

# Synthesis of Adenylyl-(3'-5')-3'-O-methylguanosine and of Guanylyl-(3'-5')-3'-O-methylguanosine

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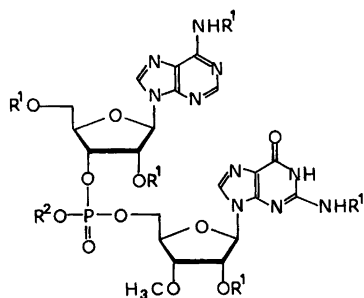
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The synthesis of adenylyl-(3'-5')-3'-O-methylguanosine and of guanylyl-(3'-5')-3'-O-methylguanosine, required for testing as potential inhibitors of replication in RNA-viruses are described.

Ribonucleotides with the 3'-end suitably blocked were required for evaluation as possible inhibitors of replication in RNA-viruses. In a previous paper we have described a straightforward synthesis of 2'-O- and 3'-O-methylguanosine.<sup>1</sup> We now report the synthesis of two dinucleotides containing 3'-O-methylguanosine.

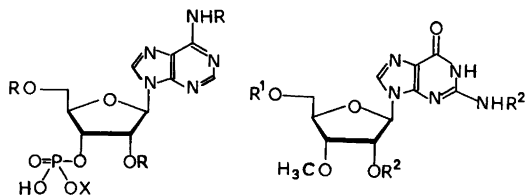
Adenosine 3'-phosphate (1) was converted into *N*<sup>6</sup>,2',5'-O-tribenzoyladenine 3'-(triethylammonium phosphate) (2) which was reacted with *N*<sup>2</sup>,2'-O-dibenzoyl-3'-O-methylguanosine (4), prepared by desilylation with fluoride anion of the corresponding silyl ether (3). The product was converted *in situ* into the triester 5, which was more readily purified by silica gel column chromatography than the corresponding phosphodiester. Blocking groups were removed from the phosphotriester 5 to yield adenylyl-(3'-5')-3'-O-methylguanosine as the triethylammonium salt 6, which was converted into the

corresponding sodium salt 7. The overall yield in the synthesis of 6 from 1 was 27%. An isomer of 4, *N*<sup>2</sup>,3'-O-dibenzoyl-2'-O-methylguanosine was prepared following the analogous synthetic route from 5'-O-*tert*-butyldiphenylsilyl-2'-O-methylguanosine.



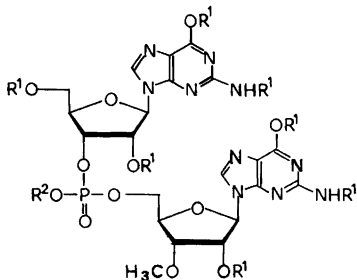
- 5  $R^1 = \text{COC}_6\text{H}_5$ ,  $R^2 = \text{CH}_2\text{CH}_2\text{CN}$   
 6  $R^1 = \text{H}$ ,  $R^2 = \text{HN}(\text{C}_2\text{H}_5)_3$   
 7  $R^1 = \text{H}$ ,  $R^2 = \text{Na}$

Guanosine-3'-phosphate disodium salt (8) was converted into *N*<sup>2</sup>,*O*<sup>6</sup>,2',5'-O-tetrabenzoylguanosine 3'-(triethylammonium phosphate) 9, which was allowed to react with the guanosine derivative 4. The product 10 was transformed into the sodium salt



- 1  $R = \text{H}$ ,  $X = \text{H}$   
 2  $R = \text{COC}_6\text{H}_5$ ,  
 $X = \text{HN}(\text{C}_2\text{H}_5)_3$   
 3  $R^1 = \text{Si}(\text{C}_6\text{H}_5)_2\text{C}(\text{CH}_3)_3$ ,  
 $R^2 = \text{COC}_6\text{H}_5$   
 4  $R^1 = \text{H}$ ,  $R^2 = \text{COC}_6\text{H}_5$   
 8  $R = \text{H}$ ,  $X = Y = \text{Na}$   
 9  $R = \text{COC}_6\text{H}_5$ ,  $X = \text{HN}(\text{C}_2\text{H}_5)_3$ ,  
 $Y = \text{H}$

of guanylyl-(3'-5')-3'-*O*-methylguanosine **12** via the intermediate **11**. The overall yield of **12** from **8** was 16 %.



