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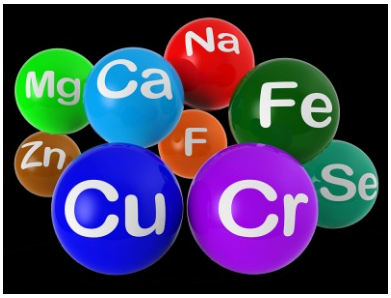

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Quote in reference to: http://bit.ly/DDDS6

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Associate Research Fellow,
Discovery Chemistry and Technology, Abbvie

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- ***Enhancing Patient Lives*** through Accelerated Drug Development
- ***Paving the Way for Precision Medicine***: Innovation & Implementation

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- ***Frederick Balagadde***, Ph.D. (K-RITH Durban, South Africa) - microfluidic systems to increase affordable healthcare access
- ***James Olson***, M.D., Ph.D. (Fred Hutchinson Cancer Research Center) - new cancer therapies for children with brain tumors
- ***Susan Hershenson***, Ph.D. (The Bill & Melinda Gates Foundation) - technical expertise & strategic guidance for the therapeutics projects

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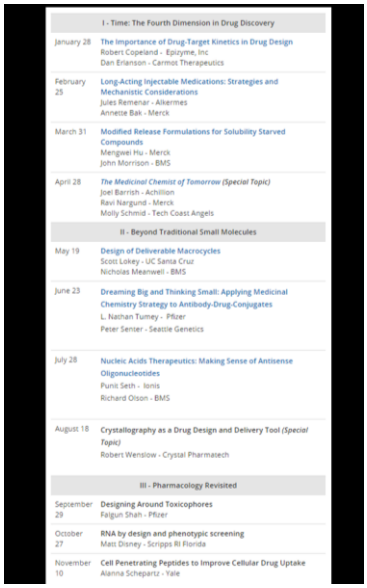
2016 Drug Design and Delivery Symposium



I - Time: The Fourth Dimension in Drug Discovery

January 28 — **The Importance of Drug-Target Kinetics in Drug Design**
Robert Copeland - Epizyme, Inc
Dan Erlanson - Carmot Therapeutics

February 25 — **Long-Acting Injectable Medications: Strategies and Mechanistic Considerations**
Jules Remenar - Alkermes
Annette Bak - Merck

March 31 — **Modified Release Formulations for Solubility Starved Compounds**
Mengwei Hu - Merck
John Morrison - BMS

April 28 — ***The Medicinal Chemist of Tomorrow (Special Topic)***
Joel Barrish - Achillion
Ravi Nargund - Merck
Molly Schmid - Tech Coast Angels

II - Beyond Traditional Small Molecules

May 19 — **Design of Deliverable Macrocycles**
Scott Lokey - UC Santa Cruz
Nicholas Meanwell - BMS

June 23 — **Dreaming Big and Thinking Small: Applying Medicinal Chemistry Strategy to Antibody-Drug-Conjugates**
L. Nathan Tumey - Pfizer
Peter Senter - Seattle Genetics

July 28 — **Nucleic Acids Therapeutics: Making Sense of Antisense Oligonucleotides**
Punit Seth - Ionis
Richard Olson - BMS

August 18 — **Crystallography as a Drug Design and Delivery Tool *(Special Topic)***
Robert Wenslow - Crystal Pharmatech

III - Pharmacology Revisited

September 29 — **Designing Around Toxicophores**
Falgun Shah - Pfizer

October 27 — **RNA by design and phenotypic screening**
Matt Disney - Scripps RI Florida

November 10 — **Cell Penetrating Peptides to Improve Cellular Drug Uptake**
Alanna Schepartz - Yale

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Meet the Organizers


Nicholas Meanwell
BMS


John Morrison
BMS

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Division of Medicinal Chemistry

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Content Advisors


Richard Connell
Pfizer


Annette Bak
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Dan Erlanson
Carmot Therapeutics


Mark Tichenor
Janssen Research and Development

http://bit.ly/2016ddds

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Upcoming ACS Webinars
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Thursday, August 4, 2016

The Chemistry of Power Free Wearable Sensors: Smart Polymeric Materials

Michele Lee, Ph.D. Student Materials Science, University of Southern California

Andrea Armani, Chair of Engineering and Associate Professor of Chemical Engineering and Materials Science, University of Southern California

Mark Jones, Executive External Strategy and Communications Fellow, Dow Chemical

Thursday, August 11, 2016

Chemophobia: How We Became Afraid of Chemicals and What to Do About It

James Kennedy, Chemistry Teacher and Blogger, Haileybury, Australia

Darren Griffen, Professor of Genetics, University of Kent, UK

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2016 Drug Design and Delivery Symposium

" Nucleic Acids Therapeutics - Making Sense of Antisense"

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Nucleic Acid Therapeutics – Making Sense of Antisense

ACS Webinar, July 28th 2016

Punit Seth, Ph.D.

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How to make an oligonucleotide drug

- Pick a mechanism
- Choose a chemical/delivery platform
- Screen, optimize drug molecule
- Clear pre-clinical tox
- Initiate clinical trials
- Register drug with FDA

How to make an oligonucleotide drug

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Pick a mechanism

- **Oligonucleotide drugs can work through multiple mechanisms**
  - Antisense oligonucleotides bind to RNA by *Watson-Crick base-pairing* and modulate RNA function to produce a pharmacological effect
  - Aptamers and immuno-modulatory oligonucleotides typically bind to protein targets and modulate their function to produce a pharmacological effect
  - mRNA drugs are translated to therapeutic proteins

- Antisense mechanisms can be broadly classified into two general categories
  - Mechanisms which promote degradation of RNA
    - RNase H – single stranded (ss) DNA ASOs
    - siRNA and miRNA – double stranded (ds) and ssRNA ASOs
  - Mechanisms which do not promote degradation of RNA
    - Translational arrest – ssASOs with variable chemistry
    - Splice modulation – ssASOs with variable chemistry
    - miRNA antagonists – ssASOs with variable chemistry

Bennett, C.F. & Swayze, E.E. RNA targeting therapeutics: molecular mechanisms of antisense oligonucleotides as a therapeutic platform. *Annu. Rev. Pharmacol. Toxicol. 50, 259-293 (**2010**).*

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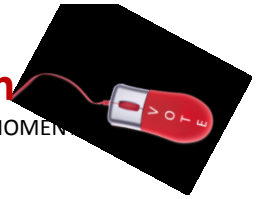
Audience Survey Question

ANSWER THE QUESTION ON BLUE SCREEN IN ONE MOMEN



How does CRISPR/Cas9 differ from "antisense" mechanisms such as RNase H and siRNA?

- RNA strand promotes cleavage of complementary RNA
- DNA strand promotes cleavage of complementary RNA
- RNA strand promotes cleavage of complementary DNA

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Pick a mechanism

- Oligonucleotide drugs can work through multiple mechanisms
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Bennett, C.F. & Swayze, E.E. RNA targeting therapeutics: molecular mechanisms of antisense oligonucleotides as a therapeutic platform. *Annu. Rev. Pharmacol. Toxicol. 50, 259-293 (**2010**).*

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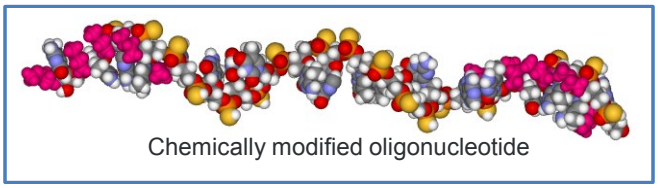
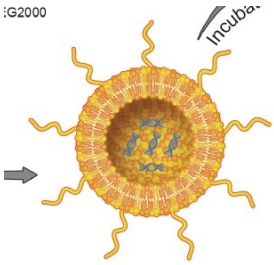
Choose a chemical / delivery platform

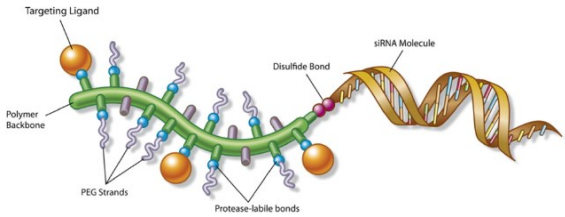


Chemically modified oligonucleotide



LNPs-siRNA

Lipid nano-particles



Dynamic poly conjugates (DPC)

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Introduction to oligonucleotide chemical modifications

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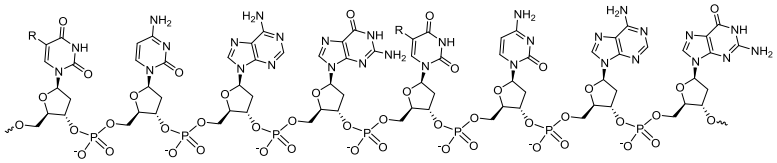
Unmodified ASOs Have Sub-Optimal "Drug-Like" Properties

- Poly-anionic macromolecules with poor cell penetration properties
- Lack sufficient bio-stability in animals
- Have poor pharmacokinetics (rapidly excreted into urine)
- Modest affinity for target RNA
- Non-specific immune stimulation



Swayze, E.E. & Bhat, B. (2007) The Medicinal Chemistry of Oligonucleotides; in Antisense Drug Technology: Principles, Strategies, and Applications, Edn. 2nd. (ed. S.T. Crooke), 143-182.

