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Synthesis and Biophysical Properties of Constrained D-Altritol Nucleic Acids (cANA)

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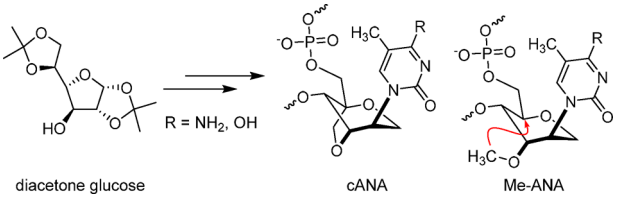
ABSTRACT



The first synthesis of constrained altritol nucleic acids (cANA) containing antisense oligonucleotides (ASOs) was carried out to ascertain how conformationally restricting the D-altritol backbone-containing ASO (Me-ANA) would affect their ability to form duplexes with RNA. It was found that the thermal stability was reduced (cANA/RNA −1.1 °C/modification) compared to DNA/RNA, suggesting the constrained system results in a small destabilizing perturbation in the duplex structure.

Some antisense oligonucleotides (ASOs)[1] modified with monomers having a conformationally constrained pentofuranosyl sugar show an increase in binding affinity with their complementary mRNA; this has been well documented in the case of LNA (**1**, Figure 1).[2−6] LNA can be thought of as a conformationally restricted 2′-OMe RNA, where the methyl group is constrained to the 4′-position, thereby locking it into its 'northern' conformation. When incorporating this conformational restricted 2′-OMe RNA, i.e. LNA, into an ASO, a large increase in affinity is observed when paired with complementary RNA. This observation also holds for a variety of other conformationally restricted analogs, such as cEt-BNA (**2**),[7] α-LNA,[8] and ENA.[9]

Herdewijn et al. have been studying anhydrohexitol-based oligonucleotides, where the pyranosyl ring system is replaced with a hexitol ring system.[10−16] The parent in this series is hexitol nucleic acid (**3**, HNA), which was incorporated into an ASO and reported to possess a strong binding affinity when duplexed with RNA, in addition to many

(1) *Antisense Drug Technology: Principles, Strategies, and Applications*, 2nd ed.; Crooke, S. T., Ed.; CRC Press: Boca Raton, FL, 2007.
(2) Lindholm, M. W.; Elmen, J.; Fisker, N.; Hansen, H. F.; Persson, R.; Moller, M. R.; Rosenbohm, C.; Orum, H.; Straarup, E. M.; Koch, T. *Mol. Ther.* **2011**, 376.
(3) Petersen, M.; Bondensgaard, K.; Wengel, J.; Jacobsen, J. P. *J. Am. Chem. Soc.* **2002**, *124*, 5974.
(4) Nielsen, C. B.; Singh, S. K.; Wengel, J.; Jacobsen, J. P. *J. Biomol. Struct. Dyn.* **1999**, *17*, 175.
(5) Braasch, D. A.; Corey, D. R. *Chem. Biol.* **2001**, *8*, 1.
(6) Petersen, M.; Wengel, J. *Trends Biotechnol.* **2003**, *21*, 74.
(7) Seth, P. P.; Vasquez, G.; Allerson, C. A.; Berdeja, A.; Gaus, H.; Kinberger, G. A.; Prakash, T. P.; Migawa, M. T.; Bhat, B.; Swayze, E. E. *J. Org. Chem.* **2010**, *75*, 1569.
(8) Hakansson, A. E.; Wengel, J. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 935.
(9) Morita, K.; Hasegawa, C.; Kaneko, M.; Tsutsumi, S.; Sone, J.; Ishikawa, T.; Imanishi, T.; Koizumi, M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 73.
(10) D'Alonzo, D.; Van Aerschot, A.; Guaragna, A.; Palumbo, G.; Schepers, G.; Capone, S.; Rozenski, J.; Herdewijn, P. *Chem.—Eur. J.* **2009**, *15*, 10121.
(11) Maier, T.; Przylas, I.; Strater, N.; Herdewijn, P.; Saenger, W. *J. Am. Chem. Soc.* **2005**, *127*, 2937.
(12) Allart, B.; Van Aerschot, A.; Herdewijn, P. *Nucleosides Nucleotides* **1998**, *17*, 1523.
(13) Allart, B.; Khan, K.; Rosemeyer, H.; Schepers, G.; Hendrix, C.; Rothenbacher, K.; Seela, F.; Aerschot, A. V.; Herdewijn, P. *Chem.—Eur. J.* **1999**, *5*, 2424.
(14) Van Aerschot, A.; Maurinsh, Y.; Allart, B.; Boudou, V.; Herdewijn, P. *Collect. Symp. Ser.* **1999**, *2*, 100.
(15) Wouters, J.; Herdewijn, P. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1563.
(16) Froeyen, M.; Wroblowski, B.; Esnouf, R.; De Winter, H.; Allart, B.; Lescrinier, E.; Herdewijn, P. *Helv. Chim. Acta* **2000**, *83*, 2153.

