Preparation of 3′-*O*-(4-Methoxytetrahydropyran-4-yl) Derivatives of 4-*N*-Benzoylcytidine and Uridine

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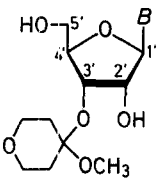
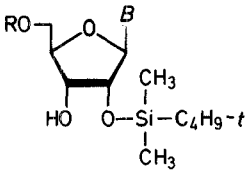
3′-*O*-(4-Methoxytetrahydropyran-4-yl)-4-*N*-benzoylcytidine (**1c**) and 3′-*O*-(4-methoxytetrahydropyran-4-yl)-uridine (**1d**) are prepared from 4-*N*,2′-*O*,5′-*O*-triacetylcytidine (**3**) in 47 and 65% overall yields, respectively.

The discovery[1] that 5′-*O*-triphosphoryladenylyl-(2′→5′)-adenylyl-(2′→5′)-adenosine (2-5A) is a powerful inhibitor of protein synthesis in a cell free system has recently stimulated much interest in the synthesis of oligoribonucleotides with 'unnatural' 2′→5′-internucleotide linkages. This has, in turn, created a need for intermediate ribonucleoside derivatives in which the 3′-hydroxy functions are protected in a suitable way. For a number of years, we have successfully used the achiral 4-methoxytetrahydropyran-4-yl (Mthp) group[2] to protect hydroxy functions vicinal to the internucleotide linkages in oligoribonucleotide synthesis. Indeed, 6-6-*N*-benzoyl-3′-*O*-(4-methoxytetrahydropyran-4-yl)-adenosine (**1a**) was used as the principal building block in our synthesis[3] of 2-5A and other workers have reported[4] the preparation of the corresponding 6-*N*-(*p*-anisoyl) derivative (**1e**). We now describe convenient preparations of the 3′-*O*-Mthp derivatives of 4-*N*-benzoylcytidine and uridine (**1c** and **1d**, respectively) which are corresponding building blocks that would be required in the synthesis of 2-5A analogues derived from pyrimidine nucleosides.

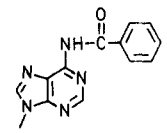
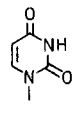
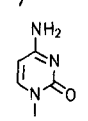
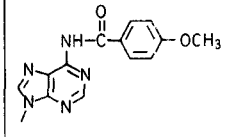
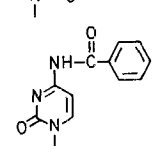


