolecular structures using DNA origami nanocages. We examined mechanical unfolding of the G-quadruplex (GQ), which has three stacked guanine quartets, in the nanocages using optical tweezers. The GQ included in the nanocage was prepared by incorporation of a GQ containing strand into a half-opened nanocage and subsequent closing. We found that mechanical and thermodynamic stabilities of the GQ inside the nanocage significantly increased with decreasing the size of nanocages. Folding rate of GQ in the confined space is dramatically accelerated compared with the uncaged state. The results suggest the possibility of cooperative folding of G-quadruplex during replication or transcription inside the polymerase machinery.

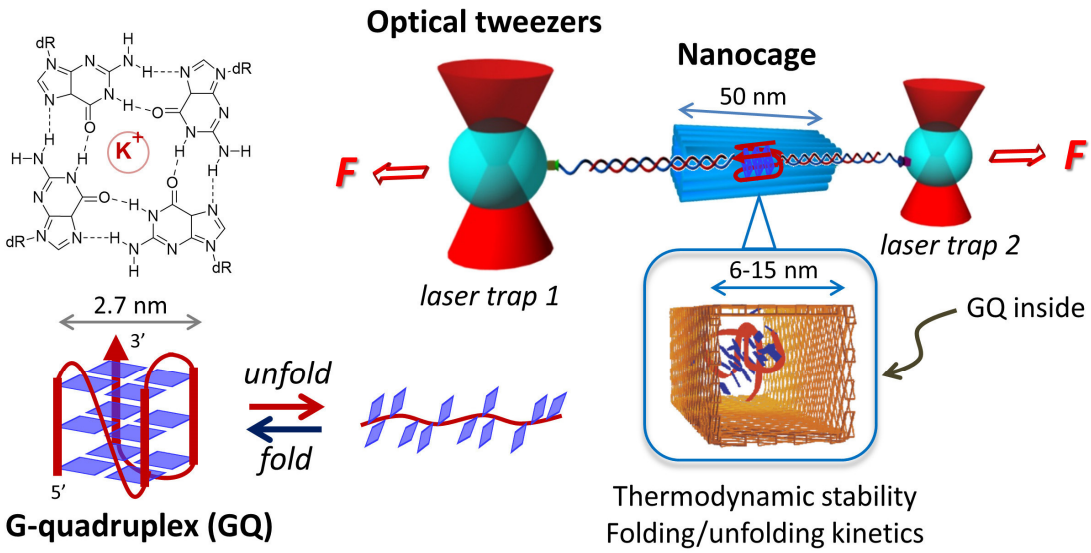



**Figure 1.** Illustration of G-quadruplex in the DNA nanocage and the method to fold/unfold G-quadruplex structure inside the nanocage using optical tweezers.

***References:*** [1] P. Shrestha, S. Jonchhe, T. Emura, K. Hidaka, M. Endo, H. Sugiyama, H. Mao, *Nature Nanotechnology*, **2017**, 12, 582–588.

*Corresponding author: E-mail: endo@kuchem.kyoto-u.ac.jp

# Novel nucleoside analogues having anti-viral activity

Hyuk-jun Jung,*[a,b] Kyungjin Kim,[b] Geunjo Lim,[b] Hakwon kim[a]

[a] *Department of Chemistry, Kyung Hee University, Yongin, Gyeonggi-do, Republic of Korea*
[b] *R&D Department, ST Pharm, Siheung-si, Gyeonggi-do, Republic of Korea*

Nucleoside analogues as a class have a well-established regulatory history, with more than 10 currently approved by the US Food and Drug Administration (US FDA) for treating human immunodeficiency virus (HIV), hepatitis B (HBV), or hepatitis C (HCV). The challenge in developing antiviral therapies is to inhibit viral replication without injuring the host cell. In HIV, a key target for drug development is reverse transcriptase (HIV-RT), a unique viral polymerase. This enzyme is active early in the viral replication cycle and converts the virus' genetic information from RNA to DNA, a process necessary for continued viral replication. Nucleoside reverse transcriptase inhibitors (NRTI) mimic natural nucleosides. In this study, we made various nucleoside analogues with 2'-methyl, 2'-fluoro pyrimidine ribonucleosides having anti-HIV activity.

***References:*** [1] Parniak MA, et al., *Adv. Pharmacol.* **2000***, 49*, 67. [2] Schinazi RF, et al. *Curr. Top. Med. Chem.* **2004**, *4*, 1035.

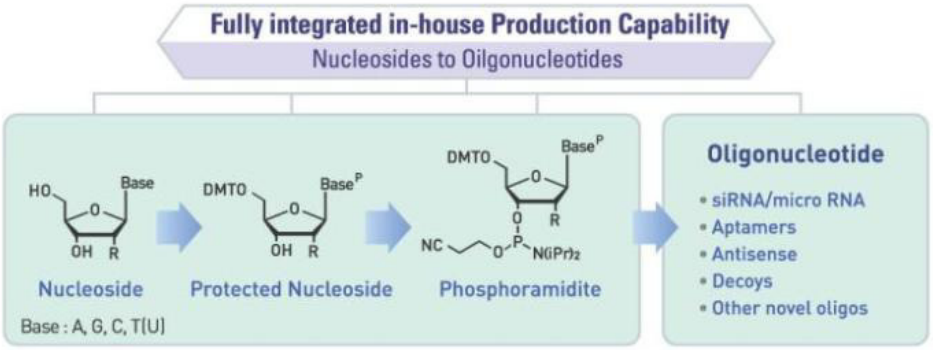
*Corresponding author: E-mail: hjjung@stpharm.co.kr

# From Lab Preparation to Commercial Production of Nucleic Acid Medicines through a Decade

Kyeong Eun Jung, Yongrack Choi, Sungwon Kim, Jeongtae Kim, Jeongryul Yun

*ST Pharm. Co. Ltd., Seoul, Korea*

We have started the oligonucleotide process research from micromole scale in 2004 and the production of oligonucleotide API (active pharmaceutical ingredient) from 2007. The journey from lab experiment to readiness of commercial scale production for oligonucleotide drugs will be presented. The development of technology from sold-phase synthesis and purification will be covered for various kinds of oligonucleotides. Evolution of analysis technology and the current requirement of quality control for oligonucleotide drugs will be described from starting materials including nucleoside and protected nucleosides.



# CHEMO-ENZYMATIC ACCESS TO THERAPEUTICALLY IMPORTANT SUGAR-MODIFIED NUCLEOSIDES

Priyanka Mangla, Vivek K. Sharma, Pallavi Rungta and Ashok K. Prasad*

*Bioorganic Laboratory, Department of Chemistry, University of Delhi, Delhi-110 007*

Nucleosides are among the most widely studied fundamental building blocks of biological system that are used as therapeutic agents to treat cancer, fungal, bacterial and viral infections. The conformational behaviour of natural or modified nucleosides has demonstrated great importance in terms of their metabolic pathways and interactions with the biological targets. This has resulted in the synthesis of chemically modified nucleoside analogues having conformationally restricted pentofuranose ring. Prominent among these are the Spironucleosides and Locked nucleic acid (LNA) monomers.

Since, the synthesis of clinically useful modified nucleosides is an arduous task and requires selective manipulation of multiple functionalities present in sugars and nucleobase moieties, the use of biocatalysts has become an attractive alternative for conventional chemical methods due to their selectivity and efficiency. We herein report the chemo-enzymatic synthesis of novel spiro and locked nucleosides (α-LNA). The full details of synthetic scheme will be presented during the poster session.

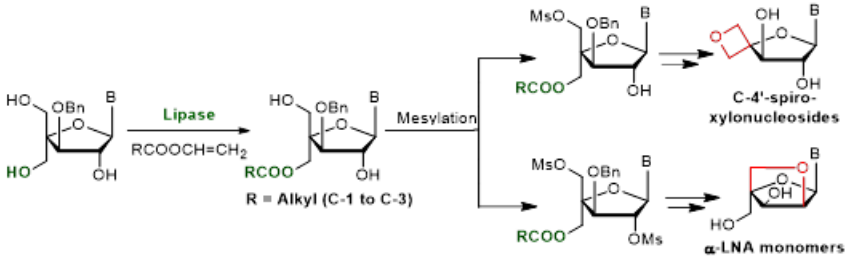



***Figure.*** *Chemo-enzymatic synthesis of C-4′-spiro-xyloucleosides and α-LNA monomers; B = nucleobase.*

***References:*** [1] Mangla, P., et. al. *Trends in Carbohydrate Research* 2017, *9*, 34. [2] Wengel, J., *Acc. Chem. Res.* 1999, *32*, 301. [3] Rajwanshi, V. K., et. al. *Angew. Chem. Int. Ed. Engl.* 2000, *39*, 1656-1659. [4] Paquette, L. A., *Aust. J. Chem.* 2004, *57*, 7. [5] Sharma, V. K., et. al. *J. Org. Chem.* 2014, *79*, 8516. [6] Sharma, V. K., et. al. *J. Org. Chem.* 2014, *79*, 6336 [7] Sharma, V. K., et. al. *RSC Adv.* 2014, *4*, 16618. [8] Kumar, P., et. al. *J. Org. Chem.* 2014, *79*, 5.

*Corresponding author: E.mail:ashokenzyme@gmail.com

# Supramolecular isoguanosine assemblies form hydrogels with excellent long-term stability

Frank Seela,[ab*] Hang Zhao,[b] Dawei Jiang ,[b] Andreas H. Schäfer[c]

[a] *Laboratory of Bioorganic Chemistry and Chemical Biology, Center for Nanotechnology, Heisenbergstraße 11, 48149 Münster, Germany*
[b] *Laboratorium für Organische und Bioorganische Chemie, Institut für Chemie neuer Materialien, Universität Osnabrück, Barbarastraße 7, 49069 Osnabrück, Germany,*
[c] *nanoAnalytics GmbH, Center for Nanotechnology, Heisenbergstraße 11, 48149 Münster, Germany*

Supramolecular nucleoside based hydrogels have the potential to be utilized in drug delivery, regenerative medicine and for the construction of nanoscopic devices. Isoguanosine, its 2'-deoxyribonucleoside**,** the 2'-deoxy-2'-fluororibonucleoside and the 8-azapurine derivative form hydrogels in alkali metal salt solutions. Gel stability depends on the metal ions ($Li^+$, $Na^+$, $K^+$, $Rb^+$, $Cs^+$).

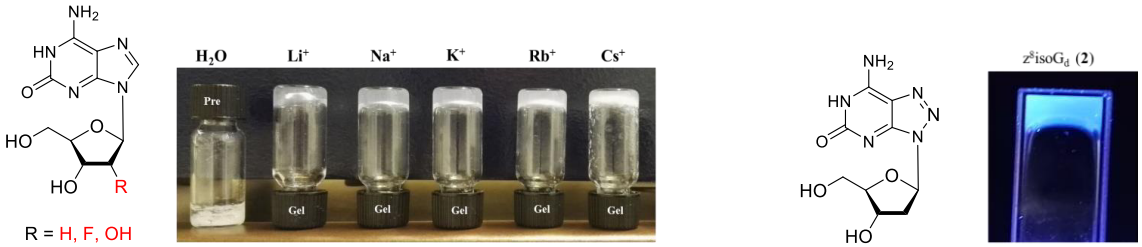



***Figure.*** *Left: Image of hydrogels obtained from isoguanosine at 1.4 mg/per 100 mL with different metal ions. Right: The fluorescent 8-aza-2'-deoxyisoguanosine hydrogel.*

All isoguanosine gels show a long-term stability of several months whereas guanosine gels collapse within minutes or hours. Rheological data confirm a 15 times higher hydrogel stability for isoG compared to G. Isoguanosine gel stability covers a broad pH range (pH 3-10), and the stability at physiological sodium ion concentration is striking. SEM images reveal that isoG self-assembles to helical stacked fibers that are interconnected while G forms discrete ribbons. Hydrogels of 8-aza-2'-deoisoguanosine are fluorescent.

***References:*** [1] Zhao, H., Schäfer, A. H. and Seela, F. *ChemPlusChem*, **2017**, 82, 826-833. [2] Zhao, H., Jiang, D. and Schäfer, A. H. and Seela, F. *ChemPlusChem*, **2017**, 82, 778-784.

*Corresponding author: E-mail: frank.seela@uni-osnabrueck.de

# New Fluorescent DNA: Syntheses and Biophysical Studies of Tropolonylated-DNA

Nagendra K Sharma *[a] Amarnath Bollu,[a] Chandrasekhar Reddy Gade,[a]

[a] *School of Chemical Science, National Institute of Science Education and Research -(NISER) Bhubaneswar, Jatani-752050, Odisha, India & HBNI-Mumbai, Mumbai-India*

DNA is genetic material and considered as gene based therapeutic candidates.[1] Tropolone is non-benzenoid aromatic naturally occurring bioactive molecule and its derivatives are considered as drug target molecules.[2] Tropolone exhibit excellent photophysical properties including fluorescence, though its quantum yield is very low.[3] Tropolone is also an excellent ligand for many transition metals.[4] Thus the incorporation of tropolone moiety in biopolymers (DNA/RNA/Peptides) would extremely be appreciated in making the fluorescent/metal binding DNA/RNA/Peptides. This report describes the syntheses of tropolonylated DNA oligo-nucleotides and their biophysical/photophysical studies. Our preliminary results show that tropolonylated DNA oligonucleotides are also weak fluorescent with quantum yeild ~1.2% (pH 7.0), but their fluorescence behavior are pH dependent as inversely proportional to pH value. Importantly the thermal stability of tropolonylated DNA duplex are remained same as of control DNA duplex. Further these modified DNA may bind with metalated peptide/protein by complexing with metal residue. Hence these modified oligonucleotides could be employed for the development of gene based therapeutic drug candidate, nanomaterials, and inhibitors of DNA binding proteins.

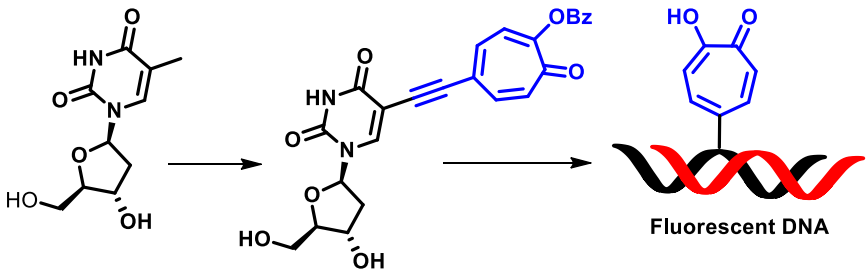



***Figure.*** *Bioactive nucleosides.*

This work was supported by grants from the SERB-DST and DBT, Govt. of India, New Delhi.

[1] (a) Watson, J. D. et. al. *Nature, **1953**, 171, 737.* [b] Deleavey, G. F. et. al., *Chem. Biol. **2012**, 19, 937.* [2] Bentley, R. et. al. *Nat. Prod. Rep*., **2008**, 25, 118. [3] E. Breheret, E. et. al. *J. Lumin*. *1978*, 17, 49. [4] Bryant, B. E. et. al. Nature, 170, 247

*Corresponding author: E-mail: nagendra@niser.ac.in

# Therapeutic Oligo Quality: Profiling and Controlling for Raw Material Impurities

Indra Pal*, Grant Fernstrum, Chandrashekar Gudise and Gary Held

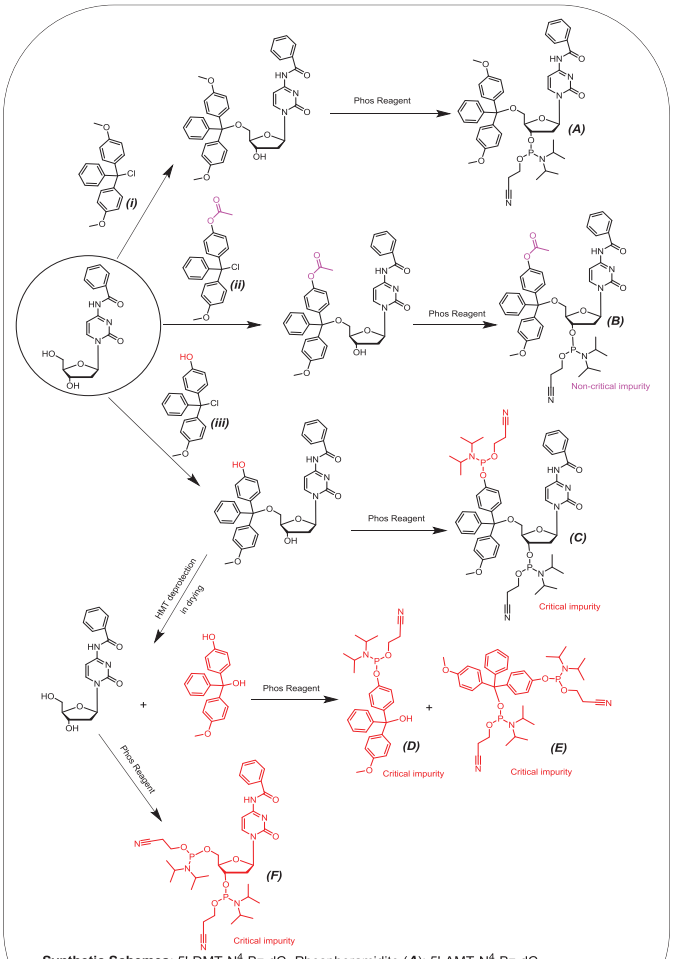
Thermo Fisher Scientific, 2202 North Bartlett Avenue, Milwaukee, WI 53202

**Introduction:** As a growing number of therapeutic oligonucleotide compounds continue to be introduced into the clinical pipeline, and advancing into larger, late phase clinical trials, an increasingly stringent demand is placed upon the phosphoramidite supply chain.

With global raw material suppliers scaling up production to meet market demands, heightened concern surrounds the increased potential for generating novel, as well as previously identified, impurities. The impurities found within the material supply chain can directly impact the quality of phosphoramidite synthesis, and thus potentially affect the quality of a therapeutic oligo.

Therefore, an increased focus has been placed upon controlling incoming raw materials, understanding the impact to phosphoramidite chemistry, including impurity profile, and subsequent effects to oligo purity. Thermo Fisher Scientific Milwaukee continues to undertake a comprehensive approach to supply chain management through partnerships with raw material suppliers, as well as customers, to define raw material specifications, including control of impurity levels to satisfy the dynamic quality requirements.

Recently, the Process Development team performed a deep investigation into the quality of an integral raw material, 4-4'-Dimethoxytrityl Chloride (***I,*** DMTr-Cl). The team capably identified the role and potential deleterious impact of two potential impurities, 4-Acetoxy-4'-methoxytrityl Chloride (***ii,*** AMTr-Cl) and 4-Hydroxy-4'-methoxytrityl Chloride (***iii,*** HMTr-Cl), in the synthesized phosphoramidite (illustrated in Synthetic Schemes, below).




**Synthetic Schemes**: 5'-DMT-N$^4$-Bz-dC- Phosphoramidite (***A***); 5'-AMT-N$^4$-Bz-dC-Phosphoramidite (***B***); 5'-HMT-N$^4$-Bz-dC-di Phosphoramidite (***C***); HMT Phosphoramidite (***D***); HMT-di Phosphoramidite (***E***); and N$^4$-Bz-dC-3',5'-di Phosphoramidite (***F***).

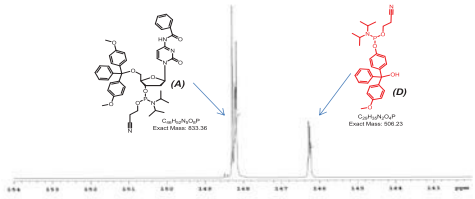


**Fig. 1**: $^{31}$P NMR Chemical shift comparison of 5'-DMT-N$^4$-Bz-dC-Phosphoramidite (***A***) and HMT Phosphoramidite (***D***)

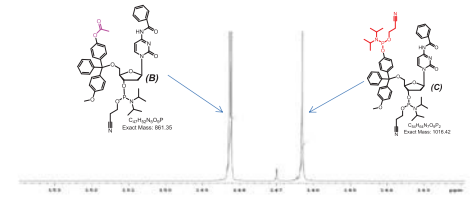
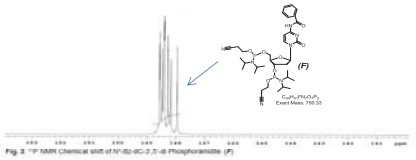


**Fig. 2**: $^{31}$P NMR Chemical shift comparison of 5'-AMT-N$^4$-Bz-dC-Phosphoramidite (***B***) and 5'-HMT-N$^4$-Bz-dC-di Phosphoramidite (***C***)



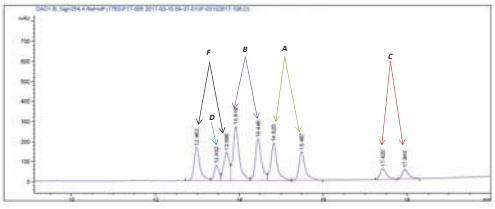



**Fig. 4:** HPLC comparisons of 5'-DMT-N$^4$-Bz-dC-Phosphoramidite (***A***), 5'-AMT-N$^4$-Bz-dC-Phosphoramidite (***B***), 5'-HMT-N$^4$-Bz-dC-di Phosphoramidite (***C***), HMT Phosphoramidite (***D***) and N$^4$-Bz-dC-3',5'-di Phosphoramidite (***F***)

**Discussion:** AMTr-Chloride in DMTr-Chloride generates non-critical impurity B. HMTr-Chloride generates the critical impurities C, D, E and F. Impurities A, B, C, D and F have been synthesized and further characterized by $^{31}$P NMR, HPLC and LC-MS.
All the critical impurities will produce deletion sequences during oligo synthesis. The number and percentage of total impurities in an oligonucleotide due to these critical impurities in phosphoramidites will depend on the sequence length. For example; a 0.1% critical impurity in the phosphoramidite, may generate up to 2.0% of impurities in a 20mer oligo.

**Conclusions:** Thermo Fisher Scientific collaborates with our suppliers and therapeutic oligo manufacturing and developmental partners to offer phosphoramidites that reflect the high standards for which our TheraPure Phosphoramidtes have been known since 2002.

Minimizing and controlling upstream single critical impurities as demonstrated in DMT-Cl, can help reduce stringency of oligo purification and increase overall yields.

Our continuous commitment to the pursuit of deeper control, analytical refinement and quantitation of the phosphoramidite supply chain will maintain Thermo Fisher Scientific as an industry leader and a sustainable partner for the continued growth and safety of oligotherapeutic medicines.

**Acknowledgement:** We thank Amal Audi, Syed Raza, Rick Matusik, Roger Knight, Amy Pasley, and Michael Singer for their support.

# Repeat-Assisted Dimerization of Thiol Modified Mismatch Binding Ligand

Takeshi Yamada,[a] Kazuhiko Nakatani*[a]

[a] *Department of Regulatory Bioorganic Chemistry, The Institute of Scientific and Industrial Research (ISIR), Osaka University, Mihogaoka 8-1, Ibaraki, Osaka 567-0047. JAPAN*

We have reported small molecule ligands **NCD** and **NA** (Figure a), which strongly bound to the G–G mismatch in CGG repeat DNAs and A–A mismatch in CAG repeat DNAs, respectively. In the CGG/CGG triad, two **NCD** molecules cooperatively bound to four guanines by disrupting Watson–Crick hydrogen bonding of two C–G base pairs and a G−G mismatch (Figure b). **NA** bound to the A–A mismatch in CAG repeat DNAs in a similar manner. In this paper, we report **NCD** and **NA** derivatives modified with various thiol linkers. Those derivatives were oxidized under aerobic conditions to give the corresponding S–S dimer *in situ*. The dimerization was selectively accelerated on the targeting repeat DNA but neither on other repeat DNAs nor on double strand DNAs. Those S-S dimers showed stronger binding to the targeting repeat DNA than unmodified **NCD** and **NA**. Preparation of thiol modified ligands, the reaction rate analysis of template-assisted disulfide formation reaction, and the binding analysis of those S-S dimers would be presented in detail.

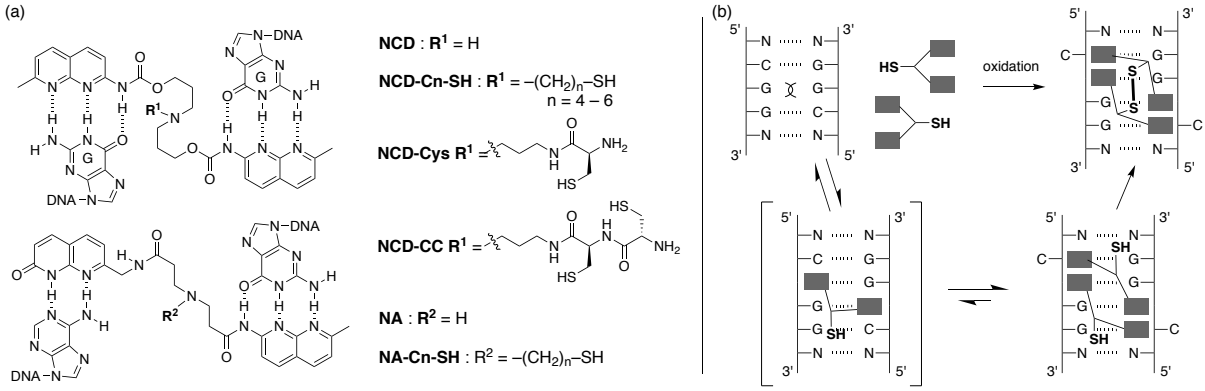



***Figure.*** *(a) Compounds used in this study. (b) Schematic illustration of template-assisted disulfide formation on CGG repeat.*

This work was supported by JSPS KAKENHI Grant-in-Aid for Specially Promoted Research (26000007) to K.N. and by Young Scientist (B) (15K17885, 17K14516) to T.Y., performed under the Research Program of “Dynamic Alliance for Open Innovation Bridging Human, Environment and Materials” in “Network Joint Research Center for Materials and Devices”.

***Reference:*** [1] Yamada, T.; Miki, S.; Ul’Husna, A.; Michikawa, A.; Nakatani, K. *Org. Lett.* **2017**, acs.orglett.7b01632.,

*Corresponding author: nakatani@sanken.osaka-u.ac.jp

# Synthesis of small molecules targeting FDTS enzyme against bio-warfare agents

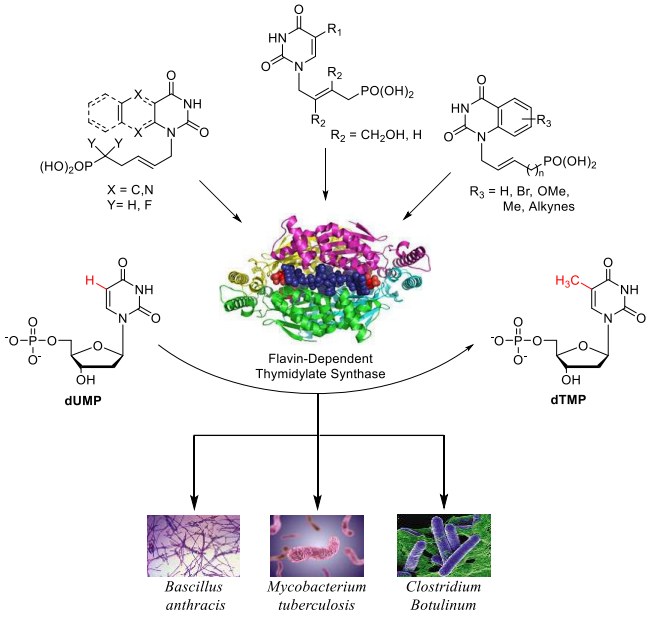
Nicolas. G. Biteau,[a] Florian Chevrier,[a] Vincent Roy,[a] Myllykallio Hannu,[b] Becker Hubert,[b] and Luigi. A. Agrofoglio.*[a]

[a] *Insitut deChimie Organique et Analytique, University of Orléans, France*

[b] *Laboratoire d'Optique et de Biosciences, University of Paris Saclay, France*

Thymidylate synthase (TSase) is an enzyme encodes by ThyA gene in eukaryotes and prokaryotes cells, in charge of the De novo synthesis of dTMP (one of the four building blocks of DNA) from dUMP, by reductive methylation. In 2002, a new class of TSase named Flavin-Dependent Thymidylate Synthase (FDTS) encoded by ThyX gene following a different mechanism was discovered only in human prokaryotes cells. FDTS uses a FAD molecules as methylene career to catalyze the mechanism, and unlike the human TSase, FDTS produces tetrahydroflate (H4folate) rather than dihydroflate (H2folate). ThyX gene wich encodes FDTS can be a new attractive antibiotics target because of the absence in the human body and the presence in several human pathogens or bio-warfare agents that can cause diseases like anthrax, tuberculosis, botulism, typhus, syphilis and more.

The aim of this project will be focused on the design and the synthesis of acyclic nucleotide analogues bearing modified uracil ring. Different series of molecules were synthesized through convergent and eco-responsible approach (green solvent, microwave, sonication..). Synthesized compounds are evaluated on ThyX from Mycobacterium tuberculosis and Helicobacter pylori.




***Scheme.*** *Aim of the project.*

N.G.B. is grateful to the Direction Generale de l'Armement (DGA), Region Centre-Val de Loire and LabEx SynOrg for a PhD scholarship.

***References:*** [1] Myllykallio, H., et al. *Science*, **2002**, *297*, 105. [2] Skouloubris, S., et al. *Open Biol*, **2015**, *5*, 15005. [3] Choi, M., et al. *Molecules*, **2016**, *21*, 654. [4] Chevrier, F., et al. *RSC Adv*, **2017**, *7*, 32282.

*Corresponding author: E-mail: luigi.agrofoglio@univ-orleans.fr

# Studies on backbone-modified oligonucleotides as prodrugs

Verena Böttner, Christian Ducho*

*Department of Pharmaceutical and Medicinal Chemistry, Saarland University, Campus C2 3, 66123 Saarbrücken, Germany*

Oligonucleotides (ON) are promising therapeutic agents, e.g., by binding the mRNA that encodes proteins which are essential for cellular functions (antisense mechanism).[1] However, ON are challenging drug candidates as they usually have poor chemical stability along with high polarity that hinders their penetration of cell membranes. Therefore, chemical modifications of the backbone structure are required.[1] A scarcely studied approach in this field is the preparation of prodrugs of antisense ON (AON), which are designed to efficiently cross biological barriers such as cell membranes and to be enzymatically deprotected inside the cell (see figure).[2] Such an approach has already been successful for siRNA.[3]

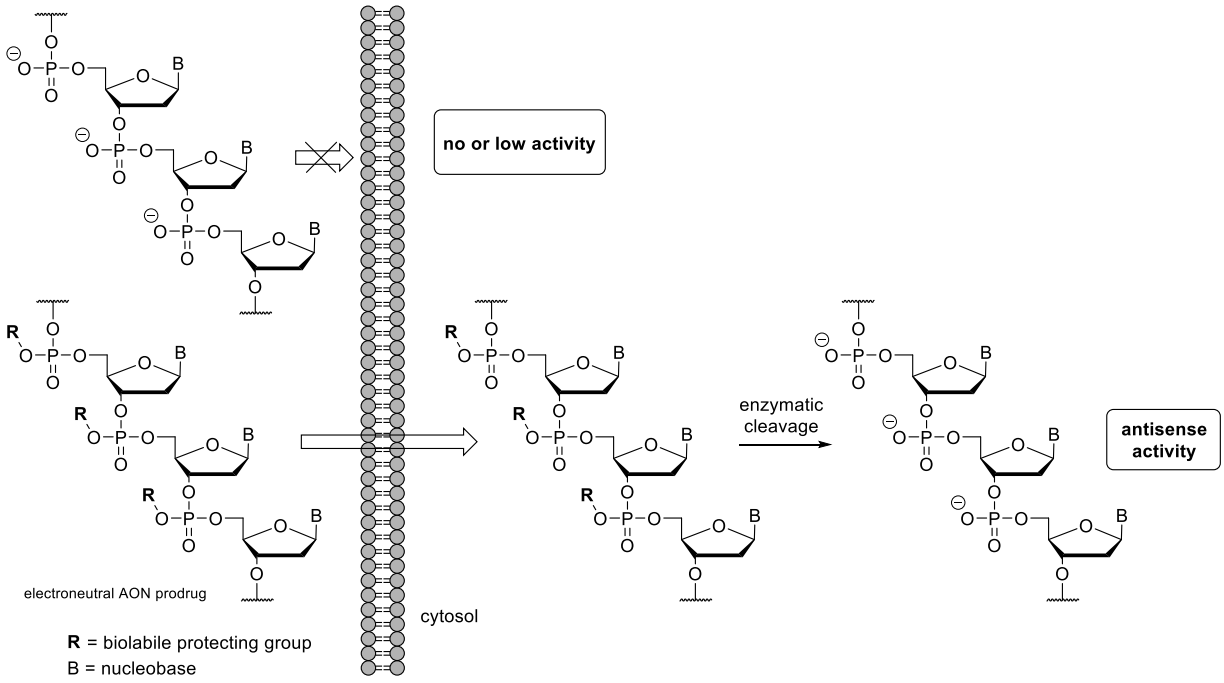



***Figure.*** *Prodrug concept for antisense oligonucleotides.*

Our overall goal is the solid phase synthesis of electroneutral AON prodrugs, composed of about 20 nucleotides with backbone-masking groups, which will allow for an esterase-mediated intracellular release of the active form. The first step has been the synthesis of model compounds, such as TxT dimers and TxTxT trimers (x = modified internucleotide linkage), to test the stability of different masking groups in several media. This work and first attempts to prepare according full-length oligonucleotide prodrugs will be presented.

***References:*** [1] Wan, W. B., et al., *J. Med. Chem.* **2016**, *59*, 9645. [2] Saneyoshi, H., et al., *Chem. Pharm. Bull.* **2018**, *66*, 147. [3] Meade, B. R., et al., *Nat. Biotechnol.* **2014**, *32*, 1256.

*Corresponding author: E-mail: christian.ducho@uni-saarland.de

# Triple helical recognition of dsRNA using PNA with artificial nucleobases

Nikita Brodyagin, Eriks Rozners*

*Department of Chemistry, Binghamton University, NY 13902, USA*

Messenger RNA (mRNA) represents quite a small portion of all RNA molecules in the living organism. Besides mRNA there are many small non-coding RNAs whose biological significance has not been understood completely yet. Here we introduce the recognition of double-stranded RNA (dsRNA) using peptide nucleic acid (PNA) forming the triple helix in the major groove of RNA. PNA is an oligonucleotide analogue that has a neutral backbone with artificial synthetic nucleobases attached to it. We use nucleobases that bind with high selectivity to A, G, C, U nucleobases of dsRNA via Hoogsteen hydrogen bonding [1]. The PNA-dsRNA triplex is stabilized by cationic nucleobase analogues, which interact with negatively charged backbone of RNA and by the improved pi-stacking of nucleobases.

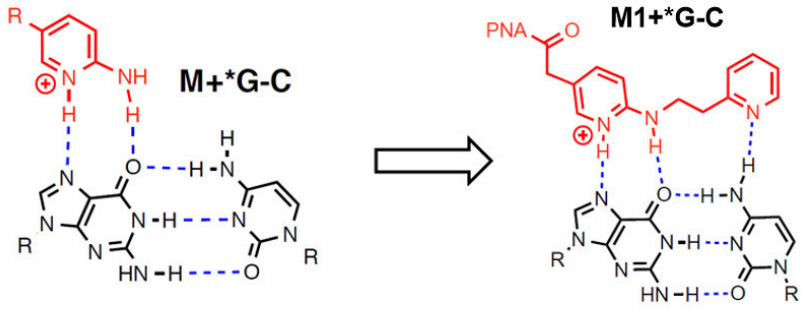



***Figure 1***. *Recognition of G-C base pair*

Recognition of guanosine (G) with cytosine (C) is problematic because of its low pKa (it has to be protonated in order to recognize G. Artificial nucleobase 2-aminopyridine (M) has a higher pKa than C and can be protonated at physiological conditions [2]. Positive charge helps to stabilize triplex since in interacts with negatively charged backbone of dsRNA. Our goal is to recognize both nucleobases in the base pair (Figure 1) with additional heterocycle. The final goal is to design heterocycles that are linked together in order to increase binding between PNA and dsRNA and also pi-stacking. This presentation will discuss our current progress towards synthesis and RNA recognition studies of the new nucleobase analogues that recognize the entire Hoogsteen face of the RNA base pair in double helix.