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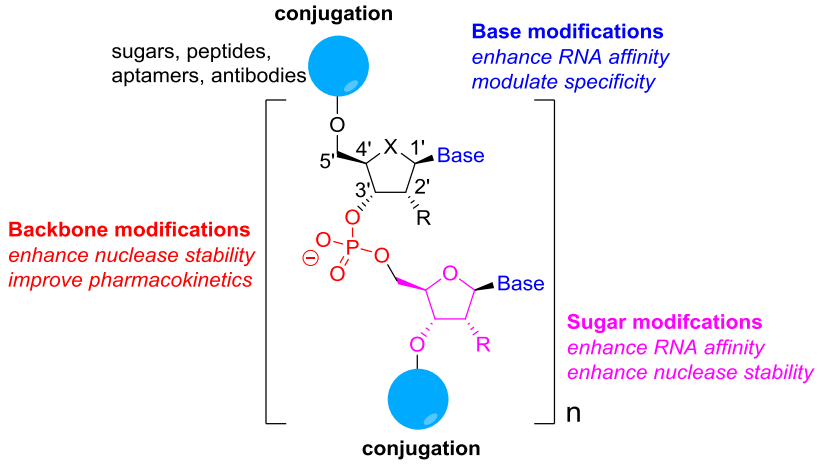
Many sites on an oligonucleotide can be chemically modified to enhance drug-like properties



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Wan et al, *J. Med. Chem.* **2016**, DOI 10.1021/acs.jmedchem.6b00551

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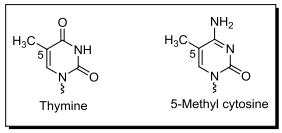
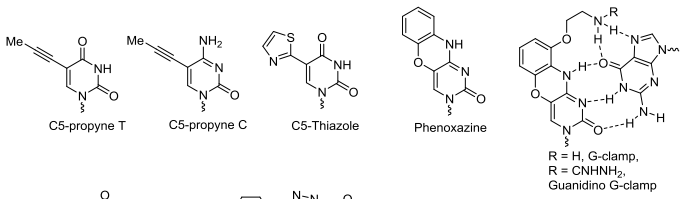
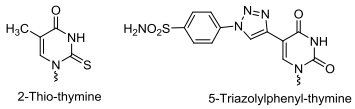
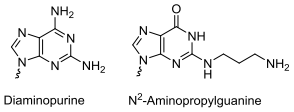
Nucleobase Modifications



Enhances affinity for RNA (ΔT_m +0.5 °C/mod.)
Enhances metabolic stability
Reduces immuno-stimulatory properties







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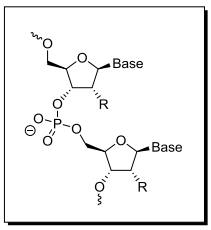
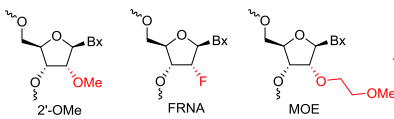
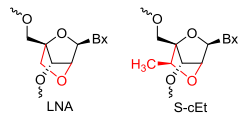
Sugar Modifications

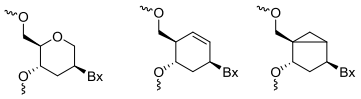




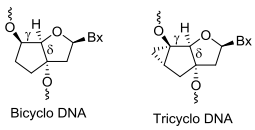
ΔT_m +1-2 °C/mod



ΔT_m +3-5 °C/mod



ΔT_m +1-3 °C/mod



ΔT_m +0-3 °C/mod

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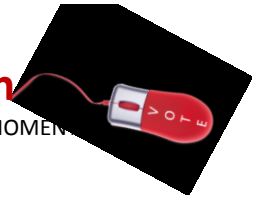
Audience Survey Question

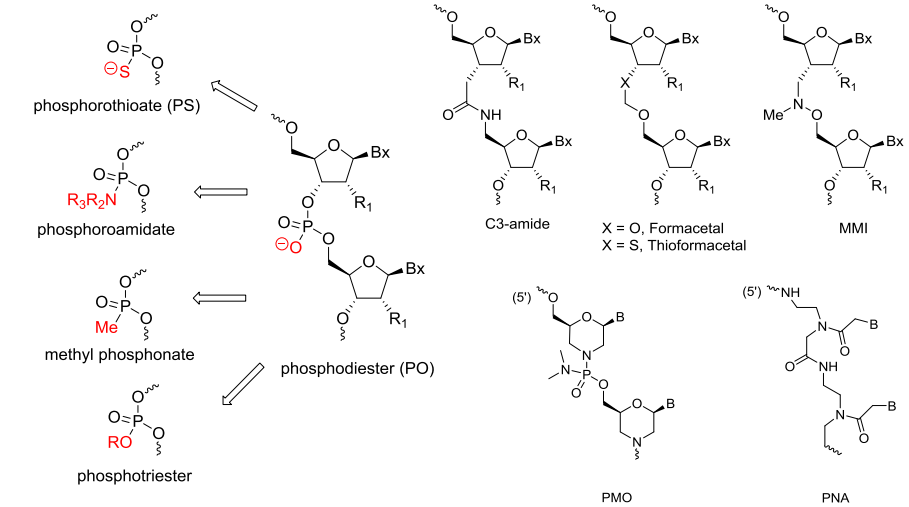
ANSWER THE QUESTION ON BLUE SCREEN IN ONE MOMEN



Which scaffold represents the boat conformation of cyclohexane?

- Hexitol nucleic acid (HNA)
- Cyclohexenyl nucleic acid (CeNA)
- N-methanocarba (NMC)

Backbone Modifications



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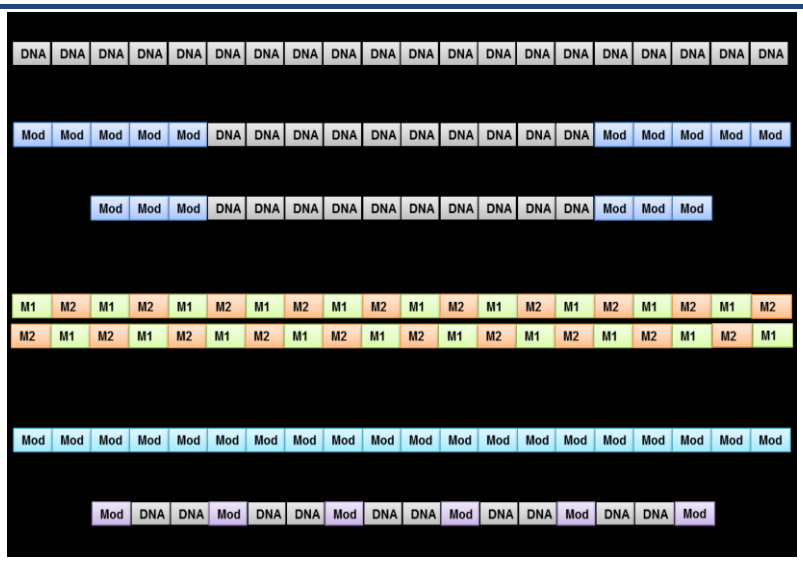
Use of modification pattern is directed by antisense mechanism being harnessed



Rigo F, Seth P, Bennett CF. Antisense Oligonucleotide-Based Therapies for Diseases Caused by pre-mRNA Processing Defects. In: Yeo GW, ed. Systems Biology of RNA Binding Proteins: Springer New York; 2014: 303-52.

