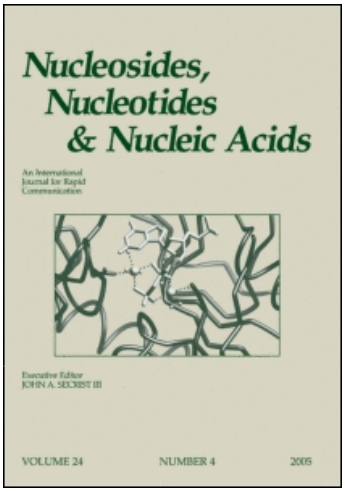

Nucleosides, Nucleotides and Nucleic Acids

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

Synthesis of Analogues of 3'-Deoxypsicothymidine

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To cite this Article Hovinen, Jari , Azhayev, Alex , Guzaev, Andrei and Lönnberg, Harri(1995) 'Synthesis of Analogues of 3'-Deoxypsicothymidine', Nucleosides, Nucleotides and Nucleic Acids, 14: 3, 329 — 332
To link to this Article: DOI: 10.1080/15257779508012374
URL: http://dx.doi.org/10.1080/15257779508012374

NUCLEOSIDES & NUCLEOTIDES, 14(3-5), 329-332 (1995)

SYNTHESIS OF ANALOGUES OF 3′-DEOXYPSICOTHYMIDINE

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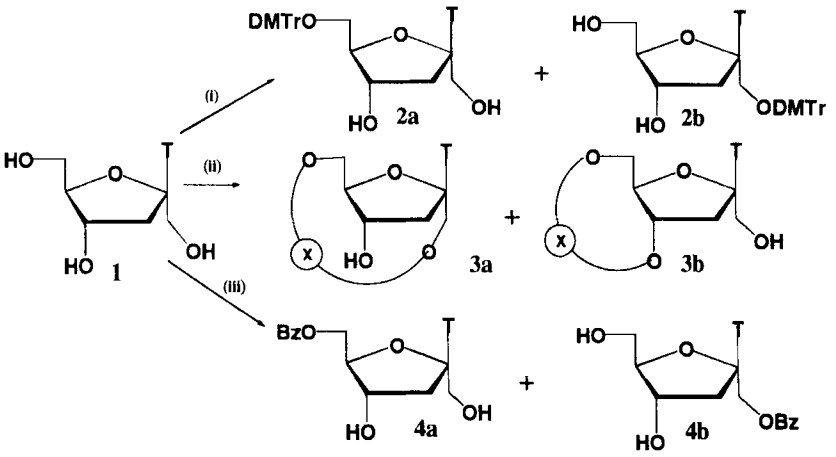
Abstract: Preparation of 3′-deoxypsicothymidines bearing a tether group at *O*1′ is described. Selective protection of the primary hydroxy functions of the starting nucleoside is briefly discussed.

Oligonucleotides and DNA fragments tethered with various reporter groups are versatile tools in studies of molecular biology. These reporters are commonly attached to the target molecules *via* aliphatic amino groups which are, in turn, introduced into polynucleotides either chemically or enzymically. The chemical approaches include either preparation of modified building blocks and their subsequent incorporation into synthetic oligonucleotides,[1] or transformation of natural DNA, for instance by transamination of cytosine residues.[2] The enzymic approach consists of preparation of nucleoside triphosphates derivatized with appropriate tethers, and their insertion into DNA by a polymerase reaction.[3] Since the tethers are often attached to the base residues, the ionic and tautomeric properties of the entire oligomer are disturbed. Therefore, nucleoside analogues possessing the ionic and tautomeric properties and all functional groups of the natural 2′-deoxynucleosides, but displaying additional functionalities for further derivatization, are of interest. We have recently focused our attention to 3′-deoxypsiconucleosides[4] (**1**) which fulfill all these requirements. Oligonucleotides bearing several 3′-deoxypsicothymidine units, labeled at their 1′-position with fluorescent groups, have been shown to serve as primers for DNA polymerization reactions catalyzed by DNA polymerases.[5]

We have previously prepared 3′-*O*-(ω-aminoalkoxymethyl)thymidine 5′-triphosphates, terminators of DNA synthesis that can be labeled at their amino function with fluorescent dyes.[6] The same synthetic strategy has now been applied to the preparation of 1′-*O*-derivatized 3′-deoxypsicothymidines.