This work was supported by the US National Institute of Health

***References*** [1] Li, M., et al. *J. Am. Chem. Soc.* **2010**, 132, 8676. [2] Zengeya, T., et al. *Angew. Chem. Int. Ed*. **2012**, 51, 12593.

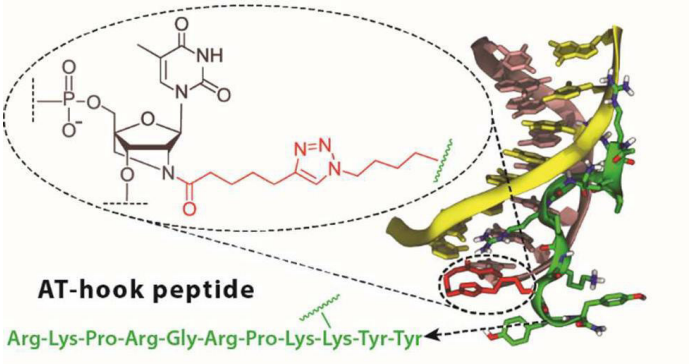
*Corresponding author: E-mail: erozners@binghamton.edu

# Synergy of two highly specific biomolecular recognition events: aligning an AT-hook peptide in DNA minor grooves via covalent conjugation to 2'-amino-LNA

Mathias Bogetoft Danielsen,‡ Maria Ejlersen,‡ Niels Johan Christensen,† Kasper K. Sørensen,† Knud J. Jensen,† Jesper Wengel‡ and Chenguang Lou*‡

*‡Biomolecular Nanoscale Engineering Center, Department of Physics, Chemistry and Pharmacy, University of Southern Denmark, Campusvej 55, 5230 Odense M, Denmark*
*†Biomolecular Nanoscale Engineering Center, Department of Chemistry, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg, Denmark*

Abstract: Two highly specific biomolecular recognition events, nucleic acid duplex hybridization and DNA-peptide recognition in the minor groove, were coalesced in a miniature ensemble for the first time. Through a copper-catalyzed azide-cycloadition was the azido-functionalized peptide covalently attached to the nucleic acid duplexes via a 2'-amino-LNA scaffold. A crystal structure of the AT-hook peptide in complex with d(CGAATTAATTCG)$_{(2)}$ was used as the starting point, with the -TTCG region truncated for modelling, to find the optimal position on the 8mer, for the AT-hook peptide to be covalently conjugated. A combination of molecular dynamics simulations and ultraviolet thermal denaturation studies, revealed that peptide-oligonucleotide conjugates (POCs) had a high sequence-specific affinity for AT-rich region in the minor grove of DNA duplexes, which were shown when no stabilization of the duplex was observed as the -AATT- cognate binding region were mutated(-AACC-) in the DNA duplex. Further proof for the correct match between peptide and the binding region was shown through the negative control, where the AT-hook binding region(-RGR-) of the peptide was changed to -SAS-. Mixing the peptide sequence with the 8mer oligonucleotide resulted in no stabilization, showing the importance of the peptide being covalently attached to the oligo. These results validate that the cooperative DNA duplex stabilization may lead the way towards further development of POCs with enhanced affinity and selectivity towards target sequences carrying peptide-binding genetic islands.




This work was supported by grants from VILLUM FONDEN

***References**: Maria Ejlersen, Niels Johan Christensen, Kasper K. Sørensen, Knud J. Jensen, Jesper Wengel and Chenguang Lou, Bioconjugate Chem. **2018**, 5, DOI: 10.1021/ acs.bioconjchem. 8b00101*

**Corresponding author: E-email: chenguang@sdu.dk*

# Splice-switching small molecules as inducers of apoptosis

Glenn A. Burley,*[a] Cyril Dominguez,[b] Ian C. Eperon,[b] Carika Weldon,[b] Andrew Jobbins, [b] Linus F. Reichenbach[a]

[a] *Department of Pure and Applied Chemistry, University of Strathclyde, Glasgow, United Kingdom.*
[b] *Leicester Institute of Chemical Biology, University of Leicester, Leicester, United Kingdom*

Alternative RNA splicing is a biological process that involves the excision (introns) and reshuffling of sequences (exons) of RNA directly after transcription to produce multiple isoforms of mature mRNA. Both the site of excision and formation of the new phosphodiester bond is catalysed by the spliceosome; a large biomacomolecular complex consisting of >150 proteins and RNA. Despite significant progress made in elucidating the key steps involved in the two transesterification steps of splicing, our understanding of the molecular determinants that define sites of phosphodiester cleavage (*i.e.,* splice site selection) is still poorly understood. This is particularly compelling when considering aberrant splicing patterns are involved a range of disease states ranging from neurodegenerative disease through to cancer.

Our research programme is aimed at addressing this gap in our knowledge of RNA splicing by developing an integrated molecular toolbox of methods to interrogate the key molecular events leading to the selection of splice sites and the development of splice-switching molecules that modulate the outcome of alternative splicing events. This presentation will highlight developments in both areas with particular emphasis in developing tools to probe RNA structure and to induce changes in RNA splicing in the Bcl-x oncogene (Figure).[1-5]

This work is currently supported by the Leverhulme Trust, EPSRC and GlaxoSmithKline.

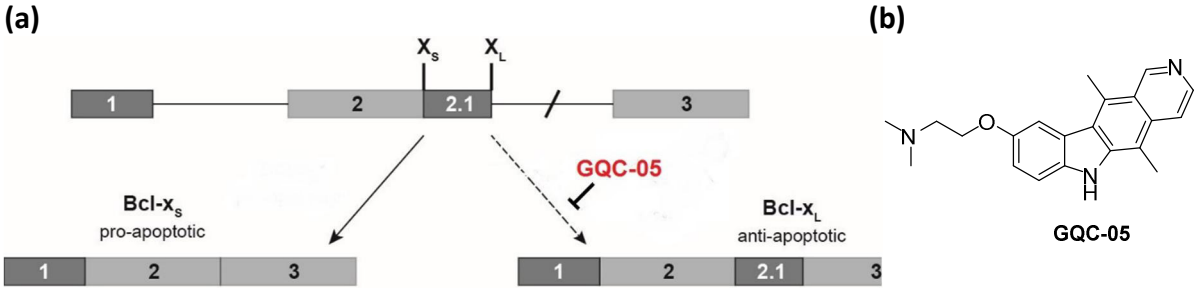



***Figure. (a)*** *Overview of the alternative RNA splicing of Bcl-x.* ***(b)*** *Structure of GQC-05.*

***References:*** [1] Jobbins, A. M. et al. *Nucleic Acids Res.* **2018***,* doi.org/10.1093/nar/gky056. [2] Weldon, C., et al. *Nucleic Acids Res.* **2018***, 46*, 886. [3] Weldon, C., et al. *Nat. Chem. Biol.* **2016***, 13*, 18. [4] Smith, L.D. et al. *Cell Rep.* **2014**, *9*, 193. [5] Lewis et al. *Angew. Chem. Int. Ed.* **2012**, *39*, 9800.

*Corresponding author: Email: glenn.burley@strath.ac.uk. www.burleylabs.co.uk

# Novel approach to therapeutic actionably RNA molecules using nearly fully automated synthesis

Annika Carstens[a], Anders Højgaard Hansen[a], Maria Taskova[a], Sofie Slott[a], Kevin Morris[b], Roslyn M. Ray[b] and Kira Astakhova*[a]

[a] Department of Chemistry, Technical University of Denmark, Lyngby, Denmark
[b] The Center for Gene Therapy, Beckman Research Institute - City of Hope, Duarte CA, USA

The field of oligonucleotide-based therapeutics has gained a lot of attention and exceptionally progressed over the last years. Anyhow, the effective delivery of oligonucleotides to their target in the cytosol or nucleus of cells within tissues and their specificity still remain major issues. [1] We hereby present a novel approach to nearly fully automated synthesis of heavily chemically altered RNA-carbohydrate conjugates, activating the low density receptor (LDLR) expression by transcriptional silencing of the LDLR regulatory lncRNA, BM450697.

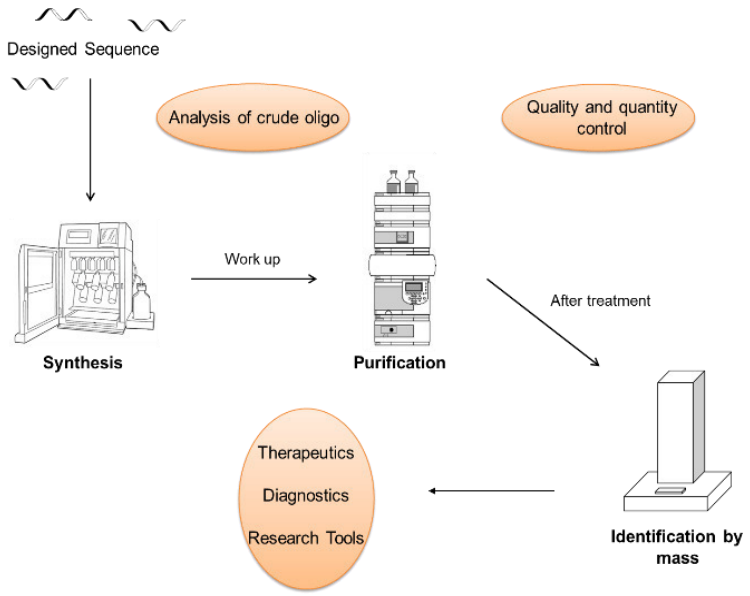



***Figure.*** *Schematic representation of the undergoing workflow of oligonucleotide synthesis*.

Using chemically modified RNA has several advantages such as enzymatic stability and specificity. [2,3] Additionally, conjugation to carbohydrate modification, e.g. GalNAc, increases the therapeutic activity of RNA, in particular for targeting hepatocytes. [4]

We will present the procedure to prepare LDLR targeting modified RNA, and discuss the issues with the preparation, purification and characterization of these RNA strands.

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* Corresponding author: Kira Astakhova: kiraas@kemi.dtu.dk

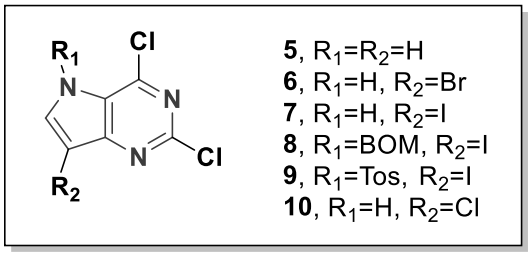
# Anticancer Properties of Halogenated Pyrrolo[3,2-*d*]pyrimidine Nucleobases with Decreased Toxicity Via N5 Substitution

Brian M. Cawrse[a], Rena S. Lapidus[b], Brandon Cooper[b], Eun Yong Choi[b], Katherine Seley-Radtke*[a]

[a] *Department of Chemistry and Biochemistry, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore MD 21250, USA*
[b] *Translational Laboratory Shared Service, University of Maryland School of Medicine, 655 W. Baltimore St., Baltimore MD 21201, USA*

Halogenated pyrrolo[3,2-*d*]pyrimidine analogues have shown antiproliferative activity in recent studies[1-3] However, the mechanism of action and pharmacokinetic (PK) profile of these compounds has yet to be determined. In this study, a series of halogenated pyrrolo[3,2-*d*]pyrimidine compounds was synthesized and first tested for activity in various cancer cell lines followed by a mouse model.[4] $EC_{50}$ values ranged from 0.014 – 14.5 µM, and maximum tolerated doses (MTD) in mice were between 5-10 mg/kg. The N-substituted compounds demonstrated comparable cell line activity ($EC_{50}$ values between 0.83 – 7.3 µM) with significantly decreased toxicity (MTD = 40 mg/kg).[4]

The PK profile of the active N5-substituted compound shows a plasma half-life of 32.7 minutes, and rapid conversion into the parent unsubstituted analogue. DNA damage assays for the histone protein H2Ax indicates direct DNA damage as a possible mechanism of action. This is supported by affinity based protein profiling (ABPP) assays which suggest that cysteine residues are likely not the mechanistic target of these electrophilic compounds.[5]




***References:*** [1] Temburnikar, K.W., et al., *Bioorg. Med. Chem.* **2015**, *23*, 4354. [2] Tari, L.W., et al., *Bioorg. Med. Chem.* **2013**, *21*, 1529. [3] Trzoss, M., et al., *Bioorg. Med. Chem. Lett.*, **2013**, *21*, 1537. [4] Cawrse, B.M., et al., *ChemMedChem*, **2018**, *13*, 178. [5] Backus, K.M., et al., *Nature*, **2016**, *534*, 570

*Corresponding author: E-mail: kseley@umbc.edu

# Enveloping DNA with a PEG Shell Enables Metal-Free Hybridization and Catalysis in Water and Organic Solvents

Gurudas Chakraborty,[a,b] Konstantin Balinin,[c] Giuseppe Portale,[a] Rafael del Villar-Guerra,[d] Tanja Weil, [c] Jonathan B. Chaires, [d] Andreas Herrmann* [a,b]

[a] *Zernike Institute for Advanced Materials, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands*
[b] *DWI-Leibniz Institute for Interactive Materials, Forckenbeckstraße 50, 52074 Aachen, Germany*
[c] *Max Planck Institute for Polymer Research, Ackermannweg 10, 55128 Mainz, Germany*
[d] *James Graham Brown Cancer Center, University of Louisville, 505 S. Hancock St., Louisville, KY 40202, USA*

Realizing DNA hybridization in the complete absence of metal ions could someday extend the applicability of functional and supramolecular structure forming DNA molecules. The potential of DNA to hybridize with its complementary sequence furnishes a plethora of opportunities for the design of programmable objects [1] and various hybrid assemblies [2]. Similarly, DNA molecules exhibiting enzymatic activity in organic solvents [3] have great importance to increase the scope of DNA nanotechnologies in non-aqueous media. However, presently metal cations are required to obtain a stable duplex state [4] and the specific binding of metal ions is indispensable for the formation of stable G-quadruplexes [5]. The complexes formed by hemin and G-quadruplexes exhibit peroxidase activity [6]. To date, no report regarding metal-free DNA duplex and G-quadruplex formation either in aqueous or in organic solvents is known. Here, we disclose for the first time a strategy to obtain thermally stable DNA duplex in aqueous medium and G-quadruplex in organic solvents devoid of metal ions. The method relies on noncovalent functionalization of DNA by PEG [7]. The DNA-PEG complex formation was confirmed by $^{1}$H-NMR. The concentrations of $Na^{+}$, $K^{+}$ and $Mg^{2+}$ in DNA-PEG solutions were determined by ICP-OES. CD and SAXS measurements were conducted to differentiate single and double-stranded DNA and also to confirm the G-quadruplex structure. The thermal stability of the duplex was determined by a melting temperature assay. Beyond structure formation, the G-quadruplex-hemin complex is capable of catalyzing oxidation reactions in organic solvents. Peroxidase activity was investigated by colorimetric and spectrophotometric methods. Moreover, quantification of the CD spectra [8] was carried out to obtain a detailed structural insight into pegylated quadruplexes.

***References:*** [1] Seeman, N. C., *Nature* **2003**, 421, 427. [2] Kwak, M., et al. *Angew. Chem. Int. Ed.* **2010**, 49, 8574. [3] Abe, H., et al. *Angew. Chem. Int. Ed.* **2012**, 51, 6475. [4] Schildkraut, C., et al. *Biopolymers* **1965,** 3, 195. [5] Lane, A. N., et al. *Nucleic Acids Res.* **2008**, 36, 5482. [6] Travascio, P., et al. *Chem. Biol.* **1998**, 5, 505. [7] Chen, W., et al. *J. Am. Chem. Soc.* **2015**, 137, 12884. [8] Del Villar-Guerra, R., et al. *Angew. Chem. Int. Ed. Engl*. **2017** Oct 26, doi: 10.1002/anie.201709184.

*Corresponding author: E-mail: herrmann@dwi.rwth-aachen.de

# Branch-PCR Constructed High Efficient Gene Nanovector for Gene Therapy

Longhuai Cheng,[a] Dejun Ma,[a] Zhen Xi*[a,b]

[a] *State Key Laboratory of Elemento-Organic Chemistry and Department of Chemical Biology, Nankai University, Tianjin, 300071, China*

[b] *National Engineering research Center of Pesticide, Nankai University, Tianjin, 300071, China*

Gene therapy can be applied to the treatment of many gene related diseases, which can recover the expression of "curative gene" in DNA level.[1] How to efficiently transfer target genes into the target tissues and cells is the key step in in gene therapy. The traditional gene cargo of nonviral vectors for gene therapy is the plasmid. However, the numerous unmethylated CpG motifs and the obstinate bacterial remnants of the plasmid can induce an adverse immune response.[2] In our work, we employed a pair of short DNA trimers (F3 and R3) to work as the branched primers,[3] and then through the PCR amplification, gene nanoparticle with target gene expression cassette was successfully constructed just in one step (Fig. 1). This construction strategy fast and efficiently produces a stable gene nanovector and supplies a useful strategy for anticancer therapy.

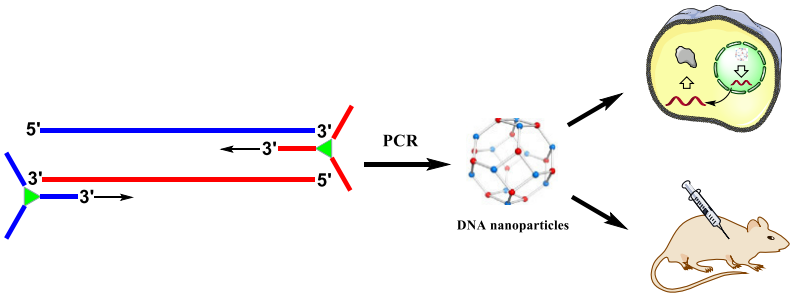



***Figure.*** *Efficient Construction of Gene Nanovector through PCR with flexible branched primers for gene delivery.*

The 2018 IRT is jointly organized by Southern California Researchers from UCSD, TriLink Biotechnologies and Rasayan Inc., on behalf of the International Society of Nucleosides, Nucleotides and Nucleic Acids. It will take place at the University of California, San Diego in La Jolla, CA, USA.

This work was supported by the MOST of China (2017YFD0200501), NSFC (21332004) and Wanxiang scholarship.

***References:*** [1] Li, S., et al. *Gene Ther.,* **2000**, *7*, 31-34. [2] Tan, Y. H., et al. *Mol. Cell*, **2014**, *54*, 212-223. [3] Liu, J., et al. *Chem. Commun.,* **2015**, *51*, 9208-9211.

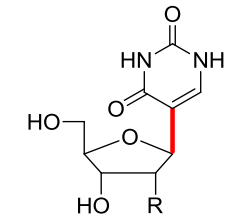
*Corresponding author: E-mail: zhenxi@nankai.edu.cn

# Synthetic Studies of Pseudouridine Derivatives

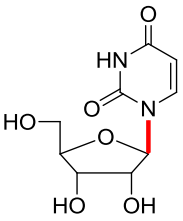
Cheng-Ping Yu, Hsin-Yun Chang, and Tun-Cheng Chien*

*Department of Chemistry, National Taiwan Normal University*
*No.88, Ting-Zhou Road, Taipei 11677, Taiwan*

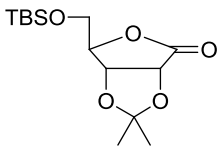
*C*-Nucleosides belong to the category of nucleoside derivatives in which the ribofuranosyl moiety is linked to the heterocyclic base with a carbon-carbon bond. The first *C*-nucleoside found in nature is pseudouridine (ψ, **1**), which is the fifth abundant nucleoside component in RNA and the most abundant *C*-nucleoside. Pseudouridine (**1**) is the structural isomer of uridine (**2**) with identical uracil base and ribosyl sugar moieties but differ only in the nucleosidic bonds. We first adopted the ribonolactone approach for the synthesis of pseudouridine (**1**). The addition of 5-lithiated 2,4-dimethoxypyrimidine to 5-*O*-*t*-butyldimethylsilyl-2,3-*O*-isopropylideneribonolactone (**4**) formed an anomeric mixture of the ribonolactols. Subsequent reductive dehydroxylation of ribonolactols followed by the deprotection afforded the desired pseudouridine accompanied with the α-isomer as the major product.[1] Alternatively, the Heck glycosylation approach was employed. The palladium-catalyzed reaction of 5-iodouracil derivatives with 3,5-di-*O*-*t*-butyldimethyl ribofuranoid glycal (**5**) followed by the removal of protecting groups gave exclusively the β-anomer of 2'-deoxypseudouridine (**3**) in a good yield.[2] Meanwhile, chemical elaboration of the Heck glycosylation adduct afforded pseudouridine derivatives.[3]



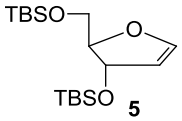

R = OH:
pseudouridine (**1**, Ψ)
R = H:
2'-deoxypseudouridine (**3**)




uridine (**2**, U)




**4**




**5**

***References***:

[1] (a) Hanessian, S.; Machaalani, R. *Tetrahedron Lett.* **2003**, *44*, 8321; (b) Grohar, P. J.; Chow, C. S. *Tetrahedron Lett.* **1999**, *40*, 2049.
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[3] Kubelka, T.; Slavětínská, L.; Hocek, M. *Synthesis* **2012**, *44*, 953.

*Corresponding author: E-mail: *tcchien@ntnu.edu.tw*

# Visualizing a Novel Checkpoint in a *Geobacillus* DNA Polymerase Crystal

Nicholas Chim,[a] Lynnette N. Jackson,[a] Anh T.M. Trinh,[a] and John C. Chaput*[a]

[a]*Department of Pharmaceutical Sciences, University of California, Irvine, CA., 92697 USA*

All branches of life are dependent on replicative DNA polymerases for faithful transfer of genetic information. Despite being categorized into several families based on sequence and topology, DNA polymerases catalyze DNA synthesis using three canonical subdomains (fingers, thumb, and palm) through a largely conserved mechanism that is tightly regulated. In many cases, the mechanistic details are still not fully understood; this is particularly true for the post-chemistry steps of nucleotide incorporation. Here, we present several X-ray crystal structures of a replicative *Geobacillus stearothermophilus* (Bst) DNA polymerase including a novel post-chemistry intermediate observed following nucleotide incorporation and protein translocation. Structural comparison of the active site reveals a new key checkpoint in the DNA synthesis pathway that is facilitated by the conserved steric gate residue, Tyr714. In the pre-chemistry structure, Tyr714 is stacked on the templating nucleobase of the final base pair and occludes the next templating base. In contrast, the post-chemistry structure shows that Tyr714 adopts a unique conformation, stacking on the base of a newly incorporated nucleotide after translocation and reorientation of the DNA duplex at the active site. Consequently, the incoming nucleotide triphosphate is prevented from entering the active site until Tyr714 returns to its pre-chemistry state.

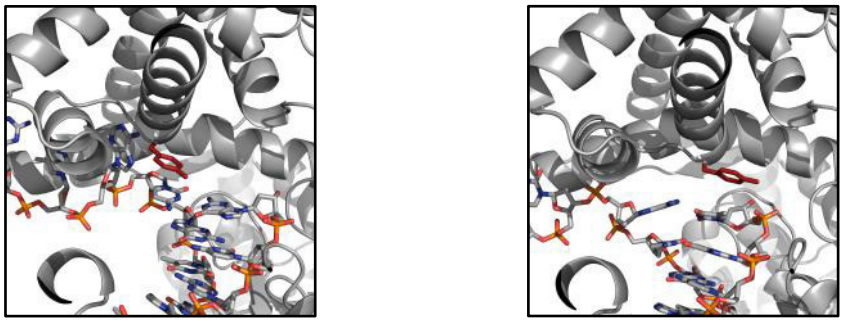


***Figure.*** *Crystal structures of the pre-chemistry (left panel) and post-chemistry (right panel) phosphodiester bonding forming steps catalyzed by Bst DNA polymerase.*

The post-chemistry conformation of Bst DNA polymerase has been observed previously in binary complexes containing non-natural and mismatched base pairs. These structures, along with the wild-type structure described above, implicate the steric gate residue as a critical checkpoint in the regulation of DNA synthesis by a replicative Family A DNA polymerase and offer a more comprehensive view of the mechanism of DNA polymerases.

This work was supported by DARPA (N66001-16-2-4061) and the NSF (1607111).

***References:*** [1] Chim, N.; Jackson, L. N.; Trinh, A. T. M.; Chaput, J. C. In preparation.

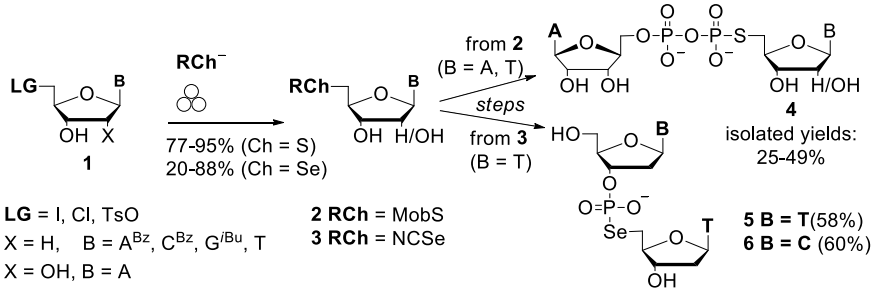
*Corresponding author: E-mail: jchaput@uci.edu

# New approaches for the synthesis of chalcogen-substituted nucleoside and nucleotide analogues

Patrick F. Conlon[a], Olga Eguaogie[a], Francesco Ravalico[a], Jamie Sweet[a], Jordan Wilson[a], Beth Cooper[a], Louis P. Conway[b], David R. W. Hodgson[b], Christopher J. Law[c] and Joseph S. Vyle[a*]

*[a]School of Chemistry and Chemical Engineering, Queen's University Belfast, David Keir Building, Stranmillis Road, Belfast BT9 5AG, UK; [b]Department of Chemistry, University of Durham, Science Labs, South Road, Durham DH1 3LE, UK; [c]School of Biological Sciences, Queen's University Belfast, Medical Biological Centre, 97 Lisburn Road, Belfast BT9 7LB, UK.*

Mechanochemistry is an attractive alternative to traditional solution-phase methods for performing nucleoside transformations especially as the use of high temperatures and involatile, toxic solvents can be avoided.[1] Using a mixer ball mill, displacement reactions of nucleoside halide or sulfonate derivatives (**1**) in the presence of 4-methoxybenzylmercaptan and base were investigated (**Scheme**). Complete and clean conversion to the corresponding 5′-thionucleoside derivatives (**2**) was achieved within one hour (and sometimes within 5 min). In contrast to solution-phase reactions of such substrates,[2] pure products could be isolated without chromatography. In contrast, grinding the same substrates with potassium selenocyanate in the presence of DMF (**1**: B = A, T) yielded the corresponding 5′-selenocyanates (**3**) in variable yields over the course of 9-11 hours. In the absence of added liquid, 5′-deoxy-5′-iodoguanosine was completely consumed in one hour. The preparation and use of hygroscopic tetrabutylammonium salts precursors was thereby avoided.[3,4]




**Scheme:** Preparation of 5′-chalcogen-nucleoside and nucleotide derivatives in a ball mill.

Michaelis–Arbuzov (M-A) reaction of activated 5′-chalcogen nucleoside precursors with trimethylsilyl-derived phosphite esters has enabled the construction of P-Ch-C moieties in high yields. In the first instance, rapid hydrolytic desilylation of the bis-TMS-derived monoester and subsequent phosphate coupling was effected in one-pot using liquid-assisted grinding to give unprecedented 5′,5′- and 3′,5′-pyrophosphorothiolate-linked dinucleoside cap analogues (**4**).[5] Alternatively, 5′-deoxythymidine-5′-selenocyanate (**3**: B = T) was selectively coupled with thymidine 3′-*H*-phosphonate in the presence of a mild base to give first *fully characterised* internucleoside phosphoroselenolate diester (**5**).[6]

Funding was provided by: The School of Chemistry and Chemical Engineering, QUB (PFC, FR); EPSRC (EP/P505488/1 – LPC) and by the authors (JSV, OE).

***References:*** [1] O. Eguaogie, J. S. Vyle, P. F. Conlon, M. A. Gîlea and Y. Liang, Beilstein J. Org. Chem., 2018, 14, 955. [2] K. J. Divakar and C. B. Reese, J. Chem. Soc., Perkin Trans. 1, 1982, 1625. [3] A. M. Belostotskii, J. Lexner and A. Hassner, Tetrahedron Lett., 1999, 40, 1181. [4] O. Eguaogie, P. F. Conlon, F. Ravalico, J. S. T. Sweet, T. B. Elder, L. P. Conway, M. E. Lennon, D. R. W. Hodgson and J. S. Vyle, Beilstein J. Org. Chem., 2017, 13, 87. [5] O. Eguaogie, L. A. Cooke, P. M. L. Martin, F. Ravalico, L. P. Conway, D. R. W. Hodgson, C. J. Law and J. S. Vyle, Org. Biomol. Chem., 2016, 14, 1201. [6] O. Eguaogie, P. F. Conlon and J. S. Vyle, Tetrahedron Lett., 2016, 57, 5000.
*Corresponding Author: E-mail: j.vyle@qub.ac.uk

# Influence of nucleotide modifications at the C2′ position on the Hoogsteen base-paired parallel-stranded duplex of poly(A) RNA

William Copp[a,b], Alexey Y. Denisov[a,b], Jingwei Xie[b,c], Anne M. Noronha[a,b], Kalle Gehring[b,c]* and Christopher J. Wilds[a,b]*

[a] Department of Chemistry and Biochemistry, Concordia University, Montréal, Québec, H4B 1R6, Canada

[b] Groupe de recherché axé sur la structure des protéines, Montréal, Québec, H3G 0B1, Canada

[c] Department of Biochemistry, McGill University, Montréal, Québec, H3G 0B1, Canada

Polyadenylate (poly(A)) has the ability to form a parallel duplex with Hoogsteen adenine:adenine base pairs at low pH or in the presence of ammonium ions. In order to evaluate the potential of this structural motif for nucleic acid based nanodevices, we characterized the effects on duplex stability of substitutions of the ribose sugar with 2′-deoxyribose, 2′-*O*-methyl-ribose, 2′-deoxy-2′-fluoro-ribose, arabinose and 2′-deoxy-2′-fluoro-arabinose. Deoxyribose substitutions destabilized the poly(A) duplex both at low pH and in the presence of ammonium ions: no duplex formation could be detected with DNA poly(A) oligomers. Arabinose and 2′-deoxy-2′-fluoro-arabinose nucleotides strongly destabilized poly(A) duplex formation. In contrast, 2′-*O*-methyl and 2′-deoxy-2′-fluoro-ribo modifications were stabilizing either at pH 4 or in the presence of ammonium ions, respectively. The differential effect suggests they could be used to design molecules selectively responsive to pH or ammonium ions. To understand the destabilization by deoxyribose, we determined the structures of RNA poly(A) duplexes with a single DNA residue by NMR spectroscopy and X-ray crystallography. The structures revealed minor structural perturbations suggesting that the combination of sugar pucker propensity, hydrogen bonding, pKa shifts, and changes in hydration determine duplex stability.

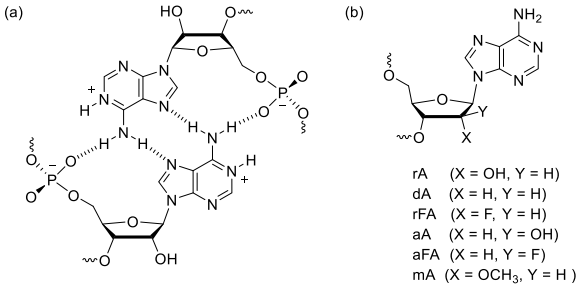



*Figure 1.* (**a**) Adenine base pairing with protonation at N1 and (**b**) 2′-modifications evaluated in this study.

***References:*** [1] Rich, A.; Davies, D. R.; Crick, F. H. C.; Watson, J. D., *J. Mol. Biol.* **1961**, *3*, 71–86. [2] Safaee, N.; Noronha, A. M.; Rodionov, D.; Kozlov, G.; Wilds, C. J.; Sheldrick, G. M.; Gehring, K., *Angew. Chem. Int. Ed.* **2013**, *52*, 10370–10373. [3] Copp, W.; Denisov, A. Y.; Xie, J.; Noronha, A. M.; Liczner, C.; Safaee, N.; Wilds, C. J.; Gehring, K., *Nucleic Acids Res.* **2017**, *45*, 10321–10331.

* Corresponding authors: e-mails: Chris.wilds@concordia.ca
Kalle.gehring@mcgill.ca

# A Biomolecular Engineering Approach for the Design and Synthesis of Polyfunctional PNAs

Andrea Rozzi,[a] Martina Neri,[a] Sasa Korom,[b] Alex Manicardi,[a] Massimiliano Donato Verona,[a] Vincenzo Verdolino,[c] Roberto Corradini[a,b] *

[a]*University of Parma, Department of Chemistry, Life Sciences and Environmental Sustainability, Viale delle Scienze 17\A Parma, Italy.*
[b]*National Institute for Biostructures and Biosystems (INBB)-Viale delle Medaglie d'Oro, 305, 00136 Roma, Italy.*
[c]*Facoltà di Informatica, Istituto di Scienze Computazionali, Università della Svizzera Italiana, 6900 Lugano,Switzerland.*

In a general project aimed at the development of Peptide nucleic acid (PNA) and their analogs, we have addressed the problems of treatment of diseases for which no effective therapy is available,[1-3] targeting of microRNA,[2-4] development of new ultrasensitive diagnostic tools,[5,6] and fabrication of complex nanosystems for personalised therapies.[7,8] During the years we have approached the design of new structures using increasingly effective tools, from simple crystal structure analysis to molecular dynamics and metadynamics (Figure).[9] At the same time we selected a series of synthetic modular strategies enabling to rationally create new PNA structures, and in particular polyfunctional PNAs with improved cellular uptake, biostability and recognition properties.[4,10,11] Recent examples of these approach will be presented and the potential impact and perspectives of molecular engineering of these new PNA-based structures in diagnostics and nano-biotechnology will be discussed.

