Synthesis of α-2'-Deoxynucleosides

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Received April 27, 1995

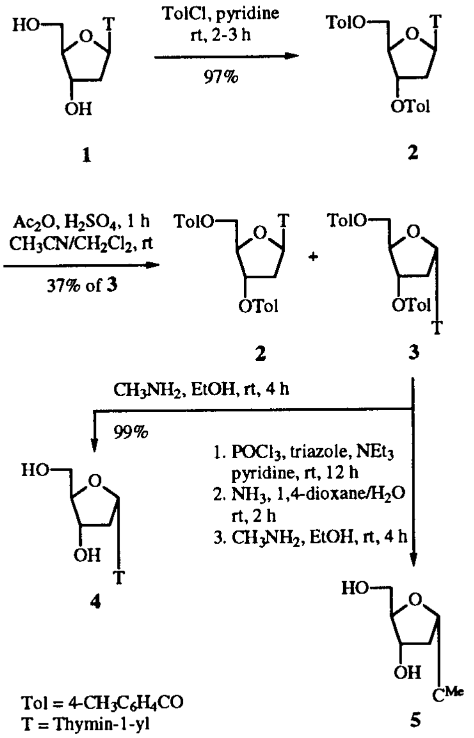
α-Thymidine (**4**) was synthesized from thymidine (**1**) in 3 steps in 36% overall yield without using chromatography and with the possibility of increasing the yield to 85% by reusing the remaining α,β-mixture. 1-(2-Deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranosyl)thymine (**3**) was further converted to 1-(2-deoxy-α-D-*erythro*-pentofuranosyl)-5-methylcytosine (**5**).

J. Heterocyclic Chem., **32**, 1645 (1995).

α-Oligonucleotides have been a subject of great interest in recent years. The reasons for this are their good hybridization properties toward DNA or RNA and stability against nuclease degradation [1-6] which make them good candidates for antisense studies. α-Oligonucleotides can form triple helixes with duplex DNA similar to those formed by β-oligonucleotides [7]. Modified β-oligonucleotides with the nucleobases thymine and cytosine displaced with 5-bromouracil and 5-methylcytosine, respectively, have shown improved triplex forming properties toward duplex DNA at physiological *p*H [8] and α-oligonucleotides modified with 5-methylcytosine have recently been investigated [9]. Only a few synthetic methods are known for preparation of the α-nucleosides, *e.g.* anomerization of pyrimidine nucleosides [10], transdeoxyribosylation of pyrimidine nucleosides with purine bases [10] and condensation of silylated nucleobases [10-13] or 2,4-dimethoxypyrimidines [14,15] with 2-deoxyribofuranosides. Even though favorable α/β-ratios may be obtained as described by Ward *et al.* [16], who achieved a 2:1 α/β-mixture from anomerization of 3',5'-di-*O*-acetylthymidine using acetic anhydride/sulfuric acid in acetonitrile, no method of separation was reported and pure anomers seem obtainable by chromatography, only. Considering the interest in α nucleosides we found it important to devise a non-chromatographic procedure for their isolation.

The starting material, thymidine (**1**) was protected at 3'-OH and 5'-OH with *p*-toluoyl chloride in pyridine. After addition of water, the protected thymidine was isolated by filtration. Anomerization of 3',5'-di-*O*-*p*-toluoylthymidine (**2**) was achieved in methylene chloride/acetonitrile using acetic anhydride/sulfuric acid as the active reagent to give a 2:1 α,β-mixture. Protected 5-bromo-2'-deoxyuridine can be used as well, although some destruction of the nucleoside occurs, but the reaction is not possible with fully protected 2'-deoxyadenosine or 2'-deoxyguanosine because of complete destruction of the nucleosides and with 3',5'-diacetyl-N^4-benzoyl-2'-deoxycytidine no reaction occurs. Crystallization from ethanol afforded the pure protected α-thymidine (**3**) in 37% yield but the yield could be improved by repeated anomerization of the anomeric mixture from the mother liquor. When 1/3 equivalent of 3',5'-di-*p*-toluoylthymidine (**2**) is added corresponding to the amount of isolated α-anomer, yield is about 85% of the deprotected α-thymidine based on the amount of thymidine added before the anomerization. In our laboratory we have done up to 5 repeated isolations of α-thymidine by this method. Benzoyl could equally well be used instead of toluoyl as the protecting group. However, with acetyl as the protecting group we did not find it possible to crystallize the α-anomer selectively. Compound **3** was deprotected with 33% methylamine in absolute ethanol (Scheme 1). The above mentioned procedures can easily be scaled up to mole syntheses with yields in the same order.

