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Conception and Synthesis of Oxabicyclic Nucleoside Phosphonates as Internucleotidic Phosphate Surrogates in Antisense Oligonucleotide Constructs

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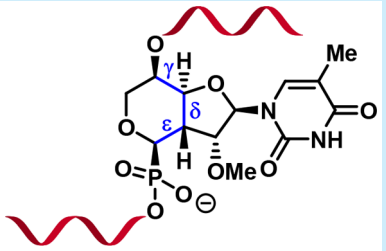
Supporting Information

ABSTRACT: The stereocontrolled synthesis of a novel oxabicyclic nucleoside phosphonate comprising a perhydrofuropyran core unit was achieved. It was incorporated in an oligonucleotide sequence as a 5′−3′ phosphonate−phosphate insert, and the stability properties of the resulting duplex were measured. The oxabicyclic nucleoside framework was designed so as to restrict rotation around angles γ, δ, and ε of a natural nucleoside.



Modified nucleic acids are used extensively for applications in biotechnology, diagnostics, and as nucleic acid-based therapeutics.[1] Conformationally restricted nucleic acids are of particular interest as they can enhance duplex stability or modulate hydrophobicity, which can impact their biological profile. Some notable examples of conformationally restricted nucleic acid analogs include locked nucleic acids (LNA),[2] tricycloDNA (tcDNA),[3] and α,β-constrained nucleic acids (α,β-CNA).[4] LNA and related analogs lock the furanose ring in the RNA-like C3′-endo sugar pucker.[5] tcDNA restricts rotation around the C4′−C5′ exocyclic bond,[6] while α,β-CNA restricts rotation around torsion angles α and β of the phosphodiester linkage.[7]

We have previously evaluated the duplex stabilizing properties of dual constrained nucleic acid analogs as components of antisense nucleoside sequences (ASOs) for applications in nucleic acid therapeutics.[8] Multiple modes of conformational restriction are enforced in such analogs to understand the contributions of individual constraint strategies toward overall duplex stability. As part of this effort, we reported the effect of restricting rotation around the C4′-C5′ exocyclic bonds in LNA and α-L-LNA.[9] Here, we report the synthesis and biophysical evaluation of oligonucleotides modified with a novel perhydrofuropyran based nucleoside scaffold, which simultaneously restricts rotation around torsion angles γ, δ, and ε (Figure 1).

In considering strategies to construct the perhydrofuropyran nucleoside phosphonate ester motif **A**, we envisaged two approaches.[10] Starting with thymidine would secure the desired stereochemistry of the nucleosidic part but would require the formation of the fused perhydrofuropyran including the stereocontrolled C−C branching at C-3′. The second approach would start with the readily available diacetone-D-glucose as a chiron, and after stereocontrolled branching at C-3, the entire carbon framework of the intended bicyclic nucleoside would be secured. We opted for this second approach.

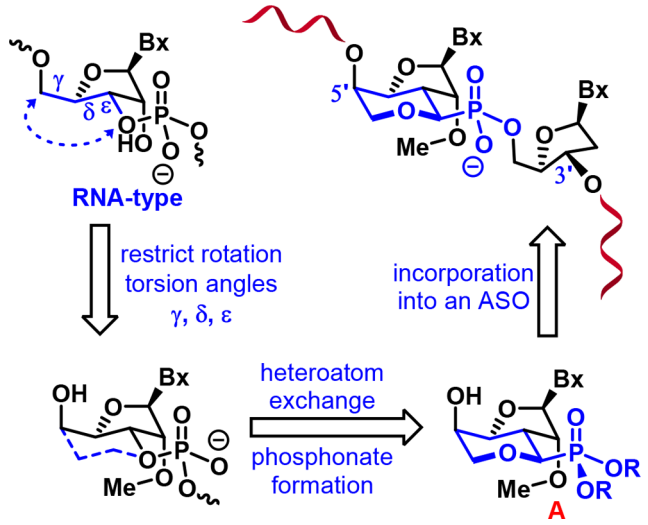


Figure 1. Perhydrofuropyran core in nucleoside **A** restricts conformational freedom around torsion angles γ, δ, and ε.

