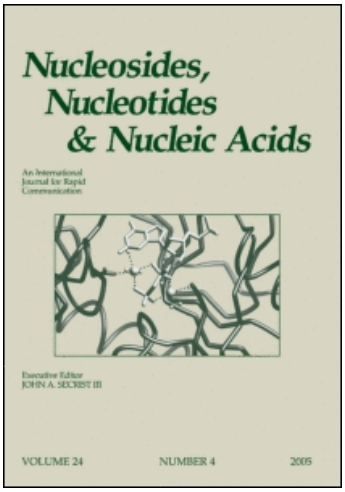

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

1,5-Anhydro-2-Deoxy-D-Altritol Oligonucleotides as Conformationally Restricted Analogues of Rna

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To cite this Article Allart, Brigitte , Van Aerschot, Arthur and Herdewijn, Piet(1998) '1,5-Anhydro-2-Deoxy-D-Altritol Oligonucleotides as Conformationally Restricted Analogues of Rna', Nucleosides, Nucleotides and Nucleic Acids, 17: 9, 1523 — 1526
To link to this Article: DOI: 10.1080/07328319808004682
URL: http://dx.doi.org/10.1080/07328319808004682

NUCLEOSIDES & NUCLEOTIDES, 17(9-11), 1523-1526 (1998)

1,5-ANHYDRO-2-DEOXY-D-ALTRITOL OLIGONUCLEOTIDES AS CONFORMATIONALLY RESTRICTED ANALOGUES OF RNA

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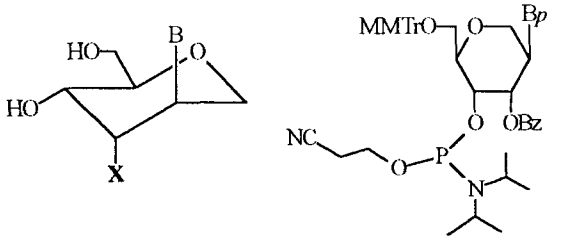
Abstract : As part of a project concerning the investigation of new hexitol nucleic acids (HNA), the 1,5-anhydro-2-deoxy-D-altritol nucleoside building blocks with a uracil, cytosine, adenine and guanine base moiety were synthesized. The uracil analogue was used for the automated synthesis of corresponding oligonucleotides. Hybridization capabilities of these altritol nucleic acids (ANA) are illustrated by the Tm values obtained for the $(a^hU)_{13}/(dA)_{13}$ duplex.

Hexitol nucleic acids (HNA) built from 1,5-anhydro-2,3-dideoxy-D-arabinohexitol nucleoside analogues **I** (X=H)[1] have proven to be one of the most efficient conformationally restricted oligonucleotides in terms of duplex formation with natural nucleic acids, and therefore are very promising candidates for antisense strategies[2]. The strong hybridizing properties of HNA could be explained by the conformational preference of the hexitol moiety to adopt a $^4C_1$ conformation, which fits very well with the 3'-endo conformation adopted by furanose nucleosides in natural nucleic acids (A form). This $^4C_1$ conformation is preserved by introducing a supplementary hydroxy group on C-3' in α-position[3]. This result prompted us to consider the synthesis of (6'→ 4') oligonucleotides built from 1,5-anhydro-2-deoxy-D-altritol nucleosides **II**.



6a : B_p = Uracil

6b : B_p = N^4-benzoylcytosine

6c : B_p = N^6-benzoyladenine

6d : B_p = N^2-isobutyrylguanine

I: **X = H** 1,5-anhydro-2,3-dideoxy-D-*arabino*-hexitol (hA, hG, hT, hC)

II: **X = OH** 1,5-anhydro-2-deoxy-D-*altrito*-hexitol (a^hA, a^hG, a^hT, a^hC)

