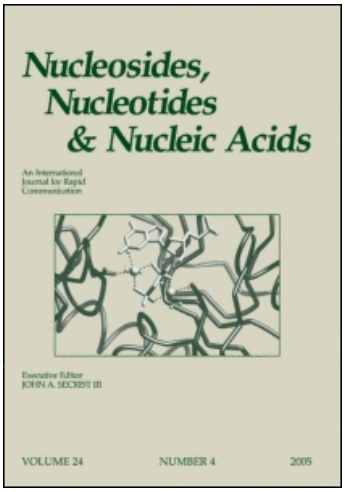

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David Sabatino[a]; Masad J. Damha[a]
[a] Department of Chemistry, McGill University, Montreal, Quebec, Canada

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SYNTHESIS AND PROPERTIES OF OLIGONUCLEOTIDES CONTAINING A 7-MEMBERED (OXEPANE) SUGAR RING

David Sabatino and Masad J. Damha □ *Department of Chemistry, McGill University, Montreal, Quebec, Canada*

□ *Herein we describe the synthesis of novel 7-membered ring (oxepane) thymine and adenine nucleosides (oT and oA) and their corresponding 5′-O-phosphoramidite derivatives. Two homopolymeric sequences (oT_{15} and oA_{15}) were prepared via conventional solid-phase synthesis. The mutually complementary strands had the ability to form a duplex (oT_{15}:oA_{15}) exhibiting a transition temperature of 12°C. The oxepane oligonucleotides were also found to associate with their respective complementary RNA strands thus forming oT_{15}:rA_{15} (13°C) and oA_{15}:rU_{15} (12°C) hybrids. The corresponding native duplexes, namely dT_{15}:dA_{15}, dT_{15}:rA_{15} and dA_{15}:rU_{15} had melting temperatures of 37°C, 32°C and 16°C, respectively. The CD spectrum of oT_{15}:rA_{15} closely resembled that of the native dT_{15}:rA_{15} hybrid and, in fact, both were found to be substrates for E. Coli RNase H. Thus the oxepane nucleic acids reported here are one of only a handful of DNA mimics capable of activating RNase H when bound to RNA.*

Keywords Oxepane; RNase; homopolymeric sequences

Pyranose nucleic acids adopt a rigid chair-like conformations that not always conform or adapt to the more flexible DNA or RNA conformations.[1,2] On the other hand, the L-(α)-threofuranosyl sugar of TNA regenerates conformational flexibility such that it is capable of pairing to a TNA complement in addition to cross-pairing with DNA and RNA.[3,4] We theorized that expanding the carbohydrate skeleton to a 7-membered heptose carbohydrate (oxepane) would provide a conformationally more versatile nucleoside and oligonucleotide structure relative to the 6-membered ring pyranose.[5] Furthermore, since flexibility of the sugar-phosphate backbone appears to be a requirement for efficient activation of RNase H by antisense constructs, we examined the ability of oxepane oligonucleotides to bind to complementary RNA and elicit its degradation through RNase H-mediated hydrolysis.