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interesting biological properties.[12] An improvement in hybridization was also demonstrated for D-altritol nucleic acids (**4**, ANAs) and its methylated analog (**5**, Me-ANA).[13,17] Due to the reported strong affinity that altritol nucleic acids have toward RNA,[14] we sought to discover if we could conformationally restrict the sugar moiety in an analogous manner to the pentofuranosyl sugars to further increase the affinity in the altritol class of nucleoside analogs. Toward this end, we prepared an ASO containing a constrained ANA monomer (**6**, cANA) and ascertained its biophysical properties.

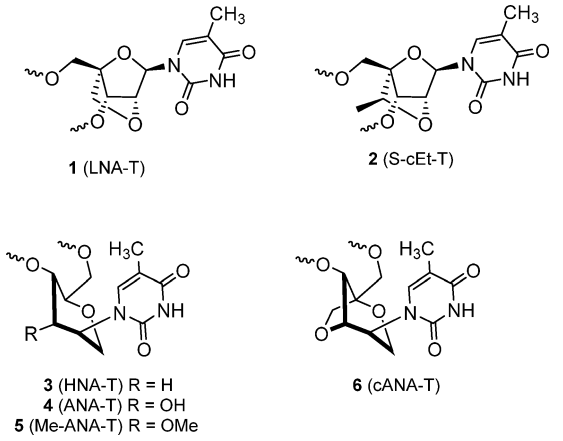
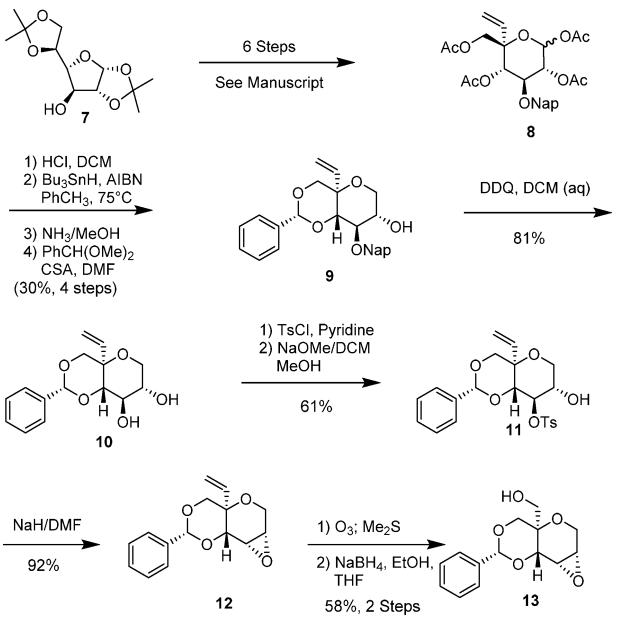


Figure 1. Several modified nucleic acid monomers.