- 10** R<sup>1</sup> = COC<sub>6</sub>H<sub>5</sub>, R<sup>2</sup> = CH<sub>2</sub>CH<sub>2</sub>CN  
**11** R<sup>1</sup> = H, R<sup>2</sup> = H<sub>3</sub>N(CH<sub>2</sub>)<sub>3</sub>CH<sub>3</sub>  
**12** R<sup>1</sup> = H, R<sup>2</sup> = Na

## EXPERIMENTAL

*General methods* were the same as those reported elsewhere.<sup>2,3</sup> <sup>1</sup>H and <sup>13</sup>C NMR spectra (at 99.55 MHz and 25.05 MHz respectively) were recorded using a Jeol JNM FX-100 instrument. Chemical shifts are given in p.p.m. downfield from internal tetramethylsilane for solutions in deuteriochloroform; for solutions in D<sub>2</sub>O, <sup>1</sup>H chemical shifts were recorded in p.p.m. downfield from internal sodium 1,1,2,2,3,3-hexadeuterio-4,4-dimethyl-4-silapentane-1-sulfonate and <sup>13</sup>C chemical shifts in p.p.m. downfield from external tetramethylsilane. Whenever necessary, spectral assignments were corroborated by homonuclear spin decoupling for <sup>1</sup>H NMR and off-resonance decoupling for <sup>13</sup>C NMR. NMR spectra were invariably in agreement with postulated structures. Only especially pertinent NMR data are given below. The coupling constants given in the <sup>13</sup>C NMR spectra (proton decoupled) refer to <sup>13</sup>C-<sup>31</sup>P couplings.

*N<sup>6</sup>,2',5'-O-Tribenzoyladenine 3'-(triethylammonium phosphate) (2)*. Benzoyl cyanide (2.15 g, 16.4 mmol) was added to a stirred solution of adenosine 3'-phosphate (monohydrate (**1**) 1.0 g, 2.7 mmol) and triethylamine (2.28 ml, 16.4 mmol) in anhydrous *N,N*-dimethylformamide (20 ml) at room temperature.<sup>4</sup> After 3 h, when TLC (CHCl<sub>3</sub> - CH<sub>3</sub>OH 7:3) indicated the presence of di- as well as of tribenzoate, more benzoyl cyanide (0.4 g, 3 mmol) and triethylamine (0.4 ml, 3 mmol) were added. After stirring for an additional 2 h, the product was precipitated in diethyl ether (1000 ml). Chromatographic purification on a Sephadex LH 20 column (CH<sub>3</sub>OH) afforded a minor, fast-moving fraction (0.52 g, 24%) consisting of *N<sup>1</sup>,2',5'-O-tribenzoyladenine 3'-(triethylammonium phosphate)*.

<sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 1.23 (9H, tr, *J* 8 Hz, CH<sub>3</sub>CH<sub>2</sub>), 3.00 (6H, q, *J* 8 Hz, CH<sub>3</sub>CH<sub>2</sub>), 5.76 (1H, m, H-3'), 6.31 (1H, dd, *J* 6 Hz), 6.51 (1H, d, *J* 6 Hz, H-1'). <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 63.4 (C-5'), 72.6 (d, C-3'), 73.8 (d, C-2'), 82.0 (d, C-4'), 86.2 (C-1'), 123.4 (C-5), 141.7 (C-8), 149.6 (C-4), 151.5 (C-6), 152.3 (C-2). The major, slower moving fraction (1.60 g, 76%) consisted of 2, [α]<sub>D</sub><sup>21</sup><sub>578</sub> - 23° (c 1.3, CH<sub>3</sub>OH). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 1.21 (9H, tr, *J* 8 Hz, CH<sub>3</sub>CH<sub>2</sub>), 3.00 (6H, q, *J* 8 Hz, CH<sub>3</sub>CH<sub>2</sub>), 6.27 (1H, dd, *J* 5 Hz, H-2'), 6.37 (1H, d, *J* 5 Hz, H-1'). <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 63.5 (C-5'), 72.7 (d, *J* 5.1 Hz, C-3'), 74.1 (d, C-2'), 81.7 (d, *J* 4.8 Hz, C-4'), 86.3 (C-1'), 119.7 (C-5), 139.1 (C-8), 149.5 (C-4), 152.8 (C-2), 155.5 (C-6).