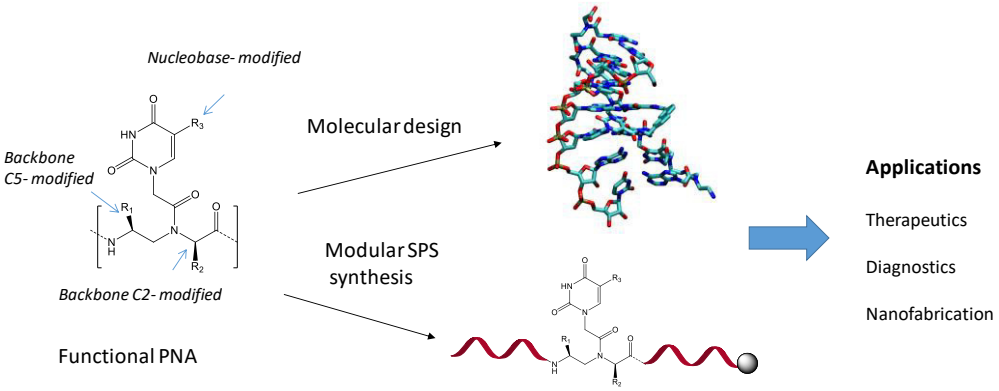



***Figure**. Present approaches for the design of new PNA-based biomolecular tools.*

***References:*** [1] Tonelli, R. et al. *Clinical Cancer Res.* **2012**, *18*,796. [2] Brognara, E. et al. *J. Neurooncol.* **2014**, *118*, 19. [3]Fabbri E. et al. *Molecules*, **2018**, *23*, 71. [4] Manicardi, A. et al. *ChemBiochem* **2012**, *13*, 1327. [5] Spoto, G., Corradini, R. Eds, Detection of non-amplified Genomic DNA. Springer, 2012. [6] Bertucci, A. et al. *Biosens. Bioelectron* **2015**, *63*, 248. [7] Bertucci, A. et al. *Adv. Healthcare Mater*. **2014**, *3*, 1812. [8] Bertucci A., et al *Small*, **2015**, *11*, 5687-5695. [9] Verona, M.D. et al. *Sci Rep.* **2017**, *7*, 42799**. [**10] Manicardi A, et al. *Beilstein J. Org. Chem.* **2014**, *10*, 1495. [11] Manicardi A. et al. *Org. Lett*, **2016**,18, 5452.

Corresponding authors: E-mail: roberto.corradini@unipr.it

# Discovery of New Nucleoside Analogue Prodrugs Against the Zika Virus

Michael Coste,[a] Jean Bernatchez,[b] Alex Clack,[b] Sungjun Beck,[b] Lucas Luna,[a] Christal Sohl,*[a] Jair Siqueira-Neto,*[b] Byron W. Purse*[a]

[a] *Department of Chemistry and Biochemistry, San Diego State University, 5500 Campanile Dr, San Diego, CA, United States of America*
[b] *Department of Chemistry and Biochemistry, University of California San Diego, 9500 Gilman Dr, La Jolla, CA, United States of America*

Starting in 2015, a pandemic of the Zika virus emerged in Latin America, causing microcephaly in newborns and Guillian-Barré Syndrome in adults. Currently there is no vaccine or treatment for ZIKV, but the FDA approved drug Sofosbuvir has been shown to inhibit Zika Virus replication in cell culture, albeit with inadequate potency.[1] Using the same modified phosphate group chemistry used to enhance drug delivery of Sofosbuvir, we synthesized a set of nucleoside analogues designed to probe three possible inhibitory mechanisms against ZIKV genomic replication.[2] We tested these analogues in Glioblastoma cell cultures and compared the results to the parent nucleoside analogues that lacked modifications for enhanced delivery. We found that attaching the ProTide onto the nucleoside analogue increased the potency of the compound when compared to the unmasked nucleoside analogue. We also discovered a modified nucleoside that shows a 5-fold increase in potency when compared with Sofosbuvir. We are currently in the process of performing mouse model studies to determine toxicity and other pharmacokinetic properties as well as trying to increase the potency of our lead compound by changing the phosphate masking group and by additional modifications to the ribose moiety with the goal of developing an even more potent and selective drug candidate against the Zika Virus.

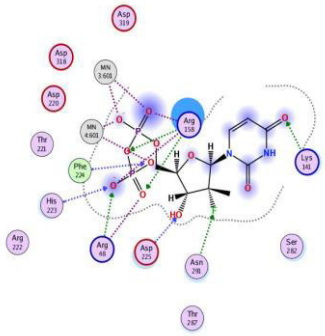


***Figure 1.*** *Key protein-ligand interactions between Sofosbuvir and Zika RNA-dependent RNA polymerase*

***References:*** [1] Mumtaz, N. et al., Cell-Line Dependent Antiviral Activity of Sofosbuvir against Zika Virus. *Antiviral Res.* **2017**, *146*, 161–163. [2] Bernatchez, J.; Yang, Z.; Coste, M.; Li, J.; Beck, S.; Liu, Y.; Clark, A.; Zhu, Z.; Luna, L.; Sohl, C.; Purse, B.; Li, R.; Siqueira-Neto, J. Development of a phenotypic high-content imaging assay for assessing the antiviral activity of small-molecule inhibitors targeting the Zika virus. *Antimicrob. Agents Chemother.* **in press**.

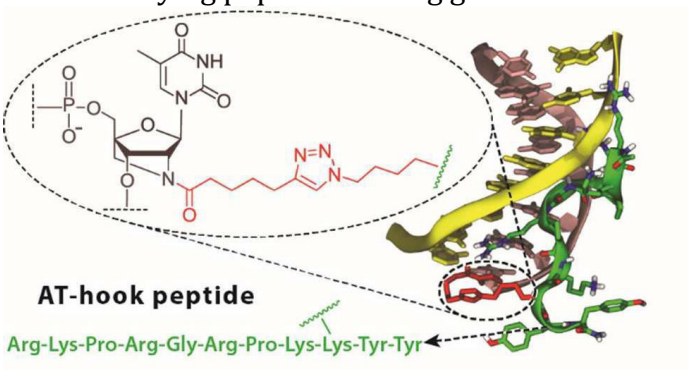
* Byron W. Purse: E-mail: bpurse@sdsu.edu

# Synergy of two highly specific biomolecular recognition events: aligning an AT-hook peptide in DNA minor grooves via covalent conjugation to 2'-amino-LNA

Mathias Bogetoft Danielsen,‡ Maria Ejlersen,‡ Niels Johan Christensen,† Kasper K. Sørensen,† Knud J. Jensen,† Jesper Wengel‡ and Chenguang Lou*‡

*‡Biomolecular Nanoscale Engineering Center, Department of Physics, Chemistry and Pharmacy, University of Southern Denmark, Campusvej 55, 5230 Odense M, Denmark*
*†Biomolecular Nanoscale Engineering Center, Department of Chemistry, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg, Denmark*

Abstract: Two highly specific biomolecular recognition events, nucleic acid duplex hybridization and DNA-peptide recognition in the minor groove, were coalesced in a miniature ensemble for the first time. Through a copper-catalyzed azide-cycloadition was the azido-functionalized peptide covalently attached to the nucleic acid duplexes via a 2'-amino-LNA scaffold. A crystal structure of the AT-hook peptide in complex with d(CGAATTAATTCG)$_{(2)}$ was used as the starting point, with the -TTCG region truncated for modelling, to find the optimal position on the 8mer, for the AT-hook peptide to be covalently conjugated. A combination of molecular dynamics simulations and ultraviolet thermal denaturation studies, revealed that peptide-oligonucleotide conjugates (POCs) had a high sequence-specific affinity for AT-rich region in the minor grove of DNA duplexes, which were shown when no stabilization of the duplex was observed as the -AATT- cognate binding region were mutated(-AACC-) in the DNA duplex. Further proof for the correct match between peptide and the binding region was shown through the negative control, where the AT-hook binding region(-RGR-) of the peptide was changed to -SAS-. Mixing the peptide sequence with the 8mer oligonucleotide resulted in no stabilization, showing the importance of the peptide being covalently attached to the oligo. These results validate that the cooperative DNA duplex stabilization may lead the way towards further development of POCs with enhanced affinity and selectivity towards target sequences carrying peptide-binding genetic islands.




This work was supported by grants from VILLUM FONDEN

***References**: Maria Ejlersen, Niels Johan Christensen, Kasper K. Sørensen, Knud J. Jensen, Jesper Wengel and Chenguang Lou, Bioconjugate Chem. **2018**, 5, DOI: 10.1021/ acs.bioconjchem. 8b00101*

**Corresponding author: E-email: chenguang@sdu.dk*

# Amidate prodrugs of cyclic and acyclic nucleoside phosphonates with potent antiviral activity

Steven De Jonghe,[a,b] Min Luo,[a] Elisabetta Groaz,[a] Chao Liu,[a] Shrinivas Dumbre,[a] Christophe Pannecouque,[b] Robert Snoeck,[b] Graciela Andrei,[b] Dominique Schols,[b] Piet Herdewijn*[a]

*[a]Medicinal Chemistry, Rega Institute for Medical Research, KU Leuven, Herestraat 49 bus 1041, 3000 Leuven, Belgium. [b]Laboratory of Virology and Chemotherapy, Rega Institute for Medical Research, KU Leuven, Herestraat 49 bus 1043, 3000 Leuven, Belgium*

Nucleoside phosphonates are a key class of antiviral agents. Structurally, they are characterized by the presence of a phosphonomethoxy functionality (P–C–O) instead of the phospho-oxymethyl (P–O–C) moiety in naturally occurring nucleoside monophosphate. Despite the favorable characteristics of the nucleoside phosphonates (such as metabolic stability and bypassing of the first phosphorylation step), they are negatively charged at physiological pH and hence are not able to easily penetrate the cell membrane, which hampers their antiviral activity. Therefore, various prodrug or pronucleotide approaches have been investigated to promote the passive diffusion through the lipophilic cell membranes and to liberate the parent nucleotide intracellularly where it can be further phosphorylated to the pharmacologically active species. The aryloxyphosphonoamidate prodrug approach[1] has been succesfully applied to nucleoside phosphonates, as demonstrated by the marketing approval of tenofovir alafenamide (TAF) for the treatment of HIV- and HBV-infected patients. Different cyclic[2] and acyclic[3] nucleoside phosphonates with a promising antiviral profile are known in literature, but no efforts have been done to boost their antiviral activity by the synthesis of prodrugs. In this poster, we describe the synthesis of amidate prodrugs of L-2′-deoxy-threose nucleoside phosphonates **1**, 3-fluoro-2-(phosphonomethoxy)propyl nucleoside phosphonates **2** and cyclic 9-(S)-[3-hydroxy-2-(phosphonomethoxy)propyl]adenine **3**. The most promising congeners within this series are endowed with low nanomolar activity against retro- (HIV) and DNA- (HBV, HSV, CMV, VZV) viruses. Besides the antiviral data, a preliminary *in vitro* ADME profiling, will also be discussed.[2-4]

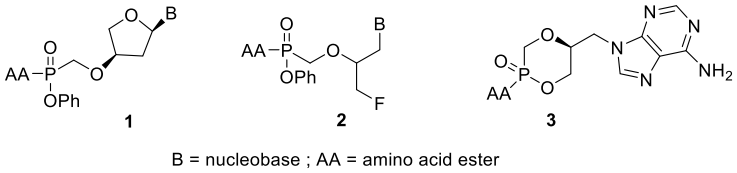



***Figure.*** *Amidate prodrugs of nucleoside phosphonates*

***References:*** [1] Slusarczyk, M. *Antiviral Chem. Chemother.* **2018**, *26*, 1. [2] Pertusat, F. *Antiviral Chem. Chemother.* **2012**, *22*, 181. [3] Holy, A. *Antiviral Res*. **2006**, *71*, 248. [4] Liu, C. *et al*., *J. Med. Chem*. **2016**, *59*, 9513. [5] Luo, M. *et al*., *J. Med. Chem*. **2017**, *60*, 6220. [6] Luo, M. *et al*., *ACS Med. Chem. Lett*. **2018**, *9*, 381.

*Corresponding author: E-mail: piet.herdewijn@kuleuven.be

# Cell internalization and gene silencing by reduction-responsive siRNAs bearing lipophilic disulfide-containing modifications at 2'-position

Florian Gauthier,[a] Sandra Claveau,[b] Jean-Rémi Bertrand,[b] Jean-Jacques Vasseur,[a] Christelle Dupouy,[a] and Françoise Debart*[a]

[a] *IBMM, UMR 5247 CNRS, Université de Montpellier, ENSCM, Montpellier, France*
[b] *UMR 8203 CNRS, Université Paris-Sud Gustave Roussy, Univ. Paris-Saclay, Villejuif, France*

Synthetic siRNAs are powerful therapeutic and biological tools for efficient recognition of mRNA resulting in its degradation and consequently in the inhibition of translation into proteins. However, low cellular penetration and enzymatic fragility of siRNAs represent a major bottleneck for medical applications. To overcome these drawbacks, for a decade we have developed a prodrug approach in which RNAs are partially masked at some 2'-OH positions by biolabile and lipophilic groups. These transient modifications such as acetalester groups have increased RNA stability towards enzymes and have improved cell penetration to reach mRNA target for inhibition of protein expression [1-3].

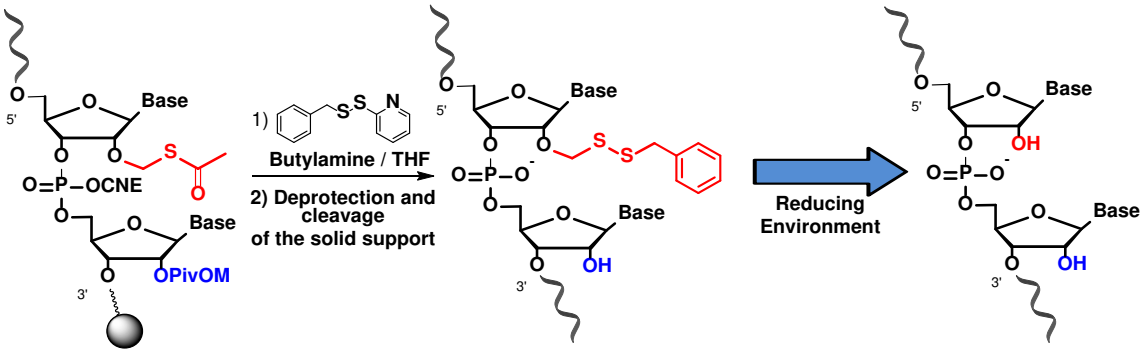



***Figure.*** *Synthesis and conversion of 2'-O-benzyldithiomethyl (2'-O-BnSSM)-modified RNA into native RNA under reducing environment*

With the same goal, here we focus on another lipophilic and biolabile modification containing a disulfide bridge prone to be reduced inside the cells, thanks to the reducing environment, in order to release an unmodified RNA. We have synthesized partially 2'-*O*-modified RNAs with some benzyldithiomethyl groups (BnSSM) using a post-synthetic method on solid support [4]. The different 2'-*O*-BnSSM RNAs were used in various siRNA constructs to study the influence of the number and the positioning of the modifications on siRNAs properties. Their thermal and enzymatic stabilities, and their unmasking under reducing conditions have been evaluated. The cell penetration without transfecting reagent and the gene-silencing activity of these 2'-*O*-BnSSM modified siRNAs were demonstrated in human Ewing's sarcoma cells [5].

***References:*** [1] Lavergne, T. et al., *J. Org. Chem.* **2011,** *76*, 5719. [2] Biscans, A. et al., *ChemBioChem* **2014,** *15*, 2674. [3] Biscans, A. et al., *ChemBioChem* **2016,** *17*, 2054. [4] Biscans, A. et al., *Org. Biomol. Chem.* **2016,** *14*, 7010. [5] Gauthier, F. et al., *submitted manuscript*.

*Corresponding author: E-mail: francoise.debart@umontpellier.fr

# siRNAzos: Photoresponsive Azobenzene-Containing siRNAs

Jean-Paul Desaulniers* and Matthew Hammill

*Faculty of Science, University of Ontario Institute of Technology, 2000 Simcoe Street North, Oshawa, ON L1H 7K4 Canada*

Fine-tuning the activity of short-interfering RNAs (siRNAs) with light could help overcome several obstacles related to potency, delivery, and off-target effects. In this study, we chemically modify siRNAs that contain azobenzene derivative spacers within the sense strand using phosphoramidite chemistry. These molecules are called siRNAzos and they are successfully accommodated within the RNAi pathway as measured by gene-silencing dose-dependent knockdown in cells. In addition to its RNAi biocompatibility, we are able to photochemically control the activity of the siRNAzos that contain the azobenzene within the central region of the sense strand. We demonstrate it is possible to both reversibly inactivate and reactivate several siRNAzos with ultraviolet and visible light, respectively (Fig. 1). Future work involves modifying the azobenzene with halogens to red-shift the wavelength needed to activate and inactivate the complex.

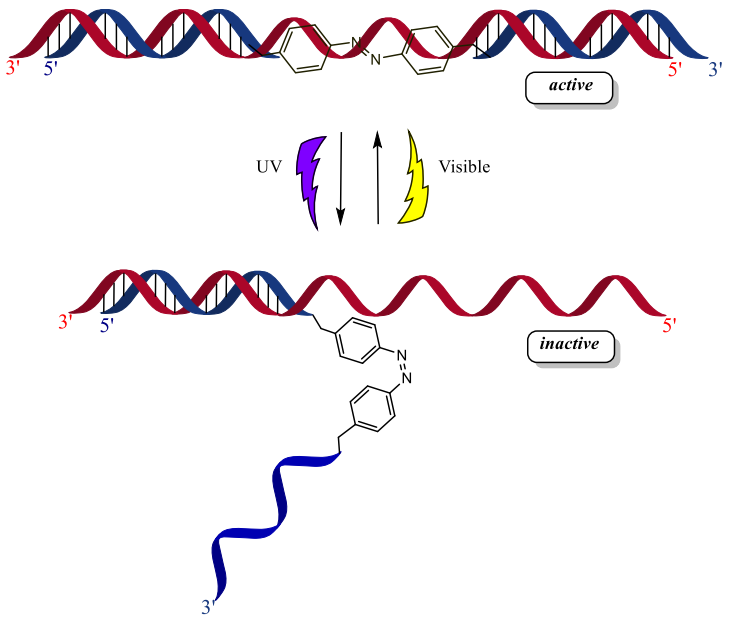



***Figure 1.*** Photoinduced inactivation and reactivation of siRNAzos.

This work was supported by the Discovery Grant from the Natural Sciences and Engineering Research Council (NSERC)

***References:*** [1] Hammill, M.; Isaacs-Trepanier, C.; Desaulniers, J.-P. *ChemistrySelect* **2017**, *2*, 9810.

*Corresponding author: E-mail: Jean-Paul.Desaulniers@uoit.ca

# Synthesis of a Neutral Cyclophane Analogue of the Second Messenger 2‘,3‘-Cyclic Guanosine Monophosphate Adenosine Monophosphate (cGAMP)

Clemens Dialer,[a] Samuele Stazzoni,[a] Felix Müller,[a] Thomas Carell*[a]

[a] *Ludwig-Maximilians-University, Department of Chemistry, Butenandtstr. 5-13, Building F, 81377 Munich, Germany*

Cytosolic DNA from pathogens can be life-threatening for multicellular organisms. The innate immune system is capable to recognize the danger and to activate a downstream signaling cascade resulting in the production of cell defending type I-interferons and cytokines.[1] As recently reported, this immune response is essentially triggered by the natural occurring second messenger 2’,3’-cGAMP (a cyclic dinucleotide) upon binding to the endoplasmic reticulum membrane protein STING (stimulator of interferon genes).[2]

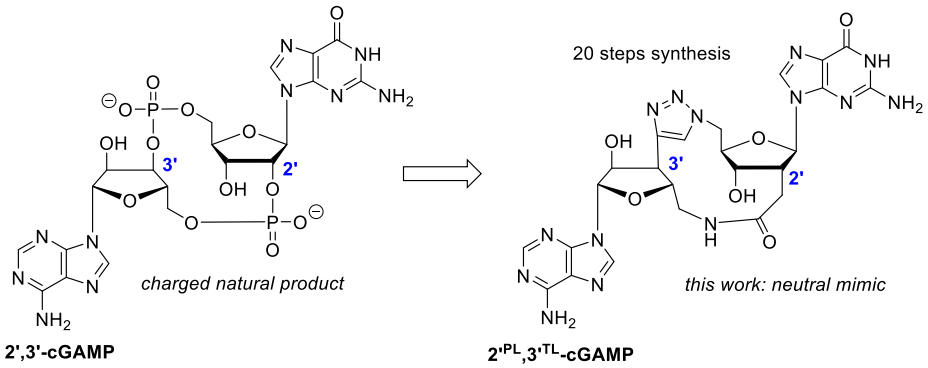



***Figure.*** *Mimicking the natural occurring second messenger cGAMP.*

Therefore, targeting STING with cGAMP analogues depicts an interesting strategy in order to regulate the immune system and to study the therapeutic potential (e.g. simplified cell membrane penetration and longer cell persistence). In this work, the synthesis and structure elucidation of a novel neutrally linked cGAMP candidate is reported.

***References:*** [1] Chen, Q., et al. *Nature Immunology* **2016**, *17*, 1142-1149. [2] Hornung, V., et al. *Nature* **2013**, *498*, 380-384.

*Corresponding author: Thomas.Carell@cup.lmu.de

# Imaging of siRNA transport and processing using FRET and FLIM microscopy

Larissa Doll,[a] J. Steinmeyer,[a] H.-K. Walter,[a] F. Rönicke,[a] Hans-Achim Wagenknecht*[a]

[a]*Institute of Organic Chemistry, Karlsruher Institute of Technology (KIT), Fritz-Haber-Weg 6, 76131 Karlsruhe, Germany*

RNA interference provides a powerful analytic tool to control gene expression in living cells. To monitor the RNA integrity and processing in real time, wavelength-shifting siRNA probes, in which a donor and acceptor dye are incorporated as energy transfer pair resulting in two distinct emission colors, were developed.[1-6] This change in emission color combined with fluorescence lifetime imaging microscopy (FLIM) reduces the risk of wrong positive or wrong negative readout in cell imaging, for example due to autofluorescence by cellular components. Herein, we modify and optimize the already existing concept of „RNA traffic lights" by using ATTO532/ATTO647N dyes. In comparison to the previous research, these dyes show higher photostability and monoexponential fluorescence lifetime. Because of these advantages, the dyes are suitable for single molecule detection and high resolution microscopy.

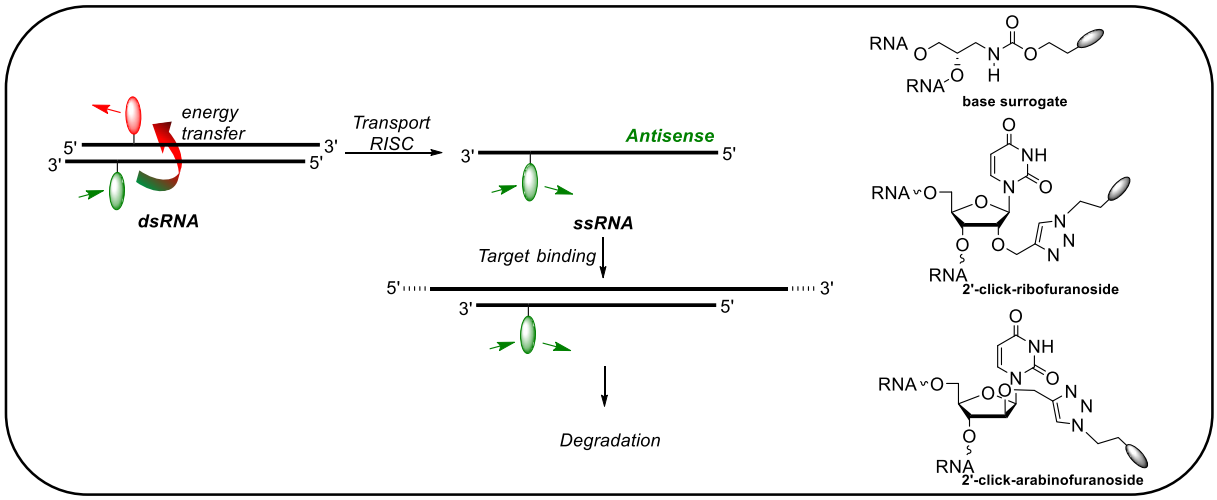



***Figure.*** *Principle of siRNA traffic lights and structures of anchor moieties.*

***References:*** [1] C. Holzhauser, H.-A. Wagenknecht, *J. Org. Chem.* **2013**, *78*, 7373 - 7379. [2] C. Holzhauser, H.-A. Wagenknecht, *Chem. Bio. Chem.* **2012**, *13*, 1136 – 1138. [3] M. Breunig, R. Liebl, A. Goepferich, H.-A. Wagenknecht, C. Holzhauser, *ACS Chem. Biol.* **2013**, *8*, 890 – 894. [4] H.-K. Walter, P. Bohländer, H.-A. Wagenknecht, *ChemistryOpen* **2015**, *2*, 92–96. [5] J. Steinmeyer, F. Rönicke, U. Schepers, H.-A. Wagenknecht, *ChemistryOpen* **2017**, *6*, 514 – 518. [6] J. Steinmeyer, H.-K. Walter, M. A. Bichelberger, V. Schneider, T. Kubar, F. Rönicke, B. Olshausen, K. Nienhaus, G. U. Nienhaus, U. Schepers, M. Elstner, H.-A. Wagenknecht, *Org. Biomol. Chem.* **2018**, DOI:10.1039/c8ob00417j.

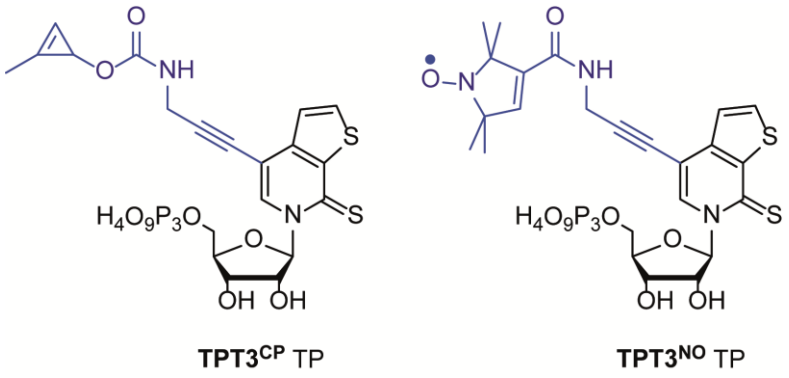
*Corresponding author: E-mail: larissa.doll@kit.edu

# Site-specific RNA labeling using functionalized nucleotides in expanded genetic alphabet transcription reactions

Frank Eggert,[a] Stephanie Kath-Schorr*[a]

[a] *LIMES-Institute, University of Bonn, Gerhard-Domagk-Str. 1, 53119, Bonn, Germany*

Investigation of functional non-coding RNAs such as ribozymes requires the introduction of reporter groups into RNA without disturbing its sensitive native structure and function.
Unnatural base pairs (UBPs) have augmented the genetic alphabet enabling increased information storage in nucleic acids. Based on the UBP (d)TPT3:(d)NaM[1,2] developed by Romesberg and coworkers we present powerful site-specific RNA labeling techniques.
Using chemically functionalized nucleotide **TPT3$^{CP}$** TP in a template-directed T7 in vitro transcription approach RNA labeling with bio-orthogonally clickable methyl cyclopropene moieties is demonstrated.[3,4] This facilitates the post-transcriptional attachment of virtually any reporter group with minimal impact on target RNA's natural performance.
The introduction of nitroxide spin label-containing nucleotide **TPT3$^{NO}$** TP into RNA further serves as nanometer range ruler employing electron paramagnetic resonance (EPR) spectroscopy. Distance measurements are performed in spin-labeled RNAs providing information about their solution structures.




**TPT3$^{CP}$** TP **TPT3$^{NO}$** TP

*Chemically functionalized triphosphates of the unnatural base TPT3.*

***References:*** [1] Li, L. et al., *J. Am. Chem. Soc.* **2014**, *136*, 826–829. [2] Zhang, Y. et al., *Nature* **2017**, *551*, 644–647. [3] Eggert, F.; Kath-Schorr, S. *Chem. Commun.* **2016**, *52*, 7284–7287. [4] Eggert, F. et al., *Methods* **2017**, *120*, 17–27.

*Corresponding author: E-mail: skath@uni-bonn.de

# Novel DNA-based Materials for Fuel Cell Catalyst Development

Klaudia Englert,[a] Ruba Hendi,[b] Neil Rees,[b] James H.R. Tucker, [a] Alex P.G. Robinson *[b]

[a] *School of Chemistry, University of Birmingham, Birmingham, United Kingdom*
[b] *School of Chemical Engineering, University of Birmingham, Birmingham, United Kingdom*

We report ongoing work towards the development of novel material precursors for fuel cell catalyst development. This is achieved through application of DNA as a material precursor for incorporation of metal complexes via covalent interactions (cisplatin) and phosphoramidite chemistry (ferrocene). The DNA backbone is used as a scaffold for the even distribution of metal centres throughout the material. Non-specific metal distribution has been studied through implementation of salmon DNA. A more specific approach is also targeted by applying DNA self-assembly to result in higher precision of metal loading. This includes DNA Holliday junctions, DNA tetrahedrons and DNA origami. These new materials are expected to exhibit enhanced catalytic behaviour due to increased surface areas around catalytic metal centres.

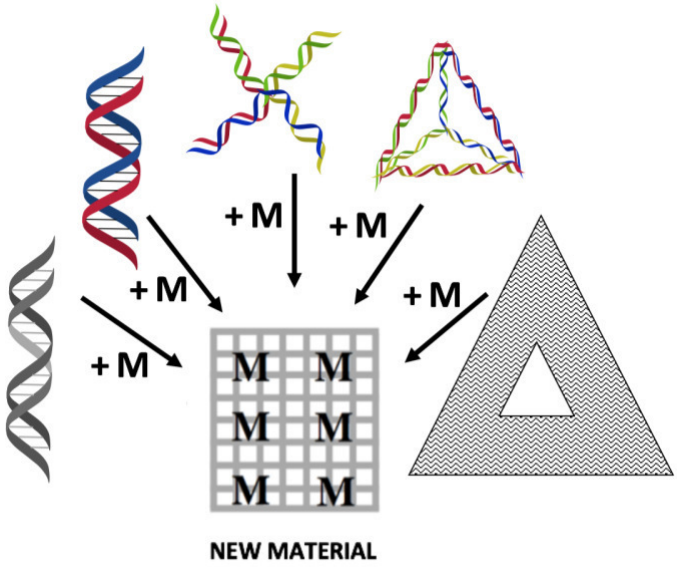



***Figure.*** *Schematic representation of the fabrication process to develop DNA-based catalytic material.*

This work is supported by DSTL.

***References:*** [1] Goodman, R. P., Berry, R. M. & Turberfield, A. J., *Chem. Comm.,* 2004, **12**, 1372-1373. [2] Malo, J., Mitchell, J. C. & Turberfield, A. J., *J. Am. Chem. Soc.,* 2009, **131**, 13574-13575. [3] Said, H. et al., *Nanoscale,* 2013, **5**, 284-290. [4] Jones, R. A., Li, W. X., Spaeth, H. & Steckl, A. J., *J. Vac. Sci. Technol. B.*, 2008, **26**, 2567–2571. [5] A. Sudik, J. Yang, D.J. Siegel, C. Wolverton, R.O. Carter and A.R. Drews, *J. Phys. Chem. C*, 2009, **113**, 2004.

*Corresponding author: E-mail: a.p.g.robinson@bham.ac.uk

# Elucidation of PAZ/3'overhang binding to improve the siRNA specificity

Andreia F. Jorge,[a] Adele Alagia,[b] Anna Avi , [b] Santiago Grijalvo, [b] Carme F brega, [b] Alberto Pais [a] and Ramon Eritja*[b]

[a] *CQC, Department of Chemistry, University of Coimbra, Rua Larga, 3004-535. Coimbra, Portugal,*
[b] *Dpt of Chemical and Biomolecular Nanotechnology. Institute for Advanced Chemistry of Catalonia (IQAC), CSIC, Barcelona, Spain and CIBER-BBN Networking Center on Bioengineering, Biomaterials and Nanomedicine, Spain.*

The discovery of the RNA interference (RNAi) pathway and the identification of the small interfering RNA (siRNA) molecule as RNAi trigger, have simplified the study of gene function paving the way to the treatment of any disease-related gene. The siRNA molecules are short double-stranded RNAs, of approximately nucleotides (nt) in length, bearing -nt overhangs at both -ends. The siRNA molecule is initially incorporated into the RISC (RNA-Induced Silencing Complex) in double stranded fashion, then a process called RISC maturation results in the dissociation of siRNA passenger strand from the guide strand, permitting the recognition of target messenger RNA (mRNA). The process of siRNA loading requires the action of the RISC- oading Complex (R C), which includes ICER, Argonaute (Ago ) and TR P (HIV- TAR RNA inding Protein) proteins. The Ago protein is a cradle-shaped protein of four main domains: MI (middle), PIWI (P-element-induced wimpy testes), PA (Piwi Argonaute wille) and N-terminal. Herein, we conduct a systematic study of Ago response by introducing chemical modifications at siRNA '-overhang by both computational and experimental techniques. Atomistic simulations and free energy calculations allow us to propose a robust and self-contained procedure for studying the factors governing PA siRNA interactions. Results from calculations based on two different crystal structures allow direct comparison and consistency checks. An overall description of the systems is thus achieved, which is followed by a comprehensive experimental study on the '-overhang structural requirements for the design of more specific siRNA molecules.

This work was supported by grants CT - 5-R, SGR , CI ER- N (C ). A. F. J. and T. F. G. G. C. acknowledge Funda ao para a Ci ncia e Tecnologia (FCT), Portugal, for financial support regarding the Post- octoral grant SFRH P 5 . C C is supported by FCT through projects PEst-OE I I and POCI- - 5-FE ER- .

***References:*** Elbashir, S.M. et al., *Nature* **2001**, *411*, . urnett, J.C. and Rossi, J,J. *Chem. Biol.* **2012**, *19*, . Alagia, A., et al. *Chem. Sci.*, **2018**, , .

*Corresponding author: E-mail: recgma@cid.csic.es

# Preparation of 1'-homo-*N*-2'-deoxy-α-nucleosides as both antiviral agents and monomers to synthesized oligonucleotides

Miguel Ferrero,*[a] Virginia Martín-Nieves,[a] Alejandro Carnero,[a] Yogesh S. Sanghvi,[b] Susana Fernández*[a]

[a] *Departamento de Química Orgánica e Inorgánica, Universidad de Oviedo, 33006-Oviedo (Asturias), Spain*
[b] *Rasayan, Inc. 2802 Crystal Ridge Road, Encinitas, California 92024-6615, USA*

Nucleoside analogues belong to an important class of antiviral and anticancer agents.[1] On the other hand, they are the structural units of antisense oligonucleotides and siRNA, which act in gene therapy.[2] Insertion of a methylene unit between a sugar moiety and a nucleobase transforms nucleosides into 1'-homonucleosides.

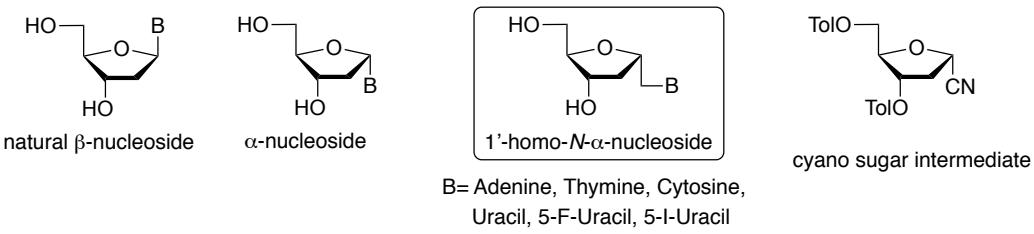



***Figure.*** *Structures of natural nucleosides and analogues, including 1'-homo-N-α-nucleosides.*

In a search for novel activities of nucleosides and/or oligonucleotides, we have synthesized 1'-homo-*N*-α-nucleosides with common natural bases as potential antiviral or antitumor agents. To date, the only one described in the literature is the thymidine derivative.[3] The new analogues were synthesized via coupling of a nucleobase and a tosylated intermediate sugar precursor, which was prepared from an easily accessible cyano sugar available on a large-scale. In addition, these important monomers will be use as building-blocks to prepare oligonucleotides. The antiviral assays are in progress.

**Acknowledgements:** Financial support by the Spanish Ministerio de Ciencia e Innovación (MICINN) (Projects CTQ2011-24237 and CTQ2014-55015-P) and Principado de Asturias (Project FC-15-GRUPIN14-002) are gratefully acknowledged.

