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# [(2-DEOXY-α- AND β-D-*ERYTHRO*-PENTOFURANOSYL)THYMIN-1-YL] METHANE DERIVATIVES AS POTENTIAL CONFORMATIONAL PROBES FOR *alt*DNA OLIGONUCLEOTIDES

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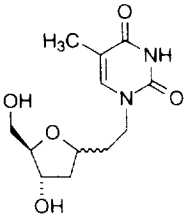
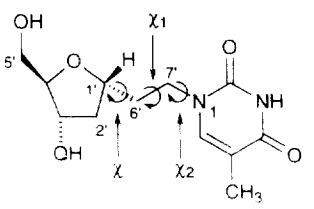
**Abstract:** The previously unknown deoxyribonucleoside analogues **2a,b** have been efficiently synthesized from commercial 1-*O*-methyl-2-deoxy-3,5-di-*O*-*p*-toluoyl-D-*erythro*-pentofuranose. The conversion of these nucleosides to the phosphoramidite derivatives **13a,b** and **14a,b** for subsequent incorporation into oligodeoxyribonucleotide analogues is also described.

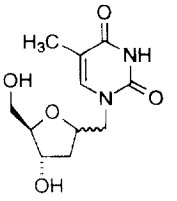
We have recently demonstrated that α,β-oligodeoxyribonucleotides with alternating (3'→3')- and (5'→5')-internucleotidic phosphodiester linkages (*alt*DNA)[1-3] exhibited higher affinity for complementary single-stranded DNA sequences than for RNA sequences.[1] The reduced thermal stability of *alt*DNA-RNA complexes may result from the limited ability of *alt*DNA to establish base-pairing within A-type helices. In this context, one can argue that a greater flexibility of *alt*DNA nucleobases may compensate for the unnatural arrangement of the internucleotidic phosphodiester motifs of these oligomers which may detrimentally affect base-pair formation. Thus, in an attempt to improve the affinity of *alt*DNA oligomers for complementary RNA oligonucleotides, we recently reported the chemical synthesis of the nucleoside analogues **1a,b** and their phosphoramidite derivatives.[4] An ethylene arm linking the nucleobase and carbohydrate moieties (for example, see **1a**) has been selected to facilitate optimal Watson-Crick base-pairing within *alt*DNA-DNA or *alt*DNA-RNA hybrids. Modeling studies of either **1a** or **1b** show that the torsion angle $\chi_1$ of energetically preferred conformers is ideal



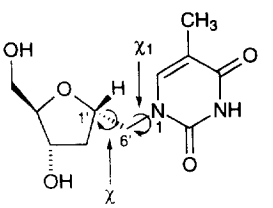
**1a** α-epimer
**b** β-epimer



**1a**



**2a,b**



**2a**

(180°) for base-pairing. These studies also suggest that rotation about the $C_6$-$C_7$ bond ($\chi_1 \neq 180°$) may introduce significant propeller twist, buckle, and opening angles to otherwise perfectly aligned base-pairs within a double helix. In this regard, nucleoside analogues with a methylene linker arm joining nucleobase and carbohydrate entities, such as **2a,b**, can also serve as a useful conformational probes for *alt*DNA-DNA and *alt*DNA-RNA hybrids. Unlike **1a,b**, these nucleoside analogues have only one additional torsion ($\chi_1$) relative to unmodified α- or β-thymidine (for example, see **2a**) and, consequently, will generate a smaller number of conformers. Modeling **2a** and **2b** in either parallel or antiparallel orientation into an A-type DNA-RNA hybrid allowed to find a helical structure that is energetically comparable to that of an unmodified DNA-RNA duplex[5] and, hence, advocates the incorporation of **2a** into *alt*DNA oligonucleotides (see Figure 1).

