ANALOGUES OF OLIGONUCLEOTIDES CONTAINING 3'-DEOXY-β-D-PSICOTHYMIDINE

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Abstract: Two different building blocks derived from 3'-deoxy-β-D-psicothymidine [1-(3-deoxy-β-D-erythro-2-hexulofuranosyl)thymidine] were prepared and used in the synthesis of modified oligonucleotides.

Dan et al.1 recently reported on the preparation of a phosphoramidite, 1, derived from 3'-deoxy-β-D-psicosourkline, 2 the O1' of which was linked to an N-protected aminoalkyl chain via a carbamate group. This building block was incorporated into oligonucleotides, and an intercalating group was attached to its amino function. The oligonucleotides obtained were shown to form stable duplexes with complementary strands, the conjugate groups being accommodated in the minor groove. We have approached the same subject in an alternative manner. The two hydroxymethyl functions of the 3'-deoxypsiconucleoside building block were protected with two different groups, both compatible with the usual phosphoramidite strategy of oligonucleotide synthesis on solid support. This enables derivatisation of the 1'-position during the course of the oligonucleotide synthesis and also selective chain elongation.

Scheme 1. 1: Lvo / Py / dioxane; ii: 2-cyanomethyl N,N,N',N'-tetraisopropylphosphorodiamidite/TetrH/ MeCN. DMTt - 4,4'-dimethoxytrityl; Lvo - levulinyl (4-oxopentanoyl).

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via either O1' or O6', i.e. preparation of branched oligonucleotides. 3'-Deoxy-β-D-psicothymidine, 3 (2),
having a normal DNA base instead of uracil, was used to construct the appropriate building blocks, 7 and 8.

The synthesis of appropriately protected psicothymidine phosphoramidites is outlined in Scheme 1.
Dimethoxytritylation of 3'-deoxy-β-D-psicothymidine (Ψ, 2)3 gave a mixture of 1'- and 6'-O-protected
nucleosides (3,4), which were separated by silica gel chromatography.4 Acylation of 3 and 4 with levulinic
anhydride5 in dioxane - pyridine led in both cases to a mixture of two monoacylated and one diacylated
product. Isolation by silica gel chromatography afforded 6'-O-(4,4'-dimethoxytrityl)-1'-O-levulinyl-3'-deoxy-β-
D-psicothymidine (5) and its regiosomer 6 in moderate yields.6 Finally, treatment of 5 and 6 with 2-cyanoethyl
N,N',N'-tetraisopropylphosphorodiamidite7 (1.25 equiv.) in acetonitrile in the presence of 1H-tetrazole (1.0
equiv.) followed by aqueous work-up and precipitation from toluene with cold hexane, gave the phosphoramidites
7 and 8 as white powders.8 5'-O-Benzoylthymidine 3'-O-(2-cyanoethyl N,N-diisopropylphosphoamidite) (9)
was prepared analogously starting from 5'-O-benzoylthymidine.9

In order to demonstrate the applicability of 7 in preparation of modified oligonucleotides, the model
sequences 5'-TpCpT(3'-p-6')W(4'-p-5')TpGpT-3' (10) and 5'-TpApTpCpT(3'-p-6')Ψ(4'-p-5')TpGpT-3' (11)
were synthesised. The oligonucleotides were assembled on an Applied Biosystems 392 DNA Synthesizer (0.2
µmol scale) according to the recommended protocol. No difference in coupling yields (>98% as determined by
trityl assay) was detected between compound 7 and commercial phosphoramidites of unmodified nucleosides,
striking contrast to the low coupling efficiency reported for the building block 1.1

Scheme 2. i: 0.5 M H₂NNH₂ in AcOH/Py (1:4); ii: continuation of oligonucleotide synthesis; iii: Aminolink 2™
TetrH/MeCN. S - solid support; p'' - 2-cyanoethyl protected internucleosidic phosphotriester unit; p - phosphodiester moiety.
5'-Tp'AbZp'T, was assembled starting from the liberated O1' of the psicothymidine residue. Analogously, condensation with aminolink 2TM completed the assembly of the aminoalkyl derivatised oligonucleotide 13.10 Phosphoramidite 8 was used also to prepare the same branched oligonucleotide 12 and the sequence 5'-TpApT(3'-p-1')Ψ(4'-p-5')TpGpT-3' (14) possessing only the O1'- branch. In contrast to 7, both 8 and the next phosphoramidite T were coupled for 6 min. The strategy of assembling 12 was similar to that described above for 7. First the chain 5'-Tp'AbZp'T(3'-p-1')Ψ(4'-p-5')TpGpT was assembled, and, after cleavage of the 6'-O-levulinyl group, 5'-Tp'CbZp'T-3' branch was attached to the 6'-O of psicothymidine residue. After the syntheses were completed, the standard procedure of ammoniolytic deprotection was used. The oligonucleotides (10-14) were isolated by an anion exchange HPLC.11 Chromatographical profile of crude 12 is shown in figure 1 as an illustrative example. After purification by RP HPLC12 and desalting by gel filtration13 the oligonucleotides were characterised by PAGE (Fig. 2.) and HPLC (Table I.).