***References:*** [1] a) Miller, P. S. *Antisense/Antigene Oligonucleotides,* in *Bioorganic Chemistry-Nucleic Acids.* (ed.); S. M. Hecht, Oxford University Press, **1996**, 347. b) Stambasky, J., et al. *Chem. Rev.* **2009**, *109*, 6729. [2] Guntaka, R. V., et al. *Int. J. Biochem. Cell Biol.* **2003**, *35*, 22. [3] Boal, J. H., et al. *J. Org. Chem.* **1996**, *61*, 8617.

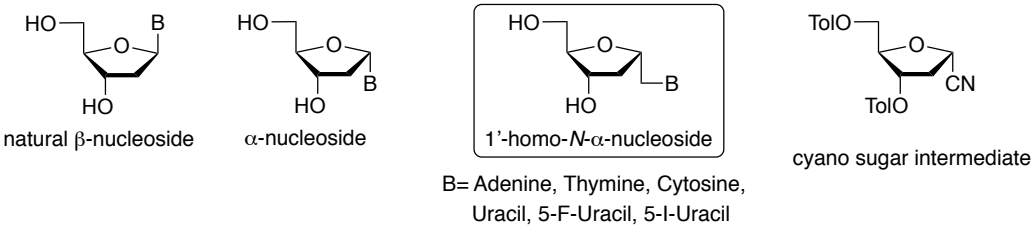
*Corresponding author: E-mail: mferrero@uniovi.es

# Preparation of 1'-homo-*N*-2'-deoxy-α-nucleosides: Potential antiviral agents and building-blocks for therapeutic oligonucleotides

Miguel Ferrero,*[a] Alejandro Carnero,[a] Virginia Martín-Nieves,[a] Olivia O. Russell,[b] Raymond F. Schinazi,[b] Yogesh S. Sanghvi,[c] Susana Fernández*[a]

[a]*Departamento de Química Orgánica e Inorgánica, Universidad de Oviedo, 33006-Oviedo (Asturias), Spain.* [b]*Center for AIDS Research, Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine, Atlanta, Ga 30322, USA.* [c]*Rasayan, Inc. 2802 Crystal Ridge Road, Encinitas, California 92024-6615, USA*

Nucleoside analogues belong to an important class of antiviral and anticancer agents.[1] On the other hand, they are the structural units of antisense oligonucleotides and siRNA, which act in gene therapy.[2] Insertion of a methylene unit between a sugar moiety and a nucleobase transforms nucleosides into 1'-homonucleosides.

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***References:*** [1] a) Miller, P. S. *Antisense/Antigene Oligonucleotides,* in *Bioorganic Chemistry-Nucleic Acids.* (ed.); S. M. Hecht, Oxford University Press, **1996**, 347. b) Stambasky, J., et al. *Chem. Rev.* **2009**, *109*, 6729. [2] Guntaka, R. V., et al. *Int. J. Biochem. Cell Biol.* **2003**, *35*, 22. [3] Boal, J. H., et al. *J. Org. Chem.* **1996**, *61*, 8617.

*Corresponding author: E-mail: mferrero@uniovi.es

# Towards the enzymatic formation of artificial metal base pairs with imidazole-modified nucleotides

Flamme Marie,[a,b] Müller Jens,[c] Hollenstein Marcel *[a,b]

[a] *Chimie ParisTech, PSL Research University, Laboratory for Inorganic Chemical Biology, F-75005 Paris, France.*
[b] *Laboratory for Bioorganic Chemistry of Nucleic Acids, Institut Pasteur, 28 rue du Dr. Roux, 75724 Paris Cedex 15, France.*
[c] *Westfälische Wilhelms-Universität Münster, Institut für Anorganische und Analytische Chemie, Corrensstr. 28/30 48149 Münster, Germany.*

The expansion of the genetic code is a long standing aim in biology that strives to create proteins with new or enhanced properties and structures as well as providing new tools for understanding the translational machinery.
In this project, we have developed new synthetic routes to generate a variety of chemically modified imidazole (Im) nucleoside triphosphates. Their potency at serving for the enzymatic construction of artificial metal base pairs has been tested. Particularly, the dependence of various metals is tested in order to improve the enzymatic construction of the Im-$M^{n+}$-Im base pair and identify the best metals cofactors.
The acceptance of those artificial base pairs is an essential prerequisite for their further use in selection experiments. The final aim of this project is to use this expanded genetic alphabet for the elaboration of aptamers. The latter are single-stranded oligonucleotides that bind to a broad variety of targets with high selectivity, affinity, and sensitivity. Thus, combinatorial selection process in presence of cancer cells will be carried out on a library of oligonucleotides containing the modified triphosphates. This process would allow the generation of a new selective therapeutic aptamer.

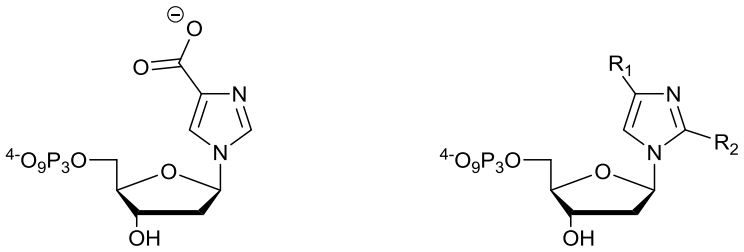



***Figure.*** *Modified imidazoles under investigation.*

This work was supported by grants from the doctoral school MTCI 5 University of Paris Descartes.

***References:*** [1] Röthlisberger, P. et al., *Org. Biomol. Chem.* **2017***, 15*, 4449. [2] Jash, B. et al., *Chem. Eur. J.* **2017***, 23*, 17166.
*Corresponding author: E-mail: marcel.hollenstein@pasteur.fr

# Inhibition of Lin28/pre-let-7 binding through short oligonucleotides

Alice Ghidini [a], Antoine Cléry [b], Timo Hagen [a], Evangelia Lekka [a], Frédéric H-T Allain [b], Jonathan Hall*[a]

[a] *Institute of Pharmaceutical Sciences, ETH Zürich, Zürich, Switzerland*
[b] *Institute of Molecular Biology and Biophysics, ETH Zürich, Zürich, Switzerland*

LIN28 is an RNA binding protein expressed in ESCs, with important roles in development and disease, which is involved in many biological processes, including development, reprogramming, pluripotency, metabolism, tissue regeneration and tumorigenesis. Humans express two isoforms of Lin28, LIN28 (Lin28A) and LIN28B (Lin28B), which bind to the let-7 primary and precursor microRNAs through bipartite recognition, close to the Dicer cleavage site, impeding let-7 biogenesis[1]. It has been shown that in cancer, the tumor suppressor function of let-7 is abrogated by overexpression of Lin28[2]. High levels of Lin28 are associated with advanced human malignancies, indicating that Lin28 acts as an oncogene.[3]

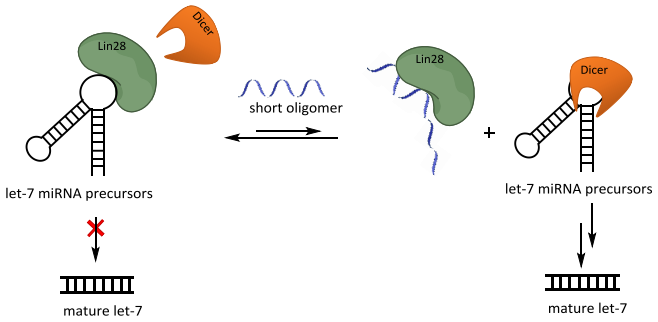



***Figure.*** *Short oligonucleotides designed to compete with let7 miRNA precursors for the active site of Lin28*

The precise mechanism of inhibition is still not completely understood. It has been shown with NMR structure that the two zinc knuckle domains of Lin28 are essential to establish a selective binding with pre-let-7 miRNAs. The structure reveals that each zinc knuckle recognizes an AG dinucleotide separated by a single nucleotide spacer[4]. Starting from the NMR structure, we designed a series of short oligonucleotides (7-12mers) able to target the Zinc fingers domain of Lin28 with the aim to inhibit the binding of the let-7 precursors, thereby de-repressing expression of let-7

This work was supported by the NCCR RNA & Disease from the Swiss National Science Foundation

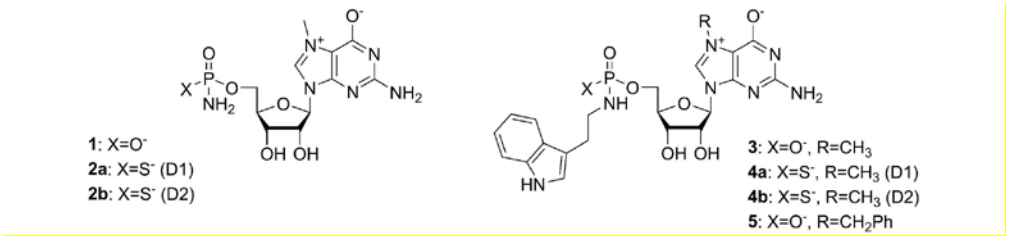
***References:*** [1] L. Wang, Y. Nam, A. K. Lee, C. Yu, K. Roth, C. Chen, E. M. Ransey, P. Sliz, *Cell Rep.* **2017**, *18*, 2664-2675; A. Desjardins, J. Bouvette, P. Legault, *Nucleic Acids Res.* **2014**, *42*, 4615-4628; S. R. Viswanathan, G. Q. Daley, R. I. Gregory, *Science* **2008**, *320*, 97-100. [2] I. Buessing, F. J. Slack, H. Grosshans, *Trends Mol. Med.* **2008**, *14*, 400-409. [3] S. Jiang, D. Baltimore, *Cancer Lett. (N. Y., NY, U. S.)* **2016**, *375*, 108-113. [4] F. E. Loughlin, L. F. R. Gebert, H. Towbin, A. Brunschweiger, J. Hall, F. H. T. Allain, *Nat Struct Mol Biol* **2011**, *19*, 84-89.

# Novel phosphoramidate and thiophosphoramidate cap analogs as potential pronucleotide translation inhibitors

Sebastian Golojuch[a,b], Michal Kopcial[b,c,d], Dominika Strzelecka[c], Joanna Kowalska[c], Jacek Jemielity*[a]

[a] *Centre of New Technologies, University of Warsaw, Banacha 2c, Warsaw, Poland*
[b] *Faculty of Chemistry, University of Warsaw, Pasteura 1, Warsaw, Poland*
[c] *Division of Biophysics, Faculty of Physics, University of Warsaw, Pasteura 5, Warsaw, Poland*
[b] *College of Inter-Faculty Individual Studies in Mathematics and Natural Sciences, University of Warsaw, Banacha 2c, Warsaw, Poland*

The mRNA cap has at least two major functions. It protects RNA from hydrolysis caused by exonucleases and it interacts with eukaryotic Translation Initiation Factor 4E (eIF4E) initiating protein biosynthesis.[1] It has been shown, that aberrant cap dependent translation in cancer cells is related to overexpression of eIF4E.[2] Furthermore it has been also shown, that inhibition of eIF4E expression reduces tumor growth without toxicity.[3] The cap structure is thereby an attractive starting point for the drug design. However, although several analogs of cap have demonstrated utility as therapeutics, their usability is limited by the low membrane permeability.[4] As one of the solutions to overcome this obstacle, development of prodrug methodology involving pronucleotides has been proposed.[4]
We report novel 7-methylguanosine monophosphate ($m^7$GMP) pronucleotide analogs bearing phosphoramidate or thiophosphoramidate moiety. Introduction of the phosphoramidate moiety was expected to increase cell permeability owing to the phosphate charge masking effect.[5] The thiophosphate modification was introduced to increase the affinity for eIF4E. We also synthesized previously reported translation inhibitor pronucleotide **5** as a reference for further biophysical and biological studies. Enzymatic activation of the pronucleotides were investigated in HEK extracts. It has demonstrated that obtained compunds are transformed enzymatically from their pronucleotide form to corresponding nucleotide in cell extracts.




***References:*** [1] Ziemniak, M., et al. Fut. Med. Chem. **2013**, *5*, 1141-1177. [2] Sun, S., et al. Cancer Res. **2005**, *65*, 7052-7059. [3] Graff, J. R., et al. J. Clin. Invest. **2007**, *117*, 2638-2648. [4] Wagner, C. R., et al. Med. Res. Rev. **2000**, *20*, 417-451. [5] Li, S., et al. Mol. Pharm. **2013**, *10*, 523-531.

*Corresponding author: E-mail: j.jemielity@cent.uw.edu.pl

# Reversible RNA acylation for CRISPR-Cas9 gene editing control in cells

Maryam Habibian, Colin McKinlay, Timothy R. Blake, Anna M. Kietrys, Robert M. Waymouth, Paul A. Wender, Eric T. Kool*

*Department of Chemistry, Stanford University, 450 Serra Mall, Stanford, CA 94305, USA*

CRISPR-Cas9 has emerged as a powerful tool that readily enables the modification of the genome. Methods for modulating CRISPR function could be powerful tools in research and in future diagnostics applications. Here, we report the development of a post-transcriptional sgRNA modification method that allows for chemical control over the CRISPR-Cas9 gene editing activity both *in vitro* and in living cells. We have recently described reversible RNA acylation of 2′-OH groups as a selective and mild chemical approach to controlling RNA hybridization, folding, and enzyme interactions. [1] Here we show that an azide-substituted acy limidazole reagent (NAI-$N_3$) efficiently acylates CRISPR single guide RNAs (sgRNAs) in 20 minutes in buffer. sgRNA that was poly-acylated ("cloaked") using this method completely inhibited the endonuclease activity of Cas9 *in vitro* and in living HeLa cells, when was efficiently delivered via CART cationic polymers. [2] sgRNA activity was, subsequently, efficiently recovered both *in vitro* and in cells by treatment with water-soluble phosphines. These phosphine agents trigger Staudinger reduction of the azide and spontaneous loss of acyl groups ("uncloaking"). Our study highlights the utility of reversible RNA acylation as a novel method for temporal control of genome-editing function.

***References:***

[1] Kadina, A. et al., *Angew. Chem. Int. Ed.* ***2018****, 57,* 3059.
[2] McKinlay, C. J. et al., *Proc. Natl. Acad. Sci.* **2017**, 114, E448.

*Corresponding author: E-mail: kool@stanford.edu

# Synthesis of Nucleoside Antibiotics Containing Emissive Nucleobase Surrogates

Kaivin Hadidi and Yitzhak Tor[a]

[a] *Department of Chemistry and Biochemistry, University of California San Diego, La Jolla, California 92093-0358, United States*

The synthesis and potential biochemical utility of fluorescent adenosine-based antibiotics incorporating a thieno[3,4-*d*]-pyrimidine heterocyclic nucleus is illustrated. Analogs of the antibiotics puromycin, cystocin, lysylaminoadenosine, and homocitrullylaminoadenosine are attained through a common ribonucleoside precursor containing an $N^6$,$N^6$ dimethylated nucleobase and an azide at the 3' position of the ribose unit (Figure 1).

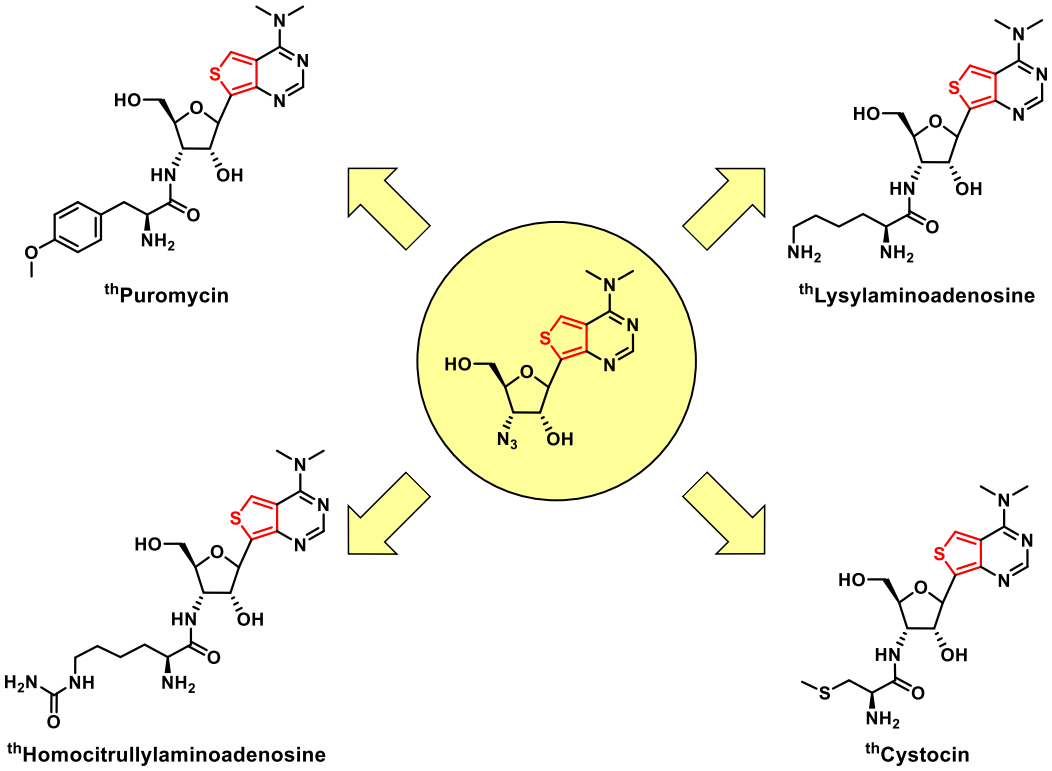



***Figure 1.*** *Fluorescent analogs of adenosine-based nucleoside antibiotics.*

This work was supported by the National Institutes of Health (grant number GM069773).

*Corresponding author: E-mail: ytor@ucsd.edu

# Novel Approach to the Determination of Nucleosome Structure Using the Highly Emissive Nucleobase $^{th}$dG-tC FRET Pair

Jihun Han[a], Soyoung Park[*a] and Hiroshi Sugiyama[*a,b]

[a] *Department of Chemistry, Graduate School of Science, Kyoto University*
[b] *Institute for Integrated Cell-Material Science (iCeMS), Kyoto University*

The structural changes of a nucleosome, in which nucleosome is basic structural unit of eukaryotic chromatin, are important key to the understanding of the mechanism of genetic process. Förster Resonance Energy Transfer (FRET) is a useful tool to investigate structural dynamics of nucleosomes such as unfolding, unwrapping and repositioning.

In the previous study, fluorophores such as Cy3 or Cy5 were conjugated to DNA base and/or histone protein via flexible linkers.[1,2] However, rotational freedom of these dyes is a drawback for accurate analysis of the conformational dynamics. Very recently, we have developed a novel FRET system that consists of 2-aminothieno[3,4-d]pyrimidine G-mimic deoxyribonucleoside ($^{th}$dG) as a donor and 1,3-diaza-2-oxophenothiazine (tC) as an acceptor.[3] In this study, we report that the $^{th}$dG–tC FRET pair was successfully incorporated to 145 bp 601 sequences, and different FRET efficiencies were obtained for the designated donor and acceptor positions in the nucleosome.

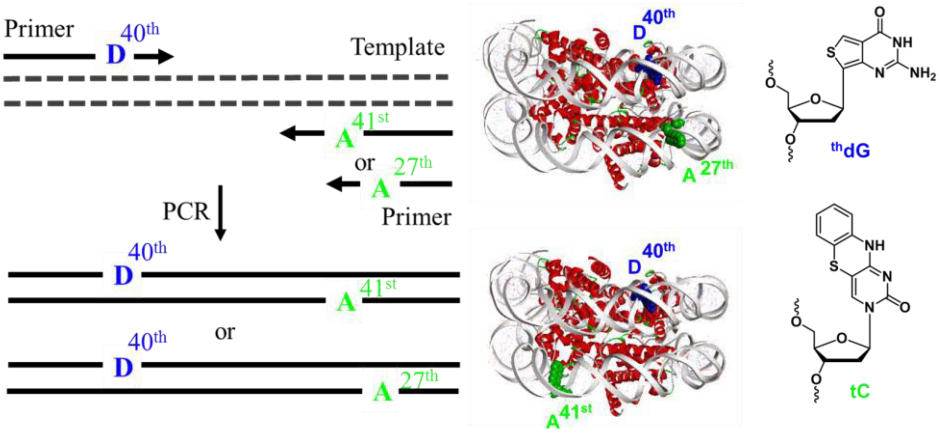



***Figure.*** *Preparation of 145bp 601 sequences containing donor ($^{th}$dG) and acceptor (tC) and schematic illustration of positions upon formed nucleosome.*

***References:*** [1] G. Li, J. Widom, *Nat. Struct. Mol. Biol.*, **2004**, 11, 763-769. [2] R. Buning, J. van Noort, *Biochimie*, **2010**, 92, 1729-1740. [3] J. H. Han, S. Yamamoto, S. Park, H. Sugiyama, *Chem. Eur. J.,* **2017**, 23, 7607-7613.

[*]Corresponding author: oleesy@kuchem.kyoto-u.ac.jp, hs@kuchem.kyoto-u.ac.jp

# Palladacyclic conjugate group promotes hybridization of short oligonucleotides

Madhuri Hande,[a] Tuomas Lönnberg*[a]

*Department of Chemistry, University of Turku, Vatselankatu 2, 20014 Turku, Finland*

Replacing a hydrogen bond by metal ion offered a high thermal stability for short oligonucleotides with cyclopalladated benzylamine moieties at their 5´-termini have been prepared to test the possibility of conferring palladacyclic anticancer agents sequence selectivity by conjugation with a guiding oligonucleotide. Hybridization of these oligonucleotides with natural counterparts was studied by UV and CD melting experiments in the absence and presence of a competing ligand (2-mercaptoethanol). Cyclopalladated benzylamine proved to be strongly stabilizing relative to unmetalated benzylamine and modestly stabilizing relative to an extra A•T base pair. The stabilization was largely abolished in the presence of 2-mercaptoethanol, suggesting direct coordination of Pd(II) to a nucleobase of the complementary strand. In all cases, fidelity of Watson-Crick base pairing between the two strands was retained. HybriPdization of the cyclopalladated oligonucleotides was characterized by relatively large negative enthalpy and entropy, consistent with stabilizing Pd(II) coordination partially offset by the entropic penalty of imposing conformational constraints on the flexible diethylene glycol linker between the oligonucleotide and the palladacyclic moiety.

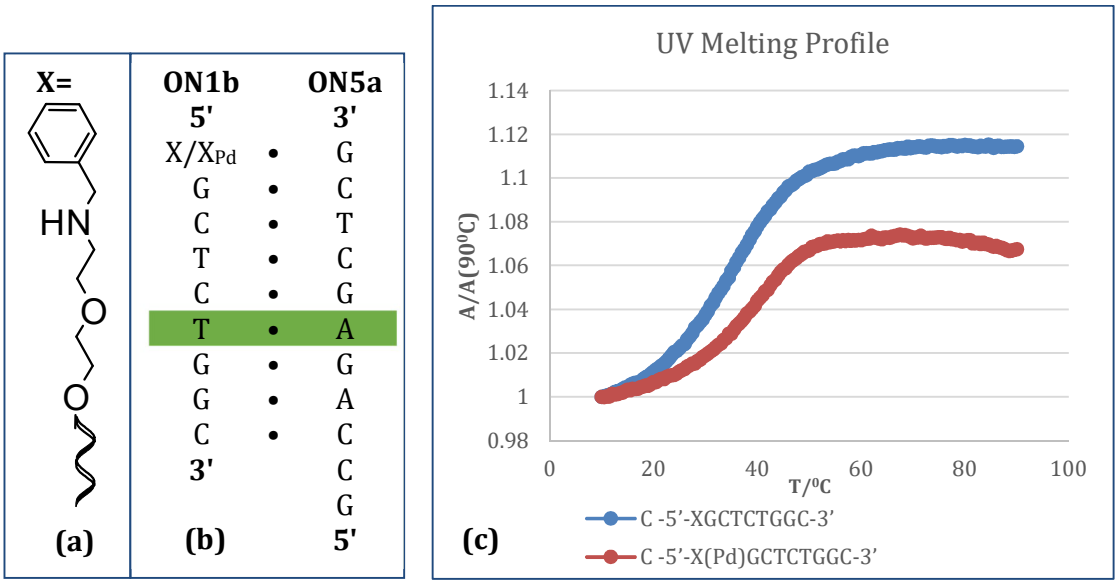



***Figure:*** a. Chemical modification of at 5'terminal end of oligonucleotide; b. duplexes used for thermal denaturation; c. UV Melting profile of palladated and non palladated oligos (ON1b) forming duplexes with ON5a.

This work was supported by MMBio – Marie Curie Training Network.

***References:*** [1] Mergny JL, Lacroix L. Analysis of thermal melting curves. Oligonucleotides. 2003 Dec 1;13(6):515-37.

*Corresponding author: E-mail: tuanlo@utu.fi

# Fluorescent nucleoside probes of DNA hybridization and cytosine deamination

Daniel A. Harki,*[a] Kellan T. Passow,[a] Ramkumar Moorthy,[a] Sydney A. Schmidt,[a] Margaret E. Olson[a]

[a] *Department of Medicinal Chemistry, University of Minnesota, Minneapolis, MN, USA*

Fluorescent nucleoside analogues are powerful chemical probes for diverse applications in nucleic acid chemistry and biology. We have recently discovered that the 4-cyanoindole-containing nucleoside, 4CIN, is a remarkably efficient isomorphic nucleoside analogue with a large Stokes shift (107 nm) and high quantum yield as the nucleoside (0.92) and in double-stranded DNA (QY = 0.15 – 0.31 depending on sequence).[1] The discovery, characterization, and some applications of 4CIN will be presented. Additionally, our group has also focused significant attention on the chemical biology of APOBEC DNA cytosine-to-uracil deaminases, which are exciting targets in antiviral and anticancer drug design.[2] To augment our arsenal of assays for measuring cytosine deamination, we have developed novel cytidine analogues that can quantify enzyme-catalyzed deamination by changes in fluorescence properties. The development and utilization of these new reagents will also be presented.

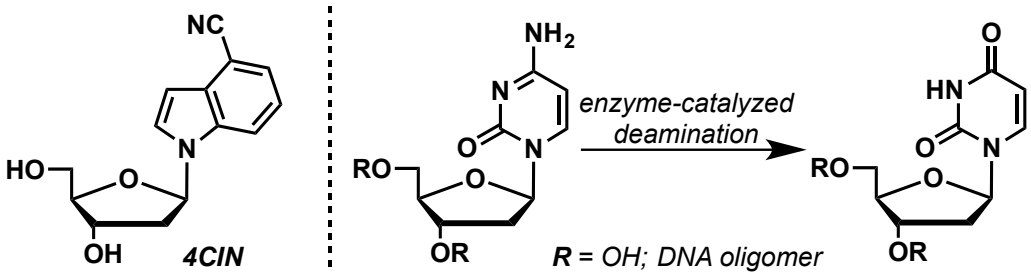



***Figure.*** *Fluorescent 4-CIN (left) and enzyme-catalyzed cytidine deamination (right).*

This work was supported by grants from the NIH (R01-GM110129 and R01-GM118000).

***Reference:*** [1] Passow, K. T. et al., *Submitted.* [2] Olson, M. E. et al., *Cell Chem. Biol.* **2018**, *25*, 36.

*Corresponding author: E-mail: daharki@umn.edu

# Synthetic Ligand for Mitochondrial DNA Sequence Recognition and Promoter-Specific Transcription Suppression

Takuya Hidaka,[a] Ganesh NAMASIVAYAM Pandian,[b] Toshikazu Bando,[a] Hiroshi Sugiyama*[a,b]

[a] *Graduate School of Science, Kyoto University, Kyoto, Japan*
[b] *Institute for Integrated Cell-Material Sciences, Kyoto University, Kyoto, Japan*

Recently, treatment of mitochondrial diseases have been gaining increasing attention and targeted alteration of mutated mitochondrial DNA is believed to have clinical prospects. We have been studying Pyrrole-Imidazole Polyamides (PIP) that can bind to DNA in a sequence-specific manner and it is promising to control replication and transcription of mitochondrial DNA basing on their DNA sequence. But the default distribution of PIP to nuclei is a problem for this purpose.
In this study, we introduced mitochondria-penetrating peptides (MPP) [1] which are composed of L-Cyclohexylalanine and D-Arginine to PIP and developed "MITO-PIP" that localizes into mitochondria and control transcription in mitochondria. [2]

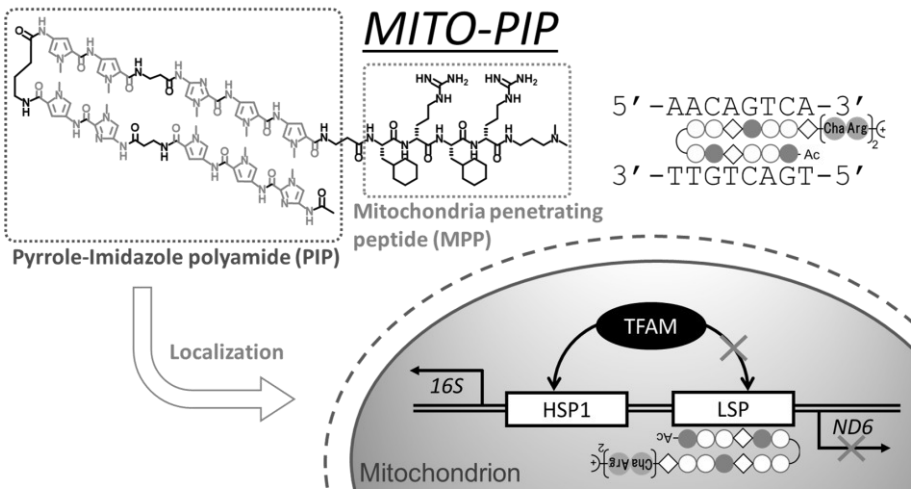



***Figure.*** *Structure of MITO-PIP and its mechanism to control mtDNA transcription.*

MITO-PIP targeting mitochondrial transcription factor A (TFAM) binding site in light-strand promoter (LSP) region successfully repressed expression of LSP downstream gene (*ND6*) while kept the expression level of HSP downstream gene (*MT-16S*).

This work was supported by grants from the Japan Society for the Promotion of Science and Kyoto University SPIRITS grant.

***References:*** [1] Horton, K. L., Kelley, S. O., *et al*., *Chem. Biol.* **2008**, *15*, 375. [2] Hidaka, T., Sugiyama, H., *et al., J. Am. Chem. Soc.* **2017**, *139*, 8444.

*Corresponding author: E-mail: hs@kuchem.kyoto-u.ac.jp

# AJIPHASE®: Development of Solution-Phase Oligonucleotide Manufacturing Process through Suppression of Impurities

Kunihiro Hirai, Taisuke Ichimaru, Ken Yamashita, Takayuki Hamada, Naoko Hirose, Daisuke Takahashi*

*Research Institute for Bioscience products and Fine chemicals, AJINOMOTO Co., Inc.*

Recent progress in various types of oligonucleotide therapeutics, such as antisense, aptamer, siRNA and miRNA, has led to growing demand for economical and/or large scale production. Conventionally-known solid-phase synthesis which has been only a method of choice over the past few decades rapidly supplies oligonucleotides in high quality but in a limited quantity. Under these circumstances, a new process for large scale manufacturing has been required to meet the future demands.

We have initially developed a unique and efficient AJIPHASE® technology as a liquid phase peptide synthesis [1-3]. And we successfully applied this technology to oligonucleotides synthesis using phosphoramidite chemistry. As a feature of AJIPHASE® technology, each reaction steps consist of coupling, sulfurization and detritylation were carried out continuously, thus isolation steps decreased to once for one elongation cycle. This one-pot reaction system was useful for enhancing yield and synthesis speed, even though not only N±1mer as major impurities but several unknown impurities were observed in the initial one-pot synthetic products.

As a result of several research, we achieved substantial improvement on our synthetic process by suppressing those impurities along with elucidation of the reaction mechanism, which led to high purity products beyond the purity of solid phase synthesis products. Moreover, the large scale manufacturing of oligonucleotide has provided conclusive proof that AJIPHASE® is a practical and efficient manufacturing method.

***References:*** [1] Takahashi, D., et al. *Tetrahedron Lett*. **2012**, *53*, 1936. [2] Takahashi, D., et al. *Organic Lett.* **2012**, *14, (17),* 4514. [3] Takahashi, D., et al. *Angew. Chem.* **2017**, *27, (56),* 7803

*Corresponding author: E-mail: ajiphase@ajinomoto.com

# siRNA-GalNAc conjugation strategy for delivery of LDLR and cholesterol modulating siRNA acting via transcriptional silencing of long non-coding RNA

Anders Højgaard Hansen[1,a], Roslyn M. Ray[1,b], Annika Carstens[a], Maria Taskova[a], Sofie Slott[a], Kevin V Morris[b] and Kira Astakhova[*,a]

[a] Department of Chemistry, Technical University of Denmark, Lyngby, Denmark
[b] The Center for Gene Therapy, Beckman Research Institute - City of Hope, Duarte CA, USA

The low-density lipoprotein receptor (LDLR) is a cell surface receptor able to bind and transport cholesterol-rich LDL into the cell thereby causing a lowering of blood cholesterol[1]. Herein, we report on small interfering RNAs (siRNA) targeted towards the long non-coding (Inc) RNA BM450697 (**si5**) or the promoter region of BM450697 (**pro5**). To increase metabolic stability and affinity for target sequences, siRNA sense strands were modified by insertion of phosphorothioates and 2'-OMe, respectively, and further conjugated to α-*N*-acetylgalactosamine (α-GalNAc) at the 3' end using copper(I)-catalyzed alkyne-azide cycloaddition to ensure effective uptake into hepatocytes (Hep3B cells) by the asialoglycoprotein receptor.

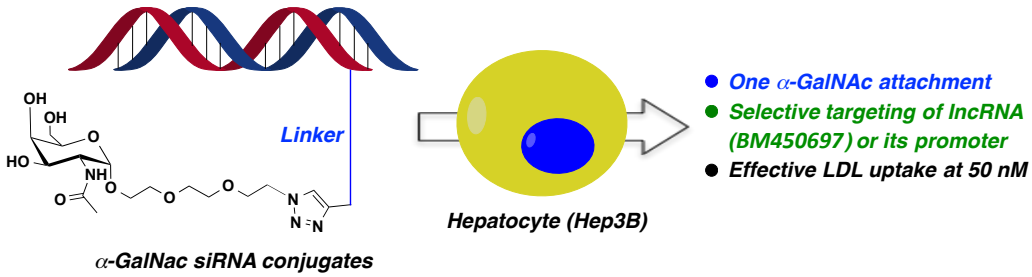



***Figure.*** *Ability of α-GalNAc siRNA conjugates **si5** and **pro5** to modulate uptake of LDL in hepatocytes.*

In Hep3B cells, α-GalNAc siRNA conjugates (**si5** and **pro5**) were found to significantly increase LDLR mRNA expression and decrease the lncRNA BM450697 in a dose dependent manner. Furthermore, 50 nM of **si5** and **pro5** were as efficacious as 1 μM lovastatin at promoting increase in LDL uptake. These results suggest that α-GalNAc siRNA conjugates **si5** and **pro5** could represent potential new therapeutics for treatment of hypercholesterolemia.