*N<sup>2</sup>,2'-O-Dibenzoyl-3'-O-methylguanosine (4)*. Benzoyl chloride (4.0 ml, 34.4 mmol) was added dropwise with stirring to an ice-cooled solution of 5'-*O-tert*-butyldiphenylsilyl-3'-*O*-methylguanosine **1** (4.0 g, 7.5 mmol) in anhydrous pyridine (50 ml). The solution was allowed to attain room temperature gradually during 4 h. Excess benzoyl chloride was decomposed by adding water and stirring for another 15 min. The product was partitioned between dichloromethane and water and the organic phase was concentrated to dryness. The product **3** (6.0 g, quant.) contained some residual pyridine. Purification by silica gel column chromatography (CHCl<sub>3</sub> - CH<sub>3</sub>OH 95:5) gave pure **3**, [α]<sub>D</sub><sup>21</sup><sub>578</sub> + 7° (c 0.75, CHCl<sub>3</sub>). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 3.38 (3H, s, OCH<sub>3</sub>), 5.87 (1H, dd, *J* 4.4 Hz H-2'), 6.20 (1H, d, *J* 4.4 Hz, H-1'). Tetrabutylammonium fluoride (30 ml, 0.5 M in anhydrous tetrahydrofuran) was added to a solution of **3** (6.0 g, 7.5 mmol) in the same solvent under dry nitrogen at room temperature.<sup>5</sup> After 15 h, the reaction mixture was concentrated and the residue was dissolved in chloroform. The chloroform solution was washed with water to remove ammonium salts and then concentrated to dryness. Crystallization of the residue from methanol yielded **4** (2.30 g). Silica gel column chromatographic purification (CHCl<sub>3</sub> - CH<sub>3</sub>OH 9:1) of the mother liquor afforded more **4** (1.18 g, combined yield 71%). Recrystallized **4** (CH<sub>3</sub>OH) had m.p. 130-135 °C (sintering begins at 70 °C), [α]<sub>D</sub><sup>21</sup><sub>578</sub> - 134° (c 1.1, CHCl<sub>3</sub>). Anal. C<sub>25</sub>H<sub>23</sub>N<sub>5</sub>O<sub>7</sub>: C, H, N. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 3.43 (3H, s, OCH<sub>3</sub>), 5.91 (1H, dd, *J* 5.7 Hz, H-2'), 6.13 (1H, d, *J* 5.7 Hz, H-1').

*N<sup>2</sup>,3'-O-Dibenzoyl-2'-O-methylguanosine* was prepared in a 79% overall yield from 5'-*O-tert*-butyl-2'-*O*-methylguanosine **1** following the analogous route to that described for **4**. The recrystallized (C<sub>6</sub>H<sub>5</sub>OH) title compound had m.p. 241-243 °C, [α]<sub>D</sub><sup>21</sup><sub>578</sub> - 102° (c 1.4, CHCl<sub>3</sub> - CH<sub>3</sub>OH 9:1). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 3.33 (3H, s, OCH<sub>3</sub>), 3.9-4.1 (2H, m, 2x H-5'), 4.46 (1H, d, *J* 1.4 Hz, H-4'), 4.70 (1H, dd, *J* 5.2 and 7.3 Hz, H-2'), 5.83 (1H, dd, *J* 1.4 and 5.2 Hz, H-3'), 5.92 (1H, d, *J* 7.3 Hz, H-1'). Anal. C<sub>25</sub>H<sub>23</sub>N<sub>5</sub>O<sub>7</sub>: C, H, N.

