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Synthesis of 1-(2,4-dideoxy-ß-D-erythro-hexopyranosyl)thymine and its incorporation into oligonucleotides

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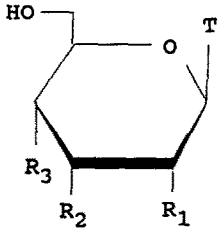
Abstract : *The synthesis of 1-(2,4-dideoxy-β-D-erythro-hexopyranosyl)thymine, starting from the well known carbohydrate precursors 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose (12 steps) or from tri-O-acetyl-D-glucal (11 steps) is described.*

The modulation of gene expression by synthetic oligonucleotides, either through an antisense or an antigene strategy, can be considered as a new concept in pharmacology with potential therapeutical applicability against infectious diseases and cancer[1].

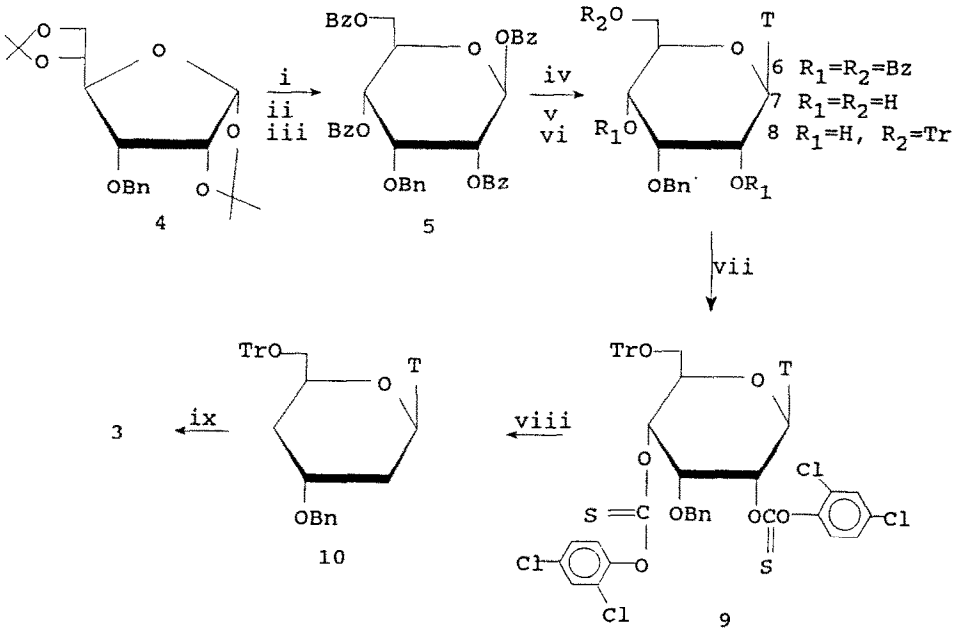
The synthetic challenge to this approach of cancer chemotherapy lies in the development of synthetic constructs which are enzymatically stable but still apt to form stable duplexes with natural DNA or mRNA. Therefore, we synthesized a series of dideoxy-D-*erythro*-hexopyranosyl nucleosides for incorporation into oligodeoxynucleotides. The synthesis of 2,3-dideoxy-β-D-*erythro*-hexopyranosyl nucleosides[2] (**1**) and of 3,4-dideoxy-β-D-*erythro*-hexopyranosyl nucleosides (**2**) is straightforward and will be reported elsewhere. However, the synthesis of 2,4-dideoxy-β-D-*erythro*-hexopyranosyl nucleosides poses a bigger challenge. Two different synthetic schemes were developped leading to the title compound starting from easily available carbohydrate precursors.