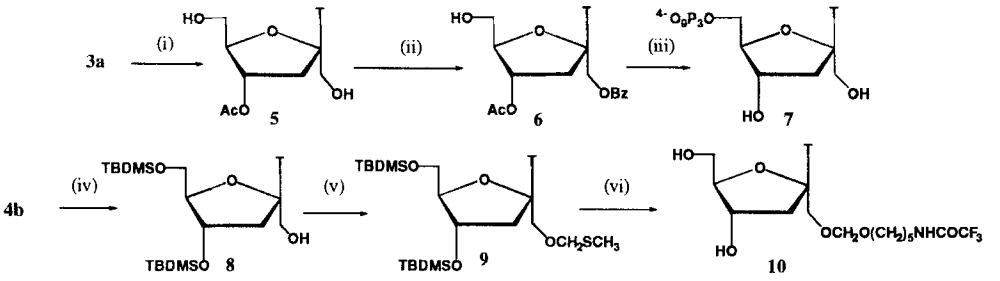


Scheme 1. (i) 1.0 eq. of 4,4′-dimethoxytrityl chloride (ii) 1.0 eq. of 1,3-dichloro-1,1,3,3-tetraisopropyl-1,3-disiloxane (iii) 1.0 eq. of benzoyl chloride. X = 1,1,3,3-tetraisopropyl-1,3-disiloxayl-

Selective protection of the primary 1′- and 6′-hydroxy functions of psiconucleosides is an arduous task due to their similar reactivity. Our previous studies have shown that **1**, when reacted with an equimolar amount of DMTr-chloride, gives predominantly the 6′-*O*-tritylated product, **2a**, while the 1′-*O*-isomer, **2b**, is formed only in 15 % yield[7] (Scheme 1). Quite recently Ono *et al.*[8] reported that 4',6'-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanodiyl)-3′-deoxypsicouridine can be prepared in a moderate yield by treating 3′-deoxypsicouridine with 1,3-dichloro-1,1,3,3-tetraisopropyl-1,3-disiloxane and imidazole in cold DMF. Surprisingly, when **1** was treated under the same conditions, a mixture of **3a** and **3b** was obtained, and the desired isomer, **3b**, was only a minor component.[9] By contrast, benzoylation of **1** occurs predominantly at 1′-OH: the monobenzoylated derivatives, **4a** and **4b**, were isolated in 16 and 55 % yield, respectively.[10]

Further derivatizations of **3a** and **4b** were performed as described in Scheme 2. Accordingly, acylation of **3a** with acetic anhydride gave, after subsequent desilylation, **5**. Its treatment with an equimolar amount of benzoyl chloride resulted in 4′-*O*-acetyl-1′-*O*-benzoyl-3′-deoxypsicothymidine, **6**, which was then converted into 3′-deoxypsicothymidine 6′-triphosphate, **7**,[11] according to the published procedure.[6]

1′-*O*-Benzoyl-3′-deoxypsicothymidine (**4b**) was easily converted into **8** by treatment with *t*-butyldimethylsilylchloride and imidazole in dry DMF, followed by debenzoylation with methanolic ammonia. **8** was then transformed to the corresponding



Scheme 2. (i) **1.** acetic anhydride/pyridine; overnight at rt. **2.** $Bu_4N^+F^-$/THF; 2 h at rt. (ii) benzoyl chloride/pyridine; overnight at rt. (iii) **1.** PO(triazole)$_3$/acetonitrile 30 min at rt. **2.** tri-n-butylammonium pyrophosphate/DMF; 2 h at rt. **3.** water; 1 h at rt; **4.** aq. NH_3; overnight at rt. (iv) **1.** *t*-butyldimethylsilyl chloride/imidazole/DMF; overnight at rt; **2.** NH_3/MeOH; overnight at rt. (v) DMSO/acetic acid/acetic anhydride; 2 d at rt. (vi) **1.** $HO(CH_2)_5NHCOCF_3$/triflic acid/dichloroethane; 2 min at 0 °C; **2.** $Bu_4N^+F^-$/THF 2 h at rt.