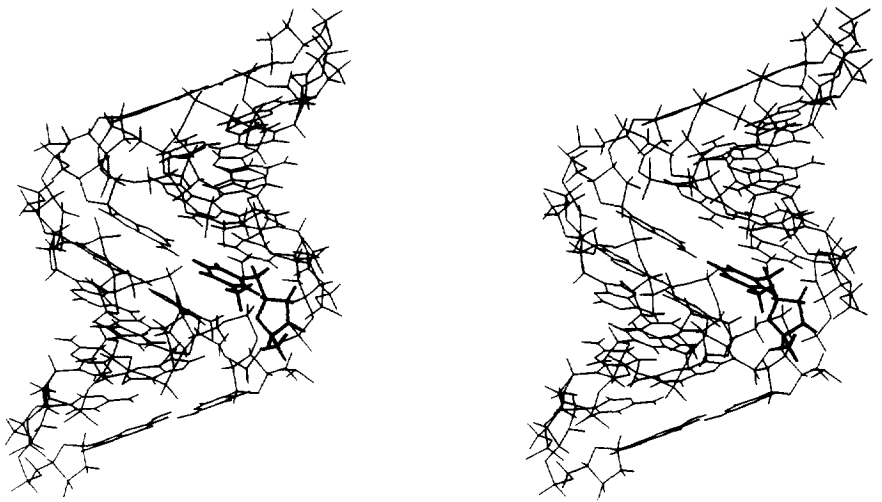
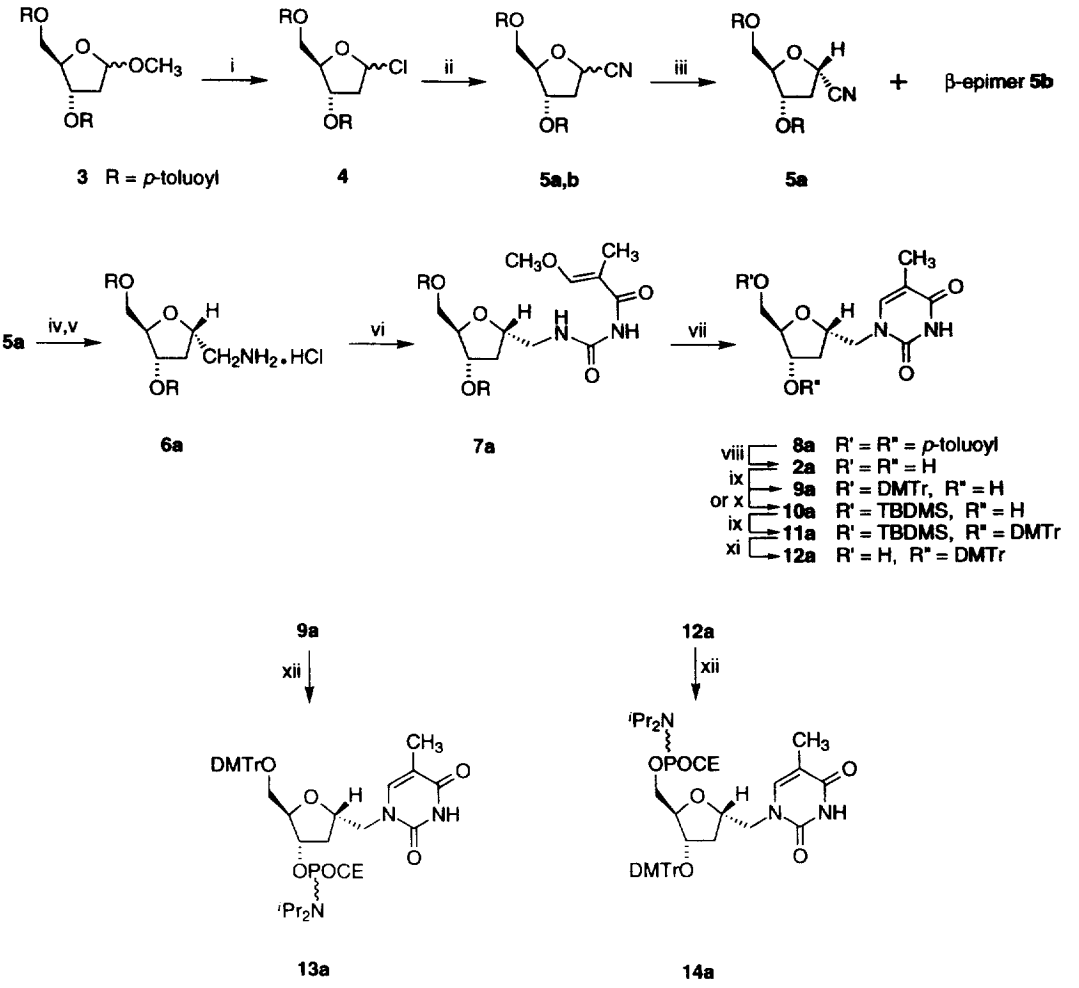