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Lessons Learned

- **Chemical modifications can enhance the RNA-binding, metabolic stability, pharmacokinetic, toxicological properties of oligonucleotide drugs**
  - All positions on an oligonucleotide can be (and have been) modified
- **Offers the opportunity to create a discrete entity which can be chemically synthesized and characterized**
  - More convenient that particulate formulations which are complex mixtures of multiple components

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RNase H active gapmer Antisense Oligonucleotides (ASOs)

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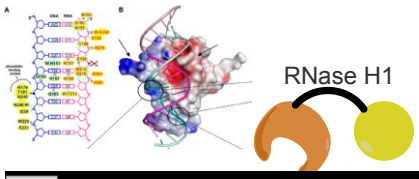
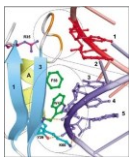
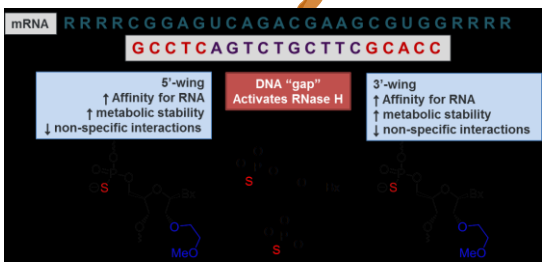
Ionis' Gen 2.0 Platform – 2'-Methoxyethyl RNA (MOE) Gapmer

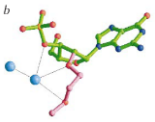
Nowotny et al 2007, *Mol. Cell 28*, 264





Nowotny et al 2008, *EMBO J. 27*, 1172





Monia et al *J. Biol. Chem.* **1993**, 268, 14514
Wu et al *J. Biol. Chem.* **2004**, *279*, 17181

Teplova, M. et al. *Nat. Struct. Biol.* **1999**, *6*, 535-539

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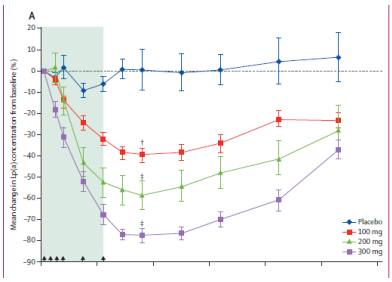
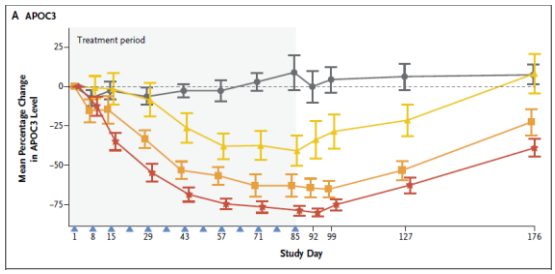
MOE ASOs Show Excellent Activity (and Pharmacology) For Suppressing Genes Expressed In the Liver in Humans



Tsimikas et al *Lancet* **2015**, *386*, 1472.



Gaudet et al *N. Engl. J. Med.* **2015**, *373*, 438.

- ApoC3 and Lp(a) are proteins synthesized primarily by hepatocytes in the liver and secreted in plasma
- Measuring reduction of protein in plasma serves as a bio-marker for ASO activity in the liver

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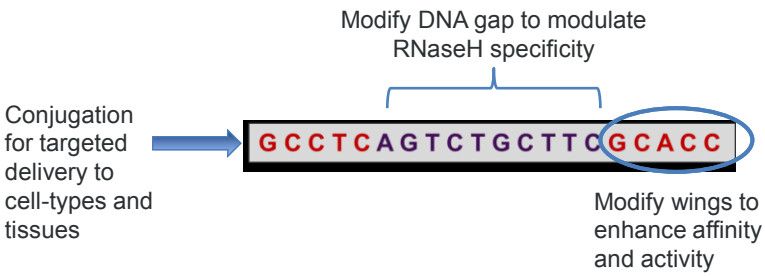
Designing the next generation ASO platform



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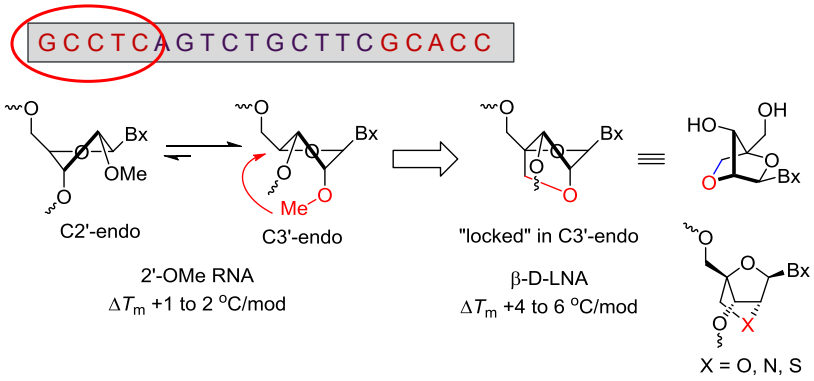
Conformationally Restricted Nucleic Acid Analogs Enhance Affinity for RNA



2'-OMe RNA
ΔT_m +1 to 2 °C/mod

β-D-LNA
ΔT_m +4 to 6 °C/mod

Imanishi et al, *Tet Lett*, **1997**, *38*, 8735
Wengel et al, *Tetrahedron*, **1998**, *54,* 3607

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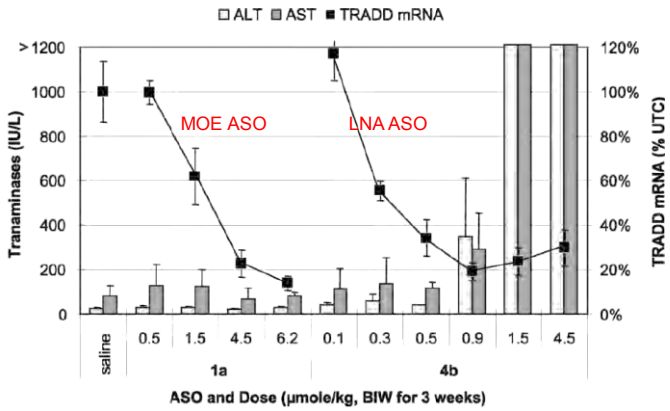
LNA ASOs Increase Potency and Risk for Hepatotoxicity



Swayze, et al *Nucl. Acids Res.* **2007,** 35, 687-700.

Can combining structural elements of LNA and MOE enhance potency while reducing toxicity?

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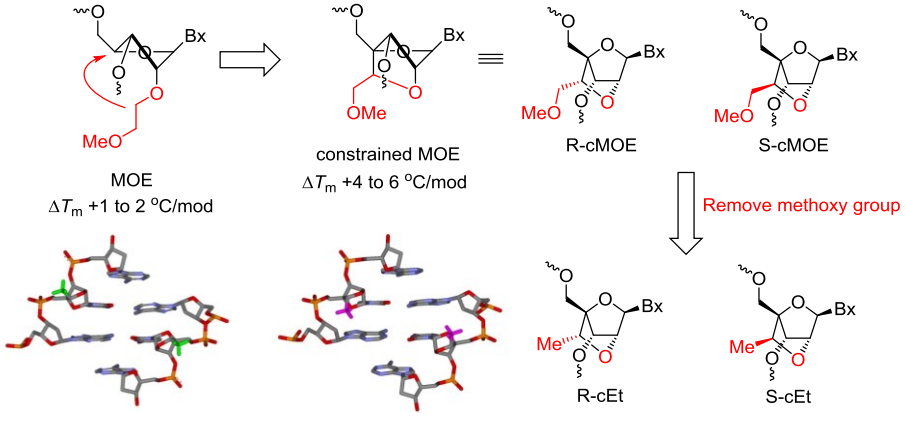
Combining Structural Elements of MOE and LNA – cMOE and cEt modifications



MOE
ΔT_m +1 to 2 °C/mod

constrained MOE
ΔT_m +4 to 6 °C/mod

constrained Ethyl (cEt)

Pallan et al *Chem. Commun* . **2012**, *48*, 8195.