We started with the transformation of the commercially available diacetone-D-glucose **1** to the known[11] hydroxymethyl analog **2**, followed by protection as a 2-naphthylmethyl ether (Scheme 1). At this juncture, we decided to exchange the 5,6-isopropylidene acetal for a more convenient pair of protecting groups. Thus, acid-mediated cleavage, selective protection of the primary hydroxyl group as the TBDPS ether was followed by benzylation and acetolysis. The installation of the nucleobase

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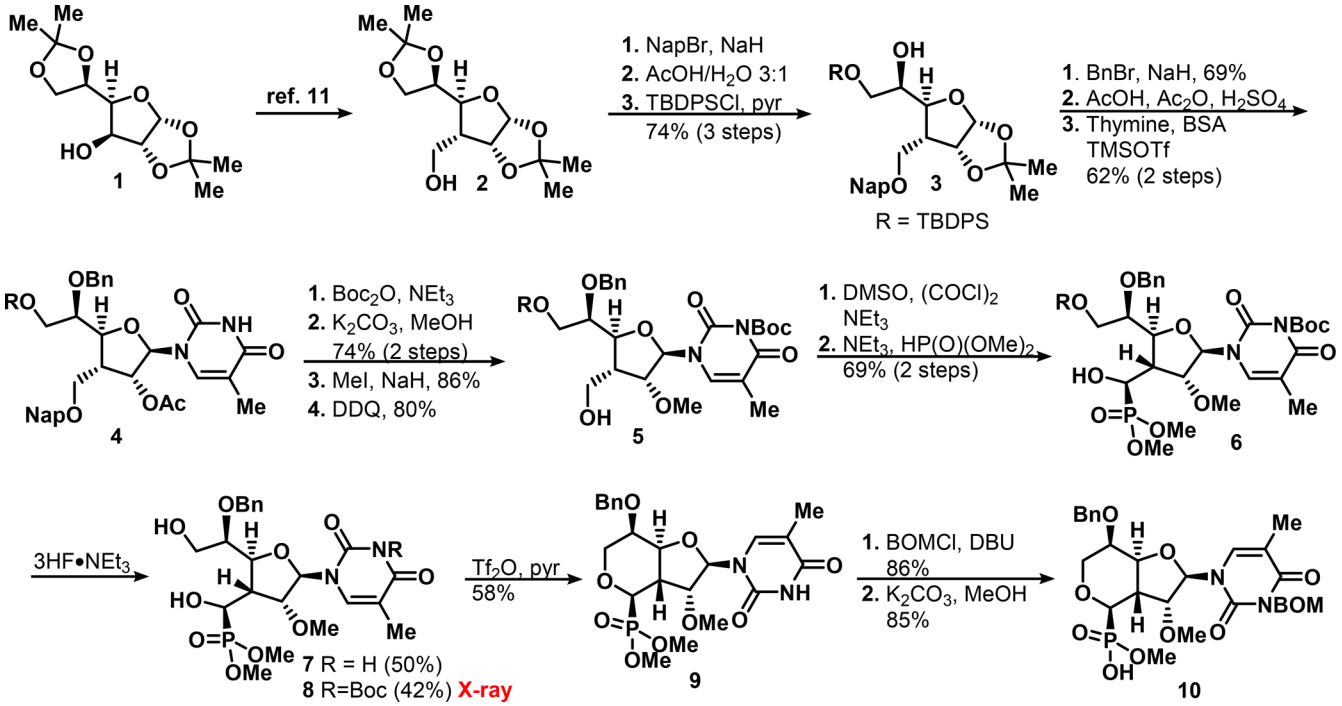
Scheme 1. Synthesis of Nucleoside Phosphonate 10