***References:*** [1] Goldstein, J. L.; Brown, M. S, *Arterioscler. Thromb. Vasc. Biol*. **2009**, *29*, 431.

[*]Corresponding author: E-mail: kiraas@kemi.dtu.dk

# Ethynylphosphonate-linked nucleic acid: A novel design for restriction of the torsion angle β

Masahiko Horiba,[a] Takao Yamaguchi,[a] Satoshi Obika *[a,b]

[a] *Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka, Japan*
[b] *National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN), 7-6-8 Saito Asagi, Ibaraki, Osaka, Japan*

In the past few decades, a lot of chemically-modified nucleic acids have been developed for oligonucleotide-based therapeutics. One of the purposes of introducing chemical modifications to oligonucleotides is to acquire high duplex-forming ability toward complementary DNA or RNA. It is well known that oligonucleotides modified with artificial nucleic acids, which mimic the structure of RNA and restrict the conformational flexibility of nucleotide, exhibit enhanced duplex-forming ability. Here, we focus on the flexibility of the phosphodiester backbone. The torsion angle β (P-O5'-C5'-C4') adopts a value near 180˚ in both the A-form duplex and the B-form duplex, meaning that the four atoms are on the same plane in the duplexes [1]. The ethynyl group has the straight and inflexible structure; thus we have expected that the ethynylphosphonate linkage can mimic the conformationally restricted phosphodiester linkage in duplexes.

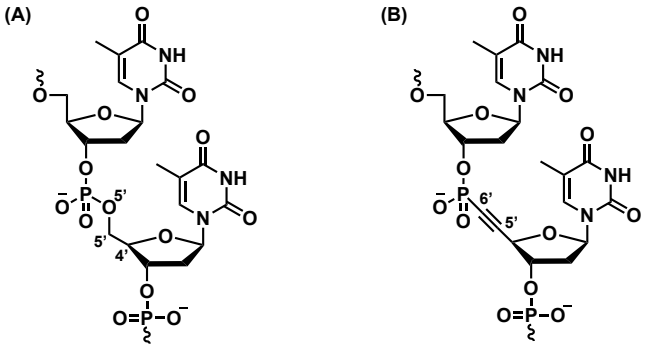



***Figure.*** *Structures of the phosphodiester (A) and the ethynylphosphonate (B) linkages.*

An ethynylphosphonate-linked thymidine dimer was synthesized by Pd(0)-catalyzed cross-coupling reaction of 3'-*O*-*H*-phosphonate-thymidine and 5'-deoxy-5'-dibromoalkenyl-thymidine. The dimer was converted to the amidite block, which was then incorporated into an oligonucleotide. Unexpectedly, the thermodynamic stability of the duplexes between the oligonucleotide modified with the ethynylphosphonate linkage at the center of the sequence and complementary DNA or RNA was lower than that of the natural DNA/DNA or DNA/RNA duplex. We would like to discuss the cause of the low duplex-forming ability from the aspect of the structure and the electron density of the ethynylphosphonate linkage.

This work was supported by the Japan Society for the Promotion of Science (JSPS), the Ministry of Education, Culture, Sports, Science, and Technology in Japan (MEXT).

***Reference:*** [1] Berman, H. M. et al., *Biopoly*. **1997***, 42*, 113.

*Corresponding author: E-mail: obika@phs.osaka-u.ac.jp

# Synthesis and properties of *N*-alkylated guanidine-bridged nucleic acids

Naohiro Horie,[a] Shinji Kumagai,[b] Hiroaki Sawamoto,[b] Takao Yamaguchi,[a] Satoshi Obika*[a,c]

[a] *Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan.*
[b] *Mitsubishi Tanabe Pharma Corporation, 1000 Kamoshida, Aoba-ku, Yokohama 227-0033, Japan.*
[c] *National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN), 7-6-8 Saito-Asagi, Ibaraki, Osaka 567-0085, Japan.*

Oligonucleotides having guanidine-bridged nucleic acid (GuNA) show attractive properties, such as high binding affinity toward complementary single-stranded RNA (ssRNA) and DNA (ssDNA), and a far superior enzymatic stability to natural oligonucleotides[1]. In our recent study, GuNA analog bearing a methyl group at the guanidine moiety (GuNA[NMe]) was successfully synthesized, and the binding affinity and the enzymatic stability of GuNA[NMe]-modified oligonucleotides were found to be comparable to those of GuNA-modified oligonucleotides. Based on these results, we expected that additional functionalities could be incorporated into the guanidine moiety of GuNA. In this study, to know if a steric bulkiness and a hydrophobicity of *N*-substituent groups affect on the duplex-forming ability and enzymatic stability, we synthesized and evaluated several *N*-alkylated GuNA analogs (GuNA[NEt], GuNA[N*i*-Pr], and GuNA[N*t*-Bu]).

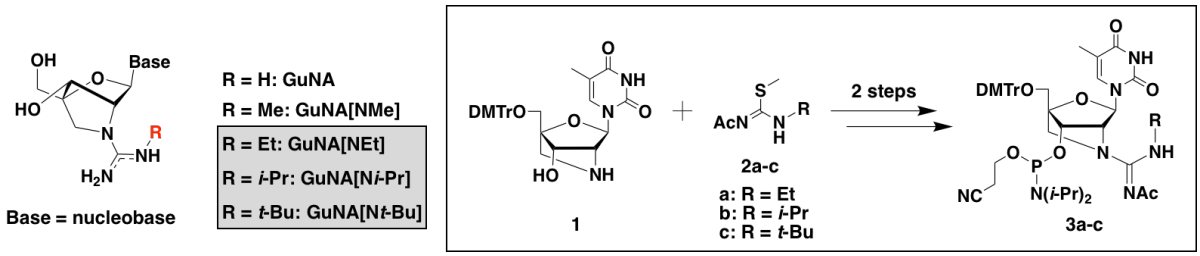



***Figure.*** *Structures of GuNA analogs and their synthetic scheme*

From amino LNA derivative **1**, GuNA[NR] building blocks **3a-c** were synthesized in two steps by using isothioureas **2a-c** as guanidination reagents. These GuNA[NR] building blocks were successfully introduced into oligonucleotides. After the synthesis, we evaluated the binding affinity of these oligonucleotides toward complementary ssRNA. To our surprise, as the steric bulkiness of *N*-substituent groups increases, the duplexes become more stable. This result importantly suggested that a chemical modification to the guanidine moiety might contribute to the duplex stability. The details of synthetic studies, binding affinities, and enzymatic stabilities of the GuNA[NR]-modified oligonucleotides will be discussed.

***References:*** [1] Shrestha A. R., Kotobuki Y., Hari Y., Obika S., *Chem. Commun.*, **2014**, *50*, 575.

*Corresponding author: E-mail: obika@phs.osaka-u.ac.jp

# Solid-Phase Sub-Monomer DNA Synthesis

Horn, Thomas *[a]

[a] *Nuatech LLC, Molecular Foundry, Lawrence Berkeley National Laboratory, Berkeley, CA.*

Solid-phase sub-monomer synthesis (SPSMS) of DNA using H-phosphonate (P III) chemistry was investigated. The approach eliminates the need for pre-made fully protected nucleoside phosphoramidites

In the first step the oligomer is extended with a H-phosphonate moiety (diphenyl H-phosphonate followed by hydrolysis [1]); in the second step the H-phosphonate moiety is activated with PyBOP and coupled to a 5'-O- or 3'-O-Fmoc nucleoside [2]; in the third step the intermediate H-phosphonate diester is oxidized to a stable phosphorothiotriester [3]; finally, in the fourth step the Fmoc protecting group is removed and the cycle repeated.

Diphenyl H-phosphonate does not react with exo-cyclic amines of nucleosides [1]. PyBOP is a powerful activator of phosphonates [2], and does not react with hydroxy groups of nucleosides, the free hydroxy group of the growing oligomer, or with unprotected exo-cyclic amines of nucleosides [2]. Further, protecting groups on exo-cyclic amino groups are not required. 5'-O-Fmoc protected nucleosides with unprotected exo-cyclic amines were prepared in three steps from the nucleoside [4]. A number of short mixed-sequence oligomers were prepared in high yields.

SPSMS can also be used to make Phosphoramitoids, a new type of sequence-controlled polymer [5]. They contain a diol/phosphoramidate back-bone displaying unique side-chain groups. In step 2 the chain is extended with unprotected trans-1,4-cyclohexanediol, a stericly constrained diol [6]. In step 3, oxidation to phosphoramidate is performed in the presence of an amine and carbontetrachloride [7]. A number of different side-chain amines were successfully incorporated in high yields.

Detailed results of the syntheses of DNA and phosphoramitoids will be presented.

Work at the Molecular Foundry is supported by the Office of Science, Office of Basic Energy Sciences, of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231.

***References:*** [1] J. Jankowska, et al., *Tet. Lett.* **1994**, *35*, 3355-3358. [2] Wada, T. et al., *J. Am. Chem. Soc.* **1997**, *119*, 12710-1272. [3] Dreef, C.E. et al., Synlett **1990**, 481-483. [4] Ohkubo, A. et al. *Org. Lett.* **2010**, *12,* 2496-2499. [5] J.-F. Lutz, et al., *Science* **2013**, *341*, 1238149. [6] T. Wada, et al, *Tet. Lett.* 1**999**, *40*, 915-918. [7] F.R. Atherton, et al., *J. Chem. Soc.* **1945**, 660-663.

*Corresponding author: E-mail: Thomas.horn@gmail.com

# Computational studies of double-headed nucleotides in DNA

Mick Hornum,[a] Julie Stendevad,[a] Michael Petersen,[a] Poul Nielsen*[a]

[a] *Department of Physics, Chemistry and Pharmacy, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark*

The four "double-headed" nucleotides $\mathbf{U_C}$, $\mathbf{U_G}$, $\mathbf{U_T}$ and $\mathbf{U_A}$ have recently been presented by us.[1,2] As dinuncleotide mimics,[3] these structures allow genetic information in DNA to be condensed to fewer phosphates; i.e. each nucleotide unit may encompass two base pairs. Since the same molecular information can be delivered using a shorter sequence and decreased polyanionic charge, this design may be used to improve cellular uptake of exogenous nucleic acids or ease drug formulation.

More recently, we have attained two new double-headed nucleotides, $\mathbf{U_D}$ and $\mathbf{U_I}$ containing diaminopurine and hypoxanthine bases, respectively. To establish the propensity of the double-headed nucleotides to communicate efficiently with a cognate strand, we have now supplemented our experimental work with detailed molecular dynamics simulations. Scrutinization of the dynamic DNA structures containing $\mathbf{U_D}$ and $\mathbf{U_I}$ in addition to $\mathbf{U_T}$, $\mathbf{U_A}$ has now allowed us to precisely identify their base-pairing behavior and level of specificity.

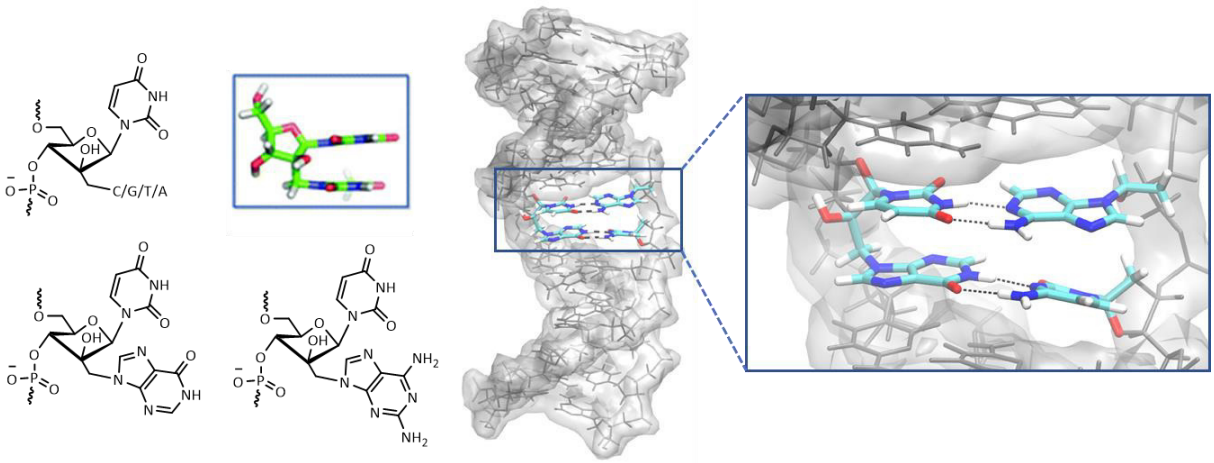



***Figure.*** *(Left) Structures of double-headed nucleotides. (Right)* $\mathbf{U_I}$ *exhibiting dual pairing to A and G in a DNA duplex.*

This work was supported by grants from the Villum Foundation and the Danish Council for Independent Research | Natural Sciences.

***References:*** [1] Kumar, P. et al. *J. Org. Chem.* **2014**, *79*, 11534. [2] Hornum, M. et al., *Chem. Commun.* **2017**, *53*, 9717. [3] Sharma, P. K. et al., *Aust. J. Chem.* **2016**, 69, 1094.

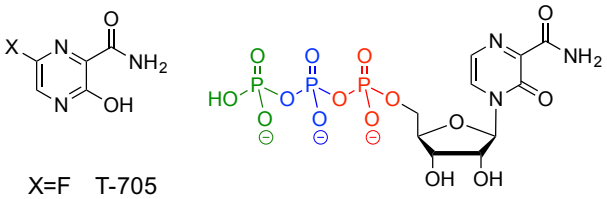
*Corresponding author: E-mail: pouln@sdu.dk

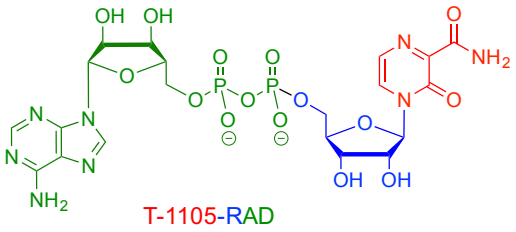
# Chemistry and Biology of Hydroxypyrazinecarboxamide Pseudobases T-705 and T-1105

Johanna Huchting,*[a,b] Evelien Vanderlinden,[b] Lieve Naesens,[b] Chris Meier[a]

[a] *Department of Chemistry, Hamburg University, Martin-Luther-King-Platz 6, D-20146, Germany;* [b] *Rega Institute for Medical Research, KU Leuven, Herestraat 49, BE-3000 Leuven, Belgium*

The hydroxypyrazine carboxamide pseudobase T-705 and its de-fluoro-analogue T-1105 exhibit promising antiviral activities against various RNA viruses.[1] Their ribonucleoside 5'-triphosphates were demonstrated to inhibit viral RNA polymerases via different mechanisms;[2-4] and it was shown that T-705/ T-1105-treatment increases the mutation frequency in replicating RNA virus genome.[5-7] Inefficient nucleoside analogue-conversion to NTP is long-known to limit antiviral potency,[8] and it was shown that phosphoribosylation by the host enzyme HGPRT of both hydroxypyrazine compounds requires high concentrations of co-substrate phosphoribosyl pyrophosphate,[9] and then still is inefficient.




We present the synthesis of ribonucleoside mono-, di- and triphosphate prodrugs of T-1105 and discuss the di- and triphosphate prodrugs' enhanced antiviral potency as well as successful metabolic bypass. Further, we show synthesis of T-1105-ribonucleoside, 5'-monophosphate (-RMP), 5'-diphosphate (-RDP) and 5'-triphosphate (-RTP), and discuss differences in the single steps of host-enzyme-catalysed phosphorylation towards the active NTP. Finally, we address the structural resemblance of the carboxamide pseudobase to nicotinamide, which has hardly been recognized in any biological study before. Synthesis of the novel NAD-analogue T-1105-RAD via the *cyclo*Sal-method[10] is presented and biological properties of this synthetic metabolite are discussed.

Presented work was supported by grants from the Deutsche Forschungsgemeinschaft (HU 2350/1-1) and the Flemish Fonds voor Wetenschappelijk Onderzoek (FWO grant no. 1509715N), and scholarships from Chu Family Foundation and from DAAD (German Academic Exchange Service).

***References:*** [1] Delang, L., et al. *Antiviral Res.* **2018,** *153*, 85. [2] Jin, Z., et al. *PLoS One* **2013,** *8*, e68347. [3] Barauskas, O., et al. *PLoS One* **2017,** *12*, e0185998. [4] Dulin, D., et al. *Cell Rep.* **2017,** *21*, 1063. [5] Baranovich, T., *J. Virol.* **2013,** *87*, 3741. [6] Vanderlinden, E., et al. *Antimicrob. Agents Chemother.* **2016,** *60*, 6679. [7] Qiu, L., et al. *PLoS Neglected Trop. Dis.* **2018,** *12*, e0006421. [8] Balzarini, J., et al. *Biochem. Pharmacol.* **1988,** *37*, 897-903. [9] Naesens, L., et al. *Mol. Pharmacol.* **2013,** *84*, 615. [10] Warnecke, S., Meier, C., *J. Org. Chem.* **2009,** *74*, 3024-3030.

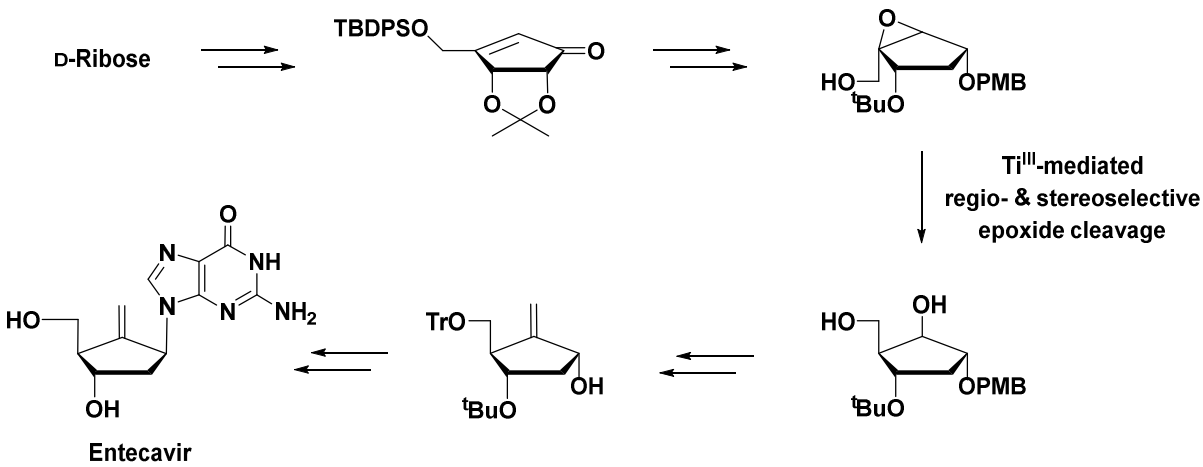
*Corresponding author: E-mail: johanna.huchting@chemie.uni-hamburg.de

# Efficient Synthesis of Anti-Hepatitis B Virus (HBV) Agent, Entecavir

Young Eum Hyun and Lak Shin Jeong*

*College of Pharmacy, Seoul National University, Seoul 08826, South Korea*

Entecavir (Baraclude®) was approved by the FDA in 2005 and has become one of the most prescribed anti-HBV drugs. It is a deoxyguanosine analogue that belongs to carbocyclic nucleosides. Bristol-Myers Squibb (BMS) was the original patent holder for entecavir, but its drug patent was expired in 2015. Because only a few synthetic methods for entecavir have been reported, we have been interested in developing alternative methodology to entecavir. Thus, we have developed efficient synthetic methodology of entecavir, using regioselective isopropylidene cleavage, stereoselective Sharpless epoxidation, and $Ti^{III}$-mediated regio- and stereoselective epoxide cleavage [1] as key steps [2].




***References:*** [1] Nugent, W. A.; Rajanbabu, T.V. *J. Am. Chem. Soc.* **1994**, *116*, 986. [2] Hyun, Y. E.; Kim, H.-R.; Choi, Y.; Jeong, L. S. *Asian J. Org. Chem.* **2017**, *6*, 1213.

*Corresponding author: E-mail: lakjeong@snu.ac.kr

# Creation of ischemia-selective oligonucleotide therapeutics system with intracellular condition-responsive Peptide Ribonucleic Acids (PRNAs): *Design and synthesis of PRNAs and its chimeras for the RNase H mediated catalytic oligonucleotide therapeutics*

Masahito Inagaki,[a] Daisuke Unabara,[a] Ryohei Uematsu,[a] Yasuyuki Araki,[a] Masaki Nishijima,[a] Satoru Ishibashi,[b] Takanori Yokota,[b] Takehiko Wada*[a]

[a] *Institute of Multidisciplinary Research for Advanced Material (IMRAM), Tohoku University, Sendai 980-8577, Japan*
[b] *Department of Neurology and Neurological Science, Tokyo Medical and Dental University, Tokyo 113-8510, Japan*

The past several decades are marked significant increase in research and interests in oligonucleotide therapeutics, such as antisense and RNAi technologies. Many kinds of modified and/or artificial nucleic acids have been reported to the application for oligonucleotide therapeutics [1]. These nucleic acid models are recognized as promising candidate, but side-effects and toxicities, namely *off-target effects*, are pointed out as one of the most crucial drawbacks for practical application. The background in minds, we have proposed intracellular condition responsible Peptide Ribonucleic Acids (PRNAs). The complexation behavior of PRNAs with target RNA could be controlled by *anti* to *syn* nucleobase orientation change induced by the synergetic effect of the cyclic borate ester formation with 2',3'-*cis* diol of the ribose moiety and the intramolecular hydrogen bonding formation between 5'-amide hydrogen atom and carbonyl oxygen atom at 6-position of the nucleobase moiety of PRNA. The stability of cyclic borate ester crucially depends on pH of the solution, thus we have successfully demonstrated the complexation behavior of PRNAs with target RNA can be controlled *off to on* by the intracellular pH difference between normal (pH 7.0–7.2) and ischemic (pH 5.8–6.2) states (**Figure 1**) [2][3]. Therefore, the ischemia selective oligonucleotide therapeutics with PRNAs would be one of the most promising strategies for the safe and secure oligonucleotide therapeutics without any *off-target effects*.

Moreover, we have succeeded design and synthesis of chimeric PRNA-DNA derivatives ($P_R$PDs) consisted of 5'-DNA modified structure with PRNAs for the RNase H mediated catalytic oligonucleotide therapeutics [4]. $P_R$PD•RNA complexes showed remarkably high RNA cleavage activity by RNase H up to >20 times compared with that of natural DNA•RNA complexes (**Figure 2**).

***References:*** [1] Wan, W. B. *et al.*, *J. Med. Chem.*, **2016**, *59*(21), 9645. [2] Wada, T. *et al.*, *J. Am. Chem. Soc.*, **2000**, *122*(29), 6900. [3] Mateika, J. H. *et al.*, *J. Appl. Physiol.*, **2015**, *118*, 520. [4] Uematsu, R.; Inagaki, M.; Wada, T. *et al.*, *Chem. Lett.*, **2016**, *45*(3), 350.

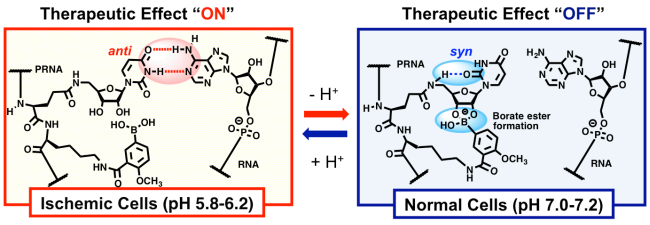



**Figure 1.** Peptide Ribonucleic Acid (PRNA).

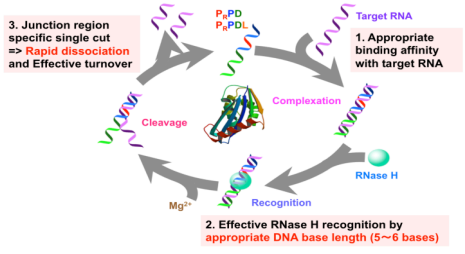



**Figure 2.** Catalytic oligonucleotide therapeutics.

*Corresponding author: E-mail: hiko@tohoku.ac.jp

# Impact of 2'-*C*-methylpyrimidine nucleosides on the stability of the i-motif and the inhibitory properties of modified siRNAs

María Dellafiore,[a] Anna Aviñó,[b],[c] Adele Alagia,[b],[c] Raimundo Gargallo,[d],[e] Carlos González,[f] Javier M. Montserrat,[g] Adolfo M. Iribarren,* [h] Ramon Eritja*[b],[c]

[a]*INGEBI (CONICET), Vuelta de Obligado 2490, 1428 Buenos Aires, Argentina*
[b] *Networking Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Jordi Girona 18-26, E-08034 Barcelona. Spain*
[c]*Institute for Advanced Chemistry of Catalonia (IQAC)Spanish Council for Scientific Research (CSIC)Jordi Girona 18-26, 08034 Barcelona, Spain*
[d]*Department of Chemical Engineering and Analytical Chemistry, University of Barcelona, Martí i Franquès, 1-11, E-08028 Barcelona, Spain*
[e]*BIOESTRAN, associated unit UB-CSIC*
[f]*Institute of PhysicalChemistry "Rocasolano", CSIC, Serrano 119, E-28006 Madrid, Spain*
[g]*Universidad Nacional de General Sarmiento, J.M. Gutiérrez 1150, 1613 Los Polvorines, Argentina*
[h]*Universidad Nacional de Quilmes, Roque Saenz Peña 352, 1876 Bernal, Argentina*

G-quadruplex and i-motif are tetraplex structures present in telomeres and promoter regions of oncogenes. The possibility of producing nanodevices with pH-sensitive functions has triggered the interest for modified oligonucleotides with improved structural properties. We synthesized C-rich oligonucleotides carrying conformationally restricted (2'*S*)-2'-deoxy-2'-*C*-methyl-cytidine units. The effect of this modified nucleoside on the stability of intramolecular i-motifs related to vertebrate telomere was investigated by means of spectroscopic methods (UV, CD and NMR). The replacement of selected positions of the C-core by the appropriate C-modified residues induces the formation of stable intercalated tetraplexes at pHs near neutrality. The study demonstrates the possibility of enhancing the stability of i-motif by chemical modifications. [1]
(2'*S*)-2'-Deoxy-2'-*C*-methyluridine and (2'*R*)-2'-deoxy-2'-*C*-methyluridine were also incorporated in the 3'-overhang region of the sense and antisense strands and in positions 2- and 5- of the seed region of siRNA duplexes directed against *Renilla* luciferase, while (2'*S*)-2'-deoxy-2'-*C*-methylcytidine was incorporated in the 6- position of the seed region of the same constructions. Dual luciferase reporter assay in transfected HeLa cells was used as model system to measure $IC_{50}$ values of 24 different modified duplexes. The best results were obtained by the substitution of one thymidine of the antisense 3'-overhang region by (2'*S*) or (2'*R*)-2'-deoxy-2'-*C*-methyluridine reducing $IC_{50}$ by half of the value observed for the natural control. Selectivity of the modified siRNA was measured, finding that modifications in positions 5- and 6- of the seed region had a positive effect on the ON/OFF activity. [2]

***References:*** [1] Stabilization of telomeric i-motif structures by (2'S)-2'-deoxy-2'-C-methyl-cytidine residues, A.Aviñó, M.Dellafiore, R.Gargallo, C.González, A.Iribarren, J.Montserrat, R. Eritja, *ChemBioChem* **2017** 18, 1123-1128. [2] siRNA modified with 2'-deoxy-2'-*C*-methylpyrimidine nucleosides, M.Dellafiore, A.Aviñó, A.Alagia, A.Iribarren, J.Montserrat, R. Eritja, *ChemBioChem* **2018** in press.
*Corresponding authors: E-mail: A. Iribarren: airibarren@unq.edu.ar, R. Eritja: recgma@cid.csic.es

# Synthesis and neuronal uptake/binding of chondroitin sulfate conjugates

Satish Jadhav,*[a,b] Prasanna Deshpande,[a] Eleanor Coffey, [a] Harri Lonnberg,[a] and Pasi Virta*[a]

[a] *Department of Chemistry, University of Turku, Vatselankatu 2, FI 20014 Turku, Finland*
[b] *Department of Cellular and Molecular Medicine, School of Medicine, UC San Diego, La Jolla, California 92093, United States (Currently post-doc in Prof. Steve Dowdy's lab)*

In the nervous system, chondroitin sulfate (CS) bound to core protein forms chondroitin sulfate proteoglycan (CSPG), a major component of the brain extracellular matrix. It functions as a regulator of plasticity and axon guidance during brain development and inhibits regeneration in the adult central nervous system.[1,2] Interestingly, CSPG has been found to bind to receptors raising the possibility that CS conjugates may be taken up into neuronal cells. To test this and to demonstrate that even CS structures with a nonregular α-glycosidic bond may show activity, preliminary cellular uptake studies with chondroitin sulfate conjugates were carried out.[3]

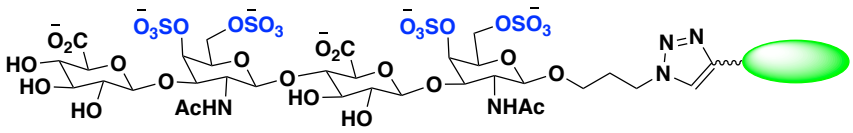



***Figure. Chondroitin Sulfate Conjugates***

Azidopropyl-modified precursors of chondroitin sulfate (CS) tetrasaccharides were synthesized, and then conjugated with alkyne-modified dye and oligonucleotides by a one-pot click-ligation. Neuronal uptake/binding of the chondroitin sulfate conjugates were studied.

This work was supported by grants from the Academy of Finland (No: 308931) and the Finnish Cultural Foundation.

***References:*** [1] Murrey, H. E., et al. *Chem. Rev*. **2008**, 108, 1708-1734. [2] Rawat, M. et al. *J. Am. Chem. Soc.* **2008**, *130*, 2959-2961. [3] Jadhav, S., et al. *Bioconjugate Chem. ASAP.* **2018**. **DOI:** 10.1021/acs.bioconjchem.8b00317

*Corresponding authors: E-mail: sgjadhav@ucsd.edu, and pamavi@utu.fi

# Synthesis and Properties of Oligonucleotides Bearing Seven-Membered Carbohydrate (Oxepane) Rings

Sunit Kumar Jana and Masad J. Damha*[a]

[a]*Department of Chemistry, McGill University, 801 Sherbrooke Street W., QC, Canada H3C0B8*

Oxapane nucleic acid (ONA) elicits RNase-H mediated degradation of ONA/RNA hetero duplex and hence may be a promising modification for antisense oligonucleotide therapy.[1] However, low duplex stability of ONA/RNA limits its application in RNA targeting approaches. Herein, we report the design and synthesis of novel ONAs as mimics of naturally occurring DNA and RNA. Both flexibility and the presence of various hydroxyl moieties in the sugar moiety may bring forward interesting properties, such as duplex stability and enzymatic resistance, both relevant to antisense and siRNA technologies.

In this presentation, we will discuss the challenges of synthesizing diastereomerically pure oxepane nucleosides as building blocks for solid phase ONA synthesis starting from readily available tri-O-acetyl-D-glucal. We will also present the physicochemical properties of ONA/RNA and ONA/DNA duplexes, and the use of ONA in gene silencing (siRNA) and gene editing (CRISPR/Cas9) approaches.

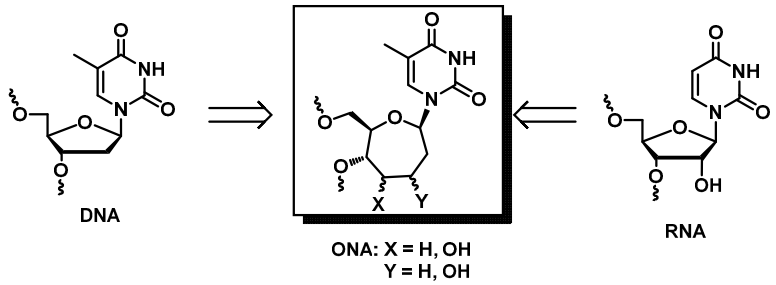



***Figure.*** *Oxapane nucleic acids (ONA) as a mimic of DNA and RNA*

***References:*** [1] Sabatino, D. and Damha, M. J., *J. Am. Chem. Soc.* **2007**, *129*, 8259.

*Corresponding author: E-mail: sunit.jana@mcgill.ca; masad.damha@mcgill.ca

# Activation of Human RNase L using various phosphonate oligoadenylates.

Tomáš Jandušík*, Ondřej Šimák, Ondřej Páv, Magdalena Petrová, Ivana Kóšiová-Markusová, Ivan Rosenberg

*Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo nám. 2, 166 10 Praha 6, Czech Republic*

The short oligoadenylates (2-5A) with unique 2′-5′-linkages play a significant role in interferon-induced antiviral defence mechanism of cells. Interferon induces expression of the dsRNA-dependent 2-5A synthetase utilizing ATP as a substrate for the synthesis of 5′-phosphorylated 2-5As, (pp)p5′Ap(Ap)$_n$A2′ (where *n* is mainly 1 or 2). These 2-5As bind to a latent endoribonuclease - RNase L and activate it. Activated form is capable of cleaving pathogenic ssRNA and thus preventing expression of viral proteins.[1]
Since the presence of the 5′-phosphate group in 2-5As is essential for the RNase L activation, several 2-5As were synthesized on solid-phase from the phosphonate monomers (Figure 1) and the ability of these oligoadenylates to stimulate human recombinant RNase L cleavage activity was evaluated. Our preliminary data suggested the substitution of 5′-terminal phosphate for 5′-terminal phosphonate in 2-5A afforded oligoadenylates with excellent activation efficiency and with improved stability against cleavage by phosphomonoesterases.

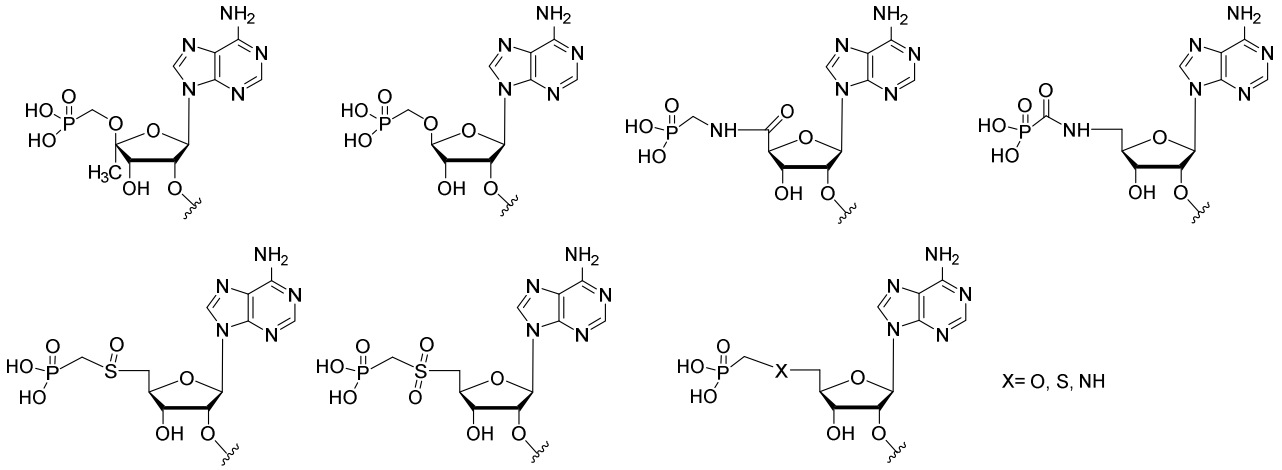



***Figure 1***. Examples of phosphonate modifications of 5′-end nucleotide incorporated into 2-5A

This work was supported by the grant # 17-12703S (Czech Science Foundation).

