A Convenient Method for the Synthesis of Adenylyl-(2′—5′)-adenylyl-(2′—5′)-adenosine Using 3′-*O*-Benzoyladenosine Derivatives

Hiroshi TAKAKU* and Souichi UEDA

Laboratory of Organic Chemistry, Chiba Institute of Technology, Tsudanuma, Narashino, Chiba 275

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N^6,3′-*O*-Dibenzoyladenosine was prepared by treating 2′,3′-*O*-(dibutylstannylene)-N^6-benzoyladenosine with benzoyl chloride in methanol. The trimeric adenylyl-(2′—5′)-adenylyl-(2′—5′)-adenosine was synthesized in a good yield using N^6,3′-*O*-dibenzoyladenosine as the starting material *via* the phosphotriester approach.

It has recently been reported[1] that 5′-*O*-triphosphoryl-adenylyl-(2′—5′)-adenylyl-(2′—5′)-adenosine (pppA$^{2'}$p$^{5'}$A$^{2'}$p$^{5'}$A) is isolated from interferon-treated cells and is acting as an inhibitor of cell-free protein synthesis. The pppA$^{2'}$p$^{5'}$A$^{2'}$p$^{5'}$A is synthesized from rabbit reticulocytes with double-stranded RNA and ATP. The core A$^{2'}$p$^{5'}$A$^{2'}$p$^{5'}$A has also been found to be an inhibitor of Concanavalin A-stimulated DNA synthesis.[2] Consequently, several groups have recently described the chemical synthesis of various structurally modified 2′—5′ linked triadenylates.[3]

In this paper, we report a convenient method for the synthesis of adenylyl-(2′—5′)-adenylyl-(2′—5′)-adenosine (2—5 core) by using N^6,3′-*O*-dibenzoyladenosine as the starting material.

Preparation of N^6,*3′*-O-*Dibenzoyladenosine.* Moffatt and his co-workers have recently reported[4] that when 2′,3′-*O*-stannylene nucleosides were treated with benzoyl chloride and triethylamine in dry methanol, 3′-*O*-benzoylnucleoside derivatives were obtained in good yields. This method may serve for the preparation of 3′-*O*-protected adenosine derivative as a key intermediate for the synthesis of 2′—5′ adenylate trimers.

We examined the preparation of N^6,3′-*O*-dibenzoyladenosione **3** by a modification of the procedure of Moffatt and his co-workers[4]. 2′,3′-*O*-(Dibutylstannylene)-N^6-benzoyladenosine (**2**) was easily obtained as a crystalline product in 56% yield by the reaction of N^6-benzoyladenosine (**1**) with dibutyltin oxide in dry methanol, and by subsequent crystallization of the product from ethanol–acetone. In this reaction, a very small ammount of debenzoylated product can be detected on TLC. However, the by-product could be separated from **2** since it was removed by crystallization from ethanol–acetone.

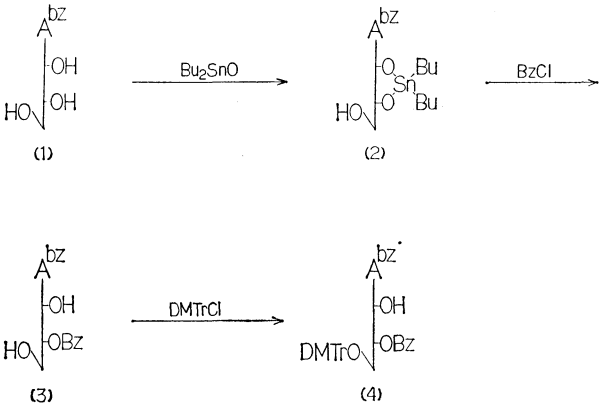
Nucleoside derivative **2** thus obtained (1.0 molar equiv.) was treated with benzoyl chloride (5.0 molar equiv.) in the presence of triethylamine (5.0 molar equiv.) in dry methanol at room temperature. After 15 min, the reaction mixture was worked up and purified to give N^6,3′-*O*-dibenzoyladenosine (**3**) in 48% yield. Product **3** was characterized by elemental analysis and NMR spectroscopy. ^{1}H-NMR spectra of **3** showed the characteristic downfield of the H-1′ and H-3′ signals, and the presence of signals due to the $C_{2'}$–OH and $C_{5'}$–OH groups. For H-1′ of **3**, the data were consistent with the rules developed by Reese and his co-workers;[5] namely 3′-substituted compounds had larger $J_{1',2'}$ coupling constants, and their H-1′ signal showed high field shifts compared to that of the 2′-isomar.

