2′,3′-*O*-Methylene Derivatives of Ribonucleosides

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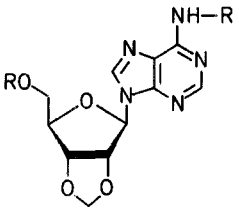
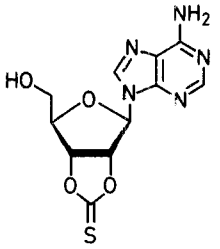
In the course of some studies concerned with the synthesis[1] and biological properties[2] of analogues of 5′-*O*-triphosphoryladenylyl-(2′ → 5′)-adenylyl-(2′ → 5′)-adenosine [2-5A], we required 2′,3′-*O*-methyleneadenosine (**1a**) as a starting material. A search of the literature revealed that the latter compound **1a** had been obtained, but only in 4.1% yield[3] as a product from the reaction between adenosine 2′,3′-*O*-thionocarbonate (**2**) and sponge nickel. As we were unable to find any other information relating to the preparation and properties of 2′,3′-*O*-methylene derivatives of ribonucleosides, we have carried out some studies in this area ourselves.



1a R = H
b R = $(C_6H_5)_3C$



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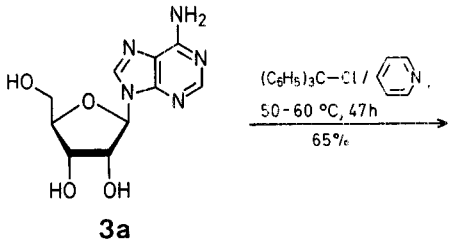
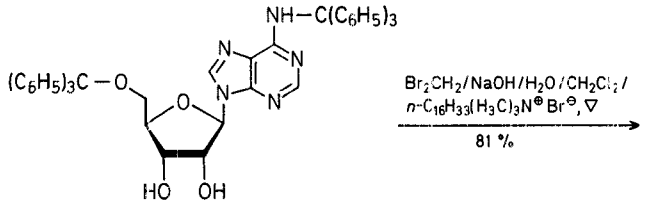
While a variety of cyclic acetal and orthoester groups have been used[4,5] to protect 1,2- and 1,3-diol systems in organic synthesis, the parent methylene group has been used[6] only very rarely for this purpose. This is due partly to the relative difficulty in preparing methylene derivatives, but more particularly to the rather drastic conditions required[6] to remove methylene protecting groups. Methylene acetals have been prepared by treating diols either (a) with formaldehyde[7] (or an equivalent, such as paraformaldehyde) in the presence of an acid catalyst or (b) with dibromomethane[8] (or dichloromethane) in the presence of base. As the acidic conditions required[7] for procedure (a) are somewhat drastic, especially for the derivatisation of adenosine, procedure (b) appeared to be more promising for the present purpose.

In the case of adenosine (**3a**), the phase transfer methylenation procedure[9] proved to be effective. Adenosine was first converted into its 5′-*O*-6-*N*-bis[triphenylmethyl] derivative **3b**, in 65% yield, by the literature procedure[10]. When a solution of **3b** and dibromomethane in dichloromethane and a large excess of 50% aqueous sodium hydroxide were stirred together in the presence of a catalytic quantity of cetyltrimethylammonium bromide, with gentle heating, 2′,3′-*O*-methylene-5′-*O*,6-*N*-bis[triphenylmethyl]adenosine (**1b**) was obtained. The latter compound **1b** was then heated in 80% acetic acid solution, under gentle reflux, for 10 min to give 2′,3′-*O*-methyleneadenosine (**1a**) as a crystalline solid isolated in 50% yield (for the two steps starting from **3b**).

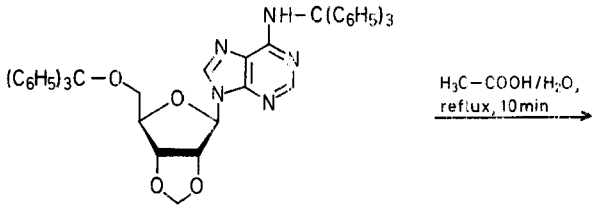
In the preparation of 2′,3′-*O*-methyleneuridine (**5a**), the readily available 5′-*O*-(triphenylmethyl)-uridine[11] (**4b**) was methylenated by allowing it to react with dibromomethane and powdered potassium hydroxide in anhydrous dimethyl sulphoxide[12] at room temperature. The intermediate 2′,3′-*O*-methylene-5′-*O*-(triphenylmethyl)-uridine (**5b**) was isolated in 49% yield and was then heated in 80% acetic acid so-



