Pergamon

Tetrahedron Letters, Vol. 36, No. 49, pp. 8953-8956, 1995
Elsevier Science Ltd
Printed in Great Britain
0040-4039/95 $9.50+0.00

0040-4039(95)01932-4

1-(2-Deoxy-α- and β-D-*erythro*-pentofuranosyl)-2-(thymin-1-yl)ethane Derivatives as Conformational Probes for *alt*DNA Oligonucleotides

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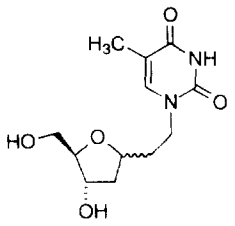
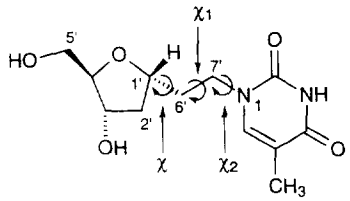
Abstract: The novel deoxyribonucleoside analogues **1a,b** have been synthesized in a straightforward manner from 2-deoxy-D-ribose. These modified nucleosides have also been converted to the phosphoramidite derivatives **13a,b** and **14a,b** for potential incorporation into oligodeoxyribonucleotides according to defined internucleotidic motifs.

α,β-Oligodeoxyribonucleotides with alternating (3'→3')- and (5'→5')-internucleotidic phosphodiester linkages (*alt*DNA) represent a unique class of synthetic oligonucleotide analogues achiral at phosphorus.[1a-c] These modified oligonucleotides exhibit superior resistance to nucleases than native β-oligodeoxyribonucleotides,[1a-c,2] and possess the ability to form stable complexes with either complementary DNA or RNA sequences.[1a-c] In this context, *alt*DNA-DNA duplexes are thermodynamically more stable than *alt*DNA-RNA complexes[1a] and, thus, demonstrate the higher affinity of *alt*DNA for single-stranded DNA sequences than for RNA sequences. The reduced thermal stability of *alt*DNA-RNA complexes may result from an inherent conformational incompatibility of *alt*DNA within the A-type helical motif of the hybrids.[1d]

A viable strategy at improving the affinity of *alt*DNA for complementary RNA oligomers would entail the substitution of the α-mononucleotides of *alt*DNA for α-mononucleotides having a distinctive linker arm between the nucleobase and carbohydrate moieties. Additional nucleobase flexibility imparted to *alt*DNA by the incorporation of, for example, **1a** may facilitate the formation of Watson-Crick base-pairs with native RNA oligonucleotides through better alignments of complementary nucleobases. This approach is supported by modeling studies of either **1a** or **1b** which indicate that the torsion angle χ_1 ($C_{1'}$-$C_{6'}$-$C_{7'}$-N_1) of energetically preferred conformers is ideal (180°) for optimal base-pairing. Thus, in order to evaluate the effectiveness of **1a,b** as conformational indicators for *alt*DNA oligonucleotides, we now report chemical syntheses of these novel nucleoside analogues and their corresponding phosphoramidite derivatives **13a,b** and **14a,b**. Scheme 1 outlines the chemical transformations involved in these syntheses even though only those pertaining to α-epimers are shown as examples.