1a-d

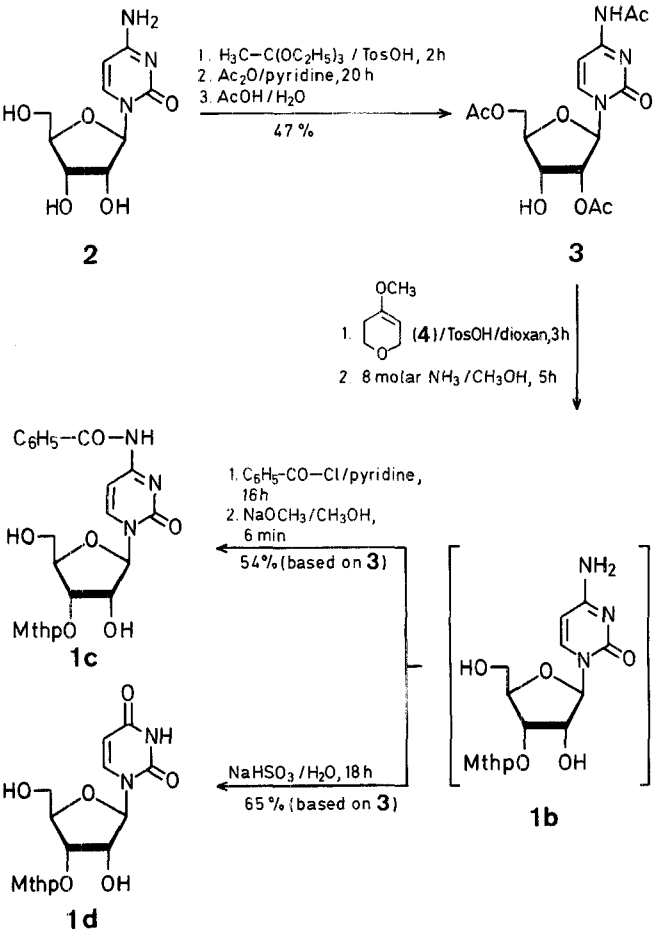


5 R = Ac

6 R = Si(CH$_3$)$_2$-C$_4$H$_9$-*t*

1,5,6	B	1,5,6	B
a		d	
b		e	
c			

Both of the above building blocks **1c** and **1d** have been prepared from a common intermediate, 4-*N*,2′-*O*,5′-*O*-triacetylcytidine (**3**). Compound **3** was readily prepared[5] from cytidine (**2**) in 47% overall yield. Treatment of **3** with an excess of 4-methoxy-5,6-dihydro-2*H*-pyran (**4**)[2,6] in the presence of *p*-toluenesulphonic acid in dioxan solution, followed by treatment of the products with methanolic ammonia, gave crude 3′-*O*-(4-methoxytetrahydropyran-4-yl)-cytidine (**1b**). When this product was allowed to react with an excess of benzoyl chloride in pyridine solution and the products then treated with sodium methoxide in methanol/dioxan, 4-*N*-benzoyl-3′-*O*-(4-methoxytetrahydropyran-4-yl)-cytidine (**1c**) was obtained as a crystalline solid in 54% overall yield, based on **3**. When the above crude 3′-*O*-(4-methoxytetrahydropyran-4-yl)-cytidine (**1b**) was treated with an excess of sodium hydrogen sulphite (pH = 5.6)[7,8] at room temperature for 18 h, 3′-*O*-(4-methoxytetrahydropyran-4-yl)-uridine (**1d**) was formed as a crystalline solid in 65% overall yield, based on **3**.



The 6-*N*-acyl-3′-*O*-(4-methoxytetrahydropyran-4-yl)-adenosine derivatives, **1a** and **1e**, referred to above have been prepared[3,4] from appropriate 2′-*O*-(*t*-butyldimethylsilyl)-adenosine derivatives **5a** and **6e**, respectively[4,9]. We have also recently prepared 3′-*O*-(4-methoxytetrahydropyran-4-yl)-adenosine (**1**; *B* = adenin-9-yl)[10] by allowing 2′,5′-di-*O*-(*t*-butyldimethylsilyl)-adenosine[11] (**6**; *B* = adenin-9-yl) to react with **4** in the presence of an acidic catalyst and then desilylating the product with tetra-*n*-butylammonium fluoride in tetrahydrofuran. Undoubtedly 2′,5′-di-*O*-(*t*-butyldimethylsilyl)-uridine[11] (**6d**) could be converted into **1d** by the same desilylation procedure. The particular merit of the present approach is that the key intermediate **3** was known[5] to crystallise in a pure state from a mixture, also containing the isomeric 4-*N*,3′-*O*,5′-*O*-triacetylcytidine. On the other hand, if 2′,5′-di-*O*-(*t*-butyldimethylsilyl)-uridine (**6d**) were used as an intermediate instead of **3**, it would first be necessary to separate it from its 3′,5′-isomer and from any other silylated uridine derivatives obtained[11].

^{1}H-N.M.R. spectra were measured at 60 MHz with a Jeol PMX60 SI, and at 250 MHz with a Bruker WM 250 spectrometer; tetrameth-

ylsilane was used as an internal standard. U.V. absorption spectra were measured with a Cary 17 recording spectrophotometer. T.L.C. was carried out on Merck silica gel 60 F_{254} plates. Merck silica gel H was used for short column chromatography[12]. Pyridine and dioxan were dried by heating, under reflux, with calcium hydride; these solvents were then distilled at atmospheric pressure.