was successfully achieved using a Vorbrüggen reaction[12] affording nucleoside **4**. Direct and selective alkylation of the 2′-OH of an unprotected nucleoside is a difficult task.[13] As an alternative, we chose to protect the thymine base as a N-3 *t*-butyl-carbamoyl derivative.[14] Subsequent base mediated cleavage of the 2′-OAc allowed methylation of the 2′-OH in the presence of NaH and iodomethane, followed by cleavage of the 2-naphthylmethyl ether with DDQ[15] releasing the primary alcohol **5** in excellent overall yield. Swern oxidation followed by a Pudovik−Abramov reaction[16] with dimethyl H-phosphonate led to the α-hydroxyphosphonate **6** as a single isomer. Cleavage of the TBDPS group was achieved by treatment of **6** with 3HF·NEt_3 to give a mixture of compounds **7** and **8**. The configuration of the newly formed stereogenic center of the α-hydroxyphosphonate **6** was confirmed to be (*R*) by single crystal X-ray analysis of compound **8**. The exclusive *Re*-face addition of the dimethoxy H-phosphonate anion appears to be sterically controlled by the 2′-OMe group of the furanose ring since the addition gave the same (*R*)-isomer as the major product in a model substrate.[17] Initial attempts to form the oxabicyclic tetrahydropyran via mesylation or tosylation of the primary alcohol in either **7** or **8** followed by DBU-mediated cyclization led to low conversion. Gratifyingly, the desired oxabicyclic compound **9** was obtained using the triflate ester of **7** in pyridine. Protection of N-3 in the nucleobase with a BOM group[8] was successfully achieved and was followed with the cleavage of the phosphonate dimethyl ester to give the hemiester **10** in 85% yield over two steps.

Our original plan for the formation of phosphonate-linked dinucleotide **12** required the activation of the acidic −OH in bicyclic nucleoside **10**, then a traditional ester coupling with 5′-OH of a 3′-OTBDPS-protected thymidine. In our attempts to optimize this process, we found that commonly utilized conditions such as the Mitsunobu reaction[18] or ester formation in the presence of BOP,[19] HATU,[20] or DEPBT[21] led only to traces of product. We reasoned that by using hemiphosphonic ester **10** as the nucleophile and a 5′-activated nucleoside as the nucleofugal partner, we could obtain the desired phosphonate dinucleotide **12** (Scheme 2). After extensive optimization,[22] we found that warming a mixture of nucleosides **10** and **11** in DMF in the presence of Cs_2CO_3 gave dinucleoside hemiphosphonic ester (not shown).[23] However, these conditions resulted in the hydrolysis of the methyl ester of the phosphonate, which was re-esterified using freshly prepared diazomethane to give **12** affording a 1:1 mixture of P-diastereomers. Hydrogenolysis of **12** in the presence of Pearlman's catalyst[24] followed by protection of the 5′-hydroxyl group as the levulinate ester[25] and deprotection of the TBDPS ether using catalytic amounts of CsF[26] in a mixture of DMSO/MeOH afforded **13** as 1:1 mixture of phosphonate P-diastereomers in 65% yield. A phosphitylation reaction provided the phosphonate phosphoramidite **14**. The GGATG**TT**CTCGA oligonucleotide incorporating **14** was then paired with matched and mismatched RNAs where it showed a slightly enhanced ability to stabilize the duplex and similar ability to discriminate mismatches as natural DNA (Table 1).

To help understand these observations, we created a structural model that depicts the preferred conformation around the internucleosidic phosphodiester linkage observed in RNA duplexes (Figure 2).

The O3′-P-O5′-C5′-H bonds form the outline of a six-membered chair, and this conformation is stabilized by anomeric effects through the phosphorus atom.[27] Presumably, these nonbonding interactions serve to preorganize the backbone in nucleic acid duplexes. Our results show that the perhydrofuropyran ring in **A** can mimic conformational preferences around γ, δ, and ε in nucleic acid duplexes..

In conclusion, we report the synthesis and the biophysical evaluation of a novel perhydrofuropyran nucleoside phosphonate analog that restricts conformational preferences around torsion angles γ, δ, and ε. The modification showed a slight enhancement in duplex stability as compared to unmodified

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Scheme 2. Synthesis of Phosphoramidite 14