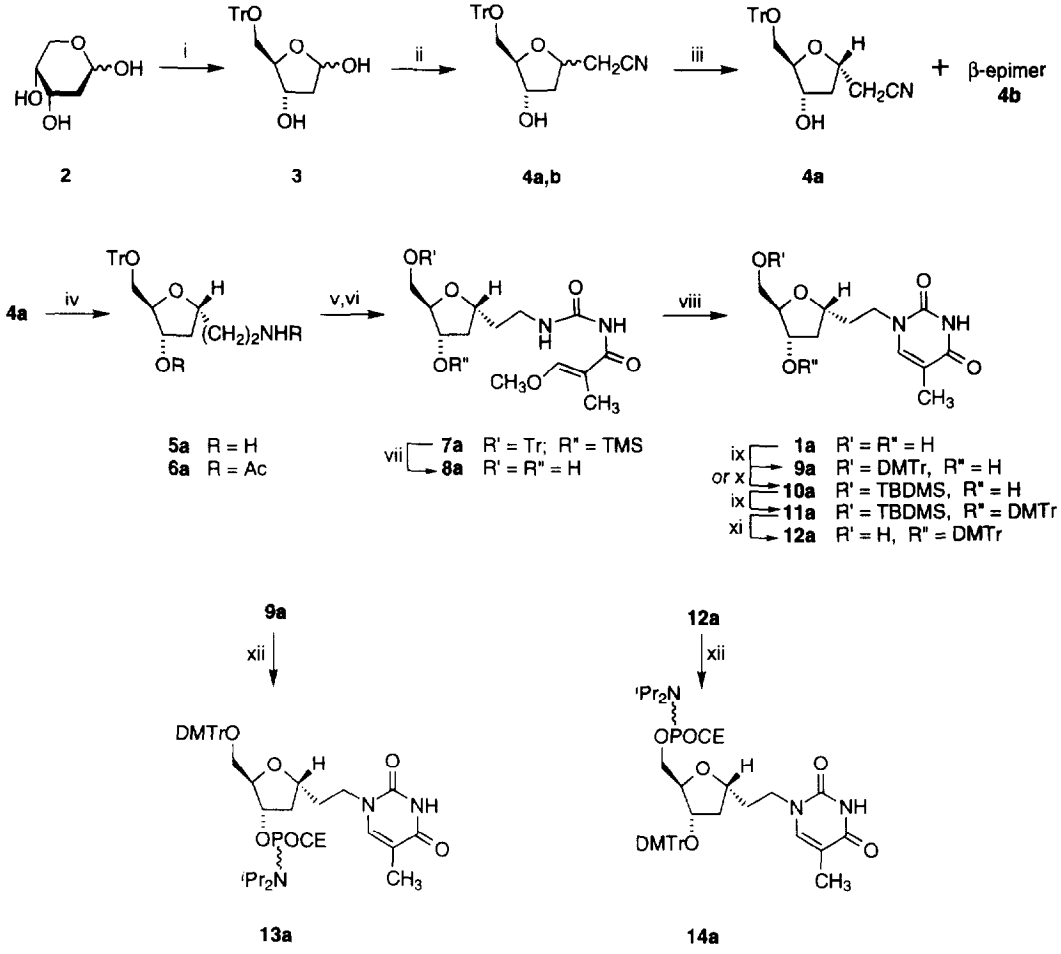


1a α-epimer
b β-epimer



1a

Scheme 1[a,b]



[a] Conditions: (i) TrCl/DMAP/C_5H_5N, 50 °C, 6 h; (ii) $(EtO)_2P(O)CH_2CN$/NaH/THF, 5 °C, 6 h; (iii) silica gel chromatography; (iv) $(CH_3)_2S{\bullet}BH_3$/THF/reflux, 40 min; (v) TMS-Cl/Et_3N/THF, 25 °C, 2 h; (vi) $CH_3OCHC(CH_3)CONCO/Et_3N/C_6H_6$, 25 °C, 3 h; (vii) 10% TFA/CH_2Cl_2, 25 °C, 2 h; (viii) conc. NH_4OH, 50 °C, 12 h; (ix) DMTrCl/DMAP/C_5H_5N, 25 °C; (x) TBDMSCl/Imidazole/DMF, 25 °C, 1 h; (xi) 1.0 M *n*-Bu_4NF/THF, 25 °C, 2 h; (xii) $(^iPr_2N)_2POCH_2CH_2CN$/cat. DIAT/CH_2Cl_2, 25 °C, 4 h. [b] Legend: Et, ethyl; Tr, triphenylmethyl; TMS, trimethylsilyl; TFA, trifluoroacetic acid; DMTr, dimethoxytrityl; DMAP, 4-dimethylaminopyridine; DIAT, *N,N*-diisopropylammonium tetrazolide; TBDMS, *tert*-butyl dimethylsilyl; CE, 2-cyanoethyl; ^{i}Pr, (1-methyl)ethyl.