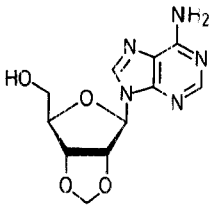
3a



3b

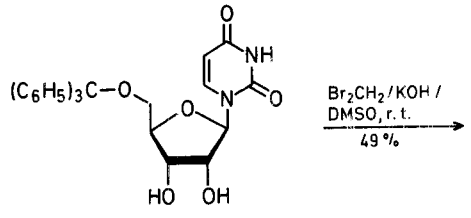


1b

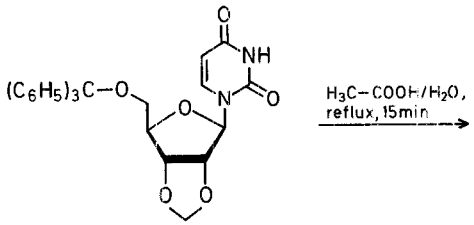


1a (50% from **3b**)

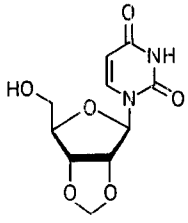
lution, unter reflux, for 15 min. The desired 2',3'-*O*-methyleneuridine (**5a**) thus obtained, was isolated in 46% overall yield (for the two steps starting from **4b**).



4b

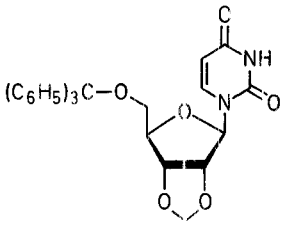


5b

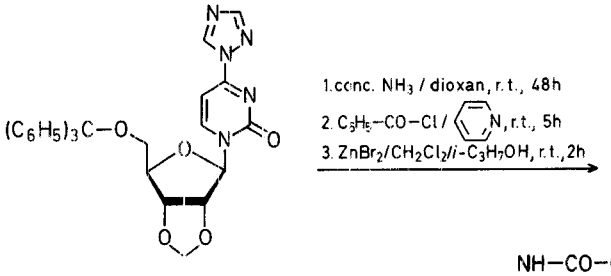


5a (46% from **4b**)

It was decided to attempt the preparation of 2',3'-*O*-methylenecytidine (**7a**), or rather its 4-*N*-benzoyl derivative **7b** from an appropriate uridine precursor. 2',3'-*O*-Methylene-5'-*O*-(triphenylmethyl)-uridine (**5b**) was treated with the products[13] of the reaction between phosphoryl chloride (~ 2 mol equiv), 1,2,4-triazole (9 mol equiv), and triethylamine (8 6 mol equiv) in acetonitrile solution. The putative triazole derivative[13] (**6**) obtained was allowed to react with concentrated aqueous ammonia in dioxan solution, and the products treated first with benzoyl chloride in pyridine solution and then with zinc bromide in dichloromethane/isopropanol (85 : 15 v/v)[14] to give 4-*N*-benzoyl-2',3'-*O*-methylenecytidine **7b**. The latter compound **7b** was isolated as a crystalline solid in 30% overall yield for the five steps starting from 5'-*O*-(triphenylmethyl)-uridine **4b**. The relatively low overall yield of **7b** is due mainly to the modest yield obtained in the conversion (see above) of **4b** into **5b**.



5b



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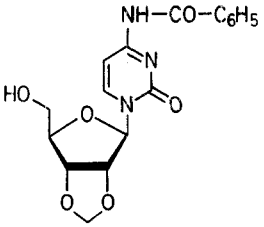

7b

The three 2',3'-*O*-methylene ribonucleoside derivatives (**1a**, **5a**, and **7b**) were characterized on the basis of microanalytical and spectroscopic data. The chemical shifts of the methylene proton and carbon resonances in the N.M.R. spectra (Table) of the derivatives are of particular note. It can be seen that the methylene protons resonate either as one or two singlets at δ = ~ 5.1 ppm, and that the methylene carbons resonate at δ = ~ 95 ppm.

