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# Nucleosides and Nucleotides

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### 3'-MODIFIED NUCLEOTIDES FOR DNA SEQUENCING WITHOUT GEL ELECTROPHORESIS

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**ABSTRACT**: Dibenzyl-3'-O-[(6-azido-2,3,6-trideoxy-4,5-di-O-benzyl) hexanoyl] thymidine 5'-yl phosphate **8a** was prepared. Catalytic hydrogenolysis removed only the benzyl esters and reduced the azido group. When the benzyl ethers were replaced by *p*-phenylbenzyl or allyl ethers, their deprotection also failed.

DNA dideoxy sequencing<sup>1</sup> is the method of choice to determine a nucleotide sequence from a single stranded DNA template. During the four enzymatic chain elongations, dideoxy nucleotides are randomly inserted instead of their corresponding deoxynucleotides. Sequencing reactions generate a complex mixture which is subsequently resolved by polyacrylamide gel electrophoresis.

We recently described a sequencing scheme<sup>2</sup> to determine the nucleotide sequence in a DNA molecule which avoids the use of both electrophoresis and harmful radiolabels. This method uses 3'-substituted-2'-deoxynucleotide 5'-triphosphates corresponding to the four nucleobases A, C, G and T. The 3'-position is esterified by a fluorescent derivative (3'-tag)<sup>2,3,4</sup> to give specific spectroscopy properties to each nucleotide. The latter would also be easily identified and could be removed rapidly under conditions compatible with DNA stability to restore an unprotected 3'-hydroxyl end. The 3'-tag would allow DNA termination and indicate which base is positioned on the template strand.

The ease of 4-oxo-pentanoate (levulinate) ester cleavage<sup>5</sup> has been used in the synthesis of oligonucleotides. The deprotection was accomplished in a quantitative yield, in 2 minutes under neutral conditions, and at room temperature. After enzymatic incorporation into DNA, followed by periodate oxidation of the cis-diol, a 2'-deoxynucleotide 5'-triphosphate esterified at the 3'-position by the 6-amino-2,3,6-trideoxy-D-erythrohexanoic acid attached to the  $\omega$ -amino-function with fluorescent probes would lead to the free tag and the DNA strand modified on the 3'-end with an analog of the levulinate. This

analog could be removed more rapidly, and under milder conditions because of the aldehyde generated by the oxidative cleavage.

The aim of this work was to synthetize these 3'-modified 2'-deoxynucleotide 5'triphosphates.

Using calcium D-gluconate as the starting material a convenient synthesis of the 6bromo-2,3,6-trideoxy-D-erythro-hexono-1,4-lactone **1** has been developed by Pedersen and his co-workers<sup>6</sup>. Nucleophilic displacement of the 6-bromo with sodium azide in DMF at 70°C afforded the 6-azido compound **2** in 70% yield. Alkylation of the potassium salt of the latter with benzyl, *p*-phenylbenzyl, *p*-methoxybenzyl or allyl bromide using sodium hydride in dry dimethylformamide gave the tri-alkylated compounds **3a,b,c,d** respectively. Saponification of these alkyl esters gave the corresponding 6-azido-2,3,6trideoxy-4,5-di-O-alkyl-D-erythro-hexanoic acids **4a,b,c,d**.



i NaN3, DMF, 70°C ; ii KOH, EtOH/H2O, r.t. then RX, NaH, DMF, 0°C to r.t. ; iii KOH, EtOH/H2O, 60°C

The selective protection<sup>7</sup> of thymidine with dimethoxytrityl chloride (DMTrCl) in pyridine gave the compound 5. Acylation of the 3'-OH of 5 with 4a and DCC in the presence of a catalytic amount of DMAP in dichloromethane at room temperature led to compound the 6a giving a 75% yield. Removal of the DMTr group by treatment with 2%



benzenesulfonic acid gave the compound **7a**. Catalytic hydrogenolysis offers the mildest method for deprotecting benzyl ethers and esters and also reduces azido group to an amino function, in one pot. For this reason, the 5'-OH group of **7a** was phosphorylated with freshly prepared dibenzylphosphorochloridate<sup>8</sup> in pyridine at -20°C. After work-up and purification on silica gel, the compound **8a**<sup>9</sup> was obtained giving a 38% yield.