***References***

1. Player, M.R.; Torrence, P.F. *Pharmacol. Ther.* **1998**, *78*, 55-113.

*Corresponding author: E-mail: jandusik@uochb.cas.cz

# Site specific, sequence independent fluorescent labeling of mRNA in living cells

Jacek Jemielity*[a], Adam Mamot[a], Pawel J. Sikorski[a], Marcin Warminski[b] Joanna Kowalska[b]

[a] *Centre of New Technologies, University of Warsaw, 02-097 Warsaw, Poland*
[c] *Division of Biophysics, Institute of Experimental Physics, Faculty of Physics, University of Warsaw, 02-093 Warsaw, Poland*

The 7-methylguanosine cap structure is a unique feature present at the 5′ ends of mRNAs that can be subjected to extensive modifications, resulting in alterations to mRNA properties, such as translability, susceptibility to degradation. It also can provide molecular tools to study mRNA metabolism, which understanding is critical for development of RNA-based therapeutics. We synthesized a series of dinucleotide cap analogs functionalized with azide groups and evaluated them as reagents for the modification of mRNA 5′ ends.[1] The transcripts were labeled with a fluorescent dye, both in vitro and in living HeLa cells, using bioorthogonal click chemistry, that allowed for the cellular localization of exogenously delivered mRNA. Importantly, the mRNAs before and after labeling procedure showed high translational activity, which differentiates our approach from previously developed approaches[2]. The potential of our azide-functionalized cap analogs is not limited to fluorescent labeling; such modified RNAs could be conjugated with different alkyne-functionalized molecules, such as D-biotin, proteins, or nanomaterials, facilitating affinity purification, pull-down experiments, transport, and many others.

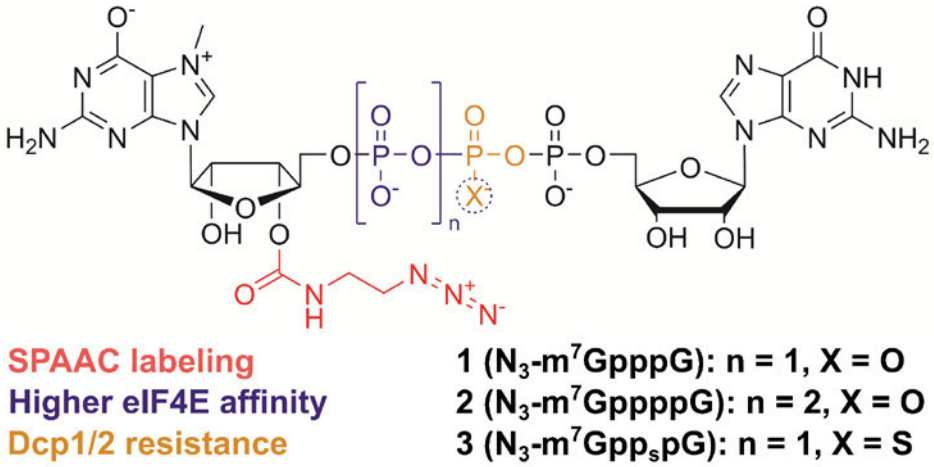



Figure 1. A) Structures of the dinucleotide mRNA cap analogues with azide moiety.

***References:***

*[1]* A. Mamot, P. J. Sikorski, M. Warminski, J. Kowalska, J. Jemielity, Angew. Chem. Int. Ed. 2017, **56**, 15628

[2] J. Holstein, L. Anhauser, A. Rentmeister, Angew. Chem. Int. Ed. 2016, **55**, 10899

This work was supported by the Foundation for Polish Science (TEAM/2016-2/13) and by the National Science Centre, Poland (UMO-2016/21/B/ST5/02556).
*Corresponding author: E-mail: jacek.jemielity@fuw.edu.pl

# Rigid nitroxide spin labels that are resistant to reduction

Haraldur Yngvi Júlíusson*[a] and Snorri Th. Sigurdsson[a]

[a] *Department of Chemistry, Science Institute, University of Iceland, Reykjavík, Iceland*

In the last years, structural studies of biomolecules in their natural (cellular) environment have begun to emerge. One technique that has been applied for such studies is electron paramagnetic resonance (EPR) spectroscopy.[1] EPR is able to provide structural information for biomolecules, such as nucleic acids, through measurements of distances between paramagnetic centers. Nucleic acids are inherently diamagnetic and thus, it is necessary to modify them with paramagnetic atoms or groups, called spin labels. The most commonly used spin labels are nitroxide radicals. The spin-labeled nucleoside **Ç** (**Figure 1A**), where the nitroxide moiety is fused to the nucleoside through a six-membered ring, is a rigid spin label that has advantageous properties as a probe for EPR studies of DNA. Not only does it give accurate information about distances between spin labels, but also information about their relative orientation.[2] However, the reductive environment of cells causes reduction of most nitroxide radicals to the corresponding EPR-inactive hydroxylamine. The spin label **Ç,** along with other tetramethyl-derived nitroxide radicals, are not stable enough to withstand these reducing conditions for long. In contrast, tetraethyl-derived nitroxide radicals, where the radical is flanked by four ethyl groups, are more resistant to reduction.[3] Therefore, tetraethyl-derived nitroxide radicals are good candidates for in-cell EPR experiments. Here we report the synthesis of **EÇ** and **EÇm** (**Figure 1A**) for spin labeling DNA and RNA, respectively**,** that combine the rigid nitroxide with stability under reducing conditions.

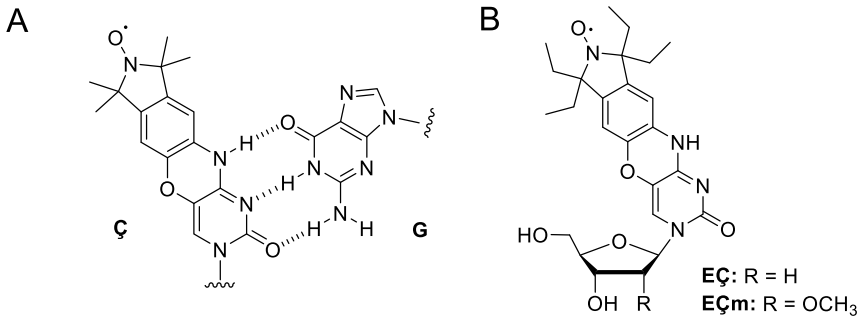



***Figure 1. A:*** *Basepairing of* **Ç** with G. ***B:*** Nucleoside **EÇ** (R=H) and **EÇm** (R=$OCH_3$).

This work was supported by a grant from the Icelandic Reasearch Fund (173727-051).

References: [1] *Angew. Chem. Int. Ed.* **2014**, 53, 10300-10314 [2] *Angew. Chem. Int. Ed.* **2009**, 48, 3292-95. [3] *Free Radical Res.* **2015**, 49, 78-85.

*Corresponding author: E-mail: hyj1@hi.is

# Oligopods of antisense oligonucleotides with boron clusters: self-assembly and biological properties

Damian Kaniowski[a], Katarzyna Ebenryter-Olbińska[a], Katarzyna Kulik[a], Milena Sobczak[a], Sławomir Janczak[b], Zbigniew J. Leśnikowski*[b], Barbara Nawrot*[a]

[a] *Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, Sienkiewicza 112, 90-363 Lodz, Poland*
[b] *Laboratory of Molecular Virology and Biological Chemistry, Institute of Medical Biology, Polish Academy of Sciences, Lodowa 106, 92-232 Lodz, Poland*

DNA nanotechnology is a branch of technology that exploits nucleic acids' ability to self-assembly in order to construct nanostructures with specific properties. There are numerous potential applications of DNA nanostructures including those in diagnostics and therapy of human disorders [1]. Based on our previous studies on boron clusters as modifying units for nucleic acids [2,3], conjugates of the epidermal growth factor receptor (EGFR)-directed antisense DNA oligonucleotides modified with boron clusters [o-carborane, $C_2B_{10}H_{12}$; dodecacarborane, $B_{12}H_{12}^{2-}$; and metallacarborane, $[Fe(C_2B_9H_{11})_2]^-$] were obtained and tested as potential agents in antisense and BNCT therapy [4,5].

In this communication, we present an application of DNA-functionalized boron clusters (oligopods) as building blocks for nano-construction of therapeutic nucleic acid systems. Thus, tri-substituted o-carborane, bis-functionalized with EGFR-targeted sense or antisense oligonucleotides were obtained by solid phase method. The complementary dipods were self-assembled to nano-structured complexes which were visualized by the non-denaturating polyacrylamide gel electrophoresis (PAGE), atomic force microscopy (AFM) and cryo-transmission electron microscopy (Cryo-TEM). Their silencing activity, stability against exo- and endo-nucleases as well as melting properties were investigated.

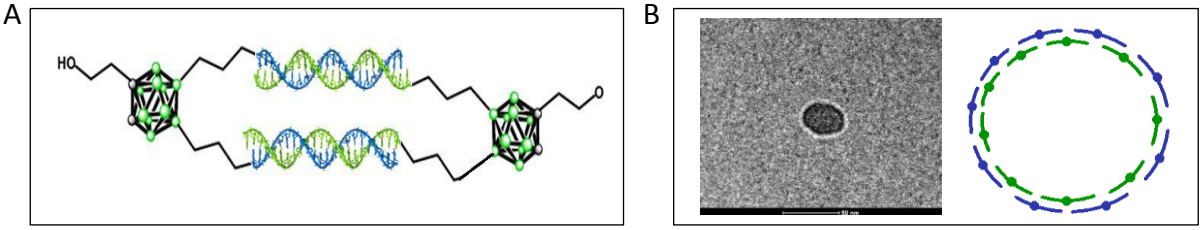



***Figure.*** *A schematic view of nanostructure formed by two complementary dipods (EGFR-sense and antisense oligonucleotides conjugated with boron-cluster)(A), and a nanostructure consisting 18-dipods; (B) visualized by Cryo-TEM.*

Acknowledgements: This research was financially supported by The National Science Centre in Poland, Grant number 2015/16/W/ST5/00413 for years 2015–2019.

***References:*** [1] DNA Nanotechnology, From Structure to Function, Springer-Verlag, **2013**. [2] Leśnikowski, Z. J., Eur. J. Org. Chem. **2003**, 4489. [3] Kwiatkowska, A. et al., Bioconjugate Chem. **2013**, 24, 1017. [4] Kaniowski, D. et al., Molecules. **2017**, 22(9), 1393. [5] Ebenryter-Olbińska, K. et al., Chem. Eur. J. **2017**, 23, 16535.

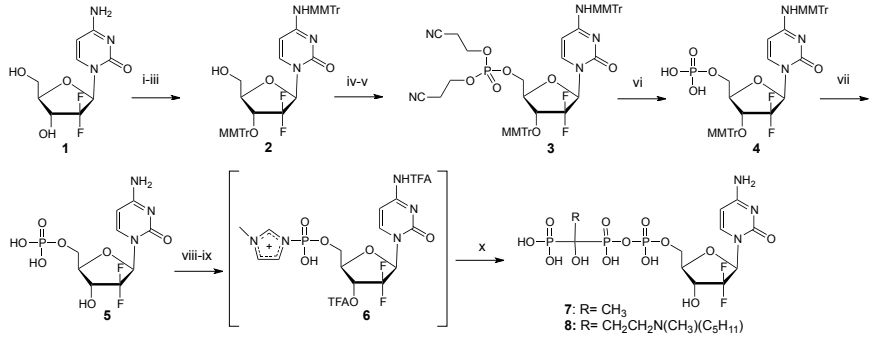
*Corresponding author: bnawrot@cbmm.lodz.pl, zlesnikowski@cbm.pan.pl

# Bisphosphonate derivatives of gemcitabine

Alexander M. Karpeisky*[a], Kristen B. Farrell[a], Douglas H. Thamm[b], Shawn P. Zinnen[a]

[a]*MBC Pharma, Inc, 12635 E. Montview Blvd., Ste 100, Aurora CO 80045, USA*
[b]*Animal Cancer Center Colorado State University, 300 W. Drake Rd, Fort Collins, CO 80523, USA*

In continuation of the development of MBC's bone-targeted therapeutics platform [1], we wish to explore the combinations of bone-targeted gemcitabine with docetaxel (DTX) in osteosarcoma therapy. Here we report highly efficient synthesis (Fig. 1) of gemcitabine-5'monophosphate (5), followed by conversion to the conjugates 7 or 8 using modified Bogachev's procedure [2].




**Reagents&Conditions:**
i)TBDMS-Cl/Py;
ii)MMtrCl/$AgNO_3$/collidine;
iii)TBAF/THF; iv)bis-cyanoethyl-N,N-diisopropyl-amino phosphoramidite;
v) t-BuOOH; vi) DBU/BSA;
vii) 80%AcOH, 60˚C;
viii) $(CF_3CO)_2O$/dimethyl-aniline; ix) NMI/$ET_3N$; then $Bu_3N$ salt of bisphosphonate
x) water

***Figure 1.*** *Synthesis of gemcitabine-bisphosphonate conjugates.*

Conjugates 7 and 8 were tested in animal model of osteosarcoma, demonstrating significant synergistic effect with docetaxel as compared to combinations using non-conjugated gemcitabine (***Figure 2***). Mice treated with gemcitabine-ibandronate conjugate **8** + DTX showed the greatest reduction in tumor growth, demonstrated by both tumor measurement and increase in survival time.

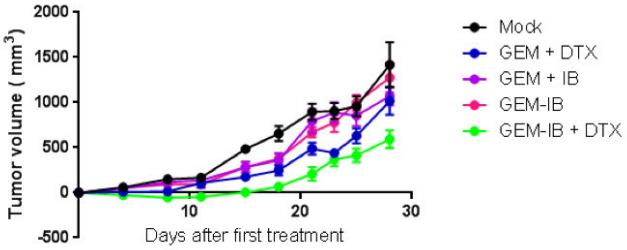



*Figure 2. Tumor volume reduction by 8+DTX treatment*

Synthetic details and in vivo efficacy data will be discussed.

This work was supported by NIH grants 1 R43 CA203166-01 and 2 R44 CA203166-02.

***References:*** [1] Shawn Zinnen, Eric Rowinsky, Alexander Alexandrov, Larisa Plekhova, Marina Roudas, and Alexander Karpeisky *J Clin. Oncol.* **2017** *35:15*_suppl, 2589
[2] Bogachev, V. S. *Bioorganicheskaya Khimia (Russ)* **1996,** *22*, 699., *345*, 12345. etc.,

*Corresponding author: E-mail: alkarp@mbcpharma.com

# RNA detection by a stem-less probe using pairing between fluorophores and quenchers

Hiromu Kashida,*[a] Kazuhiro Morimoto,[a] Hiroyuki Asanuma*[a]

[a] *Graduate School of Engineering, Nagoya University, Furo-cho, Chikusa-ku, Nagoya, 464-8603 Japan*

Fluorescent oligonucleotide probes are essential tools in biology, biotechnology, and nano-medicine research. Molecular beacons have been one of the most widely-used probes to detect DNA and RNA[1]; however, the stable base pairing of the stem portion of the molecular beacon may result in slow response to target. Linear probes that do not need stem-loop structures for quenching have also been reported.

Previously we reported stem-less probes that tether only perylenes as a fluorophore.[2] These probes have multiple perylene residues that are self-quenched through non-emissive complex formation among them in a single-stranded state. However, this strategy cannot be applied to other fluorophores such as Cy3 due to their low self-quenching efficiencies. Herein we report a new probe design based on spontaneous complex formation between Cy3 and a quencher.

Our strategy is shown in Scheme 1. We selected Nitro Methyl Red as a quencher. Two Cy3 and two quencher residues were introduced at near each end of a linear oligonucleotide. Unlike a conventional molecular beacon probe, this oligonucleotide does not have a self-complementary sequence. Nevertheless, Cy3 and the quencher spontaneously formed a complex in the absence of the target so that Cy3 emission was efficiently quenched. Upon duplex formation with a complementary target, strong Cy3 emission was observed. As a result, signal to background ratio of this probe was as high as 180. Because this probe does not have a stem-loop structure, a high response speed in the presence of a target was achieved. [3]

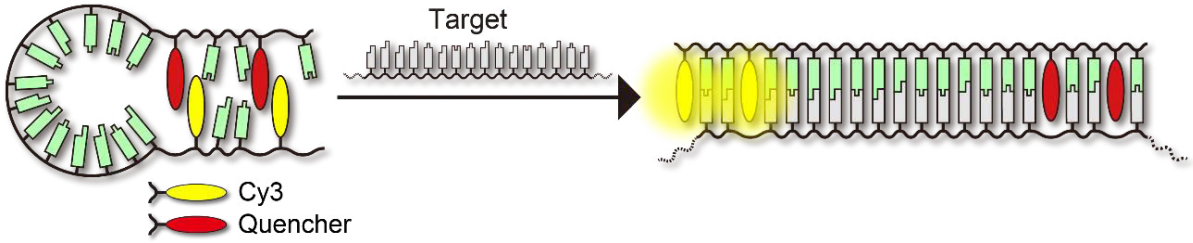



***Figure.*** *Illustration of our strategy to detect RNA by using a stem-less probe*

This work was supported by PRESTO from Japan Science and Technology Agency grant number JPMJPR14F7.

***References:*** [1] Tyagi, S. Kramer F.R., *Nat. Biotechnol.* **1996***, 14*, 303. [2] Asanuma, H., et al. *Angew. Chem. Int. Ed.* **2015**, *54*, 4315. [3] Kashida, H., et al. *Sci. Technol. Adv. Mater* **2016**, *17*, 267.

*Corresponding author: E-mail: kashida@chembio.nagoya-u.ac.jp, asanuma@chembio.nagoya-u.ac.jp

# HTS assays based on fluorescence intensity measurements for studies on proteins related to mRNA 5' end function

Renata Kasprzyk,[a,b] Beata J. Starek,[c] Sylwia Walczak,[a,b] Dorota Kubacka,[c] Joanna Kowalska[c] and Jacek Jemielity*[a]

[a] *Centre Of New Technologies, University of Warsaw, Banacha 2c 02-097 Warsaw, Poland*
[b] *College of Inter-Faculty Individual Studies in Mathematics and Natural Sciences, University of Warsaw, Banacha 2c 02-097 Warsaw, Poland*
[c] *Division of Biophysics, Institute of Experimental Physics, Faculty of Physics, University of Warsaw, Pasteura 5 02-093 Warsaw, Poland*

m$^7$G cap is an unusual nucleotide structure present at the 5' end of all eukaryotic mRNAs, consisting of 7-methylguanosine linked by 5'5'-triphosphate bridge with the first transcribed nucleotide. Cap specifically interacts with numerous nuclear and cytoplasmic proteins, thereby participating in many important biological processes essential for cell growth and function. An example is eIF4E protein, which is crucial for mRNA translation under normal conditions and if overexpressed leads to malignant transformation of cells [1]. Cap degradation by DcpS enzyme is another therapeutically relevant process, since inhibitors of DcpS enzyme are potential drugs in SMA treatment [2]. To provide small molecular probes to study eIF4E, DcpS and other biologically important cap-recognizing proteins we synthesized a series of cap-derived fluorescent probes. The mononucleotide cap analogs labelled with different fluorescent tags were obtained in CuAAC reaction between 7-methylguanine nucleotides containing 3-butynyl-C-phosphonate moiety and fluorescent tag azides [3]. We next performed spectroscopic studies to determine the influence of conjugation with 7-methylguanosine on the fluorescent properties of the dyes. We observed that while for pyrene derivatives the fluorescence is strongly quenched by 7-methylguanosine, it remains unchanged for other tested dyes. Pyrene fluorescence changes upon binding by eIF4E or hydrolysis by DcpS were used to develop binding- or activity-based assays for evaluating the affinity of chemically modified cap analogs to the proteins. To optimize the probes structure, eight new pyrene-labelled probes were synthesized and studied for their specificity to eIF4E and DcpS. Finally, using optimized probes we developed assays for fast and efficient screening and binding constants determination for eIF4E and DcpS, as well as activity assay for DcpS-catalysed hydrolysis. The utility of new assays was validated on a previously characterized libraries of eIF4E and DcpS inhibitors [4].

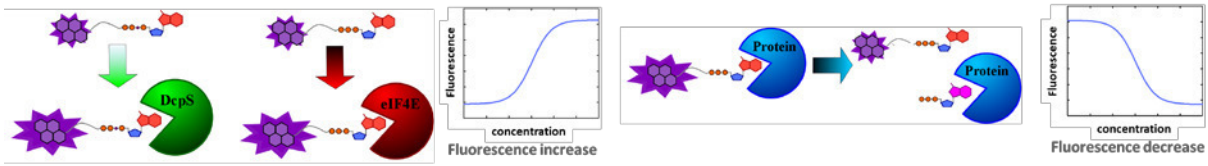



***Figure.*** Binding and activity assays for cap-binding proteins developed using fluorescently labeled m$^7$G nucleotides.

This work was supported by the Ministry of Science and Higher Education (Poland, DI2013 016143)

***References:*** [1] Lazaris-Karatzas, A. et al., *Nature* **1990**, *345*, *544*. [2] Singh, J., et al. *ASC Chem. Biol.* **2008**, *3*, 711. [3] Wanat, P., et al. *Org. Lett.* **2015**, *17*, 3062. [4] Walczak, S., et al. *Chem. Sci.* **2017**, *8*, 260.

* *j.jemielity@cent.uw.edu.pl*

# Specific detection of the target molecules using Thioflavin T derivatives

Yuka Kataoka,[a] Hiroto Fujita,[a, b] Masayasu Kuwahara*[a, b]

[a] *Graduate School of Science and Technology, Gunma University, 1-5-1 Tenjin-cho, Kiryu, Gunma 376-8515, Japan*
[b] *Graduate School of Integrated Basic Sciences, Department of Chemistry, College of Humanities & Sciences, Nihon University, 3-25-40 Sakurajosui, Setagaya-ku, Tokyo 156-8550, Japan*

Thioflavin T (ThT) is generally used to detect amyloid fibrils in the brain of neurodegenerative diseases. Previously, Mohanty *et al*. reported that ThT could selectively recognize and stabilize G-quadruplexes (G4s) [1]. In the excited states, the fluorescence intensity is greatly dependent on the molecular configuration between the benzothiazole and dimethylaminobenzene rings [2]. Therefore, ThT can provide high-contrast images when its bind to the target. We showed that the modification at the N3 position of ThT could considerably affect the selectivity for parallel G4s and induce topological changes in G4s [3]. Furthermore, using N3-hydroxyethyl ThT (ThT-HE), we successfully developed a novel, rapid, and convenient RNA detection system named signal amplification by ternary initiation complexes (SATIC) [4, 5]. We expected that N3-modified ThT derivatives with various ligand may expand the target-directing property for bioanalysis. In this study, we introduced small-molecule ligands such as d-desthiobiotin and steroid hormones at N3 position of ThT and investigated their fluorescence and binding properties for their target proteins. (Fig. 1).

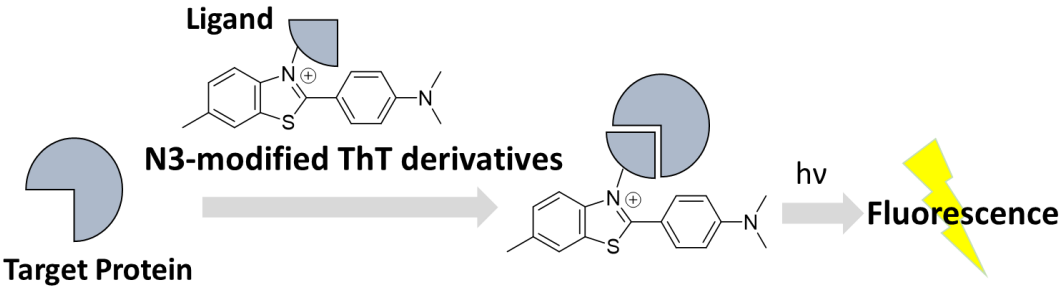



***Figure 1.*** *N3-Modified ThT derivatives with various ligands to detect target proteins.*

***References:*** [1] Mohanty, J.; Barooah, N.; Dhamodharan, V.; Harikrishna, S.; Pradeepkumar, P. I.; Bhasikuttan, A. C. *J. Am. Chem. Soc.* **2013***, 135,* 367−76. [2] Amdursky, N.; Erez, Y.; Huppert, D. *Acc. Chem. Res*. **2012**, *45*, 1548–57. [3] Kataoka, Y.; Fujita, H.; Kasahara, Y.; Yoshihara, T.; Tobita, S.; Kuwahara, M. *Anal. Chem.* **2014**, *86*, 12078–84. [4] Fujita, H.; Kataoka, Y.; Tobita, S.; Kuwahara, M.; Sugimoto, N. *Anal. Chem.* **2016**, *88*, 7137–44. [5] Fujita, H.; Kataoka, Y.; Nagano, R.; Nakajima, Y.; Yamada, M.; Sugimoto, N.; Kuwahara, M. *Sci. Rep*. **2017**, *7*, 15191.

*Corresponding author: E-mail: mkuwa@chs.nihon-u.ac.jp

# Visualization of Telomeres with Pyrrole-Imidazole Polyamides

Yusuke Kawamoto,[a] Takuya Hidaka,[b] Asuka Sasaki,[c] Kaori Hashiya,[b] Satoru Ide,[c] Toshikazu Bando,[b] Kazuhiro Maeshima,[c] Hiroshi Sugiyama*[b]

[a] *Department of Chemistry and Biochemistry, University of California, San Diego, 9500 Gilman Drive, La Jolla, California 92093, United States*
[b] *Department of Chemistry, Graduate School of Science, Kyoto University, Sakyo, Kyoto 606-8264, Japan*
[c] *Structural Biology Center, National Institute of Genetics, and Department of Genetics, School of Life Science, Graduate University for Advanced Studies (Sokendai), Mishima, Shizuoka 411-8540, Japan*

Telomeres are localized at chromosomal ends and their sequences are 5'–$(TTAGGG)_n$–3' tandem repeats. Their length is related to aging process and cancer and therefore various methods to visualize telomeres and to measure telomere length have been developed. We have worked on *N*-methylpyrrole–*N*-methylimidazole (Py–Im) polyamides bind to the minor groove of dsDNA in a sequence-specific manner without causing denaturation of dsDNA.[1] Herein we have developed a facile synthetic method of the fluorescent Py-Im polyamides targeting 12 bp in the duplex regions of telomeres.[2-4] Using the probes, we have developed the method to visualize telomeres specifically in chemically fixed cells under mild condition. Furthermore, we have designed and synthesized novel Py-Im polyamides targeting 18 bp and 24 bp in telomeres to decrease the non-specific binding.[5,6] Results of visualization studies supported their higher specificity to telomeres. We will also discuss the recent attempt to visualize telomeres in living cells.

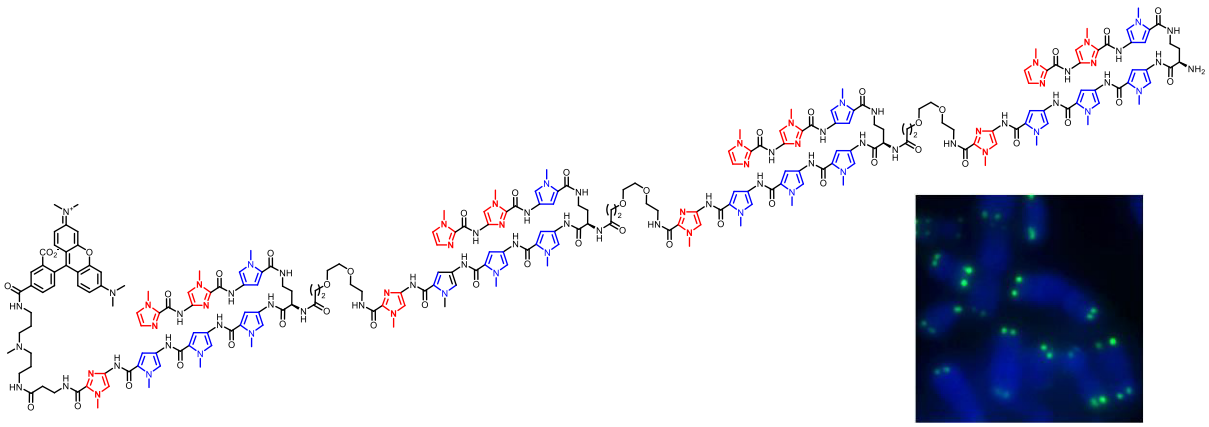


***Figure.*** *Chemical structure of the fluorescent tandem tetramer Py–Im polyamide targeting human telomere sequences and telomere staining images.*

***References:*** [1] Kawamoto, Y. et al., *Bioorg. Med. Chem.* **2018**, *263*, 1393. [2] Kawamoto, Y., et al. *J. Am. Chem. Soc.* **2013**, *135*, 16468. [3] Hirata, A., et al. *J. Am. Chem. Soc.* **2014**, *136*, 11546. [4] Sasaki, A. et al., *Sci. Rep.* **2016**, *6*, 29261. [5] Kawamoto, Y., et al. *Chem. Sci.* **2015**, *6*, 2307. [6] Kawamoto, Y., et al. *J. Am. Chem. Soc.* **2016**, *138*, 14100.

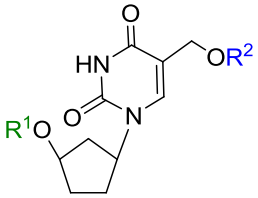
*Corresponding author: E-mail: hs@kuchem.kyoto-u.ac.jp

# Antibacterial activity of 5'-norcarbocyclic analogues of 5-substituted pyrimidine nucleosides

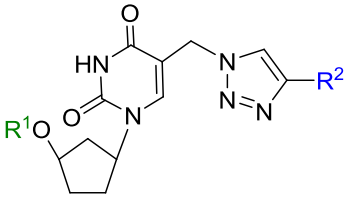
Anastasia L. Khandazhinskaya,*[a] Ludmila A. Alexandrova,[a] Elena S. Matyugina,[a] Sergey N. Kochetkov,[a] Olga V. Efremenkova,[b] Karen W. Buckheit,[c] Maggie Garvey,[c] Robert W. Buckheit Jr,[c] Larisa N. Chernousova,[d] Tatiana G. Smirnova,[d] Katherine L. Seley-Radtke,[e]

[a] *Engelhardt Institute of Molecular Biology RAS, Vavilova 32, Moscow, 119334, Russia*
[b] *G.F. Gauze Institute for Search for New Antibiotics, RAMS, Moscow, 119021, Russia*
[c] *ImQuest BioSciences, Frederick, Maryland, USA*
[d] *Central Tuberculosis Research Institute RAMS, Yauzskaya Alley 2, Moscow, 107564, Russia*
[e] *Department of Chemistry & Biochemistry, UMBC, Baltimore, MD 21250, USA*

Resistance to clinically significant antibiotics has evolved to nearly all antibiotics used.[1] Despite this, the development of new classes of antibiotics has lagged far behind the growing need, and there is the urgent need to design new drugs that can attack new targets and remain active against resistant strains of these deadly pathogens. In that regard, nucleoside derivatives have historically exhibited potent antiviral and anticancer activity[2] but it wasn't until 2000 that their anti-TB activity was reported. [3]




**1**:$R^1$=OAc, $R^2$=$C_{10}H_{21}$; **2**:$R^1$=OAc, $R^2$=$C_{11}H_{23}$;
**3**:$R^1$=OAc, $R^2$=$C_{12}H_{25}$; **4**:$R^1$=OH, $R^2$=$C_{10}H_{21}$;
**5**:$R^1$=OH, $R^2$=$C_{11}H_{23}$; **6**:$R^1$=OH, $R^2$=$C_{12}H_{25}$




**7**:$R^1$=OAc, $R^2$=$C_8H_{17}$; **8**:$R^1$=OAc, $R^2$=$C_{10}H_{23}$;
**9**:$R^1$=OAc, $R^2$=$C_{12}H_{21}$; **10**:$R^1$=OH, $R^2$=$C_8H_{17}$;
**11**:$R^1$=OH, $R^2$=$C_{10}H_{23}$; **12**:$R^1$=OH, $R^2$=$C_{12}H_{25}$

The activity of the compounds developed in our laboratories was evaluated against both gram-positive (*Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* INA 00761 (MRSA), *Mycobacterium smegmatis* $mc^2155$ and VKPM Ac 1339, *Leuconostoc mesenteroides* VKPM B-4177) and gram-negative (*Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922) bacteria. Most of the bacteria were not sensitive to the compounds, however the growth of *M. smegmatis* was completely inhibited at $MIC_{99}$ 67 μg/ml ($mc^2155$) and $MIC_{99}$ 6.7-67 μg/ml (VKPM Ac 1339). Some of the compounds also showed the ability to inhibit the growth of an attenuated strain of *M. tuberculosis* ATCC 25177 ($MIC_{99}$ 28-61 μg/ml) and *M. bovis* ATCC strain 35737 ($MIC_{99}$ 50-60 μg/ml), as well as two virulent strains of *M. tuberculosis,* a laboratory strain H37Rv ($MIC_{99}$ 20-50 μg/ml) and a clinical strain with multiple drug resistance MS-115($MIC_{99}$ 20-50 μg/ml). The results of these studies is reported herein.

This work was supported by Russian Science Foundation, project № 14-50-00060.

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*Corresponding author: E-mail: khandazhinskaya@bk.ru

# Intra-strand Cross-link Promotes Formation of *i*-motif Structures

Kenji Kikuta [a], Piao Haishun [a], John Brazier [b], Kazumitsu Onizuka [c], Fumi Nagatsugi [c], Yosuke Taniguchi [a], Shigeki Sasaki *[a]

[a]*Graduate School of Pharmaceutical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan* [b]*Reading School of Pharmacy, University of Reading, Whiteknights, Reading RG6 6AD, United Kingdom* [c]*Institute of Multidisciplinary Research for Advanced Materials, Tohoku University, 2-1-1 Katahira, Aoba-ku, Sendai 980-8577, Japan*

The *i*-motif, a four-stranded DNA secondary structure, is consisted of two parallel duplexes combined in an antiparallel fashion by intercalating hemiprotonated cytosine-cytosine base pairs. [1] The formation of the *i*-motif is of great interest under physiological conditions because of its intracellular existence and biological roles. It is difficult to develop binding molecules to the *i*-motif in cells because it transforms into a random coil conformation due to the deprotonation of cytosines at neutral pH. In this study, we hypothesized that the intra-strand cross-link in the *i*-motif structure might increase its stability in terms of both pH-dependency and thermal denaturation.

We have previously reported the 4-vinyl-substituted analog of thymidine (T-vinyl) as an efficient cross-linking nucleobase for adenine and thymine of the target strand in the close proximity.[2] T-vinyl was incorporated into the 5'-end of the human telomere complementary strand, $d(C_3TA_2)_4$, which formed the intra-strand cross-link with the internal adenine (Fig. 1).

Circular dichroism provides a convenient method to detect the formation of *i*-motifs since their spectra exhibit characteristic negative and positive bands at 254 and 286 nm, respectively. Fig. 2 shows the pH dependency of the ellipticity of the natural and cross-linked *i*-motifs at 286 nm. As previously reported, the natural *i*-motif exhibits a positive band that decreases in magnitude as the pH is raised above 6.0 (Figure 2). On the other hand, the cross-linked *i*-motif exhibited this characteristic *i*-motif CD signature at pH 6.5. The thermodynamic parameters showed that the formation of *i*-motif structures were promoted due to a favorable entropy effect of intra-strand cross-link. Thus, this study has clearly indicated the validity of the intra-strand cross-linking for stabilization of the *i*-motif structure.[3]

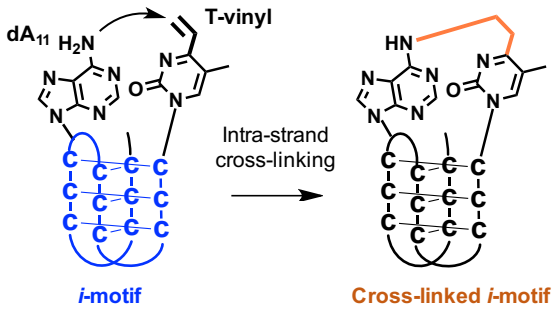



***Fig.1.*** The formation of intra-strand cross-link in the *i*-motif by the T-vinyl.

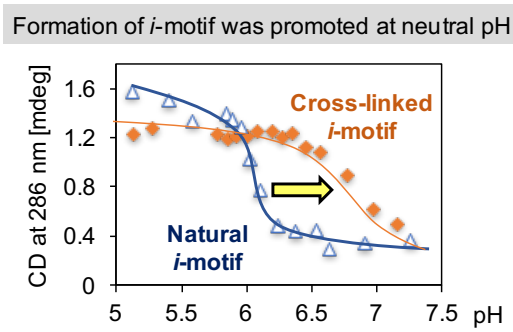



***Fig.2.*** pH-dependent CD intensity at 286 nm.

***References:*** [1] H. A Day, et al., *Bioorg. Med. Chem.* **2014**, *22*, 4407. [2] A. Nishimoto, et al., *Nucleic Acids Res.* **2013**, *41*, 6774. [3] K. Kikuta, et al., *Bioorg. Med. Chem. Lett.* **2015**, *25*, 3307.