Seth et al. *J. Med. Chem.* **2009**, *52*, 10
Seth et al, *J. Org. Chem.* **2010**, *75*, 1569

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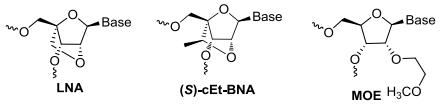
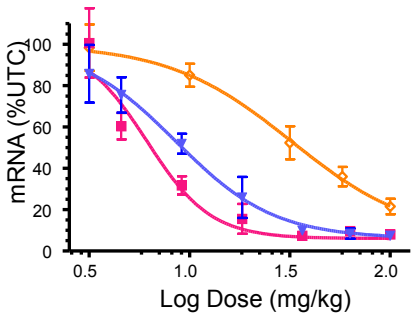
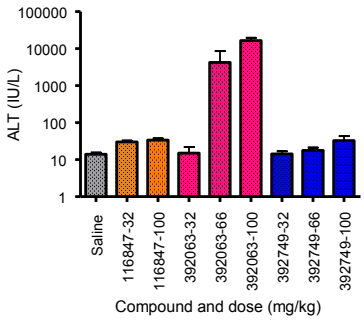
cEt BNA ASO Targeting PTEN Maintains Potency While Improving Therapeutic Profile Relative To LNA ASO

Compound	ED_{50} (mg/kg)
◇ 116847 (5-10-5 MOE)	33
■ 392063 (2-10-2 LNA)	6.1
▼ 392749 (2-10-2 cEt)	8.5

Sequence: 5'-CUtagcactggcCU (B = BNA modification)



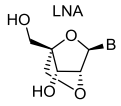




Seth et al. *J. Med. Chem.* **2009**, *52*, 10

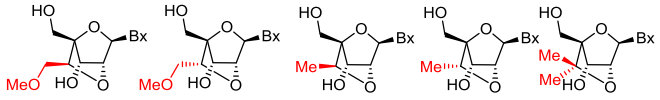
38

Beyond 2'-MOE – Examples of 2',4'-BNA Modifications Evaluated for Isis Gen. 2.5 Research Program



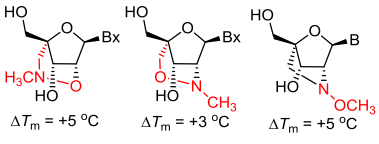
ΔT_m = +5 °C/mod.

Swayze et al *NAR*, **2007**, *35*, 687
Starrup et al *NAR*, **2010**, *38*, 7100



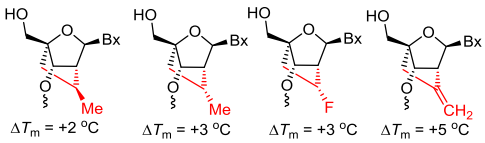
ΔT_m = +5 °C/mod.

Seth et al, *Nucleic Acid Sym. Ser.* **2008**, *52*, 553; *J. Med Chem.* **2009**, *52*, 10; *J. Org. Chem.* **2010**, *75*, 1569; *Curr. Prot. Nucleic Acids Chem.* **2011**, 1.24.1; Pallan et al, *Chem Commun.* **2012**, *48*, 8195



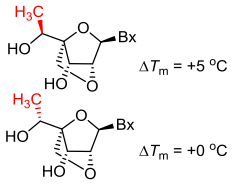
ΔT_m = +5 °C ΔT_m = +3 °C ΔT_m = +5 °C

Obika et at *J. Am. Chem. Soc.*, **2008**, *130*, 4886
Prakash, et al *J. Med. Chem.* **2010**, *53*, 1636



ΔT_m = +2 °C ΔT_m = +3 °C ΔT_m = +3 °C ΔT_m = +5 °C

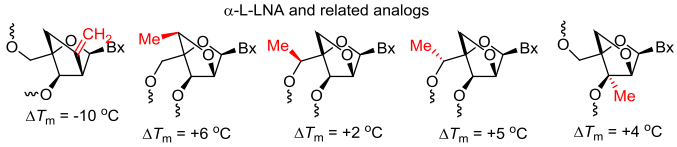
Chattopadhyaya et al, *J. Am. Chem. Soc.* **2007**, *129*, 8362
Seth et al. *J. Am .Chem. Soc.* **2010**, *132*, 14942; *ChemBioChem* **2013**, *14*, 58.



ΔT_m = +5 °C

ΔT_m = +0 °C

α-L-LNA and related analogs

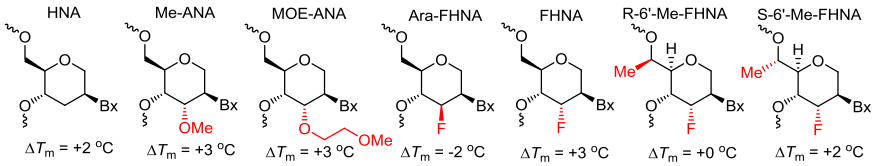


ΔT_m = -10 °C ΔT_m = +6 °C ΔT_m = +2 °C ΔT_m = +5 °C ΔT_m = +4 °C

Seth et al, *J. Med Chem*. **2010**, *53*, 8309

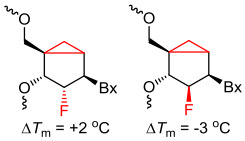
Seth et al, *BMCL* **2011**, *21*, 4690; *BMCL*, **2011**, *21*, 588; *BMCL* **2011**, *21*, 1122; *BMCL,* **2012**, *22*, 296; *Mol Ther Nucleic Acids* **2012**, *1*, e47

Examples of Other Conformationally Restricted Analogs Evaluated for Isis Gen. 2.5 Research Program



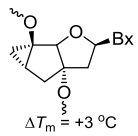
ΔT_m = +2 °C ΔT_m = +3 °C ΔT_m = +3 °C ΔT_m = -2 °C ΔT_m = +3 °C ΔT_m = +0 °C ΔT_m = +2 °C

Egli et al. *J. Am. Chem. Soc.* **2011**, *133*, 16642; Pallan et al. *Biochemistry* **2012**, *51*, 7; manuscript in preparation



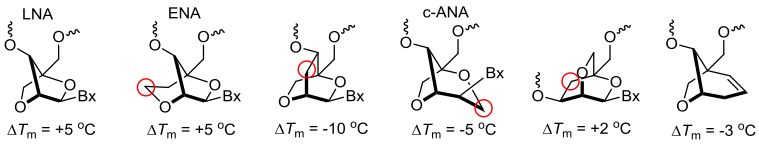
ΔT_m = +2 °C ΔT_m = -3 °C

Jung et al. *ACIEE* **2014**, 53, 9893
Oestergaard et al, *JOC* **2014**, 79, 8877



ΔT_m = +3 °C

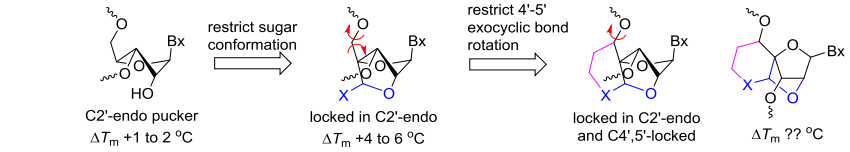
Murray et al. *NAR* **2012**, *40*, 6135

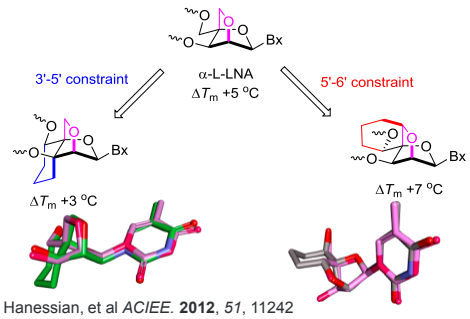


ΔT_m = +5 °C ΔT_m = +5 °C ΔT_m = -10 °C ΔT_m = -5 °C ΔT_m = +2 °C ΔT_m = -3 °C

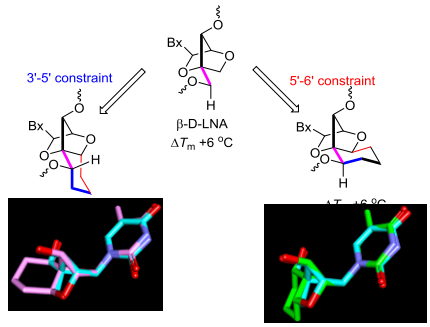
Imanishi **1997**, *Tet Lett.* 38, 8735; Koizumi *BMC* ***2003***, 11, 2211; Obika, et al. *Org. Lett.* **2011**, *13*, 6050; Kodama et al **2014**, AGDS
Migawa et al, *Org. Lett.* **2013**, *15*, 4316; manuscript in preparation