1 R_1=R_2=H, R_3=OH
2 R_2=R_3=H, R_1=OH
3 R_1=R_3=H, R_2=OH
T= thymin-1-yl

Fig. 1

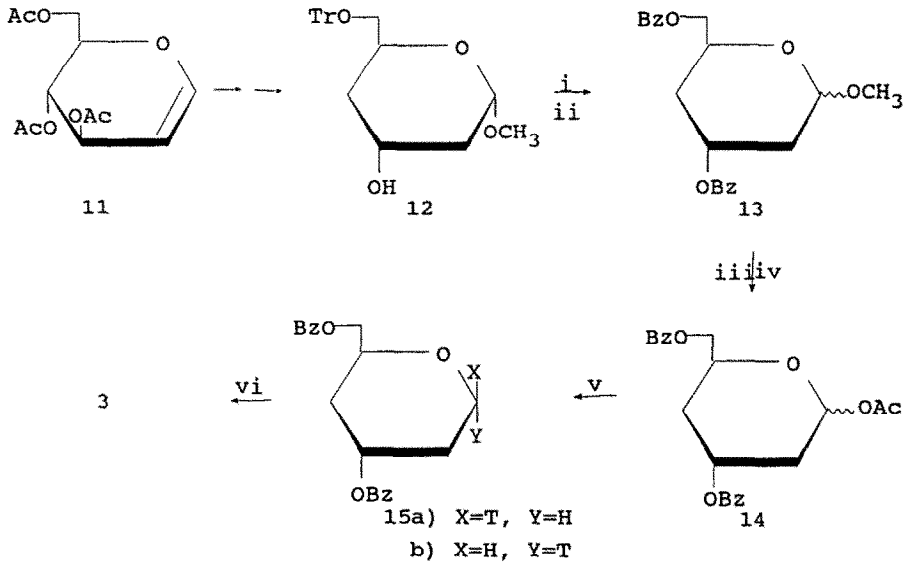
1,2:5,6-Di-*O*-isopropylidene-α-D-allofuranose[3] was treated with benzylbromide / NaH to afford the starting material 3-*O*-benzyl-1,2:5,6-di-O-isopropylidene-α-D-allofuranose (**4**). Removal of the isopropylidene functions with 80% HOAc resulted in a complex reaction mixture that contained several mono-acetates[4]. This mixture was treated with ammonia, purified by column chromatography and benzoylated. Compound **5** was isolated from the reaction mixture after extraction and crystallisation from EtOAc/EtOH in 40% yield from **4**. ^{1}H NMR analysis proved the crystalline material to be in ß-D-pyranose configuration.



i: HOAc 80%; ii: NH_3; iii: BzCl; iv: silylated thymine, $SnCl_4$; v:NH_3/MeOH; vi: TrCl; vii: a) thiophosgene, DMAP b) 2,4-dichlorophenol; viii: Bu_3SnH, AIBN; ix: 20% $Pd(OH)_2/C$, cyclohexene, ethanol

Scheme 1

Sugar-base condensation reaction of **5** with silylated thymine under standard Vorbrüggen conditions[5] ($SnCl_4$, $ClCH_2CH_2Cl$, 40°C, 4 h) yielded 80% of compound **6**. Deprotection with methanolic ammonia gave **7** (95% yield), and the primary hydroxyl function was protected with a trityl group affording **8** in 83% yield. Classical deoxygenation procedures using methoxythiocarbonyl or phenoxythiocarbonyl derivatives were unsuccessful. Complex reaction mixtures were obtained from which the desired compound **10** was isolated with great difficulty. This problem was solved by using a phenoxythiocarbonyl functionality, substituted with electron withdrawing groups. As reported by D. Barton[6], these groups increase the radicophilicity of the thione group and hence increase the speed of the deoxygenation. Compound **9** was obtained from **8** by reaction in CH_2Cl_2 with two equivalents of thiophosgene at -40°C for 2 h in the presence of DMAP (8 equiv.), followed by addition of 4 equivalents of 2,4-dichlorophenol and reaction at room temperature for 10 min. Following extraction and chromatographic purification, **9** was obtained in 63% yield. Deoxygenation went smoothly with Bu_3SnH (3 equiv.) in benzene at reflux temperature (1.5 h). Less side compounds are formed using this procedure, although the yield is still not spectacular (48%). Cleavage of both the trityl and benzyl protecting group was accomplished by transfer hydrogenation [20 % $Pd(OH)_2/C$; cyclohexene, EtOH, reflux] affording the title compound 1-(2,4-dideoxy-β-D-*erythro*-hexopyranosyl)thymine (**3**) in 53% yield.