Surprisingly catalytic hydrogenolysis of **8a** did not give the expected product **10** but **9a**<sup>10</sup>. Indeed if the benzyl ethers were easily removed and the azido function reduced, the benzyl ethers are resistant to both the standard hydrogenolysis conditions and the transfer catalytic hydrogenation. It is known that catalytic hydrogenolysis fails when the substrate contains non-aromatic amine. For this reason, the azido group of **6a** has been reduced and blocked with N-methylisatoic anhydride but this did not change the result of the hydrogenolysis. Hydrogenolysis of **7b** which was protected by *p*-phenylbenzyl ethers<sup>11</sup> which are known to be more easily removed than the benzyl group was tried without success. The isomerization of the allyl groups<sup>12</sup> on **7d** was also inefficient. The last protecting group used is the *p*-methoxybenzyl ether. The oxidative cleavage of **7c** with DDQ in dry CH<sub>2</sub>Cl<sub>2</sub> led to two products. Their structures were determined by <sup>1</sup>H, <sup>13</sup>C NMR spectroscopies and mass spectrometry. The first was partially deprotected ( $\delta_{C''5} = 71,76$  <u>C</u>H-OH and  $\delta_{C''4} = 74,93$  <u>C</u>H(OMBz); MS = 548) and the second was chlorinated ( $\delta_{C''4} = 60,19$  <u>C</u>HCl and  $\delta_{C''5} = 75,14$  <u>C</u>H(OMBz); MS = 566).

In conclusion, the benzyl, *p*-phenylbenzyl and allyl ethers of compounds **7a,b,d** are resistant to all reactions involving a catalyst. The deprotection of the *p*-methoxybenzyl ether led to a free hydroxyle at the 5"-position. As the latter doesn't lactonize, the expected molecule with a fully deprotected cis-diol seems to be viable.

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- 9 <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  : 1.85 (d, 3H, Me-T, J<sub>6</sub>, Me = 0.79 Hz); 2.17 (dd, 1H, H'2, J<sub>2',1</sub>' = 5.52 Hz, J<sub>2',2'</sub> = 13.97 Hz); 2.34 (t, 2H, CH<sub>2</sub>); 3.49 (m, 2H, 2xC<u>H</u>OBn); 3.62 (m, 2H, CH<sub>2</sub>N<sub>3</sub>); 3.98 (m, 1H, H4'); 4.16 (m, 2H, H'5, H5''); 4.70 (d, 2H, CH<sub>2</sub>Ph); 4.57 (d, 2H, CH<sub>2</sub>Ph); 5.06 (m, 5H, H'3, 2xCH<sub>2</sub>Ph); 6.31 (dd, 1H, H'1, J<sub>1',2'</sub> = 5.35 Hz, J<sub>1',2''</sub> = 9.28 Hz); 7.35 (m, 21H, NH, 4xPh); 8.82 (d, 1H, H6). <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  : 12.19 (Me-T); 25.24 (C''2); 25.46 (C''3); 36.84 (C'2); 50.71 (C''6); 66.92 (C'5); 69.66 (2x<u>C</u>H<sub>2</sub>Ph); 72.40 (2x<u>C</u>H<sub>2</sub>Ph); 74.48 (C'3); 77.07 (C''5); 79.08 (C''4); 82.55 (d, C'4); 84.82 (C'1); 111.62 (C5); 127.48-137.67 (C aromatics); 134.63 (C6); 150.20 (C2); 163.31 (C4); 172.76 (C=O). <sup>31</sup>P NMR (CDCl<sub>3</sub>)  $\delta$  : -2.50 (m, 1P).
- 10 <sup>1</sup>H NMR (D<sub>2</sub>O)  $\delta$  : 1.86 (s, 3H, Me-T); 3.22 (m, 2H, CH<sub>2</sub>NH<sub>2</sub>); 3.94 (m, 2H, H'5, H5"); 4.05 (m, 1H, H4'); 4.64 (m, 4H, 2xCH<sub>2</sub>Ph); 5.13 (m, IH, H'3); 6.11 (dd, 1H, H'1); 7.33 (s, 10H, 2xPh); 7.65 (s, 1H, H6). <sup>31</sup>P NMR (D<sub>2</sub>O)  $\delta$  : 0.78 (s, 1P). 11 Bolitt, V.; Mioskowski, C. J. Am. Chem. Soc. **1991**, 113, 6320-6321.
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