Table. N.M.R. Chemical Shifts of Methylene Proton and Carbon Resonances[a]

Compound	^{1}H-N.M.R. (DMSO-d_6) δ_{O-CH_2-O} [ppm]	^{13}C-N.M.R. (DMSO-d_6) δ_{O-CH_2-O} [ppm]
1a	5.17 (s); 5.19 (s)	95.08
5a	5.05 (s); 5.09 (s)	94.70
7b	5.08 (s)	94.77

[a] Bruker WM 250 spectrometer.

Finally, the action of dilute hydrochloric acid on 2′,3′-*O*-methyleneuridine (**5a**) was investigated. When (**5a**) was heated in 1.0 molar hydrochloric acid solution at 90 °C, it was converted mainly into uridine (**4a**). The reaction initially appeared to exhibit pseudo first-order kinetics: $t_{\frac{1}{2}}$ for the conversion of (**5a**) into (**4a**) was found to be ca. ~ 4 h. However, cleavage of the glycosidic linkage was observed before hydrolysis of the methylene acetal system was complete. Thus, after 7 h, H.P.L.C. analysis of the hydrolysate revealed the presence of starting material **5a** (~ 16%), uridine (**4a**; ~ 78%), and uracil (~ 5%). As the glycosidic linkages of pyrimidine ribosides are known[15] generally to be much more stable to acidic hydrolysis than the glycosidic linkages of purine ribosides, the methylene acetal system is unlikely to be of use a protecting group in ribonucleoside chemistry.

^{1}H-N.M.R. spectra were measured at 250 MHz with a Bruker WM 250 spectrometer; tetramethylsilane 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 which were developed in solvent system A [chloroform/methanol (9 : 1 v/v)]. Merck silica gel H was used for short column chromatography. H.P.L.C. was carried out on a Jones Apex ODS column which was eluted isocratically with water/methanol (88.5 : 11.5 v/v). Pyridine, triethylamine, and dioxan were dried by heating, under reflux, with calcium hydride; these solvents were then distilled at atmospheric pressure. Dimethyl sulphoxide was dried by distillation from calcium hydride under reduced pressure.

2′3′-*O*-Methyleneadenosine (1a):

A solution of adenosine (**3a**; 2.0 g, 7.5 mmol) and chlorotriphenylmethane (6.3 g, 22.6 mmol) in pyridine is stirred at 50–60 °C for 47 h. The cooled products are then poured into a rapidly stirred solution of sodium hydrogen carbonate (2.5 g) in ice/water (300 g). The resulting precipitate is collected by filtration, washed with cold water, and then dried. Fractionation of this material by short column chromatography on silica gel gives 5′-*O*,6-*N*-bis[triphenylmethyl]adenosine (**3b**); yield: 3.68 g (65%); R_f (solvent system A): 0.76; m.p. 210–214 °C (from ethyl acetate) (Lit.[10], m.p. 213–215 °C).

^{1}H-N.M.R. (DMSO-d_6, 250 MHz): δ = 3.20 (m, 2 H); 4.06 (m, 1 H); 4.29 (m, 1 H); 4.73 (m, 1 H); 5.24 (m, 1 H); 5.56 (m, 1 H); 5.91 (d, J = 4.6 Hz, 1 H); 7.2–7.4 (m, 30 H); 7.49 (s, 1 H); 7.82 (s, 1 H); 8.36 ppm (s, 1 H).

A solution of sodium hydroxide (30 g, 0.75 mol) in water (30 ml) is added dropwise over a period of 20 min to a stirred solution of **3b** (7.51 g, 10.0 mmol), dibromomethane (21.0 ml, 0.30 mol), and cetyltrimethylammonium bromide (0.34 g, 0.93 mmol) in dichloromethane (80 ml), under gentle reflux. After a further period of 4.5 h, the organic layer is separated, washed with water (2 × 50 ml), and dried with magnesium sulphate. The products are then concentrated under reduced pressure and the residue is purified by short column chromatography on silica gel. The appropriate fractions are combined and concentrated under reduced pressure. When a solution of the residue in chloroform (10 ml) is added dropwise to stirred petroleum ether (b.p. 30–40 °C; 250 ml), 2′,3′-*O*-methylene-5′-*O*,6-*N*-bis[triphenylmethyl]adenosine (**1b**) is precipitated; yield: 6.18 g (81%).