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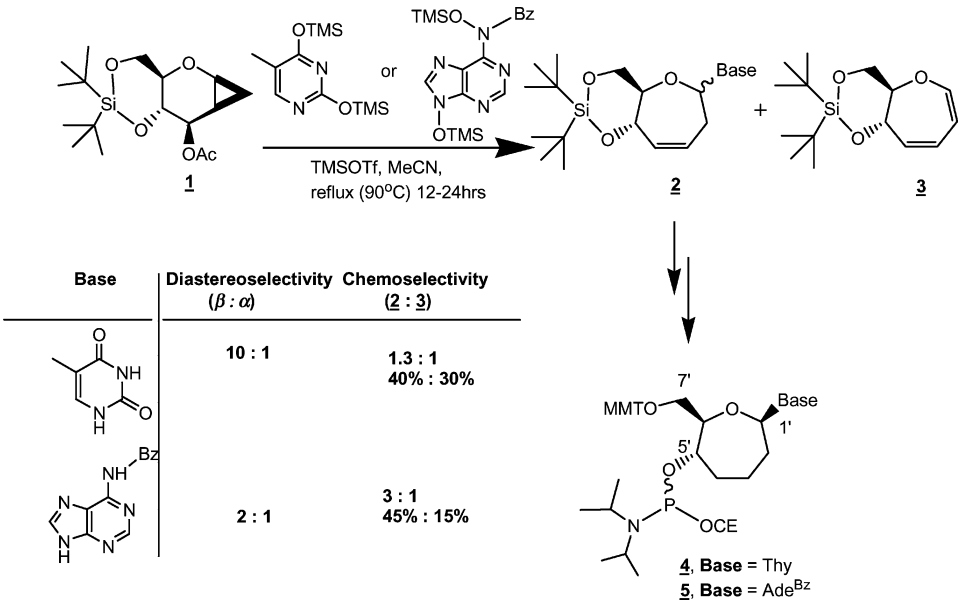
Address correspondence to Masad J. Damha, Department of Chemistry, McGill University, Montreal, QC H3A2K6, Canada. E-mail: masad.damha@mcgill.ca



SCHEME 1 Synthesis of oxepane nucleosides and their corresponding 5′-*O*-phosphoramidite derivatives.

The synthetic strategy followed for oxepane nucleoside synthesis was inspired from the elegant work of Hoberg who reported that cyclopropanated glycal sugars undergo Lewis acid catalyzed ring-expansion when treated with small nucleophiles.[6] We have expanded the scope of this reaction to nucleoside synthesis by introducing silylated purine and pyrimidine nucleobases in the ring expansion reaction (Scheme 1). Thus, Vorbruggen-type glycosylation reactions[7] with **1** afforded the desired unsaturated (oxepine) nucleosides **2**, along with the diene by-product, **3**. Coupling with adenine proceeded more rapidly compared to coupling with thymine (0.5 days versus 1 day; reflux), at the expense of a poorer anomeric diastereoselectivity (2:1 β/α ratio for A coupling and 10:1 β/α ratio for T coupling). Following reduction of the double bond, the oxepane-5′-*O*-phosphoramidite derivatives **4** and **5** were prepared and purified by standard methods, and were used as 0.05M solutions (MeCN and /or CH_2Cl_2) for solid-phase oligonucleotide synthesis.[8]

Homopolymeric oxepane oligonucleotides oT_{15} and oA_{15} were synthesized on a 0.5 μmol scale using 0.25M ethylthiotetrazole in acetonitrile as coupling reagent. The desired oligonucleotides constituted 60–70% of the crude material isolated after deprotection (HPLC analysis), indicating that under the conditions used, the monomers coupled with 98–99% efficiency. Following purification by anion exchange HPLC and/or denaturing PAGE, they were desalted by size exclusion chromatography (Sephadex G-25) and their structure confirmed by MALDI-TOF mass spectrometry (Table 1).

TABLE 1 MALDI-TOF MS data of oligonucleotides synthesized and used in this study

Sample	Theoretical M.W. (g/mol)	Experimental M.W. (g/mol)
rU_{15}	4532	4547
rA_{15}	4878	4931
dT_{15}	4503	4530
dA_{15}	4638	4640
oT_{15}	4924	4927
oA_{15}	5056	5080

Job plots (Figure 1) and melting denaturation studies showed that oT_{15} and oA_{15} formed a duplex (oT_{15}:oA_{15}) that exhibited a transition temperature (12°C) that was significantly lower than that of dT_{15}:dA_{15} (37°C). Interestingly, the oxepane oligonucleotides were found to associate with their respective complementary RNA strands thus forming oT_{15}:rA_{15} (13 C) and oA_{15}:rU_{15} (12°C) hybrids. The corresponding native hybrids dT_{15}:rA_{15} and dA_{15}:rU_{15} had melting temperatures of 32°C, and 16°C, respectively. No association was observed between the oxepane oligomers and their complementary ssDNA strands.