i: p-TsOH(cat), MeOH; ii: BzCl; iii: HOAc 80%; iv: Ac_2O;
v: thymine, BSA, $CF_3SO_3SiMe_3$; vi: NaOMe

Scheme 2

Methyl 6-*O*-trityl-2,4-dideoxy-β-D-*erythro*-hexopyranoside (**12**) can be obtained in 5 steps starting from tri-*O*-acetyl-D-glucal (**11**) as described by Corey[7a] and Yang[7b]. Detritylation with a catalytic amount of p-toluenesulfonic acid in methanol, followed by benzoylation gave compound **13** (90% yield) as a mixture of the α and ß anomer (1/3). The anomers can be separated easily but were used as such in the next step. This mixture was treated with 80% HOAc at 80°C for 5 h and acetylated with acetic anhydride. Purification by column chromatography afforded 62% of the β anomer of **14** and 7% of the α anomer. The β anomer (**14**) was condensed with silylated thymine in the presence of TMS-triflate yielding 55% of **15a** and 22% of **15b**. Deprotection of **15a** with sodium methanolate in methanol afforded the title compound **3**, which proved identical with the compound obtained following the first scheme[8].

It is clear from both schemes that the second approach affords higher amounts of the title compound in lesser steps and therefore is superior to the first one. The title compound **3** was obtained with 3% total yield in 12 steps from 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose, compared to 14% yield in 11 steps from **11**.

The synthesis of oligonucleotides containing **3** at different positions was accomplished using standard phosphoramidite chemistry. From the melting point determinations it is clear that these oligonucleotides are still able to form stable duplexes with their natural complement. Incorporation of the modified nucleoside at an internal position, however, has a more profound effect on the base pairing properties than substitution at terminal positions. These good hybridization properties are in contrast with the properties of analogous oligonucleotides containing the 2,3-dideoxy-hexose analogue (**1**)[2].

Table 1. Hybridization Properties

	Tm (°C)	ΔH (kJ/mol)
T_{13}	32.3	367
$T^*T_{11}T^*$	33.5	361
$T^*T^*T_9T^*T^*$	29.7	345
$T_6T^*T_6$	25.9	346

T : thymidine; T^* : 1-(2,4-dideoxy-β-D-*erythro*-hexopyranosyl)thymine (3)
Tm : melting temperature of the duplex consisting of the modified oligonucleotide annealed to $(dA)_{13}$ and determined as described in ref. 2.

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8. For all new compounds, satisfactory UV, ^1H and ^{13}C-NMR and mass spectral data were obtained on chromatographically homogeneous samples.
Physical data for **3** : UV (MeOH) λ_{max} = 266 nm (logε = 4.00); (0.01N NaOH) λ_{max} = 266 nm (logε = 3.79); CIMS : MH^+ (257), MNH_4^+ (274); ^1H NMR (DMSO-d_6) 1.15-2.00 (m, 4H, H-2', H-4'), 1.79 (s, 3H, CH_3), 3.39 (d, 2H, H-6'), 3.80 - 4.10 (m, 1H, H-5'), 4.20 (br s, 1H, H-3'), 4.63 (t, 1H, J=4.8Hz, 6'OH), 4.94 (br s, 1H, 3'OH), 5.94 (dd, 1H, J=2.6Hz and 11.0Hz, H-1'), 7.55 (s, 1H, H-6), 11.26 (br s, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6) 12.2 (CH_3), 33.6 and 36.5 (C-2', C-4'), 63.1 (C-3'), 64.4 (C-6'), 74.1 (C-5'), 77.4 (C-1'), 109.7 (C-5), 137.0 (C-6), 150.4 (C-2), 164.0 (C-4) ppm.