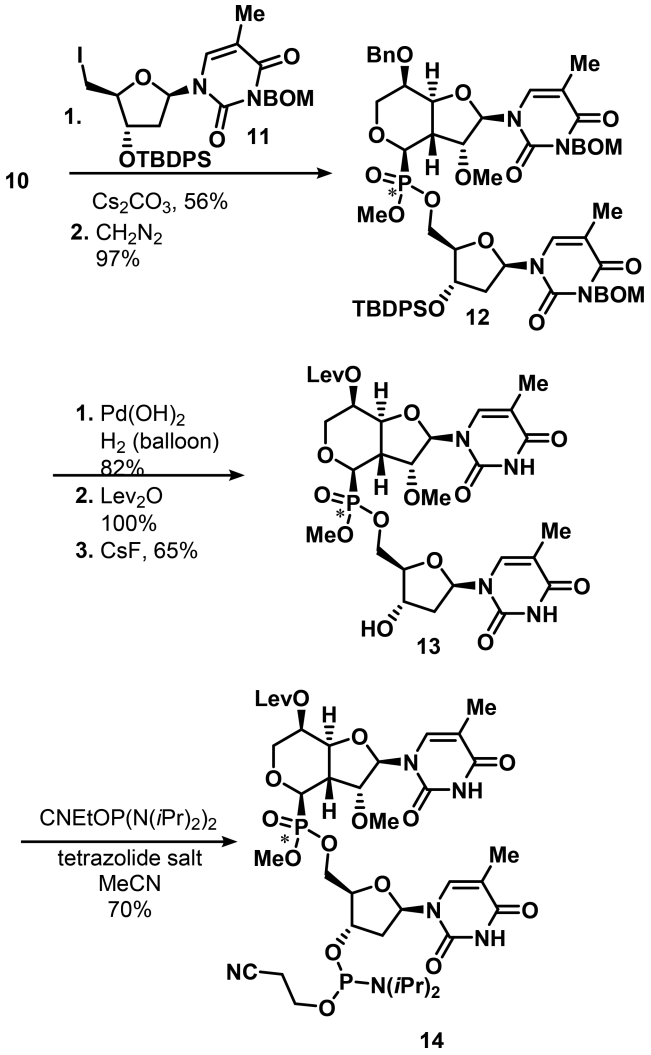

Table 1. Duplex Stabilizing and Mismatch Discrimination Properties of the Oligonucleotide Incorporating the Phosphonate Dinucleoside 13 (*TT*)[a]

	T_m °C (ΔT_m/mod)			
sequence (5′−3′)	RNA Y=A	RNA Y=C	RNA Y=G	RNA Y=U
GGATGTTCTCGA	49.7	34.5	44.0	35.8
GGATG*TT*CTCGA	50.2	34.9	46.7	35.8

[a] T_m values were measured in 10 mM sodium phosphate buffer (pH 7.2) containing 100 mM NaCl and 0.1 mM EDTA; RNA complement 3′-CCUAC*Y*AGAGCU-5′.

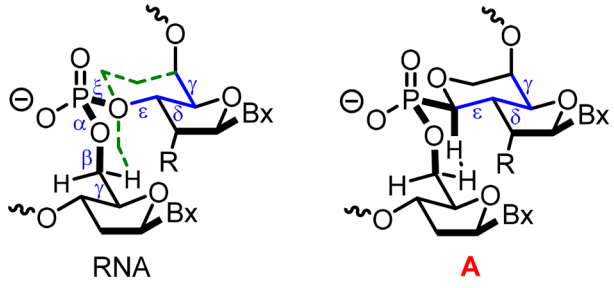

Figure 2. Conformational analysis of RNA versus phosphonate-linked nucleotide **A**.

DNA in the sequence evaluated. However, additional work using multiple sequences is required to make a complete assessment. Recent work has highlighted the ability of sugar and backbone modifications on the ASO to modulate interactions with cellular proteins, which can have profound implications on ASO behavior in biological systems.[28] Further biological evaluation of this and related oligonucleotide modifications will reveal their potential utility for applications in antisense therapeutics.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.8b02233.

Experimental details and characterization data for the products (PDF)

Accession Codes

CCDC 1827233 and 1850195 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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Notes

The authors declare no competing financial interest.

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(22) Careful selection of the protecting groups was essential in compounds **10** and **11**. Nucleobase protection with Boc resulted in complete cleavage and subsequent alkylation at the N3 position of the thymine base with another iodo nucleoside **11**. If PMB was used instead of BOM in both nucleosides, the subsequent removal was sluggish. When a 3′-OTBS was used in **11**, the silyl ether was cleaved under the reaction conditions.
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