Table 1. Retention times of 11-14 relative to 10.

<table>
<thead>
<tr>
<th>Relative retention time (min)</th>
<th>10*</th>
<th>11</th>
<th>12a</th>
<th>12b</th>
<th>13</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ion exchange11</td>
<td>18.6</td>
<td>+5.2</td>
<td>+5.2</td>
<td>+5.2</td>
<td>-0.7</td>
<td>0.0</td>
</tr>
<tr>
<td>Rev. phase12</td>
<td>29.1</td>
<td>+3.0</td>
<td>+0.9</td>
<td>+0.9</td>
<td>-0.5</td>
<td>-0.4</td>
</tr>
</tbody>
</table>

* Absolute retention time (min); a: prepared by using 7; b: prepared by using 8. 12a and 12b coeluted both on ion exchange and RP columns.

The attempts to digest the oligonucleotides 10-12 and 14 with phosphodiesterase I or II in the presence of alkaline phosphatase failed. By contrast, when 10 and 11 were treated with the mixture of these enzymes, nucleosides in the expected ratios were obtained. Under the same conditions 14 gave the expected nucleosides and an additional product which was tentatively assigned as a dimer Ψ(1'-p-3')T. Furthermore, the enzymatic digestion of 12 gave two additional products along with nucleosides expected. In all likelihood these are a "fork-like" trimer T(3'-p-6')Ψ(1'-p-3')T and a dimer Ψ(1'-p-3')T. These findings demonstrate the stability of the phosphodiester bonds of psicothymidine against nucleases.

Acknowledgments. Financial support from the Research Council for Natural Sciences, the Academy of Finland, is gratefully acknowledged.

REFERENCES AND NOTES
submitted.

4. Compound 3: 56% as colourless foam. $^1$H NMR (CDCl$_3$): 11.42 (1H, s, N3-H); 7.64 (1H, s, H-6); 7.43-6.70 (13H, m, arom.); 5.08 (1H, t, OH-1'); 5.03 (1H, d, J$_{4',4'}$-OH = 3.91 Hz, OH-4'); 4.06 (1H, m, H-4'); 4.02 (1H, m, H-5'); 3.85 (1H, dd, J$_{1',1''}$-OH = 6.34 Hz, J$_{1',1''}$ = 11.23 Hz, H-1''); 3.74 (6H, s, OCH$_3$); 3.55 (1H, dd, J$_{1',1''}$-OH = 6.34 Hz, J$_{1',1''}$ = 11.23 Hz, H-1''); 3.16 (1H, dd, J$_{5',6'}$ = 3.91 Hz, J$_{6',6''}$ = 10.25 Hz, H-6''); 3.05 (1H, dd, J$_{5',6''}$ = 3.91 Hz, J$_{6',6''}$ = 10.25 Hz, H-6''); 2.63 (1H, dd, J$_{3',4'} = 2.92$ Hz, J$_{3',3''} = 14.16$ Hz, H-3''); 2.30 (1H, dd, J$_{3',4'} = 6.48$ Hz, J$_{3',3''} = 14.16$ Hz, H-3''); 1.81 (3H, s, CH$_3$-5).

Compound 4: 16% of colourless foam. $^1$H NMR (CDCl$_3$): 11.07 (1H, s, N3-H); 7.84 (1H, s, H-6); 7.32-6.82 (13H, m, arom.); 5.01 (1H, d, J$_{4',4'}$-OH = 3.42 Hz, OH-4'); 4.88 (1H, t, J$_{6',6'}$-OH = J$_{6'',6''}$-OH = 5.13 Hz, OH-6'); 4.05 (1H, m, H-4'); 3.96 (1H, m, H-5'); 3.73 (6H, s, OCH$_3$); 3.52 (2H, m, H-6' and H-6''); 3.38 (1H, d, J$_{1',1''}$ = 9.28 Hz, H-1''); 3.19 (1H, d, J$_{1',1''}$ = 9.28 Hz, H-1''); 2.66 (1H, dd, J$_{3',4'} < 1$ Hz, J$_{3',3''} = 14.65$ Hz, H-3''); 2.22 (1H, dd, J$_{3',4'} = 6.83$ Hz, J$_{3',3''} = 14.65$ Hz, H-3''); 1.87 (3H, s, CH$_3$-5).