Figure 1. Stereoview of an energy-minimized DNA-RNA hybrid composed of 5'-d(GCGTTN*TTGCG) and 5'-r(CGCAAAAACGC), where highlighted N* is **2a** linked through (3'→3')- and (5'→5')-internucleotidic phosphodiester functions.

In order to evaluate the usefulness of **2a,b**, as conformational probes for *alt*DNA oligonucleotides, we now describe a straightforward chemical synthesis of **2a,b**,[6] and their corresponding phosphoramidites **13a,b** and **14a,b**. Synthetic simplicity has been emphasized in the preparation of **2a,b** to, ultimately, enable facile and economical syntheses of *alt*DNA oligonucleotides. Scheme 1 displays the synthetic steps involved in the synthesis of **2a,b** and its phosphoramidite derivatives. However, only those synthetic steps pertaining to the α-epimers are shown to preserve clarity.

Typically, the 1-*O*-methyl-2-deoxy-3,5-di-*O*-*p*-toluoyl-D-*erythro*-pentofuranoside **3** is converted to the halogenated glycoside **4** according to the method published by Hoffer.[7] Cyanation of **4** with diethylaluminum cyanide affords the cyanoglycosides **5a,b**, as a mixture of α- and β-epimers, in yields exceeding 95%.[8a] Each epimer is easily purified by silica gel chromatography and, then, reduced chemoselectively by borane in tetrahydrofuran to the corresponding aminomethylated glycosides **6a** and **6b** in isolated yields of *ca.* 85%.[9]

**Scheme 1**[a,b]




[a] Conditions: (i) HCl (*g*)/AcOH (ii) $Et_2AlCN$/toluene/THF, 25 °C, 7 h; (iii) silica gel chromatography; (iv) $BH_3$/THF, 25 °C, 1 h; (v) HCl (*g*)/$CH_3OH$; (vi) $CH_3OCHC(CH_3)CONCO/Et_3N/C_6H_6$, 25 °C, 16 h; (vii) AcOH/conc. HCl (10:1 v/v), 25 °C, 16 h; (viii) KOH in EtOH-$H_2O$ (2:1) and, then, AG 50W-X12 ($H^+$); (ix) DMTrCl/DMAP/$C_5H_5N$, 25 °C; (x) TBDMSCl/Imidazole/DMF, 25 °C, 1 h; (xi) 1.0 M *n*-$Bu_4NF$ in THF, 25 °C, 2 h; (xii) $(^iPr_2N)_2POCH_2CH_2CN$/cat. DIAT/$CH_2Cl_2$, 25 °C, 4 h. [b] Legend: Ac, acetyl; Et, ethyl; DMTr, dimethoxytrityl; DMAP, 4-dimethylaminopyridine; TBDMS, *tert*-butyldimethylsilyl; DIAT, *N,N*-diisopropylammonium tetrazolide; CE, 2-cyanoethyl; $^i$Pr, (1-methyl)ethyl.