The 5-*O*-trityl-2-deoxyribofuranoside **3** is prepared according to a modified literature procedure.[3] Typically, dry 2-deoxy-D-ribose (**2**) is reacted with triphenylmethyl chloride and catalytic amounts of *N,N*-dimethylaminopyridine in anhydrous pyridine at 50 °C to give **3** in 60% isolated yield. The acid-labile trityl group has been selected for the preparation of **3** because of its lipophilicity, facile ultraviolet or visible detection, and stability to Wittig reaction and reducing conditions. Thus, the Wittig-Horner condensation of **3** with the

sodium salt of diethyl cyanomethylphosphonate (3 equiv) in dry THF at 5 °C produces the (2-deoxyribofuranosyl) acetonitrile derivatives **4a,b** in almost quantitative yield, as a near equimolar mixture of α- and β-epimers. The facile separation of **4a** and **4b** by silica gel chromatography enables the isolation of relatively large amounts (> 5 g) of pure epimers.[4] The reduction of **4a** or **4b** to the corresponding amine is best achieved with aluminum hydride in dry THF[5] but large-scale preparations (> 5 g) of this reducing reagent, according to the method of Finholt *et al.*,[6] can be hazardous. To alleviate this drawback, the use of commercial boranes as alternatives to aluminum hydride in the reduction of **4a,b**, has been investigated. Specifically, the complex borane-dimethylsulfide[7] converts **4a**, within 40 min in refluxing THF, to the desired (2-deoxyribofuranosyl)aminoethane **5a** in yields exceeding 95%. The aminoethylated glycoside **5a**, which has been characterized as its diacetate derivative **6a**,[8] is pure enough for transient protection of the 3-hydroxy function with chlorotrimethylsilane, and condensation with 3-methoxy-2-methylacryloyl isocyanate.[9] Treatment of the crude reaction product **7a** with 10% trifluoroacetic acid in CH_2Cl_2 for 2 h at 25 °C affords, after work-up and purification by silica gel chromatography, the acryloylurea derivative **8a** in 40% yield with respect to **5a**.[9] Cyclization of **8a** to **1a** is effected in 90% yield upon heating at 50 °C in concentrated ammonium hydroxide.[10]

Condensation of **1a** with di-*p*-methoxytrityl chloride (DMTr-Cl) in dry pyridine generates the 5'-protected nucleoside **9a** in 75% isolated yield. Alternatively, regioselective silylation of **1a** at the 5'-hydroxy function with *tert*-butyldimethylchlorosilane gives the 5'-*O*-silylated nucleoside analogue **10a** in 90% yield. This nucleoside is reacted with DMTr-Cl in pyridine to produce the fully protected nucleosides **11a** which, without further purification, is desilylated by treatment with tetra-*n*-butylammonium fluoride in THF. The 3'-protected nucleoside derivative **12a** is isolated in 74% yield.

Phosphitylation 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.*,[11] affords the deoxyribonucleoside phosphoramidite analogues **13a** and **14a** in yields greater than 80%.[12] The application of **13a,b** and **14a,b** to the synthesis of oligonucleotide analogues, and the physicochemical properties of these oligomers will be reported elsewhere in due course.