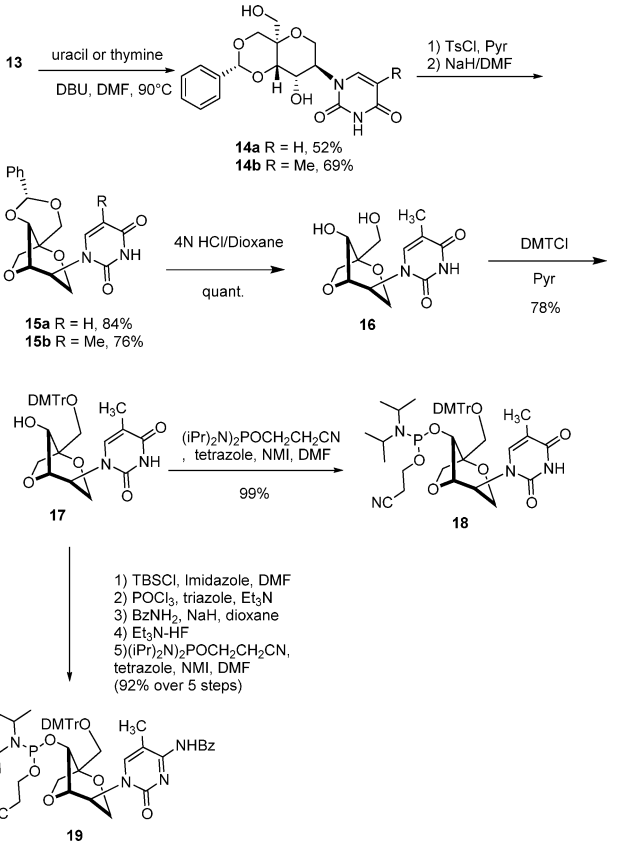
The synthesis of amidites suitable for ASO incorporation, **18** and **19**, began with the widely available and inexpensive diacetone glucose **7** (Scheme 1). Conversion to the protected hexitol (**8**) was carried out by a slight modification of known methods.[18,19] A subsequent deoxygenation of compound **8** at the anomeric position was achieved by converting the anomeric acetate to its corresponding chloride with HCl followed by a reduction of the α-chloride under radical conditions, deacetylation with methanolic ammonia, and 4,6-benzylidine formation to give the monoprotected diol (**9**) in 30% yield over four steps.[20] The 2-methylnapthyl group (Nap) was removed with DDQ in aqueous DCM in 81% yield, followed by bis-tosylation, and then selective hydrolysis gave the monotosylated alcohol **11** in 61% yield over two steps.[21]

Treatment of compound **11** with sodium hydride rapidly gave epoxide **12** in a 91% yield, which was converted into the alcohol **13** by an ozonolysis–borohydride reduction sequence. Our key intermediate, **13**, could then be coupled

Scheme 1



Scheme 2



to uracil or thymine by an epoxide ring-opening reaction under sodium salt conditions to give the corresponding nucleoside, **14a** and **14b**, in 52% and 69% yields,

(17) Van Aerschot, A.; Meldgaard, M.; Schepers, G.; Volders, F.; Rozenski, J.; Busson, R.; Herdewijn, P. *Nucleic Acids Res.* **2001**, *29*, 4187.
(18) Bleriot, Y.; Vadivel, S. K.; Herrera, A. J.; Greig, I. R.; Kirby, A. J.; Sinay, P. *Tetrahedron* **2004**, *60*, 6813.
(19) Rao, A. V. R.; Gurjar, M. K.; Devi, T. R.; Kumar, K. R. *Tetrahedron Lett.* **1993**, *34*, 1653.
(20) Auge, J.; David, S. *Carbohydr. Res.* **1977**, *59*, 255.
(21) Brockway, C.; Kocienski, P.; Pant, C. *J. Chem. Soc., Perkin Trans. 1* **1984**, 875.

respectively (Scheme 2). Tosylation of the primary hydroxyl followed by ring closure gave the corresponding protected, constrained nucleosides **15a** and **15b**.

It is known that the 5-methyl analog of ANA possesses a higher T_m than the 5-unsubstituted analog; therefore, we sought to further transform the thymidine derivative (i.e., **15b**) into an amidite suitable for ASO synthesis for T_m studies while using **15a** for X-ray crystallography studies (Figure 2). While X-ray analysis unambiguously confirmed the structure of the uracil derivative, the thymine derivative was assigned by its nearly identical NMR spectra. With the structure of **15b** verified, we removed the benzylidine to give the unprotected nucleoside **16** in quantitative yield, installed a DMTr group at the 5′-position in 78% yield, and followed this by conversion into the T and MeC amidites using well-known and previously reported methodology.[7] We were able to obtain both phosphoramidites of the pyrimidine analogs in quantities sufficient for T_m studies.

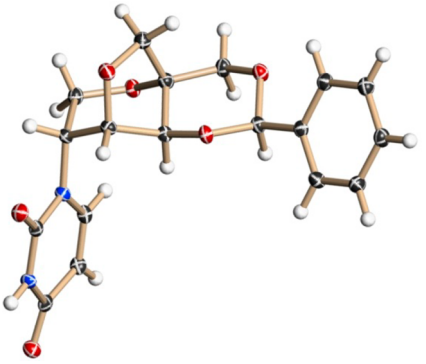


Figure 2. Structure of compound **15a** from crystal structure coordinates.