1′-*O*-methylthiomethyl ether, **9**, by treatment with the mixture of DMSO, acetic anhydride and acetic acid, as described previously for 5′-*O*-benzoylthymidine.[6] In the present case, methylthiomethylation of the primary hydroxy group of **8** was accompanied with the formation of an aldehyde as a side product (~ 15 %), while the desired monothioacetal was obtained in 50 % yield.

A method of Veeneman *et al.*,[12] was employed for the attachment of the aliphatic arm to *O*1′. Accordingly, the mixture of **9** and *N*-trifluoroacetyl-5-aminopentanol was treated with NIS in dry 1,2-dichloroethane in the presence of catalytic amount of triflic acid. After subsequent desilylation, **10** was obtained almost quantitatively.[13]

Studies on the ability of 3′-deoxypsicothymidine 6′-triphosphate, **7**, as well as its 1′-*O*-derivatized analogues to serve as substrates for DNA polymerases are in progress in our laboratory. Unfortunately, our preliminary studies on **7** are not promising.

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9. Compound **3a** (45 %). ^{1}H NMR (DMSO-d_6) 11.20 (1 H, s, H3); 7.58 (1 H, s, H-6); 5.06 (1 H, d, *J* 3.9, exch. with D_2O, 4′-OH,); 4.21 (1 H, m, H-4′); 4.11 (2 H, m, H-5′ and d, H-1′a); 3.94 (1H, dd, $J_{6',6''}$ 12.2, H-6′); 3.75 (1H, d, *J* 11.2 H-1′b); 3.70 (1 H, dd, $J_{5',6''}$ 5.9, $J_{6',6''}$ 12.2, H-6′′); 3.0 - 2.8 (2H, H-3′and H-3′′; overlapping with the signal of solvent); 1.77 (3H, s, 5-CH_3); 1.06 (24H, 4 x *i*-Pr). Found: C, 53.8; H, 7.4, N, 5.3. Calcd. for $C_{23}H_{38}N_2O_7Si_2$: C, 53.50; H, 7.50; N, 5.5 %. Compound **3b** (24 %):^{1}H NMR (DMSO-d_6) 11.18 (1H, br, H-3); 7.64 (1H, s, H-6); 5.18 (1H, t, *J* 6.4, exch. with D_2O, 1′-OH); 4.43 (1H, m, H-4′); 4.05 (1H, dd, $J_{5',6'}$ 3.4, $J_{6',6''}$ 11.7, H-6′); 3.89 (1H, dd, $J_{5',6''}$ 7.3, $J_{6',6''}$ 11.7, H-6′′); 3.82 (1H, m, H-5′); 3.63 (1H, dd, J_{AB} 11.7, $J_{1',OH}$ 6.4, d upon addition of D_2O, H-1′a); 3.52 (1H, dd, $J_{1',OH}$, 6.4, d upon addition of D_2O, H-1′b); 2.65 (1H, dd, $J_{3',3''}$ 15.0, H-3′′); 2.75 (1H, dd, $J_{3',4'}$ 5.8, H-3′); 1.87 (3H, s, 5-CH_3); 1.02 (24H, 4 x i-Pr). Found: C, 53.7; H, 7.3, N, 5.3. Calcd. for $C_{23}H_{38}N_2O_7Si_2$: C, 53.50; H, 7.50; N, 5.5 %.