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4. **4a:** ^{1}H-NMR (500 MHz, $CDCl_3$); δ 1.88 (ddd, *J* = 13.4, 5.0, 4.3 Hz, 1H), 2.47 (ddd, *J* = 13.4, 7.1, 6.5 Hz, 1H), 2.73 (d, *J* = 6.1 Hz, 2H), 3.14 (dd, *J* = 9.8, 5.7 Hz, 1H), 3.21 (dd, *J* = 9.8, 4.3 Hz, 1H), 4.11 (m, 1H), 4.37 (m, 1H), 4.45 (ddt, *J* = 6.5, 6.2, 5.0 Hz, 1H), 7.25 (t, *J* = 7.8 Hz, 3H), 7.31 (t, *J* = 7.8 Hz, 6H), 7.44 (d, *J* = 7.8 Hz, 6H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 24.8, 39.4, 64.3, 74.1, 74.4, 85.6, 86.9, 116.9, 127.1, 127.9, 128.6, 143.6.
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8. **6a:** ^{1}H-NMR (300 MHz, $CDCl_3$); δ 1.67 (ddd, *J* = 13.7, 6.7, 4.3 Hz, 1H), 1.79 (m, 2H), 1.93 (s, 3H), 2.03 (s, 3H), 2.58 (ddd *J* = 13.7, 7.2, 6.9 Hz, 1H), 3.25 (m, 3H), 3.57 (m, 1H), 4.17 (ddd, *J* = 4.5, 4.5,

3.1 Hz, 1H), 4.27 (m, 1H), 5.17 (ddd, J = 7.3, 4.3, 4.2 Hz, 1H), 6.25 (t, J = 5.8 Hz, 1H), 7.24 (t, J = 7.0 Hz, 3H), 7.30 (t, J = 7.7 Hz, 6H), 7.44 (d, J = 6.9 Hz, 6H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 21.1, 23.4, 34.7, 38.0, 38.4, 64.4, 76.2, 78.7, 82.8, 86.8, 127.1, 127.8, 128.6, 143.8, 170.0, 170.6.

9. To a cold solution (0 °C) of **5a** (3.6 g, 8.9 mmol) and Et_3N (3.0 mL, 2.2 g, 22 mmol) in anhydrous toluene (50 mL) was added trimethylsilyl chloride (2.0 mL, 1.7 g, 16 mmol), dropwise, over a period of 10 min. The reaction mixture was then allowed to stir at 25 °C for 2 h before being cooled to −10 °C. Meanwhile, dry silver cyanate (12.0 g, 80 mmol) was added, under an inert atmosphere, to a solution of 3-methoxy-2-methylacryloyl chloride[13] (2.0 g, 15 mmol), in dry benzene (54 mL).[14] The suspension was stirred under reflux for 30 min and, then, allowed to settle at ambient temperature. The supernatant was cannulated into an addition funnel, and added over a period of 30 min, to the solution of silylated **5a** kept at −10 °C. Upon completion of the addition, the reaction mixture was removed from the cold bath and stirred at 25 °C for 3 h. The solution was cooled to 0 °C, and H_2O (20 mL) was added to destroy unreacted acrylolyl chloride and acryloyl isocyanate derivatives. After removal of the solvents, the material left was dissolved in $CHCl_3$ (200 mL) and extracted, successively, with aqueous 5% $NaHCO_3$ (200 mL) and H_2O (200 mL). The organic phase was dried over anhydrous Na_2SO_4 and evaporated to dryness to give an amorphous solid. Without further purification, the product was treated with a solution of 10% trifluoroacetic acid in CH_2Cl_2 (80 mL) for 2 h at 25 °C. The solution was cooled to 0 °C, and brought to neutral pH upon addition of a basic anion exchange resin (Amberlite IRA-93, free base, 30 g). The resin was filtered off and washed with CH_2Cl_2 (200 mL). The filtrates were combined, and evaporated under reduced pressure. The oily product was purified by silica gel chromatography using, as eluent, a gradient of CH_3OH (3-10%) in CH_2Cl_2. Pure **8a** (1.1 g, 3.6 mmol) was isolated as a powder in 40% yield. ^{1}H-NMR (300 MHz,$CDCl_3$): δ 1.70 (m, 1H), 1.76 (s, 3H), 1.85 (m, 2H), 2.38 (ddd, J = 12.3, 6.3, 6.3 Hz, 1H), 3.31 (ddd, J = 17.7, 8.7, 4.4 Hz, 2H), 3.61 (m, 2H), 3.74 (m, 1H), 3.85 (s, 3H), 4.15 (m, 2H), 7.35 (s, 1H), 8.42 (bs, 1H), 9.15 (t, J = 4.7 Hz, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 8.7, 34.8, 38.0, 41.0, 61.5, 63.2, 72.4, 76.9, 84.8, 107.1, 154.3, 158.7, 169.5.