5. Levulinic anhydride was prepared in situ by overnight treatment of levulinic acid (10 mmol) with DCC (5 mmol) in dry dioxane (10 ml).

6. Compound 5: 30.8% as colourless foam. $^1$H NMR (CDCl$_3$): 9.07 (1H, s, N3-H); 7.62 (1H, s, H-6); 7.43-6.83 (13H, m, arom.); 4.64 (1H, d, J$_{4',4'}$-OH = 11.48 Hz, H-1'); 4.44 (1H, m, H-4'); 4.35 (1H, d, J$_{4',4'}$-OH = 11.48 Hz, H-1''); 4.26 (1H, m, H-5'); 3.80 (6H, s, OCH$_3$); 3.31 (1H, dd, J$_{5',6'}$ = 4.40 Hz, J$_{6',6''}$ = 10.25 Hz, H-6'); 3.26 (1H, dd, J$_{5',6''}$ = 4.40 Hz, J$_{6',6''}$ = 10.25 Hz, H-6''); 3.09 (1H, dd, J$_{3',4'} = 2.44$ Hz, J$_{3',3''} = 14.65$ Hz, H-3''); 2.65 (1H, dd, J$_{A,M} = 6.35$ Hz, J$_{A,L} = 7.32$ Hz, J$_{A,B} = 18.55$ Hz, CH$_3$COC-H$_A$); 2.59-2.53 (2H, m, H-3', CH$_3$COC-H$_B$); 2.46 (1H, dd, J$_{B,L} = 5.86$ Hz, J$_{A,L} = 7.32$ Hz, J$_{L,M} = 17.57$ Hz, OCOC-H$_L$); 2.33 (1H, dd, J$_{B,M} = 6.35$ Hz, J$_{A,M} = 6.35$ Hz, J$_{L,M} = 17.57$ Hz, OCOC-H$_M$); 2.09 (3H, s, CH$_3$CO); 1.91 (3H, s, CH$_3$-5).

Compound 6: 32.6% as colourless foam. $^1$H NMR (CDCl$_3$): 11.40 (1H, s, N3-H); 7.50 (1H, s, H-6); 7.45-6.75 (13H, m, arom.); 4.43 (1H, m, H-4'); 4.10 (1H, m, H-5'); 3.80 (6H, s, OCH$_3$); 3.51 (1H, d, J$_{1',1''}$ = 9.28 Hz, H-1''); 3.34 (1H, d, J$_{1',1''}$ = 9.28 Hz, H-1''); 3.05-3.20 (8H, m, H-6', H-3', H-3'', (CH$_2$)$_2$); 2.21 (3H, s, CH$_3$CO); 1.97 (3H, s, CH$_3$-5).


8. Compound 7: $^{31}$P NMR (CDCl$_3$): 148.87 (0.5 P); 148.64 (0.5 P); TLC (Kieselgel 60 F$_{254}$, CH$_2$Cl$_2$-MeOH 19:1): $R_f$ 0.34, 0.40. Compound 8: $^{31}$P NMR (CDCl$_3$): 149.15 (0.5 P); 149.03 (0.5 P); TLC (Kieselgel 60 F$_{254}$, CH$_2$Cl$_2$-MeOH 19:1): $R_f$ 0.43, 0.48.


11. Ion exchange HPLC was performed on a Synchropak AX-300 column (6.5 μm, 4.6x250 mm) at flow rate 1.0 ml/min using a linear gradient from 0 to 40% of B for 40 min. ($A = 0.03$ M KH$_2$PO$_4$ in 50% aqueous formamide, pH 5.60; $B = 0.6$ M (NH$_4$)$_2$SO$_4$ in buffer A, pH 5.60).

12. Reversed phase HPLC was performed on a Nucleosil 120-5C$_{18}$ column (5 μm, 4.0x250 mm) at flow rate 1.0 ml/min using a linear gradient from 0 to 40% B for 40 min. ($A = 0.1$ M ammonium acetate buffer; $B = 0.1$ M ammonium acetate buffer containing 50% acetonitrile).

13. TSKgel G2000SW (7.5 x 300 mm, Toso Haas); flow rate 1.0 ml/min of water.

(Received in UK 5 July 1993; accepted 23 July 1993)