Treatment of **6a** with 3-methoxy-2-methylacryloyl isocyanate,[10] generated *in situ* from the reaction of the parent acryloyl chloride[11] with silver cyanate, gives the acryloylurea derivative **7a** in 80% yield. Pure **7a** cyclizes efficiently only under acidic conditions to produce the thymine nucleoside analogue **8a** in 90% yield.[12] Saponification of **8a** with aqueous potassium hydroxide gives **2a** in near quantitative yields.[13]

Conversion of **2a** to the phosphoramidite derivatives **13a** and **14a** begins with the reaction of **2a** with di-*p*-methoxytrityl chloride to generate the 5'-protected nucleoside **9a** in 85% yield. On the other hand, silylation of **2a** at the 5'-hydroxy function with *tert*-butyldimethylchlorosilane affords the 5'-*O*-silylated nucleoside analogue **10a** in 94% isolated yield. The latter nucleoside is further condensed with di-*p*-methoxytrityl chloride to produce the fully protected nucleoside **11a**. Without further purification, **11a** is desilylated by treatment with tetra-*n*-butylammonium fluoride to the 3'-protected nucleoside derivatives **12a** in isolated yields of 68% with respect to **10a**. Treatment of **9a** and **12a** with *O*-(2-cyanoethyl)-*N,N,N',N'*-tetraisopropylphosphordiamidite and catalytic amounts of *N,N*-diisopropylammonium tetrazolide, according to the procedure of Barone *et al.*,[14] affords the deoxyribonucleoside phosphoramidite analogues **13a** and **14a** in yields exceeding 88%.[15] The incorporation of **13a,b** and **14a,b** into oligonucleotide analogues (24-mers) is achieved in a manner similar to that described earlier.[1] Preliminary results indicate that an oligonucleotide modified by the incorporation of **14a** through (3'→3')- and (5'→5')-internucleotidic phosphodiester linkages at selected positions, has, unlike previously studied *alt*DNA oligonucleotides,[1] higher affinity for a complementary RNA oligomer than for its DNA complement. The physicochemical properties of oligodeoxyribonucleotides modified by the incorporation of **13a,b** and **14a,b** according to defined internucleotidic motifs will be reported elsewhere.

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3. Koga, M.; Moore, M. F.; Beaucage, S. L. *J. Org. Chem.* **1991**, *56*, 3757.
4. Scremin, C. L.; Boal, J. H.; Wilk, A.; Phillips, L. R.; Zhou, L.; Beaucage, S. L. *Tetrahedron Lett.* **1995**, *36*, 8953.
5. Hybrid simulations were achieved using QUANTA version 4.0 with helix constraints in vacuum. The lowest energy conformation was further energy-minimized using the Adopted Basis Newton-Raphson method. Figure 1 should be viewed with a stereoviewer.
6. In spite of structural homologies between **1a,b** and **2a,b**, the preparation of these nucleoside analogues has been achieved according to quite different synthetic strategies.
7. Hoffer, M. *Chem. Ber.* **1960**, *93*, 2777.