A solution of **1b** (3.77 g, 4.94 mmol) in acetic acid/water (4 : 1 v/v; 40 ml) is heated, under reflux, for 10 min. The cooled products are then poured into ice/water (110 g). The resulting mixture is extracted with chloroform/pyridine (9 : 1 v/v; 6 × 70 ml), and the combined extracts are washed with saturated aqueous sodium hydrogen carbonate (70 ml), and then dried with magnesium sulphate. After evaporation of the solvents under reduced pressure, the residual glass is triturated with petroleum ether (b.p. 40–60 °C, 150 ml) and then crystallized from methanol to give **1a** as a crystalline solid; yield: 0.86 g (50%, based on **3b**); m.p. 205–207 °C (Lit.[3], m.p. 201.5–205 °C); R_f (solvent system A): 0.46.

$C_{11}H_{13}N_5O_4$	calc.	C 47.31	H 4.69	N 25.08
(279.3)	found	47.4	4.7	25.1

U.V. (95% C_2H_5OH): λ_{max} = 259 (ε = 15000); λ_{min} = 228 nm (ε = 2000).

^{1}H-N.M.R. (250 MHz, DMSO-d_6): δ = 3.57 (m, 2 H); 4.16 (m, 1 H); 4.91 (dd, J = 3.7 Hz, 6.4 Hz, 1 H); 5.14 (s, 1 H); 5.17 (s, 1 H), 5.19 (m, 1 H); 5.31 (dd, J = 3.2 Hz, 6.4 Hz, 1 H); 6.13 (d, J = 2.8 Hz, 1 H); 7.38 (br.s, 2 H); 8.16 (s, 1 H); 8.35 ppm (s, 1 H).

2′,3′-*O*-Methyleneuridine (5a):

Dry powdered potassium hydroxide (3.58 g, 64 mmol) is added to a solution of 5′-*O*-(triphenylmethyl)-uridine (**4b**; 3.876 g, 7.97 mmol) and dibromomethane (2.78 g, 16.0 mmol) in anhydrous dimethyl sulphoxide (16 ml) at room temperature. After 1.5 h, dichloromethane (40 ml) is added and the products are filtered through celite. The filtrate is washed with water (2 × 30 ml) and saturated brine (30 ml), and then dried with magnesium sulphate. After evaporation of the solvent, the residue is purified by short column chromatography on silica gel. The appropriate fractions are combined and concentrated under reduced pressure. When a chloroform (10 ml) solution of the residue obtained is added dropwise to stirred petroleum ether (b.p. 30–40 °C, 200 ml), 2′,3′-*O*-methylene-5′-*O*-(triphenylmethyl)-uridine (**5b**) is precipitated; yield: 1.95 g (49%).

A solution of **5b** (0.499 g, 1.0 mmol) in acetic acid/water (4 : 1 v/v; 20 ml) is heated, under reflux, for 15 min and the products are concentrated under reduced pressure. After the residue has been evaporated from ethanol (3 × 20 ml) solution, it is fractionated by short column chromatography on silica gel. The appropriate fractions are combined, evaporated under reduced pressure, and the residue crystallized from ethanol to give **5a** as a colourless crystalline solid; yield: 0.241 g (46%, based on **4b**); m.p. 157.5 °C; R_f (solvent system A): 0.46.

$C_{10}H_{12}N_2O_6$	calc.	C 46.88	H 4.72	N 10.93
(256.2)	found	46.65	4.8	10.8

U.V. (95% C_2H_5OH): λ_{max} = 260 (ε = 9700); λ_{min} = 229 nm (ε = 2300).

^{1}H-N.M.R. (250 MHz, DMSO-d_6): δ = 3.61 (m, 2 H); 4.01 (m, 1 H); 4.71 (dd, J = 4.1 Hz, 6.9 Hz, 1 H); 4.83 (dd, J = 3.2 Hz, 6.9 Hz, 1 H); 5.05 (s, 1 H); 5.09 (s, 1 H); 5.1 (m, 1 H); 5.66 (d, J = 8.3 Hz, 1 H); 5.81 (d, J = 2.8 Hz, 1 H); 7.76 (d, J = 8.3 Hz, 1 H); 11.40 ppm (br.s, 1 H).