*Adenylyl-(3'-5')-3'-O-methylguanosine sodium salt*

(7). All reagents were previously dried *in vacuo* over phosphorus pentoxide. The phosphate 2 (550 mg, 0.72 mmol) and the nucleoside 4 (550 mg, 1.08 mmol) were condensed in the presence of 2,4,6-triisopropylbenzenesulfonyl chloride (TPS) (660 mg, 2.2 mmol) in anhydrous pyridine (5 ml) under dry nitrogen for 6.5 h at room temperature. 3-Hydroxypropionitrile (0.52 ml, 7.2 mmol) was added and the reaction mixture was left at room temperature overnight. Additional TPS (400 mg) was added and the reaction mixture was left for another 24 h at room temperature.<sup>6,7,8</sup> The product was precipitated in diethyl ether (400 ml) and chromatographically pure 5 (400 mg, 50%) was obtained by means of silica gel column chromatography (CHCl<sub>3</sub>-CH<sub>3</sub>OH 9:1). The dinucleotide 5 (320 mg, 0.27 mmol) was treated with concentrated aqueous ammonia in methanol (8 ml of a 1:1 mixture) and pyridine (2 ml) at room temperature for 2 days. Benzamide was removed by partitioning between water and diethyl ether. The aqueous phase was concentrated. TLC CHCl<sub>3</sub>-CH<sub>3</sub>OH 7:3 and <sup>1</sup>H NMR (D<sub>2</sub>O) showed the presence of residual benzoyl and cyanoethyl groups. The mixture was treated with methanol-concentrated aqueous ammonia (10 ml of a 1:1 mixture) for 4 days. The mixture still contained cyanoethyl group, but only traces of benzoate. After concentration, the mixture was dissolved in methanolic sodium methoxide (30 mol, containing methoxide from the addition of 30 mg sodium) and left overnight at room temperature. The product was converted into the triethylammonium salt 6 by treatment with Dowex 50 W (X8) in the triethylammonium form, analogously to that described for the conversion of deoxynucleoside phosphates into the pyridinium salts.<sup>6</sup> <sup>1</sup>H NMR on 6 thus obtained (200 mg) showed the presence of 1 ml of triethylammonium ion. Purification on a diethylaminoethylcellulose column using a linear gradient of 0→0.2 M aqueous triethylammonium hydrogen carbonate as eluant afforded pure 6 (130 mg). This was dissolved in methanol (25 ml) and treated with a 0.5 M solution of sodium iodide in acetone (1 ml). A slight turbidity was removed by filtration through glass wool. The filtrate was concentrated. The residue was suspended in acetone and washed several times with acetone by centrifugation and decantation. The product 7 was obtained as white amorphous powder (95 mg), [α]<sub>D</sub><sup>25</sup><sub>78</sub> -20° (c 1.0, H<sub>2</sub>O). <sup>1</sup>H NMR (D<sub>2</sub>O, 85 °C): δ 3.49 (3H, s, OCH<sub>3</sub>), 5.80 (1H, d, J 4.6 Hz, H-1' guanosine residue), 5.97 (1H, d, J 4.9 Hz, H-1' adenosine residue). <sup>13</sup>C NMR (D<sub>2</sub>O, ambient temperature): δ for adenosine residue: 62.2 (C-5'), 74.3 (J 4.3 Hz, C-2') 75.4 (J 4.9 Hz, C-3'), 85.4 (J 4.2 Hz, C-4'), 90.2 (C-1'), 120.0 (C-5), 141.4 (C-8), 149.0 (C-4), 113.4 (C-2), 156.3 (C-6); δ for guanosine residue: 59.2 (OCH<sub>3</sub>) 66.3 (J 4.2 Hz, C-5'), 73.7 (C-2'), 80.2 (C-3'),

84.4 (J 9.2, C-4'), 89.1 (C-1'), 117.3 (C-5), 138.2 (C-8), 152.4 (C-4), 154.9 (C-2), 159.6 (C-6).