8. (a) Iyer, R. P.; Phillips, L. R.; Egan, W. *Synth. Commun.* **1991**, *21*, 2053. The synthesis of **5a,b** has also been achieved by treatment of **4** with sodium cyanide in 1,2-dimethoxyethane.[8b] (b) Kolb, A.; Huynh Dinh, T.; Igolen, J. *Bull. Soc. Chim. France* **1973**, 3447.
9. In our hands, the chemoselective reduction of **5a,b** with borane in tetrahydrofuran produced **6a,b** in higher yields and faster rates than with sodium trifluoroacetoxyborohydride under conditions reported in the literature.[8a] For example, 80 mL of 1.0 M borane in tetrahydrofuran (80 mmol) were added, dropwise, over a period of 15 min to a cold solution (0 °C) of the α-cyanoglycoside **5a** (8.5 g, 22.4 mmol) in tetrahydrofuran (40 mL). The solution was then removed from the cold bath and stirred at 25 °C for 1 h. TLC analysis indicated the absence of starting material and no further reaction. A saturated solution of hydrogen chloride in methanol (20 mL) was added slowly to the reaction mixture cooled to 0 °C. Ten minutes later, the solution was evaporated under reduced pressure to give an oily residue. The oil was dissolved in dichloromethane (300 mL) and extracted with water (300 mL). The organic layer was collected while the aqueous phase was extracted twice with methylene chloride (200 mL). The organic extracts were combined, dried over anhydrous sodium sulfate, and evaporated to dryness. The material left was dissolved in a minimum amount of dichloromethane, and the hydrochloride salt of **6a** was precipitated upon addition of twenty volumes of *n*-hexane. The hygroscopic salt was isolated in 85% yield (8 g, 19.1 mmol), and was pure enough to be used in the next synthetic step. $^1$H-NMR (300 MHz, $CDCl_3$): δ 1.95 (m, 1H), 2.36 (s, 3H), 2.38 (s, 3H), 2.60 (m, 1H), 3.25 (m, 2H), 4.41 (m, 1H), 4.58 (m, 2H), 4.78 (m, 1H), 5.50 (m, 1H), 7.16 (d, $J$ = 8.0 Hz, 2H), 7.22 (d, $J$ = 8.0 Hz, 2H), 7.86 (d, $J$ = 8.0 Hz, 2H), 7.91 (d, $J$ = 8.0 Hz, 2H), 8.50 (bs, 3H). $^{13}$C-NMR (75 MHz, $CDCl_3$): δ 21.7, 35.5, 43.3, 64.1, 75.5, 75.9, 82.4, 126.5, 126.7, 129.2, 129.6, 143.9, 144.2, 165.9, 166.4.
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11. Shaw, G.; Warrener, R. N. *J. Chem. Soc.* **1958**, 153.
12. A solution of **7a** (11.0 g, 20.9 mmol) in fresly distilled glacial acetic acid (200 mL) and concentrated hydrochloric acid (20 mL) was stirred overnight at 25 °C in a stoppered round bottom flask. The solution was then evaporated under reduced pressure, and the foamy material dissolved in dichloromethane (300 mL). Aqueous 5% sodium bicarbonate (200 mL) was slowly added to the solution to prevent vigorous evolution of carbon dioxide. The organic layer was washed further with water (200 mL), dried over anhydrous sodium sulfate, and evaporated to dryness to give **8a** (10.1 g, 20.5 mmol). The crude product was purified by silica gel chromatography using a gradient of methanol (2-5%) in methylene chloride as eluent. Pure **8a** was isolated as an amorphous white solid (8.2 g, 16.7 mmol, 80%). $^1$H-NMR (300 MHz, $CDCl_3$): δ 1.88 (s, 3H), 2.1 (m, 1H), 2.44 (s, 3H), 2.45 (s, 3H), 2.70 (q, $J$ = 7.16 Hz, 1H), 3.84 (dd, $J$ = 14.4, 7.7 Hz, 1H), 4.10 (dd, $J$ = 14.4, 2.7 Hz, 1H), 4.50 (m, 4H), 5.52 (m, 1H), 7.17 (s, 1H), 7.28 (m, 4H), 7.92 (m, 4H), 8.63 (s, 1H). $^{13}$C-NMR (75 MHz, $CDCl_3$): δ 12.5, 21.9, 35.3, 51.5, 64.5, 76.5, 77.8, 82.5, 110.5, 127.0, 127.1, 129.4, 129.5, 129.8, 129.9, 141.6, 144.2, 144.6, 151.3, 164.1, 166.3, 166.5. FAB-HRMS: Calcd for $C_{27}H_{29}N_2O_7$ ($MH^+$): 493.1975. Found: 493.1977.
13. To a solution of **8a** (7.0 g, 14 mmol) in ethanol (150 mL) was added a solution of potassium hydroxide (4.5 g, 80 mmol) in ethanol (20 mL) and water (7 mL) at 25 °C. Upon completion of the reaction (30 min), a cation exchange resin (BioRad AG 50W-X12, hydrogen form) was added to the basic solution, in small increments, until neutrality according to pH paper. The suspension was filtered off, and the resin carefully