Dual Constrained Nucleic Acids – Improving Affinity Beyond LNA





Hanessian, et al *ACIEE.* **2012**, *51*, 11242
Hanessian et al *J. Org. Chem.* **2013**, *78*, 9064
Hanessian et al *J. Org. Chem.* **2013**, *78*, 9051



Giacometti et al *Org. Biomol. Chem.* **2016**, *14*, 2034

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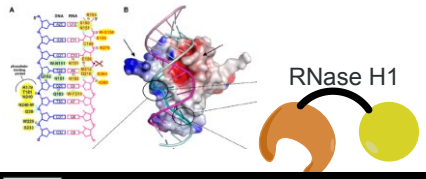
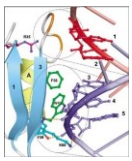
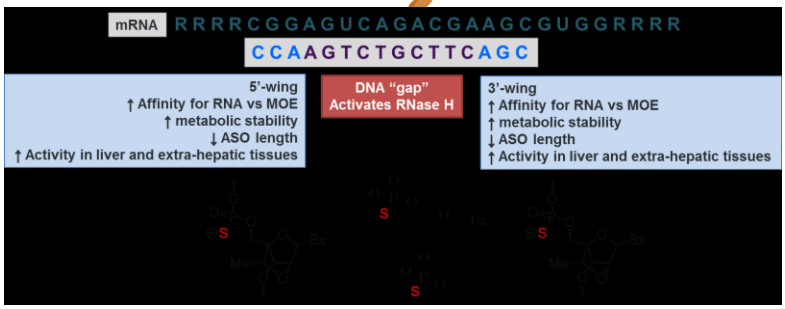
Ionis' Gen 2.5 Platform – cEt BNA Gapmer

Nowotny et al 2007, *Mol. Cell 28*, 264





Nowotny et al 2008, *EMBO J. 27*, 1172



Seth et al *J. Med. Chem.* **2009**, *52*, 10; *J. Org. Chem.* **2010**, *75*, 1569; Pallan et al *Chem. Commun* . **2012**, *48*, 8195; Burel et al *Nucleic Acid Ther.* **2013,** 23, 213-27; Pandey et al *J. Pharmacol. Exp. Ther.* **2015,** 355, 329-340; Hong, et al *Sci. Transl. Med.* **2015,** 7, 314ra185

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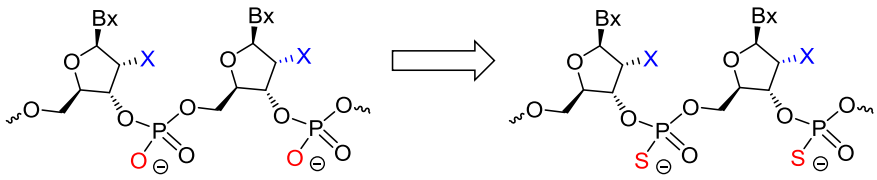
Modulating Pharmacokinetics Properties of Oligonucleotides by Targeted Delivery

IONIS PHARMACEUTICALS

Phosphorothioate Modification Enhances Nuclease Stability and Pharmacokinetic Properties



- **Phosphorothioate (PS) Linkage**
  - Improves metabolic stability
  - Improves plasma protein binding and facilitates ASO distribution to tissues
  - Supports RNase H activity and also compatible with RISC mechanism
  - Reduces affinity for RNA
  - Activates immune system (sequence dependent)

Eckstein, F., Phosphorothioate oligodeoxynucleotides: what is their origin and what is unique about them? Antisense Nucleic Acid Drug Dev. 2000, *10*, 117-21.

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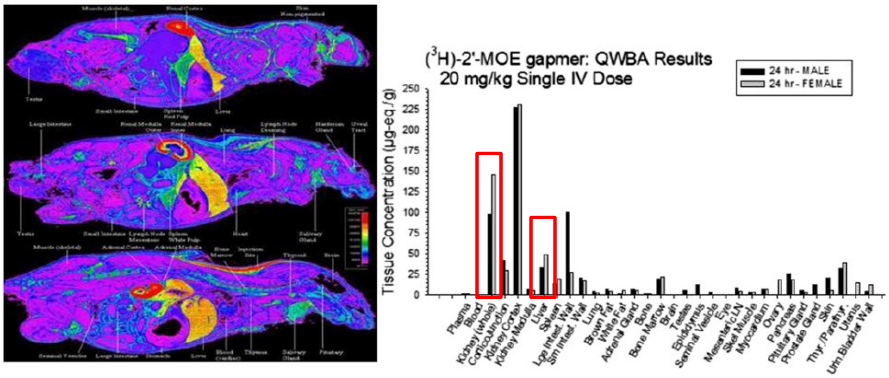
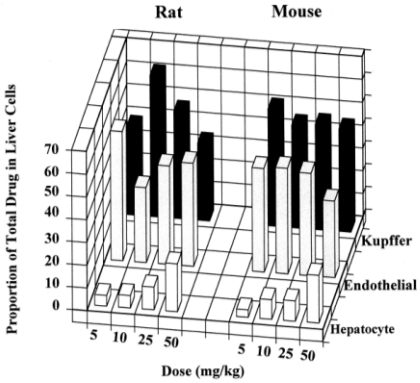
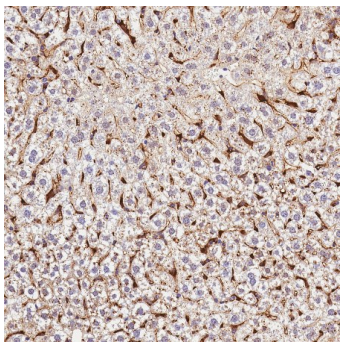
PS ASOs Distribute Broadly After Systemic Injection



Geary et al *Adv. Drug Delivery Rev.* **2015**, *87*, 46

PS ASOs Accumulate Preferentially in Non-Parenchymal Cells in The Liver



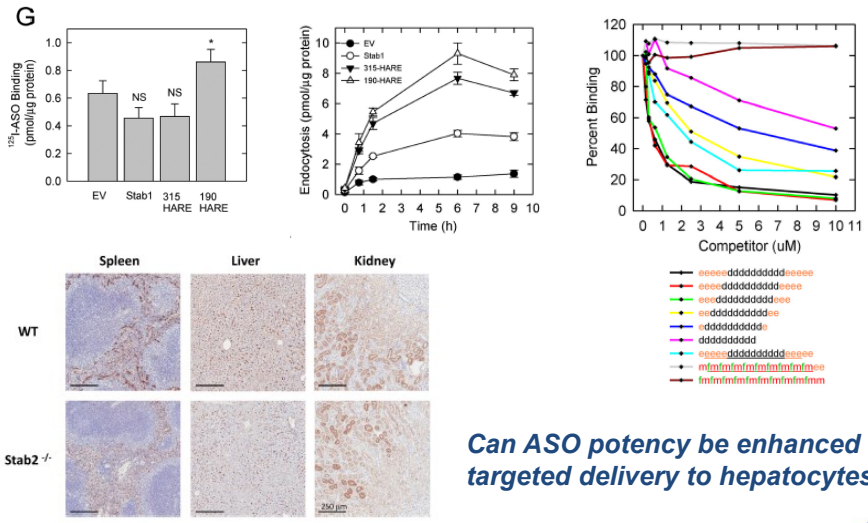


Graham et al *Biochem. Pharmacol.* **2001**, 62, 297
Butler et al *Lab. Invest.* **1997**, *77*, 379.
Bijsterbosch et al *Nucleic Acids Res.* **1997**, *25*, 3290.

NPC's constitute <10% of liver mass
Hepatocytes account for 80% of liver mass

Why do PS ASOs accumulate in non-parenchymal cells in the liver?

Stabilin-1 and Stabilin-2 are specific receptors for the cellular internalization of PS ASOs in liver sinusoidal cells



Miller, et al. *Nucleic Acids Res.* **2016**, 44, 2782-94

Can ASO potency be enhanced by targeted delivery to hepatocytes?

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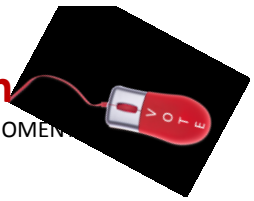
Audience Survey Question

ANSWER THE QUESTION ON BLUE SCREEN IN ONE MOMEN



How can one achieve targeted delivery to hepatocytes?