The synthesis of the ASO was carried out (mCTTAGCACTGGCmCT), and the T_m of this ASO duplex with complementary RNA was determined using previously reported procedures.[7] As shown in Table 1, the cANA ASO (**20**) T_m was reduced by −1.1 °C/modification compared to DNA (**23**), while the HNA (**22**) and Me-ANA (**21**) were stabilizing, resulting in 1.6 °C/mod and 0.1°/mod, respectively.

Table 1. Biophysical Data of ASOs Containing Me-ANA, HNA, and cANA Modifications[a]

no.	sequences (5′-3′)	chemistry	T_m (°C)
20	d($^{m}C_{B1}T_{B1}$TAGCACTGGC$^{m}C_{B1}T_{B1}$)	cANA	44
21	d(C_AU_ATAGCACTGGCC_AU_A)	Me-ANA[23]	48.9
22	d($^{m}C_HT_H$TAGCACTGGC$^{m}C_HT_H$)	HNA[23]	54.9
23	d(CTTAGCACTGGCCT)	DNA	48.5

[a] All internucleosidic linkages are phosphorothioate, $^{m}C_{B1}$ = cANA-5-methyl-cytidine, T_{B1} = cANA thymidine, C_A = 3′-*O*-methyl-ANA-cytidine, U_A = 3′-*O*-methyl-ANA-cytidine uridine, $^{m}C_H$ = HNA-5-methylcytidine, T_H = HNA-5-methylcytidine.

To explain the destabilizing behavior of the cANA incorporation, we speculated that the conformation of the cANA monomer in the duplex differs somewhat from that of Me-ANA. Recently, it has been suggested that very slight perturbations in the lean of the nucleobase or the rotation angle of the glycosidic bond axis can result in a large destabilizing effect.[22] While the crystal structure of **15a** suggests that a nearly perfect overlay with Me-ANA or HNA is possible, it is also likely that the addition of the 3′,5′-CH_2O- bridge may introduce additional steric effects modifying the preferred conformation. As shown in Figure 3a, the Me-ANA monomer prefers to adopt a conformation that situates the nucleobase in an axial orientation which is necessary for Watson−Crick base pairing in the duplex. However, as seen in Figure 3b, an additional 1,6 steric interaction could render this conformation undesirable, with the six-membered ring adopting the preferred chair conformation, while the outer seven-membered prefers a chair or twist-chair conformation. This would situate the nucleobase in an equatorial position, preferentially, resulting in a destabilizing, rather than stabilizing, effect in the context of the ASO/RNA duplex. Additionally, in the case of Me-ANA, the methyl group lies 'outside' the main pyranosyl ring, whereas in the constrained system the methyl group lies 'underneath' the ring. This gives rise to additional steric bulk being added underneath the pyranosyl ring which may be unfavorable within the duplex structure.

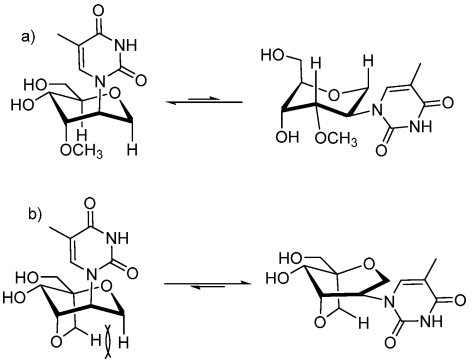


Figure 3. Conformational analysis of Me-ANA (a) and cANA (b).

In conclusion, we were able to report the first synthesis of a constrained altritol nucleoside and incorporate it into an ASO to determine that it is a destabilizing modification as compared to the unconstrained hexitol nucleic acids. Additional work will need to be carried out to determine the precise nature of the destabilizing effect of cANA and determine if alternative modes of conformational

(22) Mori, K.; Kodama, T.; Obika, S. *Org. Lett.* **2011**, *13*, 6050.
(23) Egli, M.; Pallan, P. S.; Allerson, C. R.; Prakash, T. P.; Berdeja, A.; Yu, J.; Lee, S.; Watt, A.; Gaus, H.; Bhat, B.; Swayze, E. E.; Seth, P. P. *J. Am. Chem. Soc.* **2011**, *133*, 16642.

constraint will result in a stabilizing effect when paired with RNA.

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Supporting Information Available. Experimental procedures, spectroscopic data for new compounds, and crystallographic data for compound **15a** are available. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.