N<sup>2</sup>,O<sup>6</sup>-2',5'-Tetrabenzoylguanosine 3'-(triethylammonium phosphate) (9). Guanosine 3'-phosphate (8) was converted into the corresponding bis(triethylammonium) salt by treatment with Dowex 50 W (X8) in the triethylammonium form analogously to the conversion of deoxynucleoside phosphates into the corresponding pyridinium salts.<sup>6</sup> The bis(triethylammonium) salt (1.25 g, 2.2 mmol) in *N,N*-dimethylformamide (20 ml) was benzoylated with benzoyl cyanide (4.5 g, 34.4 mmol) and triethylamine (1 ml) with stirring at room temperature for 3 h. The starting material was only sparingly soluble, but dissolution occurred during the reaction. The product was precipitated in diethyl ether (1000 ml) and then purified by chromatography on a Sephadex LH 20 column (methanol) to yield chromatographically homogeneous 9 (1.80 g, 92%) (TLC, CHCl<sub>3</sub>-CH<sub>3</sub>OH 4:1) as an amorphous solid, [α]<sub>D</sub><sup>25</sup><sub>78</sub> +52° (c 1.4, CH<sub>3</sub>OH). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 6.44 (1H, J 4.9 Hz, H-1'). <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 61.2 (C-5'), 71.3 (d, C-3'), 74.8 (C-2'), 78.5 (d, C-4'), 121.1 (C-5), 139.2 (C-8), 147.7 (C-2), 155.4 (C-6).

Guanylyl-(3'-5')-3-O-methylguanosine sodium salt (12). All reagents were previously dried *in vacuo* over phosphorus pentoxide. The phosphate 9 (1.934 g, 2.2 mmol) and the nucleoside 4 (1.520 g, 3.0 mmol) were condensed in the presence of TPS (2.73 g, 9.0 mmol) in anhydrous pyridine (14 ml) under dry nitrogen for 6 h at room temperature. 3-Hydroxypropionitrile (1.37 ml, 20 mmol) was added and the reaction mixture was left at room temperature for 24 h.<sup>7-9</sup> The product was precipitated in diethyl ether (1000 ml), purified twice by column chromatography on Sephadex LH 20 (CH<sub>3</sub>OH) and then partitioned between chloroform and water to give rather impure 10 (2.95 g). Column chromatography of most of this material (2.3 g) on silica gel (CHCl<sub>3</sub>-CH<sub>3</sub>OH 9:1) yielded chromatographically pure but somewhat discoloured 10 (450 mg corresponding to a yield of 20%). Compound 10 (420 mg, 0.32 mmol) in methanol (20 ml) was treated with *n*-butylamine (20 ml) at room temperature for 3 days.<sup>7</sup> The product was concentrated to yield crude 11 (225 mg) after partitioning between water and diethyl ether to remove *N*-butylbenzamide. Attempted purification of 11 on a diethylaminoethylcellulose column failed due to irreversible absorption. The crude 11 was therefore purified by column chromatography on Sephadex G-25 (1% aqueous pyridine) to give 11 (182 mg). This material was dissolved in methanol (30 ml) and treated with a 0.5 M solution of sodium iodide in acetone (1 ml). The product was worked up as described for 7 above to yield 12 (174 mg, 74% from 10), [α]<sub>D</sub><sup>25</sup><sub>78</sub> +37° (c 2.0, H<sub>2</sub>O). <sup>1</sup>H NMR (D<sub>2</sub>O, 85 °C): δ 5.74 and 5.80 (each 1H, J 4 Hz and 6 Hz respectively, H-1' for two guanosine residues). Due to gelation

over extended periods in water, a  $^{13}\text{C}$  NMR spectrum was unobtainable.

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