- Target the Mannose Receptor
- Target the Asialoglycoprotein Receptor
- Target the Hyaluronic Acid Receptor for Endocytosis

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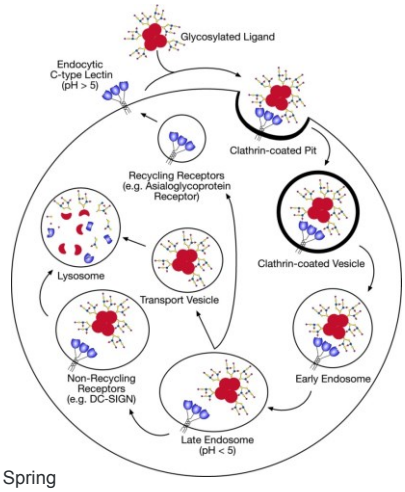
The Asialoglycoprotein Receptor (ASGPR) Is a High Capacity Receptor Specific for Hepatocytes

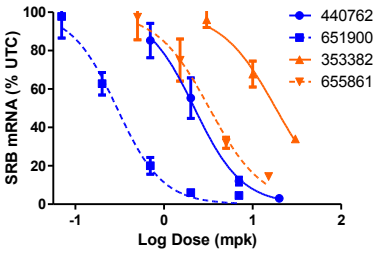
- ASGPR is a C-type lectin expressed exclusively on hepatocytes
  - 500,000 copies per cell
- ASGPR clears glycoproteins from circulation through receptor mediated endocytosis
- ASGPR binds Galactose and *N*-acetyl-galactosamine (Gal-NAc) terminated oligosaccharides with high affinity
  - Sia-2,6-Gal modified glycoproteins are physiological ligands for ASGPR

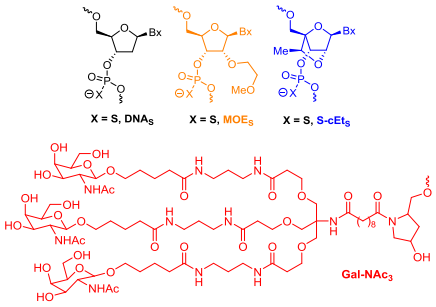


Cummings et al Essentials of Glycobiology. 2nd Ed. Cold Spring Harbor Laboratory Press; 2009. Chapter 31.

IONIS PHARMACEUTICALS

Targeted Delivery Enhances Potency of Gen 2.0 and Gen 2.5 ASOs In The Liver





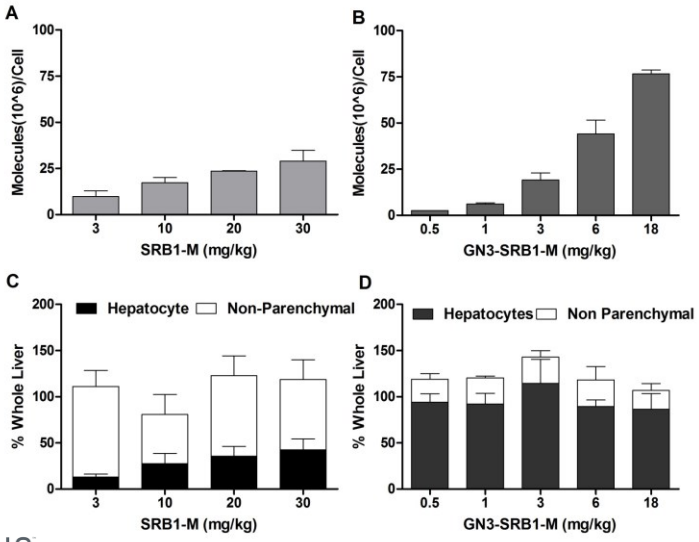
Rensen et al. *J. Biol. Chem.* **2001**, 276, 37577
Nair et al *J. Am. Chem. Soc.* **2014**, 136, 16958

ISIS #	Single Dose (mpk)	ASO (5'-3')	SRB1 mRNA ED_{50}
353382	3, 10, 30	$G_sC_sT_sT_sC_sA_sG_sT_sC_sA_sT_sG_sA_sC_sT_sT_sC_sC_sT_sT$	18.3
655861	0.5, 1.5, 5, 15	$G_sC_sT_sT_sC_sA_sG_sT_sC_sA_sT_sG_sA_sC_sT_sT_sC_sC_sT_sT_oA_oGalNAc_3$	3.3
440762	0.7, 2, 7, 20	$T_sC_sA_sG_sT_sC_sA_sT_sG_sA_sC_sT_sT_sC$	2.2
651900	0.07, 0.2, 0.7, 2, 7	$T_sC_sA_sG_sT_sC_sA_sT_sG_sA_sC_sT_sT_sC_oA_oGalNAc_3$	0.3

DNA, MOE, *S*-cEt, Phosphorothioate (s), Phosphodiester (o), Triantennary Gal-NAc

Prakash et al, Nucleic Acids Res. **2014**, *42*, 8796-8807 NAR Breakthrough Article

GalNAc Enhances ASO Accumulation in Hepatocytes Relative to Non-Parenchymal Cells



IONIS PHARMACEUTICALS

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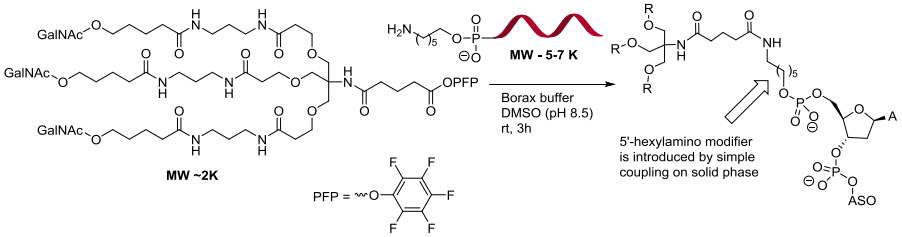
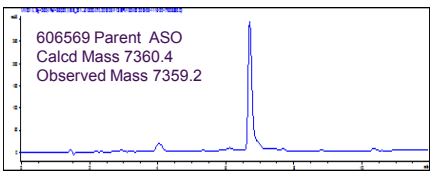
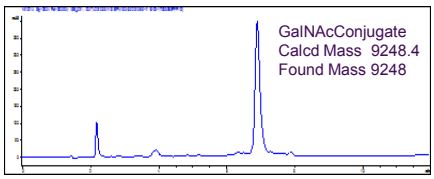
5'-Hexylamino-ASOs Can Be Efficiently Conjugated to PFP Activated GalNAc Clusters In Aqueous Solution







Ostergaard *et al. Bioconjug. Chem.* **2015**, 26, 1451-1455
St-Pierre et al *Bioorg. Med. Chem.* **2016,** 24, 2397-409.

IONIS PHARMACEUTICALS

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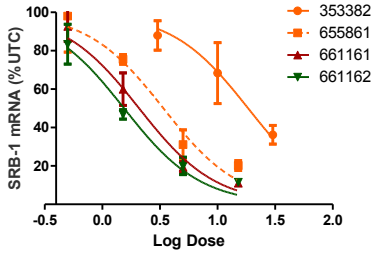
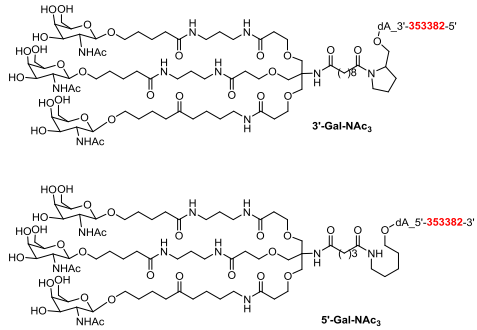
5’-GalNAc Mixed Backbone ASOs Further Improve Potency





Isis #	Sequence (5’-3’)	Design	SRB1 mRNA ED_{50} (mpk)
353382	$G_sC_sT_sT_sC_sA_sG_sT_sC_sA_sT_sG_sA_sC_sT_sT_sC_sT_sT$	None	18.8
655861	$G_sC_sT_sT_sC_sA_sG_sT_sC_sA_sT_sG_sA_sC_sT_sT_sC_sC_sT_sT_oA_o$GalNAc$_3$	3’-GalNAc$_3$ PS	3.4
661161	GalNAc$_{3o}A_oG_sC_sT_sT_sC_sA_sG_sT_sC_sA_sT_sG_sA_sC_sT_sT_sC_sC_sT_sT$	5’-GalNAc$_3$ PS	2.1
661162	GalNAc$_{3o}A_oG_sC_oT_oT_oC_sA_sG_sT_sC_sA_sT_sG_sA_sC_sT_sT_oC_oC_sT_sT$	5’-GalNAc$_3$ MBB	1.6