washed with water. The filtrates were extracted twice with methylene chloride to remove most of *p*-toluic acid contaminants. The aqueous phase was evaporated to dryness, and the material left was repeatedly coevaporated with dry toluene to give **2a** as a white foam (3.5 g, 14 mmol). An analytical sample was recrystallized from ethanol:chloroform (1:6) under slow (24 h) diffusion of ethyl ether (mp 143-144 °C). UV ($H_2O$, pH 7): $\lambda_{max}$ 273 nm (ε 8,900), $\lambda_{min}$ 239 nm (ε 2,100). $[\alpha]_D^{25}$ +108.7 (*c* 0.6, $CH_3OH$). FAB-HRMS: Calcd for $C_{11}H_{17}N_2O_5$ ($MH^+$): 257.1137. Found: 257.1144. $^1$H-NMR (500 MHz,$CDCl_3$): δ 1.69 (ddd, *J* = 13.6, 5.5, 4.7 Hz, 1H), 1.81 (d, *J* = 1.1 Hz, 3H), 2.36 (ddd, *J* = 13.9, 7.6, 6.9 Hz, 1H), 3.51 (dd, *J* = 12.2, 5.5 Hz, 1H), 3.58 (dd, *J* = 12.2, 4.0 Hz, 1H), 3.84 (dd, *J* = 14.5, 3.4 Hz, 1H), 3.89 (dd, *J* = 14.5, 7.8 Hz, 1H), 3.90 (ddd, *J* = 5.5, 4.1, 4.0 Hz, 1H), 4.24 (ddd, *J* = 6.9, 4.7, 4.1 Hz, 1H), 4.36 (dddd, *J* = 7.9, 7.8, 5.5, 3.5 Hz, 1H), 7.43 (q, *J* = 1.1 Hz, 1H). $^{13}$C-NMR (75 MHz, $CDCl_3$): δ 13.8, 39.0, 54.3, 63.9, 74.2, 79.0, 87.9, 112.8, 146.2, 154.8, 169.2. The saponification of **8b** was performed under conditions identical to those described for **8a**, and produced a similar yield of **2b** which was recrystallized from acetone (mp 174-175 °C). UV ($H_2O$, pH 7): $\lambda_{max}$ 272 nm (ε 9,300), $\lambda_{min}$ 238 nm (ε 1,900). $[\alpha]_D^{25}$ −38.1 (*c* 1.2, $CH_3OH$). FAB-HRMS: Calcd for $C_{11}H_{17}N_2O_5$ ($MH^+$): 257.1137. Found: 257.1146. $^1$H-NMR (500 MHz,$CDCl_3$): δ 1.81 (ddd, *J* = 13.6, 9.8, 5.8 Hz, 1H), 1.81 (d, *J* = 1.1 Hz, 3H), 1.99 (ddd, *J* = 13.7, 5.9, 2.2 Hz, 1H), 3.47 (dd, *J* = 12.1, 5.6 Hz, 1H), 3.53 (dd, *J* = 12.1, 4.6 Hz, 1H), 3.79 (dd, *J* = 14.6, 7.3 Hz, 1H), 3.84 (ddd, *J* = 5.5, 4.6, 2.4 Hz, 1H), 3.95 (dd, *J* = 14.6, 2.9 Hz, 1H), 4.24 (ddd, *J* = 5.6, 2.6, 2.3 Hz, 1H), 4.36 (dddd, *J* = 9.7, 7.3, 5.9, 2.9 Hz, 1H), 7.41 (q, *J* = 1.1 Hz, 1H). $^{13}$C-NMR (75 MHz, $CDCl_3$): δ 13.7, 39.1, 53.9, 64.5, 74.8, 79.2, 89.4, 113.0, 146.2, 155.0, 169.4. The unambiguous identification of the deoxyribonucleoside analogues **2a** and **2b** is based on their respective NOESY spectra at 300 MHz. Specifically, the structural proximity of $C_{1'}$-H to $C_{2'}$-H' in the α-deoxyribonucleoside **2a** is revealed by the presence of a strong NOE crosspeak. In the case of the β-deoxyribonucleoside **2b**, however, a strong NOE crosspeak indicates the proximity of $C_{1'}$-H to $C_{2'}$-H". $C_{2'}$-H' and $C_{2'}$-H" are defined according to Wood, D. J.; Hruska, F. E.; Ogilvie, K. K. *Can. J. Chem.* **1974**, *52*, 3353.

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15. **13a:** $^{31}$P-NMR (121 MHz, $CDCl_3$); δ 146.9 and 146.7 ppm. **14a:** $^{31}$P-NMR (121 MHz, $CDCl_3$); δ 147.2 and 147.1 ppm.

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