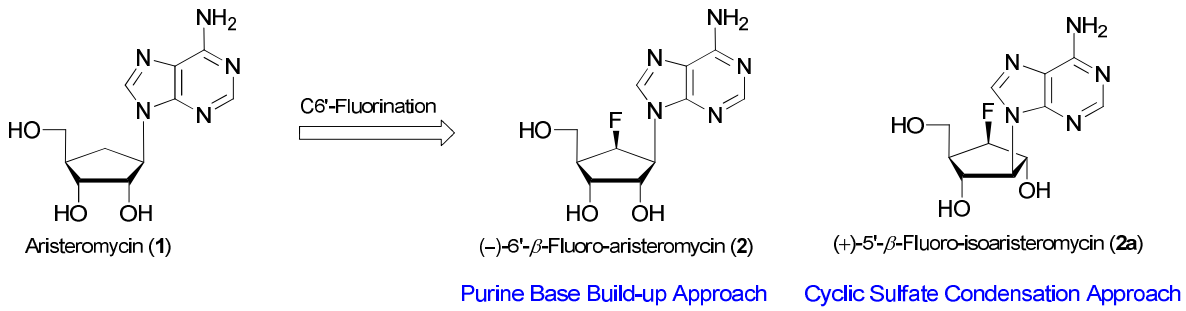
*Corresponding author: E-mail: sasaki@phar.kyushu-u.ac.jp

# Asymmetric Synthesis of (–)-6'- $\beta$ -Fluoro-aristeromycin via Stereoselective Electrophilic Fluorination

Hong-Rae Kim, Ji-seong Yoon, Gyudong Kim, and Lak Shin Jeong*

*College of Pharmacy, Seoul National University, Seoul 08826, South Korea*

(–)-Aristeromycin (**1**), is a naturally occurring carbocyclic nucleoside, which was originally isolated as a metabolite of *Streptomyces citricolors* in 1968.[1] This compound is a potent inhibitor of *S*-adenosylhomocysteine (SAH) hydrolase and has potent antiviral activities against several RNA and DNA viruses, but it has cytotoxicity. Base on the bioisosteric rationale, introducing fluorine at C6' position also potent inhibitor of SAH hydrolase. Herein, we synthesized (–)-6'-$\beta$-fluoro-aristeromycin via stereoselective electrophilic fluorination followed by a purine base build-up approach. Alternatively, we condensed the purine base using a cyclic sulfate[2] to avoid the build-up approach, resulted in a (+)-5'-$\beta$-fluoro-isoaristeromycin (**2a**). Computational analysis indicates that the fluorine atom controlled the regioselectivity of the purine base substitution during the cyclic sulfate approach.




***References:*** [1] Kusaka, T.; Yamamoto, H.; Shibata, M.; Muroi, M.; Kishi, T.; Mizuno, K. *J. Antibiotics* **1968**, 21, 255. [4] Naik, S. D.; Chandra, G.; Sahu, P. K.; Kim, H.-R.; Qu, S.; Yoon, J.-S.; Jeong, L. S. *Org. Chem. Front.* **2016**, 3, 1472.

*Corresponding author: E-mail: lakjeong@snu.ac.kr

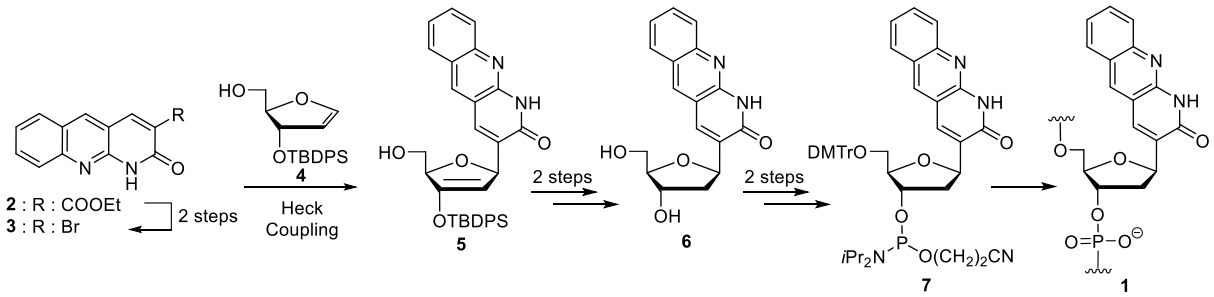
# Synthesis and Properties of Oligonucleotide Containing Tricyclic Thymine Analogue, 2-Oxobenzo[*b*][1,8]naphthyridine

Yuki Kishimoto, Akane Fujii, Natsumi Nozaki, Osamu Nakagawa, Satoshi Obika*

*Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan.*

Artificial nucleobases have been developed to improve duplex-forming ability and exert selective recognition with targeted nucleobases. Tricyclic cytosine analog, phenoxazine, is known to form selective hydrogen bonding with guanine, and enhance duplex-forming ability by effective π-π stacking interaction.[1] Phenoxazine derivatives are expected to apply to not only nucleic acids therapeutics but also gene diagnosis[2] because of its fluorescent properties.

In this study, we designed and synthesized a novel thymidine analogue with 2-oxobenzo[*b*][1,8]naphthyridine as an artificial tricyclic nucleobase (**1**). We expected that **1** could form selective hydrogen bonding with adenosine, and oligodeoxynucleotides (ODNs) containing **1** showed effective duplex-forming ability via π-π stacking interaction.




***Scheme.*** *Synthesis of ODN containing 2-oxobenzo[b][1,8]naphthyridine nucleobase.*

Using the known compound **2**,[3] the 2-oxobenzo[*b*][1,8]naphthyridine precursor (**3**) was constructed in two steps. Compound **3** was introduced into glycal **4** [4] by Heck reaction to obtain **5**, which was successfully converted to the desired nucleoside **6** over two steps. Then, **6** was tritylated and phosphitylated into **7**, which was incorporated into ODN by an automated DNA synthesizer. ODNs containing **1** showed effective duplex-forming ability with targeted RNA rather than with DNA. Furthermore, DNA/RNA duplexes with consecutive incorporation of **1** were highly stable. The structures of duplexes containing **1** were evaluated by circular dichroism spectroscopy and molecular modeling. In this paper, the attractive fluorescent properties of **1** will be discussed.

***References:***

[1] Matteucci, M. *et al.*, *J. Am. Chem. Soc.* **1995**, *117*, 3873.
[2] Wilhelmsson, L. M. *et al.*, *J. Am. Chem. Soc.* **2008**, *131*, 4288.
[3] Jachak, M. N. *et al.*, *J. Phys. Org. Chem.* **2011**, *24*, 203; Jachak, M. N. *et al.*, *J. Fluoresc.* **2011**, *21*, 1033.
[4] Lan, T. and McLaughlin, L. W., *Bioorg. Chem.* **2001**, *29*, 198.

*Satoshi Obika: E-mail: obika@phs.osaka-u.ac.jp

# Ligand supported approach for cellular uptake of functionalized 5'cap mRNA

Natalia Kleczewska,[a] Paweł J. Sikorski,[a] Joanna Kowalska,[b] Jacek Jemielity [a]
Corresponding Author Jacek Jemielity *[a]

[a] *Centre Of New Technologies, University of Warsaw, Banach St 2c, 02-097 Warsaw, Poland*
[b] *Division of Biophysics, Institute of Experimental Physics, Faculty of Physics, University of Warsaw, Pasteur St 5, 02-093 Warsaw, Poland*

Messenger RNA (mRNA) belongs to RNA molecules that convey genetic information from DNA and direct the assembly of proteins on ribosomes. At the 5' end of mature mRNA in eukaryotic organisms is present a unique structure called mRNA cap. It consist of N7-methylguanosine moiety linked by a 5'–5' triphosphate chain to the first transcribed nucleotide. The unusual chemical structure of the cap is essential for all stages of mRNA metabolism: synthesis, splicing, nucleocytosolic transport, intracellular localization, translation, and turnover. Moreover, the cap mRNA is responsible for 5' mRNA end protection from early degradation step and plays a crucial role in recognition of translation factors. Therefore, some chemical modifications can increase stability of cap and elongate its lifetime or/and better affinity to translation factors. Moreover, synthetic cap analogs as small molecule inhibitors of cap-related processes could be considered as potential therapeutic tools. [1]

To deliver chemically modified cap mRNA from environment into cell, we adjusted ligand supported approach, which involves molecular recognition of small molecules. The cap analogs were fluorescently functionalized as molecular probes to visualization of cellular uptake. [2]

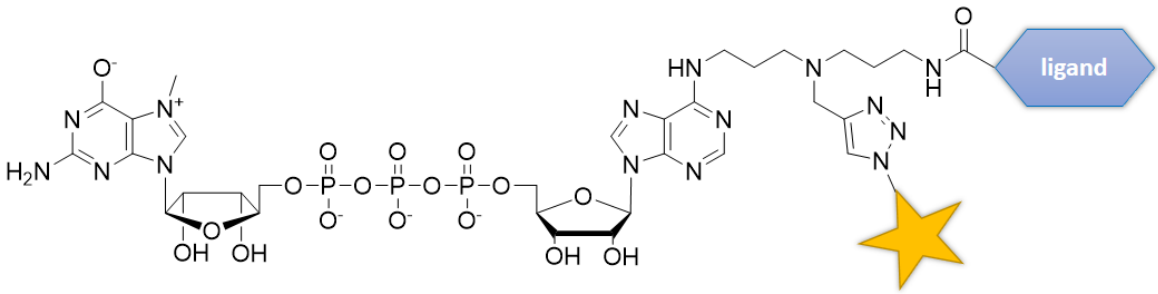



***Figure 1. Cap-ligand analog probe***

This work was supported the *National Science Centre* Poland (UMO-2016/20/S/ST5/00364)

***References:*** [1] Warminski M., Sikorski P. J., Kowalska J., Jemielity J. *Topics in Current Chemistry* 375, **2017**, 16. [2] Srinivasarao M., Low P. S. *Chem. Rev*. **2017**, 117, 12133.

*Corresponding author: E-mail: j.jemielity@cent.uw.edu.pl

# Rational design of thiazole orange probes for detection of DNA and RNA

Piotr Klimkowski,[a] Sara De Ornellas[a,b], Daniel Singleton[d], Afaf H. El-Sagheer[a,c] and Tom Brown[a*]

*[a]Department of Chemistry, University of Oxford, 12 Mansfield Road, Oxford, OX1 3TA, UK [b]Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Headley Way, Oxford, OX3 9DS, UK [c]Chemistry Branch, Department of Science and Mathematics, Faculty of Petroleum and Mining Engineering, Suez University, Suez 43721, Egypt [d]ATDBio, School of Chemistry University of Southampton, SO17 1BJ, UK*

Thiazole orange (TO) is an unsymmetrical cyanine dye which becomes highly fluorescent upon intercalation into nucleic acids due to restricted rotation about the methine bridge.[1] Oligonucleotides (ONs) conjugated to thiazole orange can be used as probes for the detection of target nucleic acids.[2] Another important advantage of TO-containing oligonucleotide probes is the potential for significant increase in duplex stability imparted by intercalation of TO.[3]

ON Probes can be modified with TO at the ribose sugar or the nucleobase. A key variable is the position of attachment of the thiazole orange to the probe; the fluorophore can be linked either via its benzothiazole nitrogen or its quinoline nitrogen. In order to investigate the effects on fluorescence and duplex stability of the mode of attachment of thiazole orange to the probe, three nucleosides, 5-propargylamino-dU (PA), amino C6-dT (C6) and 2′-aminoethoxy-T (AE) were used as phosphoramidite monomers to introduce TO into DNA and 2′-OMe oligonucleotides (Figure 1). OMe-modified probes displayed favourable fluorescence properties compared to their DNA analogues, and have potential for diagnostic applications and as live cell imaging of RNA.

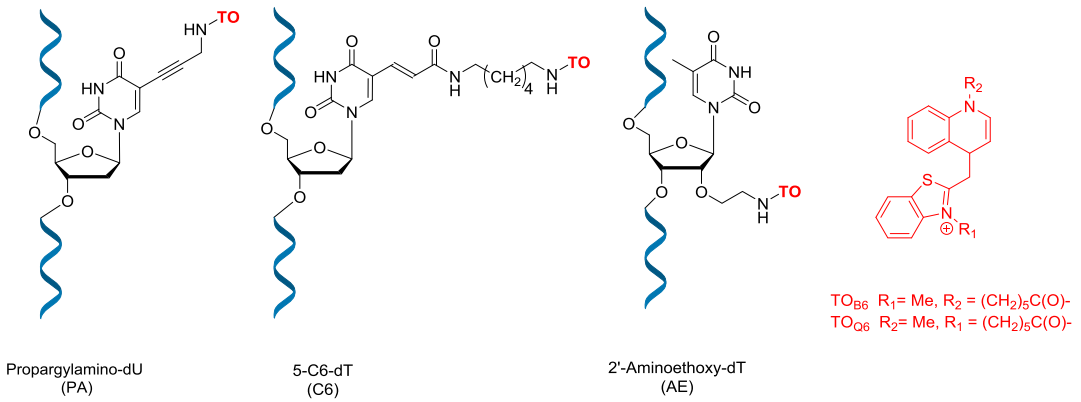



Figure 1 Structures of TO-modified nucleotides PA, C6, AE (left), and the TO moieties used for labelling (right).

This work was supported by the Marie Skłodowska-Curie Innovative Training Network (ITN) ClickGene (H2020-MSCA-ITN-2014-642023).

***References:*** [1] Nygren, J.; Svanvik, N.; Kubista, M., *Biopolymers* **1998,** *46* (1), 39-51. [2] Bethge, L.; Singh, I.; Seitz, O., *Org Biomol Chem* **2010,** *8* (10), 2439-48. [3] Usui, K. et al., *Biochemistry* **2012,** *51* (31), 6056-6067.

*Email: tom.brown@chem.ox.ac.uk

# Design, Synthesis and Biological Evaluation of Dinucleotide mRNA Cap Analog Containing Propargyl Moiety

Anilkumar R. Kore,* Muthian Shanmugasundaram, Irudaya Charles, and Annamalai Senthilvelan

Life Sciences Solutions Group, Thermo Fisher Scientific, 2130 Woodward Street, Austin, TX 78744-1832, USA.

The first example of the synthesis of new dinucleotide cap analog containing propargyl group such as $m^{7,3'\text{-}O\text{-propargyl}}G[5']ppp[5']G$ will be presented. The effect of propargyl cap analog with standard cap was evaluated with respect to their capping efficiency, *in vitro* T7 RNA polymerase transcription efficiency, and translation activity using cultured HeLa cells. It is noteworthy that propargyl cap analog outperforms standard cap by 3.1 fold in terms of translational properties. The propargyl cap analog forms a more stable complex with translation initiation factor eIF4E based on the molecular modeling studies. Details of the finding will be presented during IRT meeting.

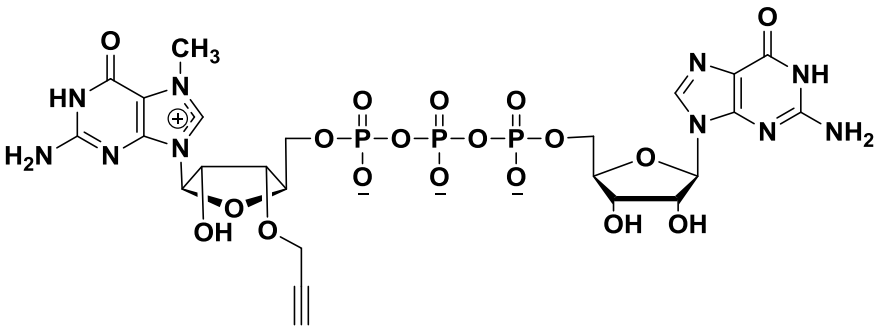


***Figure.*** *Structure of dinucleotide cap analogues i.e.* $m^{7,3'\text{-}O\text{-propargyl}}G[5']ppp[5']G$

***References:*** [1] Muthian, S. et al., *Current Organic Chemistry* **2017**, 21(25), 2530. [2] Muthian, S. et al., *Bioorganic & Medicinal Chemistry* **2016**, 24(6), 1204.

*Corresponding author: E-mail: anil.kore@thermofisher.com

# N - e plated N e - l o ya ine lycosylation

Tommi sterlund,[a] Heidi Korhonen,[a] Pasi Virta*[a]

[a] *Department of Chemistry, University of Turku, Vatselankatu 2, 20014 Turku, Finland*

Kinetically rapid, enzyme-free methods for ligation of NA fragments in aqueous solution are scarce. We have recently reported the potential of alkoxyamine glycosylation as a new NA-templated ligation method. The reaction is exceptional since it operates without any additional catalysts, occurs in slightly acidic conditions and the products are virtually stable at neutral pH. In addition, high anomeric selectivity may be observed.

igation experiments were first carried out at monomeric level without a NA catalyst at a millimolar concentration of the substrates. Accordingly, 5'-*O*-(methylamino)thymidine was prepared and mixed with buffered solutions of -glucose (see Figure) at pH , 5, and . Consistent with previous findings, lowering the pH from to accelerated the reaction while the equilibrium yield decreased. The half-life of the N-glycosylation was h and the equilibrium yield was ca. 5 at pH 5. Only β-anomer was detected in each experiment.

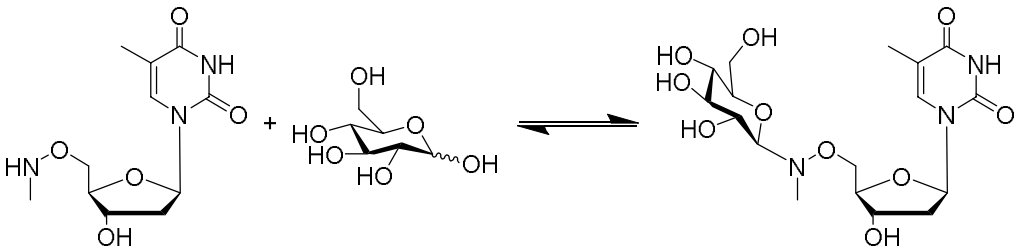


***Figure.*** *N-Glycosylation of 5'-O-(methylamino)thymidine with D-glucose.*

Templating the ligation through Watson-Crick base-pairing offers selectivity and can hasten the chemical reaction by bringing the reactive groups to close proximity and, hence, increasing the local concentration. Therefore, a hairpin stem-template model with 5'-*N*(Me)aminooxy- and '- -glucose-modified oligonucleotides was prepared and the progress of the ligation at a micromolar substrate concentration was followed by an ion-exchange chromatography. A notable rate enhancement and an increased equilibrium yield were observed at pH 5. The half-life of this NA-templated N-glycosylation was ca. h, and the equilibrium yield was ca. . The N-glycosidic linkage was dynamic at pH 5, whereas it became irreversible at pH .

This work was supported by the Academy of Finland.

***References:*** sterlund, T., Korhonen, H., Virta, P. *Org. Lett.* **2 1** , , . audendistel, O. R. et al. *Chem. - Eur. J.* **2 16**, , 5 .

*Corresponding author: E-mail: pamavi@utu.fi

# Oligonucleotides with isopolar nonisosteric 5'-*O*-ethylphosphonate internucleotide linkages.

Ivana Kóšiová*, Miloš Buděšínský, Pavel Novák, Ivana Dvořáková, Šárka Rosenbergová, Ivan Rosenberg

*Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo nám. 2, 166 10 Prague 6, Czech Republic*

Antisense modified oligodeoxynucleotides composed of natural and nucleoside 5'-*O*-methylphosphonate (**1**) units exhibited at 2 to 1 ratio of units a significant increase of *E. coli* RNase H cleavage rate of RNA strand of the modified heteroduplex [1]. In addition these antisense compounds discriminate between RNA and DNA targets, i.e., they increase the thermodynamic stability of DNA*RNA heteroduplex and decrease the stability of DNA*DNA duplex.

Based on the previously obtained results we prepared oligodeoxynucleotides containing various ratio of 5'-*O*-ethylphosphonate (**2**) and 5'-*O*-(hydroxy)ethylphosphonate (**3**) units to examine their ability to (i) hybridize with complementary RNA and DNA and (ii) induce *E. coli* RNase H activity. The advanced phosphotriester method [2] was used for solid phase oligonucleotide synthesis, and starting 5'-*O*-ethyl- and 5'-*O*-(hydroxy)ethylphosphonates were prepared according to the procedure described earlier [3].

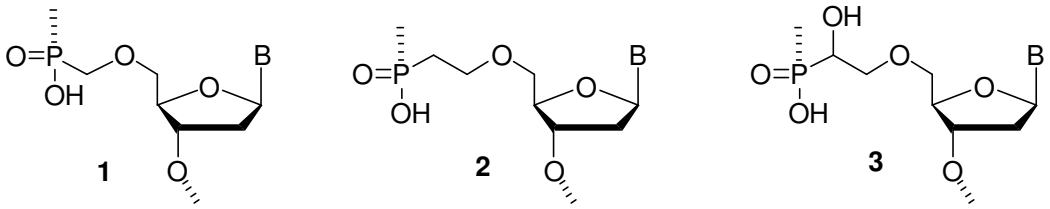



***Figure.*** Structures of the nucleoside phosphonate units

This work was supported by grant # 17-12703S (Czech Science Foundation).

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*Corresponding author: E-mail: kosiova@uochb.cas.cz

# Study on reactivity of H-phosphinate internucleotide linkage.

Ondřej Kostov*, Ondřej Páv, Ondřej Šimák, Magdalena Petrová, Miloš Buděšínský, Pavel Novák, Ivana Dvořáková, Šárka Rosenbergová, Ivan Rosenberg

*Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo nám. 2, 166 10 Praha 6, Czech Republic*

Based on our previous work[1,2], where we presented synthesis of nucleoside H-phosphinate monomers and corresponding oligonucleotides with a mixed H-phosphinate and H-phosphonate linkages, we have continued in further evaluation of the reactivity of the H-phosphinate linkage (Figure 1) in comparison with that of H-phosphonate. For the oxidation of the linkages at the end of synthetic cycle we have elaborated non-aqueous conditions because of sensitivity of the H-phosphinate linkage to the presence of water. We have also elaborated oxidation and sulfurization procedure in each synthetic cycle (oxidative coupling). This would open up possibility combining the H-phosphonate method with the phosphoramidite method while replacement of the oxygen atom with sulfur at selected positions. Finally, we have found conditions for selective oxidation, sulfurization, and amidation of H-phosphonate and H-phosphinate linkages in each synthetic cycle. Thanks to these findings, we are able to prepare different sequences of such modified oligonucleotides and examine their physicochemical and biological properties. In addition, we have found a method purely transforming P(=S) or P(-N) into P(=O) oligonucleotides.

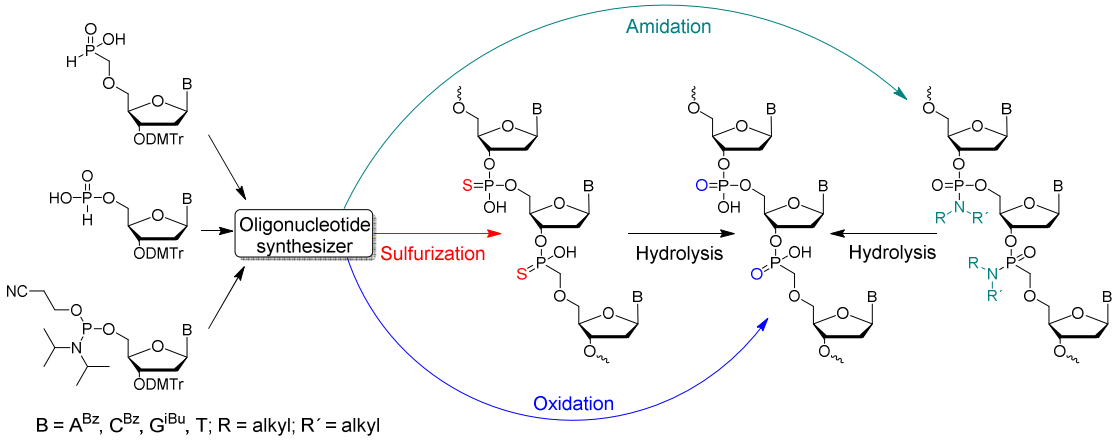



***Figure 1.*** *Synthetic approaches for synthesis of modified oligonucleotides*

This work was supported by grant # 15-31604A (Ministry of Health, CR, all rights reserved).

*Corresponding author: E-mail: kostov@uochb.cas.cz

***References:***

*1. Kostov, O.; Páv, O.; Buděšínský, M.; Liboska, R.; Šimák, O.; Petrová, M.; Novák, P.; Rosenberg, I., Org.Lett. 2016, 18 (11), 2704-2707.*

*2. Kostov, O.; Páv, O.; Rosenberg, I., Current Protocols in Nucleic Acid Chemistry*, 2017, 70, 4.76.1–4.76.22.

# Syntheses and RNAi activity of amide-modified siRNAs

Venubabu Kotikam* and Eriks Rozners*

*Department of Chemistry, Binghamton University, NY 13902, USA*

Recent developments in antisense, RNA interference (RNAi) and, most recently, CRISPR-Cas9 technologies continue driving the interest in synthetic chemistry of oligonucleotides. Our group has been developing amide linkages as replacement of phosphates in RNA. Recently, our group demonstrated that siRNA bearing amide linkages at certain positions were more active than unmodified siRNAs in RNAi mediated mRNA degradation [1,2]. However, a detailed investigation pertaining to the position and number of amide linkages that are required to exhibit the optimum effect required optimization of synthetic route to the monomeric building blocks and coupling of these monomers to prepare the amide-RNAs with satisfactory yields. This presentation will discuss a novel and concise stereo selective synthesis of 3'-homologated ribonucleoside amino acids, building blocks for solid-phase synthesis of consecutive amide linkages in RNA. Our strategy utilized an atom-economical hydrogenation for the transformation of three different functional groups in a penultimate step with excellent conversion (>90%). Fine-tuning of the substrates and solvent systems led to a synthetic route that does not require separate deprotection step to reach the final building blocks [3].

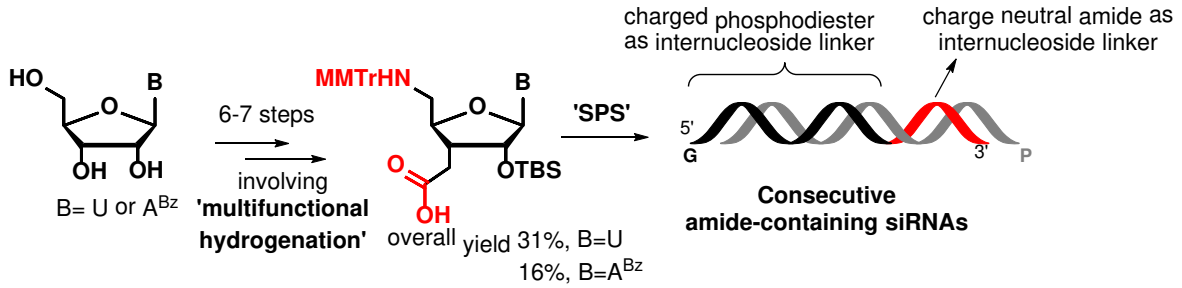



***Figure.*** *Syntheses of consecutive amide-containing siRNAs.*

This presentation will also discuss the syntheses of a series of siRNA guide strands containing up to seven consecutive amide linkages at the 3'-end. Preliminary results on their in vitro RNAi activity will be presented.

This work was supported by the US National Institutes of Health (R01 GM071461).

***References:*** [1] Mutisya, D., et al. *Nucleic Acid Res.* **2014***, 42,* 6542. [2] Mutisya, D., et al. *Nucleic Acid Res.* **2017**, *45*, 8142. [3] Kotikam, V., et al. *Org. Lett.* **2017***, 19,* 4122.

*Corresponding author: E-mail: vkotikam@binghamton.edu/erozners@binghamton.edu

# Synthesis of novel m$^7$GMP analogues and evaluation as the inhibitors of cNIIIB nucleotidase

Mateusz Kozarski,[a] Dorota Kubacka,[b] Blazej A. Wojtczak,[b] Renata Kasprzyk,[b,c] Marek Baranowski,[a] Joanna Kowalska*[a]

[a] *Faculty of Physics, Institute of Experimental Physics, Division of Biophysics, University of Warsaw, Pasteura 5, 02-093 Warsaw, Poland*
[b] *Centre of New Technologies, University of Warsaw, Banacha 2c, 02-097 Warsaw, Poland*
[c] *College of Inter-Faculty Individual Studies in Mathematics and Natural Sciences, University of Warsaw, Banacha 2c, 02-097 Warsaw, Poland*

5'-nucleotidases are enzymes responsible for hydrolysis of nucleosides 5'-monophosphates to corresponding nucleosides and inorganic phosphate. 5'-Nucleotidases are involved in the regulation of cellular levels of nucleoside 5'-monophosphates. Some members of 5'-nucleotidases are also involved in deactivation of certain nucleoside-derived drugs [1]. A recently identified 5'-nucleotidase cNIIIB shows preference towards 7-methylguanosine monophosphate (m$^7$GMP) as a substrate, which suggests its potential involvement in mRNA degradation [2]. However, the biological functions of cNIIIB are still unknown. Here, we synthesized a series of m$^7$GMP analogues that could be used to modulate processes related to cNIIIB activity.

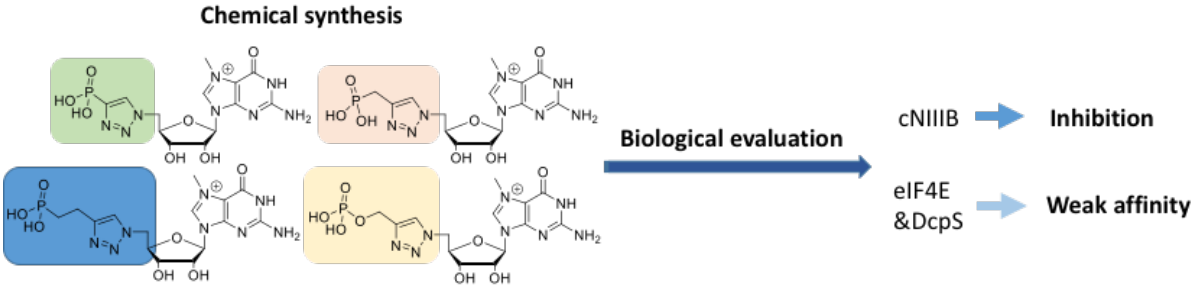



***Figure.*** *The main aim of the project*

The novel m$^7$GMP analogues that contain modifications 5' positions of 7-methylguanosine were synthesized. Obtained m$^7$GMP analogues were characterized by nuclear magnetic resonance spectroscopy (NMR) and high-resolution mass spectrometry (HRMS) and evaluated as inhibitors of cNIIIB enzyme. Also the affinities for other proteins that are involved in m$^7$G-nucleotides recognition (eIF4E and DcpS) were determined [3].

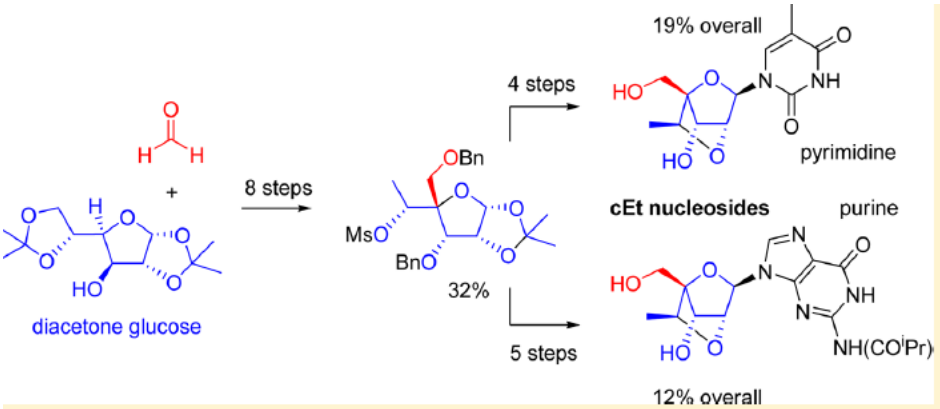
This work was supported by the National Science Center in Poland (UMO-2017/24/C/NZ1/00169)

***References:*** [1] Mammalian 5'-Nucleotidases, Bianchi V. et al., *JBC* **2003**, *278*, 47. [2] Crystal Structures of the Novel Cytosolic 5'-Nucleotidase IIIB Explain Its Preference for m$^7$GMP, Monecke T., et al. *PLOS* **2014**, *9*, 3. [3] 7-Methylguanosine monophosphate analogues with 5'-(1,2,3-triazoyl) moiety: Synthesis and evaluation as the inhibitors of cNIIIB nucleotidase, Kozarski M., Kubacka D. et al. *BMC* **2018**, *26*, 1.
*Corresponding author: E-mail: jkowalska@fuw.edu.pl

# Development and Scale-Up leading to a Modular Synthesis of Constrained Ethyl (cEt) Purine and Pyrimidine Nucleosides.

Venkata Krishnamurthy, Ph.D., Associate Principal Scientist, AstraZeneca, Waltham, USA.

**Abstract Title:** AstraZeneca have developed a modular and scalable approach to pyrimidine- and purine-containing constrained ethyl (cEt) nucleosides. Minimizing stereochemical adjustments and protecting group manipulations, diacetone glucose was converted to two representative cEt nucleosides via a functionalized, common intermediate. By comparison to the existing synthetic route, this approach offers i) reduction in the step count from 23 steps to 17 steps ii) increased yield iii) greater atom efficiency iv) an improved purity profile. The retrosynthetic approach to this complex class of drug precursors offers clear benefits over existing routes that will ultimately help provide new oligonucleotide based medicines to patients.




For reference see Blade, H., et. al. J. Org. Chem. 2015, Vol 80, 5337-5343

Email: Venkata.krishnamurthy@AstraZeneca.com

# Structural insights into the processing of modified nucleotides and primers by A- and B-family DNA polymerases

Heike M. Kropp,[a] Andreas Marx*[a]

[a] *Department of Chemistry, University of Konstanz, Universitätsstraße 10, 78464 Konstanz, Germany*

Thermostable A- and B-family DNA polymerases (DNA pols) are core aspects of various biochemical applications like next-generation sequencing. [1] Here, their capability of accepting chemically modified nucleotides as substrates for the DNA synthesis is of outstanding importance. It was shown that A- and B-family DNA pols accept modified nucleotides differently, where B-family DNA pols are mostly superior and show a broader substrate range. [2, 3] But as the structural knowledge about the interactions between a modified triphosphate and the polymerase are so far only observed from A-family DNA pols (KlenTaq DNA pol), [4] no conclusion can be made, on which features this superiority of B-family DNA pols relies on.