IONIS PHARMACEUTICALS

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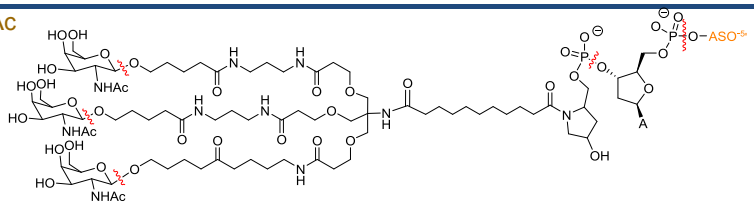
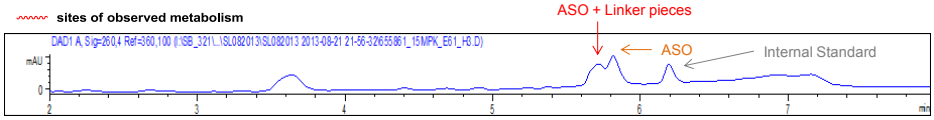
5’-GalNAc Cluster Is Cleanly Metabolized to Parent ASO in Liver

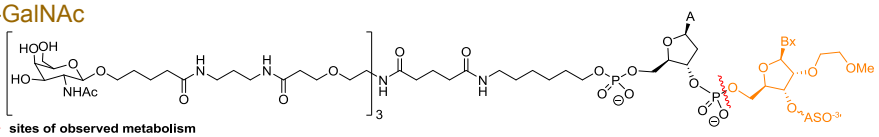
3’-GalNAc



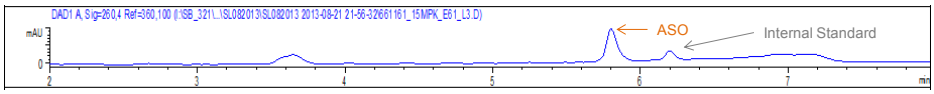
sites of observed metabolism



5’-GalNAc



sites of observed metabolism



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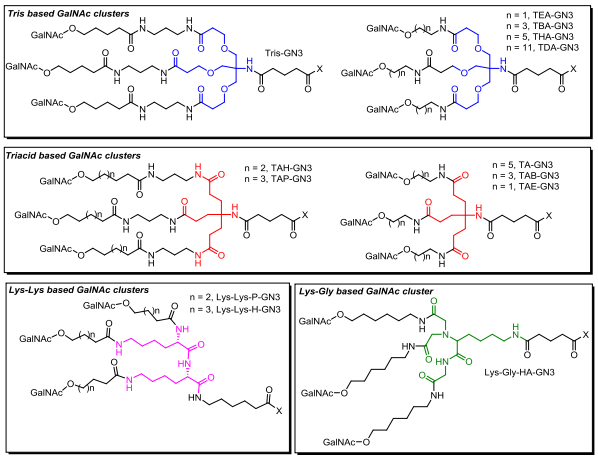
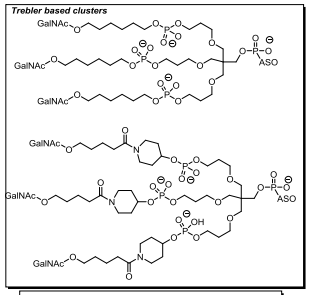
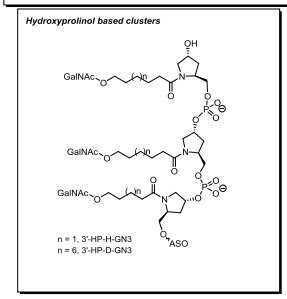
SAR Scaffolds Evaluated For ASGR Mediated ASO Delivery To Hepatocytes







Prakash et al *J. Med. Chem.* **2016,** 59, 2718-33

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Most Tri-GalNAc Clusters Show Similar ASGR Binding And Activity In Hepatocytes and Mice

Table 1. ASGR Binding and Potency of SRB-1 ASOs Containing Tris (32−36), Triacid (37−41), Lys_Lys (42), Lys-Gly (43), Trebler (44 and 45), and Hydroxylprolinol (46−48) Scaffolds[a]

ASO No.	X:trivalent GalNAc	atoms in linker[b]	ASGPR *Ki* (nM)	IC_{50} (nM)	ED_{50} (mg kg^{-1})
31	none	none		250.3 ± 1.8	18.3 ± 1.1
Tris Based GalNAc Clusters					
32	5′-tris-GN3	16	8.0 ± 1.2	40.2 ± 1.1	2.4 ± 1.1
33	5′-TEA-GN3	7	6.8 ± 1.6	10.4 ± 1.2	4.2 ± 1.3
34	5′-TBA-GN3	9	7.0 ± 1.3	10.3 ± 1.2	3.5 ± 1.4
35	5′-THA-GN3	11	6.1 ± 1.4	20.1 ± 1.1	3.7 ± 1.2
36	5′-TDA-GN3	17	23.0 ± 1.5	149.8 ± 6.1	7.2 ± 1.5
Triacid Based GalNAc Clusters					
37	5′-TAH-GN3	15	10.3 ± 1.2	30.1 ± 1.4	2.2 ± 1.1
38	5′-TAP-GN3	14	10.0 ± 1.3	70.3 ± 1.1	2.4 ± 1.0
39	5′-TA-GN3	11	6.2 ± 1.2	30.0 ± 1.3	2.6 ± 1.2
40	5′-TAB-GN3	9	8.4 ± 1.2	20.4 ± 1.1	3.8 ± 1.3
41	5′-TAE-GN3	7	26.0 ± 1.5	20.4 ± 1.1	3.2 ± 1.3
Lys-Lys and Lys-Gly Based GalNAc Clusters					
42	5′-Lys-Lys-H-GN3	11, 12, 15	10.1 ± 1.4	30.3 ± 1.2	2.1 ± 1.2
43	5′-Lys-Gly-HA-GN3	11, 12	6.3 ± 1.2	6.3 ± 1.2	2.3 ± 1.1
Trebler Based GalNAc Clusters					
44	5′-PGN3	15	20.0 ± 1.3	60.3 ± 1.2	3.1 ± 1.3
45	5′-Pip-PGN3	18	23.0 ± 1.2	149.6 ± 8.4	9.8 ± 1.6
Hydroxyprolinol Based GalNAc Clusters					
46	3′-HP-H-GN3	7	12.0 ± 1.6	40.2 ± 1.1	2.9 ± 1.2
47	3′- HP-D-GN3	13	40.0 ± 1.8	55.3 ± 1.2	4.8 ± 1.4
48	3′-HP-HAH-GN3	14	9.6 ± 1.2	30.3 ± 1.2	2.2 ± 1.1

[a]SRB-1 ASO: 5′-X-$_s$$A_{ds}$$G_{es}^{m}C_{es}$$T_e$$T_e$$_s^{m}C_{es}$$A_{ds}$$G_{ds}$$T_{ds}^{m}C_{ds}$$A_{ds}$$T_{ds}$$G_{ds}$$A_{ds}^{m}C_{ds}$$T_{ds}$$T_{es}^{m}Ce_s^{m}C_{es}$$T_{es}$$T_e$; e, 2′-*O*-MOE; d, DNA; ^{m}C, 5-methylcytidine; s, phosphorothioate; o, phosphodiester. [b]Number of atoms in the linker

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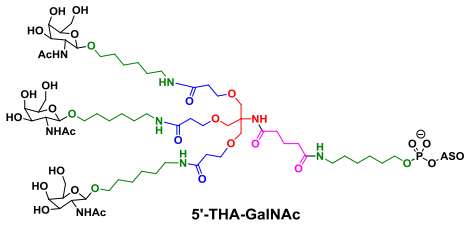
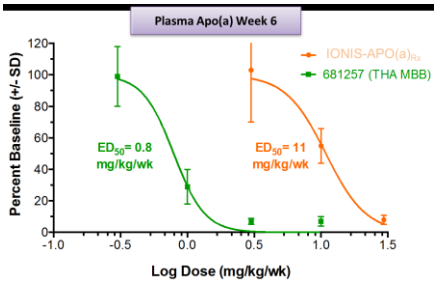
Gal-NAc Conjugate Targeting Human Apo(a) Shows >10-Fold Improved Potency in Transgenic Mice





Yu, at al *Mol. Ther. Nucleic Acids* **2016**, 5, e317
Shemesh, et al *Mol .Ther. Nucleic Acids* **2016,** 5, e319.