The CD spectra of oT_{15}:oA_{15} and oT_{15}:rA_{15} were then compared to those of dT_{15}:dA_{15} and dT_{15}:rA_{15} (Figures 2a and b). The data showed that the completely modified oxepane duplex, oT_{15}:oA_{15}, exhibits a very unique CD profile that is somewhat the mirror image of the dT_{15}:dA_{15} profile (Figure 2a). By contrast, cross-pairing between oT_{15} and rA_{15} provides a hybrid whose helical conformation resembles that of the dT_{15}:rA_{15} hybrid (Figure 2b). Furthermore, the fact that oT_{15}:rA_{15} is a substrate of *E. Coli* RNase H suggest that oT_{15} adopts a flexible "DNA-like" structure when hybridized to rA_{15} (data not shown). The oxepane oligonucleotides, oT_{15} and oA_{15} were also found to be completely resistant to nucleases present in

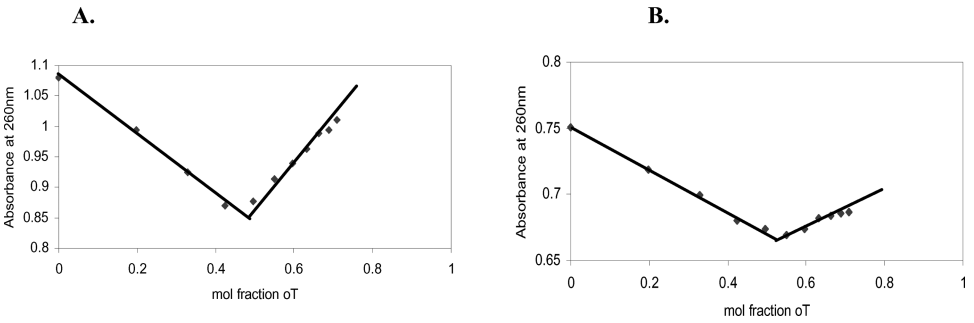


FIGURE 1 UV mixing curves (Job Plots) obtained by titrating (A). oT_{15}-oA_{15} (5 μM per strand) or (B). oT_{15}–rA_{15} (5 μM per strand) in a buffer consisting of 10 mM Na_2HPO_4, 100 mM NaCl, pH 7.2. Titrations were carried out at 5°C.

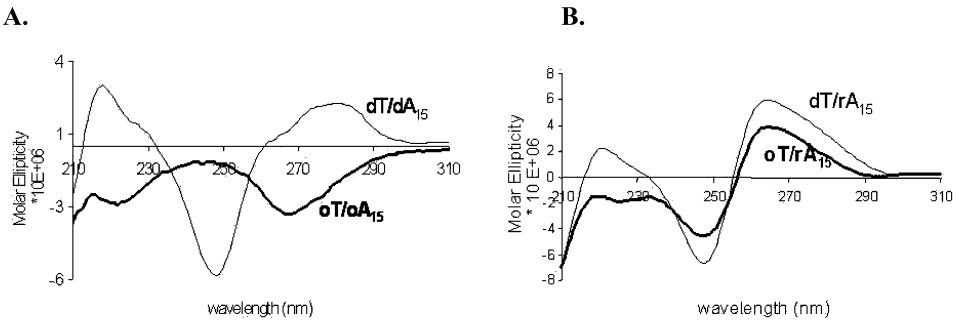


FIGURE 2 Comparison of the CD spectral signatures at 5°C for (A). dT_{15}-dA_{15} and oT_{15}-oA_{15}; (B). dT_{15}-rA_{15} and oT_{15}-rA_{15}. Duplex concentration is 3.04 μM; buffer: 140 mM KCl, 1 mM $MgCl_2$, 5 mM Na_2HPO_4 pH = 7.2.

fetal bovine serum (FBS) even after 24 hours incubation at 37°C (data not shown).

In summary, the oxepane carbohydrate modification generates potentially useful nucleoside and oligonucleotide analogues for therapeutic applications.[9,10] Current studies are aimed towards modifying the oxepane carbohydrate structure to improve binding to target RNA sequences.

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