To close the knowledge gap on this biotechnologically important class of enzymes, we succeeded in obtaining the first high resolution crystal structures of two B-family DNA pols (KOD and 9°N DNA pols), which will be presented. [5]

Furthermore, I will present partially unpublished data of an A- and B-family DNA pol in complex with chemically modified nucleotides. My presentation will feature differences among the DNA polymerases in the interaction patterns at the incorporation site as well as within the primer template duplex.

This work was supported by the Deutsche Forschungsgemeinschaft and the European Research Council.

***References:*** [1] Bentley, D.R., et al., *Nature*, 2008. **456**(7218): p. 53-59. [2] Kuwahara, M., et al., *Nucleic Acids Res*. , 2006. **34**(19): p. 5383-5394. [3] Jäger, S., et al., *J. Am. Chem. Soc.*, 2005. **127**(43): p. 15071-15082. [4] Hottin, A. and A. Marx,. *Acc. Chem. Res*., 2016. **49**(3): p. 418-427. [5] Kropp, H.M., et al., *PLOS ONE*, 2017. **12**(12): p. e0188005.

*Corresponding author: andreas.marx@uni-konstanz.de

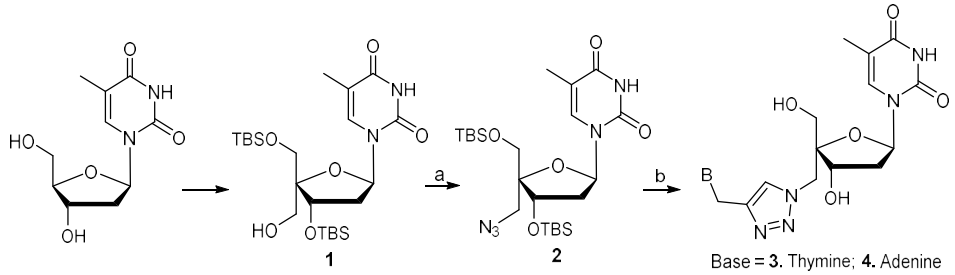
# Synthesis of double-headed nucleosides via click chemistry approach

Surender Kumar

*Department of Chemistry, University College, Kurukshetra University, Kurukshetra, Haryana, 136119, India*

The chemically modified nucleosides and oligonucleotides have great potentials in nucleic acid nanotechnology,[1-2] oligonucleotide-based diagnostics and as antisense drugs[3]. In search of novel nucleic acid structures, double-headed nucleotides (defined as nucleotides with additional nucleobases) were synthesized and interesting structural motifs has been found. By adding an additional nucleobase in nucleic acid sequence, both stacking and hydrogen-bonding interactions with target are possible. To investigate the effect of additional nucleobase for base-base recognition and for interactions in nucleic acid secondary structures, we have synthesized two double headed nucleosides (3,4) bearing additional thymine and adenine nucleobase.

Thymidine was converted into nucleoside **1** in eight steps in 12% overall yield (Figure 1). The desired azido derivatives **2** was synthesized and further double headed nucleosides bearing additional thymine and adenine nucleobase were synthesized by using Cu–catalysed alkyn-azide cycloaddition (CuAAC) reaction. These double headed nucleosides (3,4) would be converted into phosphoramidite and hybridizing properties would be studied.




**Reagents and conditions:** (a) i. MsCl, $CH_2Cl_2$, 2,6-lutidine, 84%; ii. $NaN_3$, DMF, 145°C, 53%; (b) i. Na ascorbate, $CuSO_45H_2O$, *t*-BuOH, $H_2O$, 73%, (B = Benzoylatedadenine); ii. TBAF, THF, 63% ( B = Adenine), 41%, B = Thymine after 2 steps.

***Figure 1.*** *Synthesis of double-headed nucleosides.*

Both the double-headed nucleosides bearing additional thymine and adenine nucleobase were synthesized from thymidine as a starting material by linear strategy. The hybridizing properties and other biological applications would be studied later.

The Department of Science and Technology (DST), New Delhi is highly acknowledged for providing the financial assistance for this project.

***References:*** [1] H. Yan, Science, **2004**, *306*, 2048. [2] Wengel, J. *Org. Biomol, Chem.*, **2004**, *2*, 277. [3] Kurreck, J. *Eur. J. Biochem.*, **2003**, *270*, 1628.

Corresponding author: E-mail: skjchem@yahoo.com

# DNA Nanocubes Interfacing with Biological Systems

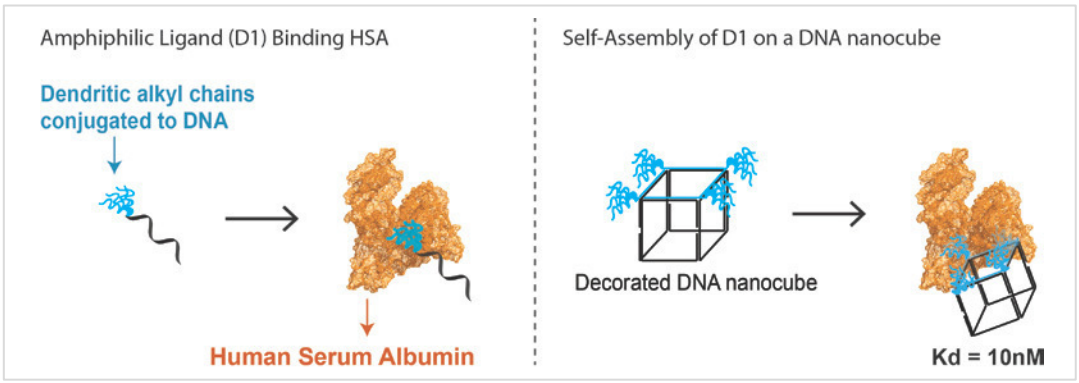
Aurélie Lacroix,[a] Empar Vengut-Climent,[a] Thomas G. W. Edwardson,[b] Hassan Fakih,[a] Mark A. Hancock,[c] Michael D. Dore,[a] Hanadi F. Sleiman,[a] hanadi.sleiman@mcgill.ca

[a] *Department of Chemistry and Centre for Self-Assembled Chemical Structures (CSACS), McGill University, Montréal, Québec H3A 0B8, Canada*
[b] *Laboratory of Organic Chemistry, ETH Zurich, 8093 Zurich, Switzerland*
[c] *SPR-MS Facility, McGill University, Montréal, Québec H3G 1Y6, Canada*

DNA-minimal nanostructures have been of great interest for biological applications such as bio-sensing or drug delivery, because of their low cost, high yielding assembly and retained functionality. [1]

We describe a new strategy to engineer DNA structures with strong binding affinity to human serum albumin (HSA). [2] HSA is the most abundant protein in the blood and has a long circulation half-life (19 days). It has been shown to hinder phagocytosis, is retained in tumors, and aids in cellular penetration. We show that conjugating dendritic alkyl chains to DNA creates amphiphiles that exhibit high-affinity binding (Kd in the low nanomolar range) to HSA. Notably, complexation with HSA did not hinder the activity of silencing oligonucleotides inside cells, and the degradation of DNA strands in serum was slowed. We also show that, in a site-specific manner, altering the number and orientation of the amphiphilic ligand on a self-assembled DNA nanocube can modulate the affinity of the DNA cage to HSA. The serum half-life of the amphiphile bound to the cage and the protein was shown to reach up to 22 hours. Finally, we will present preliminary results on cellular uptake and biodistribution in mouse models of the amphiphilic ligand.

In a second part, we investigated the cellular uptake of DNA nanocubes in mammalian cancer cells, and their cellular fate. Most of the studies have relied on attaching cyanine dyes (positively charged) to nanostructures, such as Cy3- and Cy5-, because of the availability of their phosphoramidite derivatives commercially. These dyes can direct the uptake of DNA strands themselves and interact strongly with mitochondria.




***References:*** [1] Bujold, K. et al., *Chem,* **2018***, 4 (3)*, 495-521
[2] Lacroix, A. et al., *J. Am. Chem. Soc.,* **2017***, 139 (21)*, 7355-7362

*Corresponding author: E-mail: hanadi.sleiman@mcgill.ca

# Assembly and Applications of Tetraplex DNA/RNA Foldamers

Thomas Lavergne*, Laureen Bonnat, Alexandre Devaux, Laure Bar, Yang Si, Camille Van Wesemael, Jérôme Dejeu, Eric Defrancq

*Département de Chimie Moléculaire, Université Grenoble Alpes-CNRS, Grenoble, France*

Tetraplexes are unusual nucleic acid structures formed through non-canonical nucleobase interactions within G-rich (G-quadruplex, (G4)) and C-rich (i-motif) DNA and RNA (Fig. 1A). There are now compelling evidences demonstrating that those structures form in key regulatory regions of genomes and transcriptomes where they can be involved in normal and pathological cellular processes. Thereby, the identification of chemical probes and drug candidates capable of specifically binding and/or stabilizing those structures is of great interest. On the other hand, the structural features of synthetic tetraplexes has been exploited to develop potent aptamers, catalysts and probes.

Herein, we will report on our recent efforts to assemble bioconjugates that fold into stable mimics of G4 structures derived from telomeric and viral sequences and on our on-going efforts to assemble stable mimics of i-motif structures (Fig. 1B). We will discuss the synthesis of those molecular objects via orthogonal ligations from heterofunctionalized cyclopeptides and oligonucleotides. We will also briefly discuss the use of those mimics for - the evaluation and development of structure-selective G4 binding ligands, - the identification of their cellular binding partners via pull-down assays - the design of G4 aptamers with antiviral activities.

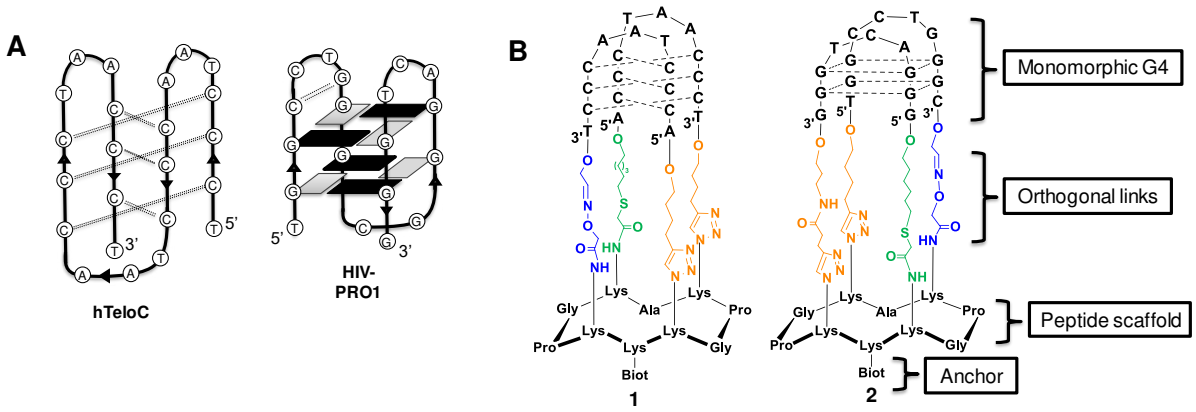



***Figure 1. A**/ Schematic drawing of an i-motif structure formed by the human telomeric DNA sequence (hTeloC) and a G4 structure formed by the HIV-1 promoter sequence (HIV-PRO1) **B**/ Template assembled structural foldamers mimicking i-motif and G4 folds formed by the human telomere sequence (1) and the HIV-1 promoter sequence (2)*

This work is supported by grants from the Labex Arcane (ANR-11-LABX-0003-01), the ANR (ANR-16-CE11-0006-01 and ANR-16-TERC-0021-01) and the Région Auvergne Rhône-Alpes

***References:*** [1] Bonnat, L ; Dejeu, J ; Bonnet, H ; Gennaro, B ; Jarjayes, O ; Thomas, F ; Lavergne, T ; Defrancq, E Chem. Eur. J. **2016**, *22*, 3139 [2] Bonnat, L ; Bar, L ; Bonnet, H ; Jarjayes, O ; Thomas, F ; Dejeu, J ; Defrancq, E ; Lavergne, T ; Chem. Eur. J. **2017**, *23*, 5602

*Corresponding author: E-mail: Thomas.lavergne@univ-grenoble-alpes.fr

# Molecular dynamics simulations of the structure and binding strength of siRNA-peptide complexes

Hwankyu Lee*[a]

[a] *Department of Chemical Engineering, Dankook University, Yongin-si, South Korea*

All-atom (AA) and coarse-grained (CG) molecular dynamics (MD) simulations were performed to determine the effect of the size of arginine(Arg)-rich peptides on the structure and binding strength of the siRNA-peptide complex. At a fixed peptide/siRNA mole ratio of 5:1 or 10:1, the siRNA complexes with peptides longer than 9 Arg residues are more easily decomplexed by heparin than are those with 9 Arg residues. At these mole ratios, peptides longer than 9 Arg residues have cationic/anionic charge ratios in excess of unity, and produce more weakly bound complexes than 9-Arg residue ones do. AA simulations of mixtures of peptides with a single siRNA show formation of an electrostatically-induced complex, and the longer peptides produce a larger complex, but with no significant increase in the number of Arg residues bound to the siRNA. Larger-scale CG-MD simulations show that multiple siRNAs can be linked together by peptides into a large complex, as observed in the experiments. The peptides longer than 9 residues, which at mole ratio 5:1 yield a peptide/siRNA charge ratio in excess of unity, include many non-interacting Arg residues, which repel each other electrostatically. This leads to a less dense complex than for 9-residue peptides, which can explain why these longer complexes are more easily decomplexed by heparin molecules, as observed in the experiments. The key role of the charge ratio is supported by simulations that show that at a mole ratio of 2.5 peptides per siRNA, the longer 18-residue peptide has a charge ratio of roughly unity, and also shows a tight complex, just as the 9-residue peptide does at a 5:1 mole ratio, where its charge ratio is also unity.

*Corresponding author: E-mail: leeh@dankook.ac.kr

# Post-synthetic approach to the chemical preparation of tRNA fragments modified with 5-substituted 2-thiouridines, geranylated 2-thiouridines and cyclic form of $t^6A$

Grazyna Leszczynska,[a] Karolina Bartosik,[a] Michal Matuszewski,[a] Klaudia Sadowska,[a] Katarzyna Debiec,[a] Agnieszka Dziergowska,[a] Barbara Nawrot,[b] Elzbieta Sochacka*[a]

[a] *Institute of Organic Chemistry, Lodz University of Technology, Zeromskiego 116, Lodz;* [b] *Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, Sienkiewicza 112, Lodz, Poland*

Modified nucleosides in tRNA are mostly located in the anticodon loop domain, particularly at positions 34 (wobble) and 37 (3'-adjacent to the anticodon). Modifications within the anticodon domain are known to be essential for accuracy and efficiency of protein biosynthesis, however their contribution to the codon-anticodon interactions and the translation process is still not fully understood. Relevant studies are limited mainly due to the difficult access to the model modified oligomers.

Herein, we report new methods for chemical preparation of modified tRNA fragments containing wobble hypermodified 5-substituted-2-thiouridines ($nm^5s^2U_{34}$, $mnm^5s^2U_{34}$, $cmnm^5s^2U_{34}$, $\tau m^5s^2U_{34}$) and *S*-geranylated 2-thiouridines ($ges^2U$, $mnm^5ges^2U_{34}$) as well as recently discovered cyclic form of N6-threonylcarbamoyladenosine ($ct^6A_{37}$) *via* post-synthetic transformations of suitable precursor oligoribonucleotides [1-3] (Fig.).

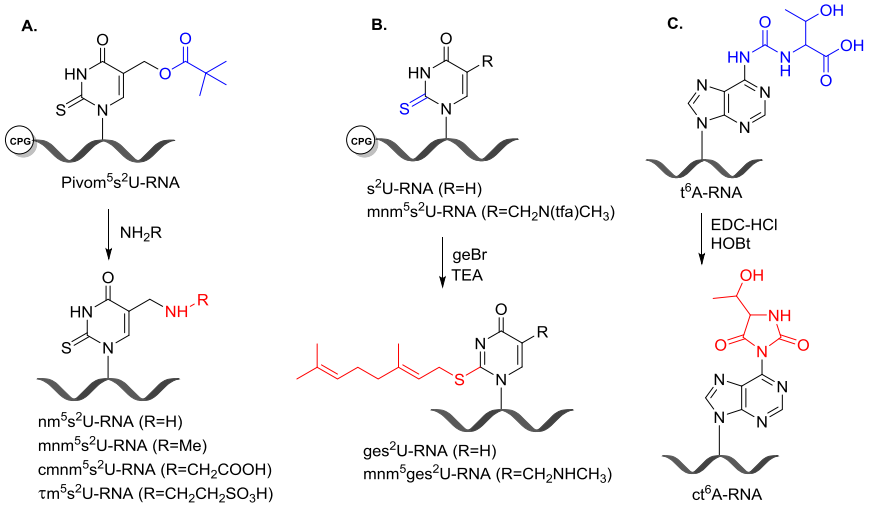



***Figure.*** *General routes for the post-synthetic preparation of RNAs modified by 5-substituted 2-thiouridines (A); S-geranylated 2-thiouridine (B) and a cyclic form of $t^6A$ (C).*

This work was financially supported by the National Science Centre, Poland, grant No. UMO-2017/25/B/ST5/00971 to E.S.

***References:*** [1] Bartosik, K. et al., *Org. Biomol. Chem.* **2017**, *15*, 2097. [2] Sierant, M., Leszczynska, G. et al., *Nucleic Acids Res.* **2016**, *44*, 10986. [3] Matuszewski, M. et al. *Chem.Commun.* **2017**, *53*, 7945.

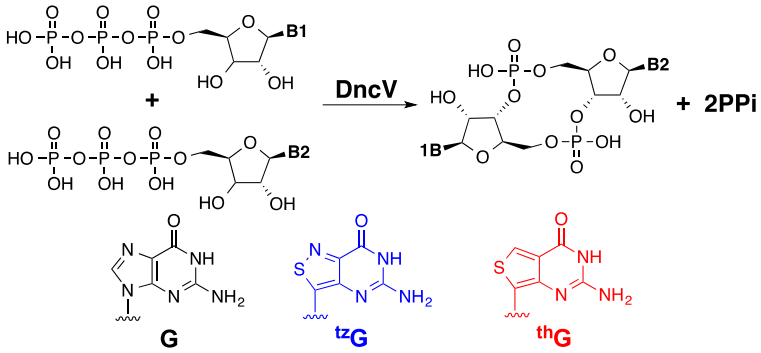
*Corresponding author: E-mail: elzbieta.sochacka@p.lodz.pl

# Tuning the Innate Immune Response to Cyclic Dinucleotides with Atomic Mutagenesis

Yao Li,[a] Andrea Fin,[a] Paul Ludford III,[a] Yitzhak Tor*[a]

[a] *Department of Chemistry and Biochemistry, University of California, San Diego, 9500 Gilman Dr, La Jolla, CA 92093, USA*

The discovery that cyclic dinucleotides (CDNs), bacterial second messengers, trigger the innate immune response in eukaryotic cells through the STING-TBK1 pathway has provided new insight into their biology and applications.[1,2] We report the enzymatic preparation, as well as biophysical and biochemical application of a novel group of c-di-GMP analogs. By employing an "atomic mutagenesis" strategy, which allowed us to change one specific atom in a molecule at a time, we obtained valuable mechanistic insights into reactions mediated by cyclase and phosphodiesterase (PDE), which play important roles in c-di-GMP metabolism and signaling transduction. Moreover, these c-di-GMP analogs that feature a gradual change in their atomic structures display variations in their ability to induce type-I interferon (IFN) production, with several derivatives being more potent than their native archetype.




| | B1 | B2 |
|---|---|---|
| c-di-GMP | G | G |
| c-di-$^{tz}$GMP | $^{tz}$G | $^{tz}$G |
| c-di-$^{th}$GMP | $^{th}$G | $^{th}$G |
| c-G$^{th}$GMP | $^{th}$G | G |
| c-G$^{tz}$GMP | $^{tz}$G | G |

***Figure.*** *Enzymatic preparation of c-di-GMP analogs. DncV is able to convert two NTPs (any combination GTP and GTP surrogate) into the corresponding cyclic dinucleotides.*

This work was supported by the National Institutes of Health (grant number GM069773).

***References:*** [1] Danilchanka, O. et al., *Cell* **2013***, 154,* 962-970. [2] McWhirter, S. M. et al., *J. Exp. Med.* **2009**, *206*, 1899-1911.

*Corresponding author: E-mail: ytor@ucsd.edu

# Synthesis and Evolution of a TNA Aptamer Bearing 7-Deaza-7-Substituted Guanosine Residues

Jen-Yu Liao,[a] Hui Mei,[a] Randi Jimenez,[a] Yajun Wang,[a] Saikat Bala,[a] Cailen McCloskey,[a] Christopher Switzer,[b] John C. Chaput*[a]

[a] *Department of Pharmaceutical Sciences, University of California, Irvine, California 92697, United States of America*
[b] *Department of Chemistry, University of California, Riverside, California 92521, United States of America*

Threose nucleic acid (TNA) is an unnatural genetic polymer capable of undergoing the *in vitro* selection, a process that requires faithful enzymes that can transcribe and reverse transcribe between DNA and TNA molecules. Previously, we have shown that Kod-RI, an engineered polymerase developed to transcribe single-stranded DNA template into TNA, can function with high fidelity in the absence of manganese ions. However, the transcriptional efficiency of this enzyme significantly reduces when working with DNA library pools. With the addition of manganese ions to the transcription mixture, G to C transversion mutations were observed following the reverse transcription of TNA into DNA. Here we report the synthesis and fidelity of TNA replication using 7-deaza-7-modified guanosine base analogues in the DNA template and incoming TNA nucleoside triphosphate.

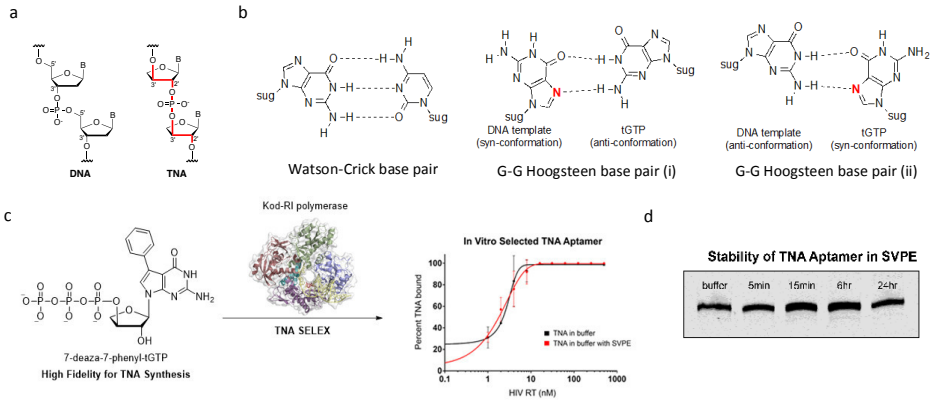



***Figure.*** *TNA SELEX containing 7-deaza-7-phenyl-GTP*

Our findings reveal that tGTP misincorporation occurs via a Hoogsteen base pair in which the incoming tGTP residue adopts a *syn* conformation with respect to the sugar. Substitution of tGTP for 7-deaza-7-phenyl tGTP enabled the replication of TNA polymers with >99% overall fidelity. A TNA library containing the 7-deaza-7-phenyl tGTP analogue was used to evolve a biologically stable TNA aptamer that binds to HIV reverse transcriptase with 10nM affinity.
This work was supported by DARPA (N66001-16-2-4061) and the NSF (1607111).

***References:*** Paper under review.

*Corresponding author: E-mail: jchaput@uci.edu

# Colorimetric detection of DNA amplicons using the rapid disassembly of DNA-gold nanoparticle aggregates at room temperature

Yuning Liu,[a] B. Safeenaz Alladin-Mustan,[a] Julianne M. Gibbs *[a]

[a] *Department of Chemistry, University of Alberta, Edmonton, Alberta, T6G 2G2 Canada*

One of the requirements of an ideal point-of-care (POC) detection strategy according to the World Health Organization is that be instrument-free, so direct visualization is an optimal method for DNA diagnostics at the point of care. Our group has developed a rapid colorimetric detection method based on the disassembly of aggregated DNA-modified gold nanoparticles, which can operate over a wide range of temperatures.[1] The gold nanoparticle aggregates are formed over hours through DNA hybridization of DNA bound to the nanoparticles with a linker DNA in solution. However, upon adding target DNA that is complementary to this linker, disassembly is accomplished within 10 minutes. Moreover, by adding a nucleotide overhang region on the both sides of the linker, we were able to perform colorimetric detection at room temperature. Here I will describe our recent efforts to combine this approach with our group's DNA amplification technique that works at one temperature which we call lesion induced DNA amplification (LIDA) [2,3].

LIDA has proven to be a general method for DNA amplification of short DNA sequences. [2] Our group has shown that we can perform DNA LIDA over a covering wide range of room temperature, and on the benchtop without any equipment. [3] We are now expanding LIDA to the detection of RNA biomarkers. For the latter, the first step consists of transcribing the RNA to complementary DNA (cDNA), which can then be amplified and detected using LIDA. The set up of the experiment consists of only two Eppendorf tubes (with and without the target RNA) with the required reagents (15 µL total volume) on a tube rack placed on the bench top at room temperature. Then, the resulting DNA amplicons can be detected colorimetrically also at room temperature by DNA-modified gold nanoparticles.

With this combination we achieved rapid, isothermal RNA triggered DNA amplification on the bench top without heating equipment. Additionally, we reduced the limit of detection (LOD) for this colorimetric detection method by combining it with our amplification technique.

***References:*** [1] Lam, M. K., et al., *Langmuir,* **2016***, 32 (6)*, 1585–1590. [2] Kausar, A., et al. *Angew. Chem. Int. Ed.* **2013**, 52, 10577 –10581. [3] Alladin-Mustan, B. S., et al. *Chem. Commun.* **2015**, *51*, 9101-9104.

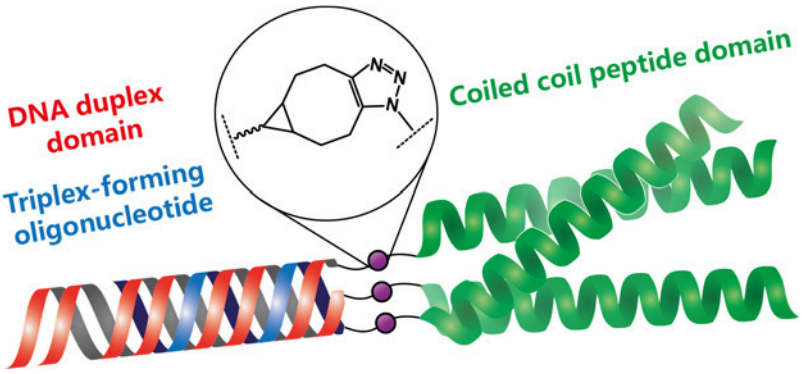
*Corresponding author: E-mail: julianne.gibbs@ualberta.ca

# *De novo* protein design: templating artificial coiled coil structures by an oligonucleotide triplex

Chenguang Lou,[a]* Manuel C. Martos-Maldonado,[b] Charlotte S. Madsen,[b] Rasmus P. Thomsen,[c] Søren R. Midtgaard,[d] Niels J. Christensen,[b] Jørgen Kjems,[c] Peter W. Thulstrup,[e] Jesper Wengel[a] & Knud J. Jensen[b]

[a] *Biomolecular Nanoscale Engineering Center, Department of Physics, Chemistry and Pharmacy, University of Southern Denmark, Campusvej 55, 5230 Odense M, Denmark*
[b] *Biomolecular Nanoscale Engineering Center, Department of Chemistry, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg, Denmark*
[c] *Biomolecular Nanoscale Engineering Center (iNANO) and Department of Molecular Biology and Genetics, University of Aarhus, Gustav Wieds Vej 14, 8000 Aarhus C, Denmark*
[d] *Niels Bohr Institute, University of Copenhagen, Universitetsparken 5, 2100 Copenhagen Ø, Denmark*
[e] *Department of Chemistry, University of Copenhagen, Universitetsparken 5, 2100 Copenhagen Ø, Denmark*

Abstract: Two orthogonal self-assembly principles, oligonucleotide triple helix and a coiled coil protein domain formation, have for the first time been combined for *de novo* construction of artificial proteins with unique folding patterns. The results demonstrated a significant stabilizing cooperativity between the two disparate domains, portraying the formation of the desired triple helix and coiled coil domains at low concentrations, while a dimer of trimers was dominating at high concentration. Furthermore, the oligonucleotide triplex was employed to template the peptide folding of a short but stable coiled coil and the formed ensemble structurally differed from the individual peptide-oligonucleotide conjugate strands and the unconjugated peptide. Besides the geometric templating effect, chirality propagation was also investigated using stereoisomeric oligonucleotides and peptides, as was effect of peptide orientation. These results validate the use of orthogonal self-assembly principles as a paradigm for de novo protein design scaffolded by nucleic acid hybridization.




This work was supported by grants from VILLUM FONDEN.

***References:*** [1] Lou, C., et al., *Nat. Commun.* **2016**, 7, 12294. [2]. Lou, C., et al., *Chem. Eur. J.* **2017**, 23, 9297.

*Corresponding author: E-mail: chenguang@sdu.dk

# Fluorescing Isofunctional Ribonucleosides: Assessing Adenosine Deaminase Activity and Inhibition

Paul Ludford, Alex R. Rovira, Andrea Fin, Yitzhak Tor*[a]

[a] *Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla, California, 92093-0358, United States*

The conversion of isothiazolo[4,3-d]pyrimidine based adenosine ($^{tz}$A) and 2-aminoadenosine ($^{tz}$2-AA) analogues to the corresponding isothiazolo[4,3-d]pyrimidine based inosine ($^{tz}$I) and guanosine ($^{tz}$G) derivatives by Adenosine Deaminase (ADA) is evaluated and compared to the conversion of native adenosine to inosine (**Figure**).[1] A foundation for a high throughput screening assay is established and the efficacy of the assay showcased via fluorescence-based analysis of $^{tz}$A conversion to $^{tz}$I in the presence of known and newly synthesized inhibitors.

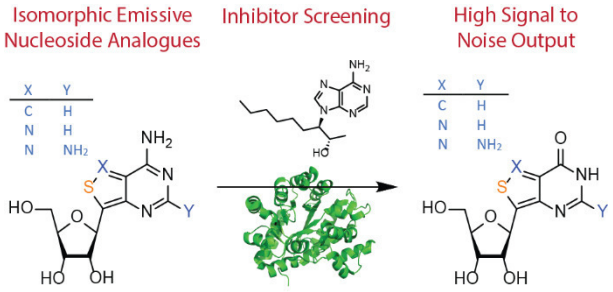



***Figure.*** *Adenosine Conversion to Inosine Analogues in the Presence of Adenosine Deaminase.*

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***References:*** [1] A. R. Rovira, A. Fin and Y. Tor, *J. Am. Chem. Soc.*, 2015, **137**, 14602–14605.

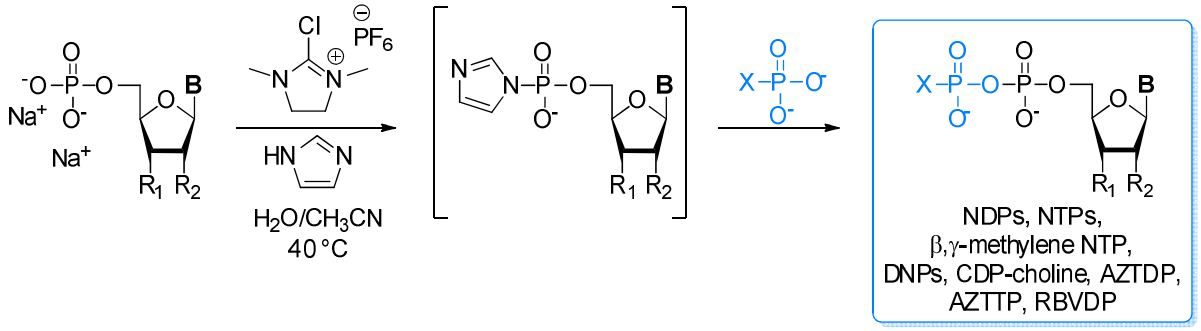
*Corresponding author: E-mail: ytor@ucsd.edu

# One-Pot synthesis of nucleotides in water-medium

Suzanne Peyrottes,* Anaïs Depaix, Béatrice Roy

*Institut des Biomolécules Max Mousseron (IBMM), UMR 5247 CNRS, Université de Montpellier, ENSCM, Campus Triolet, cc 1705, Place Eugène Bataillon, 34095 Montpellier, France*

Given the importance of nucleotides and their derivatives in biological processes, numerous methods have been developed to access to these compounds and their structural analogues [1]. Phosphoramidates, in general, and phosphorimidazolides in particular, have been extensively used as intermediates for pyrophosphate bond formation in anhydrous organic solvents. We recently developed a one-pot approach, in a mixture of water and acetonitrile, to obtain nucleoside 5'-di- and 5'-triphosphates, dinucleoside 5',5'-polyphosphates as well as some nucleotide analogues modified either on the nucleosidic or on the phosphate moieties [2,3]. The attractive features of this strategy include absence of protecting groups on the starting material and convenient set-up. The experimental results demonstrated the applicability of the reported method for the synthesis of a variety of nucleotides.




This work was supported by a grant from the University of Montpellier.

***References:*** [1] Roy, B. et al., *Chem. Rev.* **2016***, 116*, 7854. [2] Depaix, A., et al. *Eur. J. Org. Chem.* **2017**, 241. [3] Depaix, A., et al. *Current Protocols in Nucleic Acid Chemistry*, **2017**, *69*, 13.16.1-13.16.11.

*Corresponding author: E-mail: suzanne.peyrottes@umontpellier.fr

# Cellular Delivery of Peptide Nucleic Acid (PNA) by Calixarene Derivatives

Roberto Corradini,[a] Alessia Finotti,[b] Jessica Gasparello,[b] Alex Manicardi,[a] Alessandro Casnati,[a] Francesco Sansone,*[a] Roberto Gambari*[b]

[a] *University of Parma, Department of Chemistry, Life Sciences and Environmental Sustainability, Viale delle Scienze 17\A Parma, Italy.*
[b] *University of Ferrara, Department of Life Sciences and Biotechnology, Via Fossato di Mortara 74, 44121 Ferrara, Italy.*

Peptide nucleic acids (PNAs), have been used for antisense, anti-gene, anti-miR therapy in a number of studies,[1-3] and, more recently, they have been used for precise gene editing.[4] For these applications, however, one of the most important issues is the low uptake by target cells. In order to solve this drawback, in the past years several approaches have been used such as conjugation with carrier peptides and use of biodegradable polymer nanoparticles.[3,4] In our effort to exploit PNA as anti-miR agents,[5-8] we have used several approaches, such as conjugation with cationic peptides,[5-7] use of modified PNA backbones,[8] and incorporation into inorganic nanocarriers, which allow to obtain multifunctional materials and perform co-delivery with specific drugs.[9,10] Currently, most of the carrier systems require special fabrication procedures or covalent modification of the PNA, whereas and ideal PNA transfecting agent for cellular systems would require a simple mixing of the carrier with chosen PNA sequence, either naked or fluorescently labelled. To this end, we recently explored the use of cationic calix[4]arenes[11] as PNA vectors.[12] Some of them evidenced high efficiency in delivery to glioma U251 cells; negligible toxicity was observed and the biological activity of the delivered PNA resulted maintained. These findings indicate these calixarene derivatives as potential universal vectors for the transport into cells of unmodified PNAs, drastically simplifying the study and application of these nucleic acid mimics as anti-miRs. The nature of the PNA-calixarene interactions and of their complexes will be discussed in this presentation.

This work was supported by grants from the Foundation AIRC (IG 13575: peptide nucleic acids targeting oncomiR and tumor-suppressor miRNAs: cancer diagnosis and therapy).

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Corresponding authors: E-mail: francesco.sansone@unipr.it; gam@unife.it