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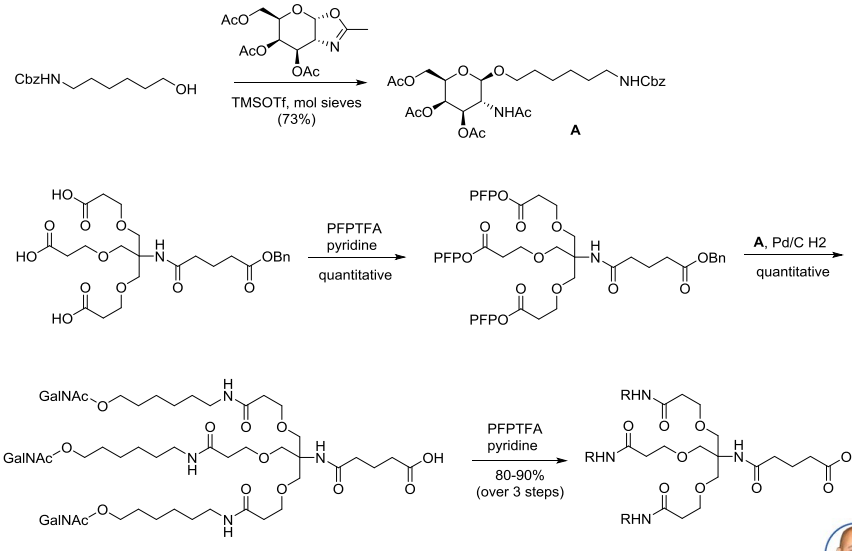
Simplified Synthesis Of GalNAc Cluster For Solution Phase Conjugation



Migawa et al, *Bioorg. Med. Chem. Lett.* **2016**, 26, 2194-7

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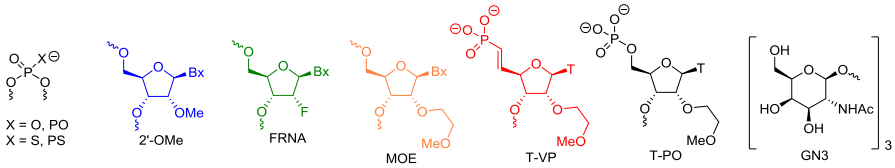
Enhancing activity of chemically modified siRNA

siRNA	Sense (5'-3')/Antisense (3'-5')	IC_{50} (nM) hepatocytes	ED_{50} (mg/kg) liver
1	AsCsCoUoGoAoUoCoAoUoUoAoUoAoGoAoUsAsA AsAsUoGoGoAoCoUoAoGoUoAoAoUoAoUoCoUoAsUsT-PO	957	--
2	AsCsCoUoGoAoUoCoAoUoUoAoUoAoGoAoUsAsA AsAsUoGoGoAoCoUoAoGoUoAoAoUoAoUoCoUoAsUsT-VP	305	53
3	AsCsCoUoGoAoUoCoAoUoUoAoUoAoGoAoUsAsA-GN3 AsAsUoGoGoAoCoUoAoGoUoAoAoUoAoUoCoUoAsUsT-PO	7.6	2.3
4	AsCsCoUoGoAoUoCoAoUoUoAoUoAoGoAoUsAsA-GN3 AsAsUsGsGsAsCsUoAsGoUsAoAsUoAsUoCsUoAsUsT-VP	8.5	0.5



Allerson, et al *J. Med. Chem.* **2005**, 48, 901-4
Lima, et al *Cell* **2012**, 150, 883-894
Prakash et al *Nucleic Acids Res.* **2015**, 43, 2993-3011
Prakash et al *Bioorg. Med. Chem. Lett.* **2016**, 26, 2817-2820

Summary

- Chemical modification is a viable strategy to enhance the drug-like properties of oligonucleotide therapeutics
  - Improves RNA-binding affinity, metabolic stability, pharmacological, pharmacokinetic and toxicological properties
- >30 chemically modified oligonucleotide drugs encompassing multiple mechanisms are currently in mid to late-stage development
- Targeted delivery represents a new platform to further enhance potency of oligonucleotide therapeutics

Acknowledgements

- **Ionis Medicinal Chemistry**
  - E. Swayze, T. Prakash, M. Oestergaard, M. Migawa, G. Vasquez, Jeff Yu, B. Wan
- **Ionis bio-analytical Team**
  - H. Gaus, A. Berdeja, K. Schmidt, A. Chappell, R. Gupta
- **Ionis Core Research and Drug Discovery**
  - S. Murray, A. Siwkowski, A. Low, H. Murray, W. Lima, A. Jazayeri, B. Monia, F. Bennett, S. Crooke
- **Ionis Collaborators**
  - Hanessian Lab (U Montreal), Egli Lab (Vanderbilt), Jung Lab (UCLA), Leumann Lab (U Bern), Herdewijn Lab (Rega Institute), Harris Lab (U Nebraska)
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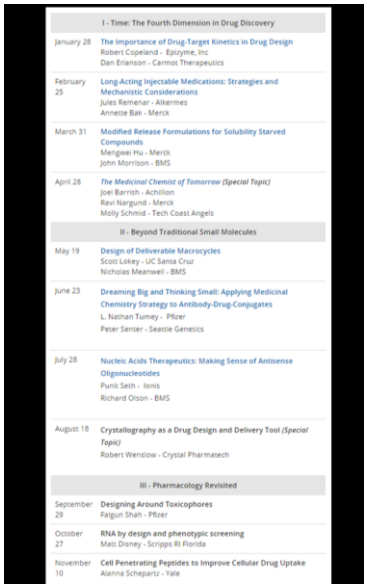

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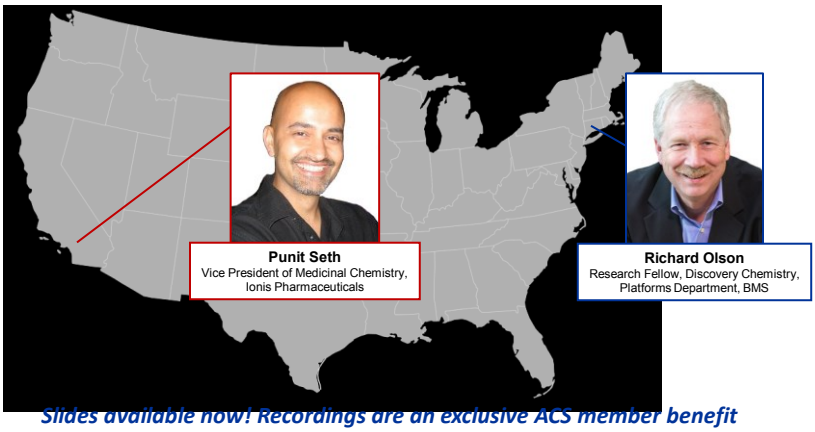

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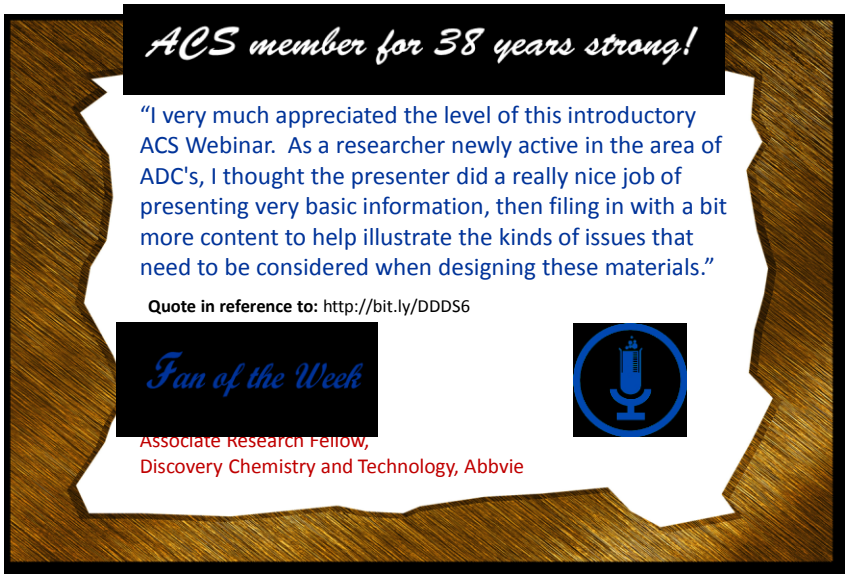

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