4-*N*,2′-*O*,5′-*O*-Triacetylcytidine (3):
Cytidine (**2**; 5.83 g, 24.0 mmol), triethyl orthoacetate (17.0 ml, 92.7 mmol), and *p*-toluenesulphonic acid, monohydrate (5.70 g, 30.0 mmol) are stirred together at room temperature. After 2 h, an excess of 8 molar methanolic ammonia is added to the resulting solution, and the products are concentrated under reduced pressure. Chloroform (20 ml) is then added and the mixture is filtered. The residue is washed with chloroform (2 × 10 ml) and the combined filtrate and washings are concentrated under reduced pressure. The syrup obtained is dissolved in anhydrous pyridine (20 ml) and acetic anhydride (5.7 ml, 60.4 mmol) is added. After the reactants have been stirred at room temperature for 18 h, an additional quantity of acetic anhydride (2.5 ml, 26.5 mmol) is added. After a further period of 2 h, saturated aqueous sodium hydrogen carbonate (5 ml) is added and the resulting mixture is stirred for 15 min. The products are then poured into saturated aqueous sodium hydrogen carbonate (150 ml) and the mixture obtained is extracted with chloroform (5 × 30 ml). The combined, dried (magnesium sulphate) chloroform extract is concentrated under reduced pressure and the residue is dissolved in water (60 ml). Acetic acid is then added until the pH drops to 3.5 and the solution is evaporated under reduced pressure. After the residue has been evaporated from ethanol (5 × 30 ml) solution, it crystallises very slowly from ethanol to give **3** as fine colourless prisms; yield (in several crops): 4.15 g (47%, based on cytidine); m.p. 183–186 °C (Lit.[5], m.p. 182–185 °C).

^{1}H-N.M.R. [60 MHz, DMSO-d_6/1 molar HCl/D_2O (9:1 v/v)]: δ = 2.07 (s, 3 H); 2.10 (s, 3 H); 2.13 (s, 3 H); 4.0–4.5 (m, 4 H), 5.25 (dd, J = 3.5, 5.3 Hz, 1 H); 5.86 (d, J = 3.5 Hz, 1 H); 7.21 (d, J = 7.5 Hz, 1 H); 8.06 ppm (d, J = 7.5 Hz, 1 H).

4-*N*-Benzoyl-3′-*O*-(4-methoxytetrahydropyran-4-yl)-cytidine (1c):
4-*N*,2′-*O*,5′-*O*-Triacetylcytidine (**3**; 1.49 g, 4.03 mmol) and then 4-methoxy-5,6-dihydro-2*H*-pyran (**4**; 2.28 g, 20.0 mmol) are added to a magnetically stirred solution of *p*-toluenesulphonic acid monohydrate (0.38 g, 2.0 mmol) in anhydrous dioxan (20 ml) at room temperature. After 1.5 h, an additional quantity of 4-methoxy-5,6-dihydro-2*H*-pyran (0.91 g, 8.0 mmol) is added. After a further period of 1.5 h, the products are poured into saturated aqueous sodium hydrogen carbonate (100 ml) and the resulting mixture is extracted with chloroform (5 × 20 ml). The combined chloroform extract is dried with magnesium sulphate and concentrated under reduced pressure. The residue is fractionated by short column chromatography on silica gel (eluent = chloroform/ethanol, 98:2 to 96:4, v/v). The appropriate fractions are combined, concentrated under reduced pressure and the residual yellow oil is dissolved in 8 molar methanolic ammonia (50 ml). The solution is allowed to stand at room temperature for 5 h and is then concentrated under reduced pressure. When the residue is triturated with petroleum ether (b.p. 40–60 °C), the putative 3′-*O*-Mthp derivative of cytidine (**1b**) is obtained as a pale yellow solid.