Scheme 1



Tol = 4-$CH_3C_6H_4CO$
T = Thymin-1-yl

Protected α-thymidine **3** was converted to 1-(2-deoxy-α-D-*erythro*-pentofuranosyl)-5-methylcytosine (**5**) with phosphorus oxychloride/1,2,4-triazole [9,17], the triazole ring being displaced with ammonia using ammonia/dioxane/water [9,17], followed by chromatographic workup, and finally deprotection accomplished with 33% methylamine in ethanol.

EXPERIMENTAL

3',5'-Di-*O*-*p*-toluoylthymidine (**2**).

Thymidine (**1**) (2.42 g, 10 mmoles) was dissolved in dry pyridine (10 ml), *p*-toluoyl chloride (4.00 g, 21 mmoles) was added in one portion and the reaction mixture stirred for 3 hours at room temperature. Water (50 ml) was added, the precipitate filtered off, washed with cold water, cold ethanol and cold ether, and dried *in vacuo* to give compound **2** as a colorless solid, yield 5.30 g (97%), mp 147-148° (lit [14] 147.5-149°).

1-(2-Deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranosyl)thymine (**3**).

Anomerization: Compound **2** (5.50 g, 10 mmoles) was dissolved in a mixture of methylene chloride/acetonitrile/acetic anhydride/sulfuric acid (45 ml, v/v/v/v 20/20/4/1) and stirred for 1 hour. After neutralization with saturated aqueous sodium bicarbonate (150 ml), the resulting mixture was partially evaporated in order to remove methylene chloride and acetonitrile. The precipitated mixture of compound **2** and **3** could then be separated from the aqueous phase by decanting. Pure compound **3** was obtained by crystallization from ethanol (96%). The remaining ethanolic solution of **2** and **3** from the crystallization could be cooled to -20° overnight and an α,β-mixture filtered off and reused for the anomerization procedure above. The ethanolic solution could similarly be reused for the crystallization to obtain the title compound **3** as a colorless solid, yield 1.80 g (37%), mp 146-148° (lit [10] 145-146°).

1-(2-Deoxy-α-D-*erythro*-pentofuranosyl)thymine (**4**).

Compound **3** (1.39 g, 3 mmoles) was dissolved in 33% methylamine in absolute ethanol (20 ml), stirred for 4 hours and evaporated under reduced presure. Addition of methylene chloride (25 ml) with stirring, filtration of the mixture and washing the white solid with methylene chloride (10 ml) gave after drying *in vacuo* compound **4** as a colorless solid, yield 0.72 g (99%), mp 186-187° (lit [13] 187°).

1-(2-Deoxy-α-D-*erythro*-pentofuranosyl)-5-methylcytosine (**5**).

Triethylamine (13.9 ml, 0.10 mmole) was added dropwise to a solution of 1-*H*-1,2,4-triazole (7.0 g, 100 mmoles) and phosphorus oxychloride (3.1 ml, 34 mmoles) in acetonitrile (60 ml) at 0°. Compound **3** (4.80 g, 10 mmoles) in dry acetonitrile/pyridine (60 ml, v/v 1:1) was added and the reaction mixture stirred at room temperature overnight. Water (2.5 ml) was added, the solvents evaporated *in vacuo* and the resulting residue partitioned between chloroform (200 ml) and saturated aqueous sodium bicarbonate (200 ml). The organic phase was dried over anhydrous sodium sulfate and evaporated *in vacuo* followed by addition of aqueous ammonia (20%, 20 ml) and 1,4-dioxane (80 ml). After 2 hours the mixture was evaporated under reduced pressure, the residue partitioned between chloroform (200 ml) and saturated aqueous sodium bicarbonate (200 ml). The organic phase was dried over anhydrous sodium sulfate and evaporated *in vacuo* to give after silica gel (200 g) chromatographic workup the 3',5'-diprotected 1-(2-deoxy-α-D-*erythro*-pentofuranosyl)-5-methylcytosine. After deprotection using the method for compound **4** the title compound was obtained as a colorless solid, yield 1.6 g (68%), mp 185-186° (lit [14] 186-188°).

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