10. (a) **1a**: ^{1}H-NMR (500 MHz,$CDCl_3$); δ 1.70 (ddd, J = 13.3, 7.5, 6.0 Hz, 1H), 1.89 (d, J = 1.0 Hz, 3H), 1.99 (dddd, J = 14.1, 7.8, 7.1, 4.8 Hz, 1H), 2.05 (dddd, J = 14.1, 7.8, 7.8, 6.1 Hz, 1H), 2.47 (ddd, J = 13.3, 6.9, 6.9 Hz, 1H), 3.61 (dd, J = 12.1, 6.3 Hz, 1H), 3.70 (dd, J = 12.1, 3.7 Hz, 1H), 3.83 (ddd, J = 14.8, 7.8, 7.1 Hz, 1H), 3.90 (ddd, J = 14.8, 7.8, 6.1 Hz, 1H), 3.93 (ddd, J = 6.3, 5.0, 3.7 Hz, 1H), 4.16 (dddd, J = 7.8, 7.5, 6.9, 4.8 Hz, 1H), 4.30 (ddd, J = 6.9, 6.0, 5.0 Hz, 1H), 7.54 (q, J = 1.0 Hz, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 13.7, 36.5, 41.8, 48.4, 63.7, 74.3, 78.2, 87.2, 113.1, 145.7, 154.7, 169.3. **1b**: ^{1}H-NMR (500 MHz,$CDCl_3$); δ 1.81 (ddd, J = 13.5, 10.2, 6.3 Hz, 1H), 1.84 (d, J = 1.2 Hz, 3H), 1.89 (dddd, J = 13.8, 8.1, 7.9, 5.9 Hz, 1H), 1.95 (dddd, J = 13.8, 8.2, 6.7, 2.3 Hz, 1H), 2.00 (ddd, J = 13.5, 5.6, 2.0 Hz, 1H), 3.55 (dd, J = 12.0, 5.8 Hz, 1H), 3.62 (dd, J = 12.0, 4.3 Hz, 1H), 3.77 (ddd, J = 14.0, 8.1, 6.7 Hz, 1H), 3.83 (ddd, J = 5.8, 4.3, 2.7 Hz, 1H), 3.86 (ddd, J = 14.0, 8.2, 5.9 Hz, 1H), 4.17 (dddd, J = 10.2, 7.9, 5.6, 2.3 Hz, 1H), 4.25 (ddd, J = 6.3, 2.7, 2.0 Hz, 1H), 7.48 (q, J = 1.2 Hz, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 13.8, 36.2, 42.1, 48.4, 64.6, 75.0, 78.7, 89.1, 113.2, 145.6, 154.7, 169.4. The structure of the α-deoxyribonucleoside **1a** is differentiated from that of **1b** on the basis of their respective NOESY spectra at 300 MHz which revealed, by the presence of strong crosspeaks, the proximity of C_1'-H to C_2'-H' or C_2'-H" in the case of **1a** or **1b**, respectively. 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*, 3353-3366. (b) Shealy, Y. F.; O'Dell, C. A. *J. Het. Chem.* **1976**, *13*, 1041-1047.

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12. **13a:** ^{31}P-NMR (121 MHz, $CDCl_3$); δ 147.4 and 147.1 ppm. **14a:** ^{31}P-NMR (121 MHz, $CDCl_3$); δ 147.9 and 147.6 ppm.

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(*Received in USA* 1 *September* 1995; *revised* 28 *September* 1995; *accepted* 6 *October* 1995)