The above material is dried by evaporation from anhydrous pyridine (20 ml) solution, redissolved in pyridine (15 ml) and allowed to react with benzoyl chloride (1.85 g, 13.2 mmol) at 0 °C. The stirred reactants are allowed to warm up to room temperature and, after 16 h, saturated aqueous sodium hydrogen carbonate (2 ml) is added to the mixture. After a further period of 15 min, the mixture is concentrated under reduced pressure, partitioned between chloroform (100 ml) and saturated aqueous sodium hydrogen carbonate (30 ml) and the dried (magnesium sulphate) chloroform layer evaporated under reduced pressure. The residue is then fractionated by short column chromatography using chloroform/ethanol (98:2 to 96:4, v/v) as eluent. The appropriate fractions are combined and evaporated under reduced pressure to give a glass. The material is dissolved in dioxan/methanol (1:1 v/v, 15 ml), and the stirred solution is cooled to 0 °C (ice/water bath). 4.6 Molar methanolic sodium methoxide (5 ml, 23 mmol) is added and the cooling bath is then removed. After 6 min, the products are neutralised with an excess of Dowex 50 (pyridinium form) cation exchange resin and then concentrated under reduced pressure. The resultant material is fractionated by short column chromatography on silica gel (eluent: chloroform/ethanol, 95:5 to 91:9, v/v). The appropriate fractions are combined, evaporated under reduced pressure, and the residue crystallised from aqueous ethanol to give **1c** as colourless crystals; yield: 1.00 g (54%); m.p. 198–199 °C; R_f [chloroform/methanol (9:1 v/v)]: 0.42.

$C_{22}H_{27}N_3O_8$	calc.	C 57.26	H 5.90	N 9.11
(461.5)	found	57.4	5.8	9.1

U.V. (95% C_2H_5OH): λ_{max} = 260, 305 nm (ε = 19000, 8800); λ_{min} = 230, 285 nm (ε = 8700, 6800).

^{1}H-N.M.R. (250 MHz, DMSO-d_6): δ = 1.77 (m, 4 H); 3.19 (s, 3 H); 3.42 (m, 2 H); 3.65 (m, 3 H); 3.84 (m, 1 H); 4.09 (m, 2 H); 4.23 (dd, J = 4.7, 6.9 Hz, 1 H); 5.30 (m, 1 H); 5.55 (d, J = 5.6 Hz, 1 H); 5.79 (d, J = 2.6 Hz, 1 H); 7.33 (d, J = 7.3 Hz, 1 H); 7.52 (m, 2 H); 7.63 (m, 1 H); 8.01 (m, 2 H), 8.55 (d, J = 7.5 Hz, 1 H); 11.28 ppm (br. s, 1 H).

3′-*O*-(4-Methoxytetrahydropyran-4-yl)-uridine (1d):
A solution of sodium hydrogen sulphite is prepared by dissolving sodium metabisulphite (3.8 g, 20 mmol) in water (20 ml) and adjusting the pH to 5.6 by the addition of 2 molar aqueous sodium hydroxide. Crude 3′-*O*-(4-methoxytetrahydropyran-4-yl)-cytidine (**1b**), prepared from 4-*N*-2′-*O*,5′-*O*-triacetylcytidine (**3**; 1.49 g, 4.03 mmol) in the manner described above, is added to the stirred sodium hydrogen sulphite solution at room temperature. After 18 h, the pH of the solution is adjusted to ~ 7 by the addition of aqueous sodium hydrogen carbonate, and the product is continuously extracted with dichloromethane. The latter extract is dried with magnesium sulphate and concentrated under reduced pressure. Crystallisation of the residue from ethyl acetate gives **1d** as colourless crystals; yield: 0.94 g (65%, based on **3**); m.p. 157.5 °C; R_f [chloroform/methanol (9:1 v/v)]: 0.19 [R_f of 2′-*O*-(4-methoxytetrahydropyran-4-yl)-uridine[2]: 0.15].

$C_{15}H_{21}N_2O_8$	calc.	C 50.27	H 6.19	N 7.82
(358.3)	found	50.4	6.05	7.8

U.V. (95% C_2H_5OH): λ_{max} = 262 nm (ε = 9700); λ_{min} = 231 nm (ε = 2300).

^{1}H-N.M.R. (250 MHz, DMSO-d_6): δ = 1.77 (m, 4 H); 3.19 (s, 3 H); 3.43 (m, 2 H); 3.56 (m, 1 H); 3.70 (m, 3 H); 3.98 (m, 1 H); 4.07 (m, 1 H); 4.22 (m, 1 H); 5.20 (br., 1 H); 5.40 (d, J = 6.3 Hz, 1 H); 5.65 (d, J = 8.1 Hz, 1 H); 5.76 (d, J = 5.1 Hz, 1 H); 7.92 (d, J = 8.1 Hz, 1 H); 11.34 ppm (br., 1 H).

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