4-*N*-Benzoyl-2′,3′-*O*-methylenecytidine (7b):

Triethylamine (2.4 ml, 17.2 mmol) is added dropwise to a stirred solution of 1,2,4-triazole (1.243 g, 18.0 mmol) and phosphoryl chloride (0.36 ml, 3.9 mmol) in anhydrous acetonitrile (10 ml) at 0 °C. The products are allowed to warm up to room temperature and a solution of **5b** (0.997 g, 2.0 mmol) in dry acetonitrile (6 ml) is added with constant stirring. After 90 min, triethylamine (1.66 ml, 11.9 mmol) and water (0.42 ml, 23.3 mmol) are added and, after a further period of 10 min, the products are concentrated under reduced pressure. The residue obtained is dissolved in chloroform (20 ml), the solution extracted with saturated aqueous sodium hydrogen carbonate (30 ml), dried with magnesium sulphate, and then evaporated under reduced pressure to give the solid *triazole derivative* **6**.

The latter material is dissolved in dioxan (10 ml), and concentrated aqueous ammonia (*d* 0.88; 1.6 ml, ~ 29 mmol) is added. The resulting solution is stirred at room temperature for 48 h, concentrated under reduced pressure, and then dried by evaporation from ethanol (3 × 10 ml) solution. The residue obtained is purified by short column chromatography on silica gel. The appropriate fractions are combined and concentrated under reduced pressure. When a chloroform (10 ml) solution of the purified material is added dropwise to stirred petroleum ether (b.p. 30–40 °C, 150 ml), *2′,3′-O-methylene-5′-O-(triphenylmethyl)-cytidine* is precipitated; yield: 0.776 g (78%).

Benzoyl chloride (0.281 g, 2.0 mmol) is added to a stirred solution of the latter compound (0.70 g, 1.4 mmol) in anhydrous pyridine (15 ml) solution at room temperature. After 5 h, saturated aqueous sodium hydrogen carbonate solution (1 ml) is added, and the solution is stirred for a further period of 10 min. The products are

concentrated under reduced pressure and partitioned between chloroform (20 ml) and saturated aqueous sodium hydrogen carbonate (30 ml). The dried (magnesium sulphate) chloroform layer is concentrated under reduced pressure, and the residue is dissolved with stirring in a 1 molar solution of zinc bromide in dichloromethane/isopropanol (85 : 15 v/v; 40 ml) at room temperature. After 2 h, the products are poured slowly into stirred 1.0 molar aqueous ammonium acetate solution (100 ml). The layers are separated and the aqueous layer is extracted with dichloromethane (5 × 30 ml). The combined organic layers are dried with sodium sulphate and concentrated under reduced pressure. The residue obtained is purified by short column chromatography on silica gel. The appropriate fractions are combined and evaporated under reduced pressure. Crystallization of the residue from ethanol/ethyl acetate gives **7b** as colourless crystals; yield: 0.402 g (30%, based on **4b**); m.p. 214–215°C; R_f (solvent system A): 0.63.

$C_{17}H_{17}N_3O_6$	calc.	C 56.82	H 4.77	N 11.70
(359.3)	found	56.6	4.8	11.7

U.V. (95% C_2H_5OH): λ_{max} = 303, 260 (ε = 9600, 24500), λ_{min} = 287, 230 nm (ε = 8100, 9600).

^{1}H-N.M.R. (250 MHz, DMSO-d_6): δ = 3.67 (m, 2H); 4.17 (m, 1H); 4.75 (dd, J = 3.6 Hz, 6.5 Hz, 1H); 4.87 (dd, J = 2.3 Hz, 6.5 Hz, 1H); 5.08 (s, 2H); 5.87 (d, J = 2.3 Hz, 1H); 7.34 (d, J = 7.4 Hz, 1H); 7.54 (m, 2H); 7.61 (m, 1H); 8.01 (m, 2H); 8.26 (d, J = 7.5 Hz, 1H); 11.33 ppm (br.s, 1H).

Acidic Hydrolysis of 2′,3′-*O*-Methyleneuridine (5a):

2′,3′-*O*-Methyleneuridine (**5a**; 0.002 g) is dissolved in 1.0 molar hydrochloric acid (5 ml) and the solution is maintained at 90°C. After suitable intervals of time aliquots are removed, neutralized with aqueous triethylammonium hydrogen carbonate buffer (pH 7.5), and analyzed by H.P.L.C. The hydrolysis raction is found initially to display pseudo first order kinetics: $t_{\frac{1}{2}}$ for the conversion of the substrate **5a** (R_T: 6.3 min) into uridine (R_T: 2.7 min) is found to be ~ 4 h. A very small quantity of uracil (R_T: 2.3 min) is detected after 45 min; after 7 h, H.P.L.C. analysis reveals the presence of starting material **5a** (~ 16%), uridine (~ 78%), and uracil (~ 5%).

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