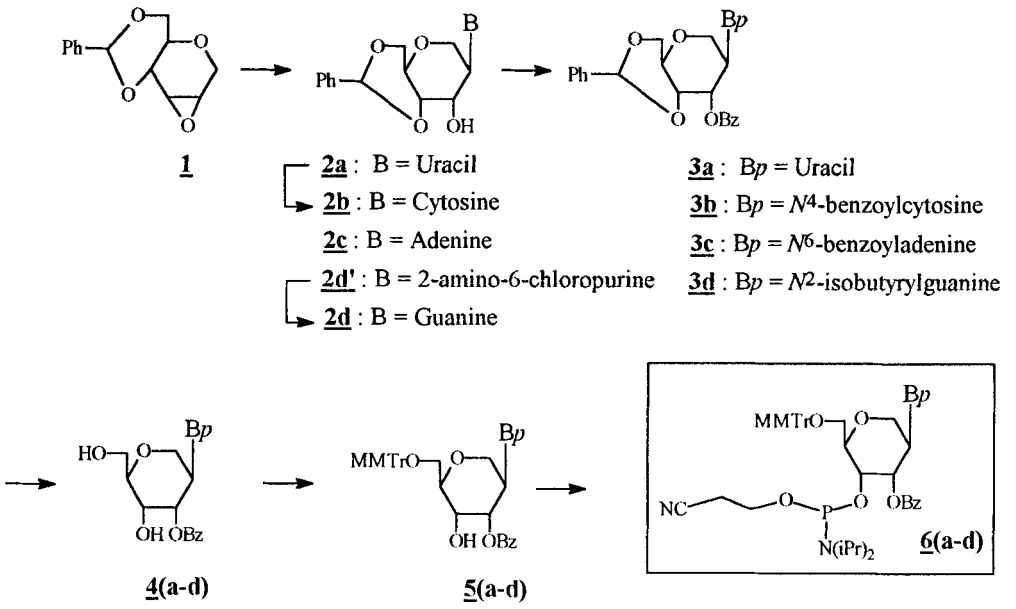


FIG. 1

General scheme for the synthesis of the *altritol* phosphoramidite building blocks

In this communication we describe the general synthetic route to prepare the uracil phosphoramidite building block **6a** (a^hU) and the synthesis of the all-uracil 13-mer (a^hU)$_{13}$. Compound **6a** was synthesized in 10 steps starting from tetraacetyl-α-D-bromoglucose (**FIG. 1**) as follows :

Introduction of the base moiety was achieved by the regiospecific opening of 1,5:2,3-dianhydro-4,6-*O*-benzylidene-D-allitol **1** (prepared in 5 steps from tetraacetyl bromoglucose according to the procedure of Kocienski[4]) by the sodium salt of uracil. On ribonucleosides, the tert-butyldimethylsilyl group is most commonly used for protection of the free 2'-OH position which is necessary for RNA synthesis. Because of the steric hindrance protection of the 3'-OH group of **2a** with a tert-butyldimethylsilyl group proved to be difficult. Therefore we used a benzoyl protecting group which is, however, prone to migration reaction. Treatment of **3a** with 90% aq. CF_3CO_2H (1h), careful evaporation at T<40°C followed by precipitation in diethyl ether afforded the crude product **4a** in 70% yield. Compound **4a** was then monomethoxytritylated without further purification. Since benzoyl migration from the 3'- to the 4'- position was found to occur more quickly under the classical conditions (MMTrCl/pyridine/4,4'-dimethylaminopyridine as catalyst), the reaction was performed in DMF, using silver nitrate as activator (1 eq) in presence of a small quantity of 2,4,6-collidine (1.5 eq) [4] and led to **5a** in 74% yield. Compound **5a** was converted into the corresponding 2-cyanoethyl-*N*,*N*-diisopropyl phosphoramidite **6a** by the general procedure in 94% yield[5].

TABLE I : Compared Tm values obtained for pairing of *altrito*-(a^h) and *arabino*-hexitol (**h**) oligomers with their DNA complement

mixture	Tm[a]	
(4 µM of each strand)	0.1M NaCl	1M NaCl
$(a^hU)_{13}/(dA)_{13}$	30	48
$(hU)_{13}/(dA)_{13}$	22	36-39

(a) Tm were measured in a buffer containing 0.02M KH_2PO_4, pH 7.5, 0.1 mM EDTA with 0.1 or 1M NaCl

Compounds **5b**, **5c**, and **5d** were synthesized following a similar synthetic scheme, with an additional step for protection of the base moiety (N^6-benzoyl, N^4-benzoyl or N^2-isobutyryl for adenine, cytosine and guanine derivatives respectively) using conventional procedures and their conversion into the phosphoramidites **6b**, **6c** and **6d** is under way.

Oligonucleotide synthesis : Making use of compound **6a**, a (all-uracil) homopolymer was synthesized with the aim to optimize the conditions of oligomer deprotection and to evaluate the hybridization capabilities of the altritol nucleic acids.

Oligonucleotide synthesis was carried out on an automated DNA-synthesizer using the phosphoramidite approach, with some modifications for final deprotection of the oligomer. After assembly, the 2- cyanoethyl groups were selectively cleaved by a 30 min. treatment of the support with a piperidine/dioxane (1:4) solution, before final treatment with concentrated ammonia (50°C, 16h). The (all-uracil) 13-mer prepared from **6a** was found to be >95% homogeneous after purification.

Hybridization Properties : The UV-melting curves at 260 nm (and 284 nm) of the all-uracil altritol oligomer with complementary DNA $(dA)_{13}$ demonstrate the formation of a $(a^hU)_{13}/(dA)_{13}$ complex. According to Tm values (**TABLE I**), the $(a^hU)_{13}/(dA)_{13}$ duplex is more stable than the $(hU)_{13}/(dA)_{13}$ duplex. This may be attributed to a better hydration of the altritol nucleic acid/DNA duplex, which is now under investigation. Incorporation of the other phosphoramidite derivatives **6b**, **6c** and **6d** and the study of the pairing properties of this new construct with DNA and with RNA is in progress. The preliminary studies, however, demonstrate that altritol nucleic acids might form more stable duplexes with natural nucleic acids than HNA itself.

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