10. Compound **4b** ^{1}H NMR (DMSO-d_6): 11.17 (1H, br, H-3); 8.03 (2H, 2xd, arom.); 7.71 (1H, t, arom.); 7.64 (1H, s, *J*<1, H-6); 7.58 (2H, t, arom.); 5.22 (1H, d, *J* 3.9, exch. with D_2O, 4′-O*H*); 4.45 (1H, dd, $J_{5',6'}$ 3.9, $J_{6',6''}$ 11.7, H-6′′); 4.38 (1H, dd, $J_{5',6'}$ 4.4, $J_{6',6''}$ 11.7,H-6′); 4.24 (1H, m, H-4′); 3.75 (1H, dd, J_{AB} 11.2, d upon addition of D_2O, H-1′a); 3.57 (1H, dd, J_{AB} 11.2, d upon addition of D_2O H-1′b); 3.35 (1H, m, H-5′); 2.67 (1H, dd, $J_{3',3''}$ 14.1, $J_{3'',4'}$ 2.9, H-3′′); 2.44 (1H, dd, $J_{3',4'}$ 6.4, H-3′); 1.80 (3H, s, 5-Me). Found: C, 57.43; H, 5.47; N, 7.10. Calcd. for $C_{18}H_{20}O_7N_2$: C, 57.44; H, 5.36; N, 7.44. Compound **4a** ^{1}H NMR (DMSO-d_6): 11.23 (1H, br, H-3); 7.88 (2H, 2xd, arom.); 7.73 (1H, d, *J*<1, H-6); 7.65 (1H, m, arom); 7.52 (2H, t, arom.); 5.09 (1H, d, *J* 3.4, exch. with D_2O, 4′-O*H*); 4.90 (1H, t, exch. with D_2O, 6′-OH); 4.90 (1H, d, *J* 11.2, H-1′a); 4.47 (1H, d, *J* 11.2, H-1′b); 4.19 (1H, m, H-4′); 4.02 1H, m, H-5′); 3.48 (2H, m, H-6′, H-6′′); 2.77 (1H, dd, H-3′′); 2.49 (1H, dd, H-3′); 1.78 (3H, s, 5-Me). Found: C, 56.91; H, 5.68; N, 7.28. Calcd. for $C_{18}H_{20}O_7N_2$: C, 57.44; H, 5.36; N, 7.44 %.
11. Compound **7**:^{1}H NMR (D_2O): 7.71 (1H, s, H-6); 4.32 (1H, m, H-4′); 4.14 (1H, m, H-5′); 3.97 (2H, m, H6′/H-6′′); 3.85 (1H, d, *J* 12.2, H-1′a); 3.60 (1H, d, *J* 12.2 H-1′b); 2.55 (1H, dd, $J_{3',3''}$ 14.6, $J_{3'',4'}$ 2.4, H-3′′); 2.37 (1H, dd, $J_{3',4'}$ 6.4, H-3′); 1.72 (3H, s, 5-Me). ^{31}P NMR (D_2O): -24.32; -11.84; -8.93.
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13. Compound **10**: ^{1}H NMR (DMSO-d_6) 11.17 (1H, s, H-3), 9.40 (1H, s, CONH), 7.63 (1H, s, H-6), 5.01 (1H, d, *J* 3.4, exch. with D_2O, 4′-OH), 4.86 (1H, t, *J* 5.4, exch. with D_2O, 6′-OH), 4.57 (2H, AB, *J* 6.4 OCH_2O), 4.12 (1H, m, H-4′), 4.01 (1H, d, *J* 10.7, H-1′a), 3.95 (1H, m, H-5′), 3.61 (1H, d, *J* 10.7 H-1′b), 3.48 (2H, t, *J* 5.4, OCH_2), 3.38 (2H, m, H-6′and H-6′′), 3.32 (2H, m, CH_2NH), 2.65 (1H, dd, $J_{3',3''}$ 14.2, $J_{3'',4'}$<1, H-3′′); 2.27 (1H, dd, $J_{3',4'}$ 6.3, H-3′); 1.80 (3H,s, 5-Me); 1.48 (2H, m, CH_2); 1.25 (4H, m, 2 x CH_2). Found, C, 47.0 H, 6.0. N, 8.2. Calcd. for $C_{19}H_{28}F_3N_3O_8$: C, 47.2, H, 5.8, N, 8.7 %.