The reaction of **3** with dimethoxytrityl chloride in dry pyridine gave corresponding 5′-*O*-dimethoxytrityl-N^6-dibenzoyladenosine (**4**) in 86% yield.

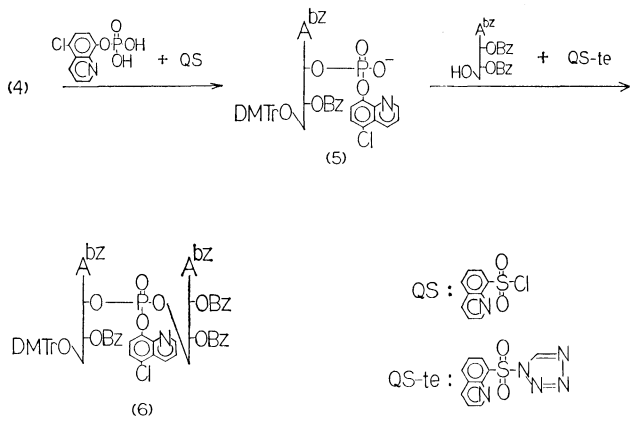
When **3** was treated with methanolic ammonia for 16 h at 25 °C, the corresponding adenosine was obtained in 97% yield.

Synthesis of Adenylyl-(2′—5′)-adenylyl-(2′—5′)-adenosine (Core). The synthesis of 2′—5′ linked triadenylates (2—5 core) **8** by using **4** is as follows: The nucleoside derivative **4** was phosphorylated with 5-chloro-8-quinolyl phosphate[6] in the presence of 8-quinolylsulfonyl chloride (QS)[7] in dry pyridine for 2 h to give correspondong phosphodiester **5** in almost quantitative yield. The ^{31}P NMR spectrum of **5** (pyridine) consists of one resonance signal at δ+4.68.

Phosphodiester intermediate **5** thus obtained (1.5 molar equiv.) was now allowed to react together with N^6, 2′,3′-*O*-tribenzoyladenosine (1.0 molar equiv.) and 1-(8-quinolylsulfonyl)-1*H*-tetrazole (QS-te)[8] After 1 h, no starting material, N^6,2′,3′-*O*-tribenzoyladenosine



Scheme 1.



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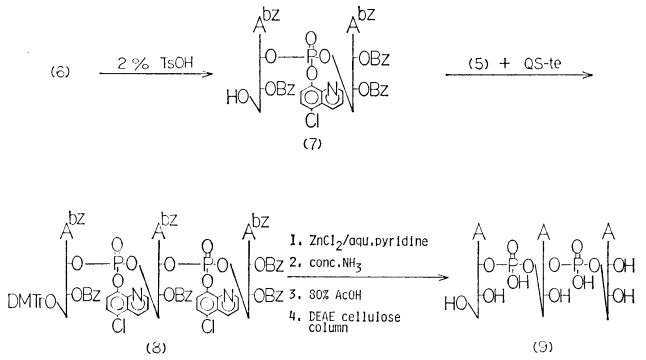

Scheme 3.

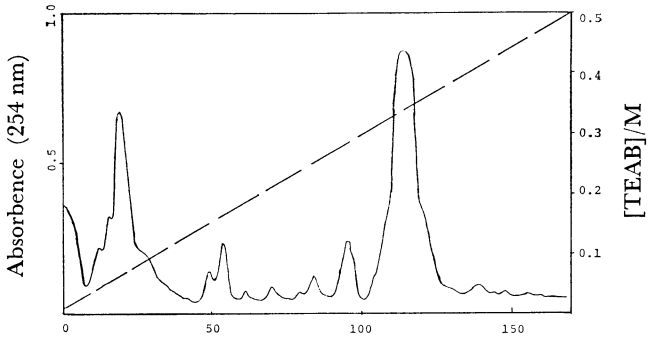

Fig. 1. Chromatography of the trinucleotide on a column (1.0×55 cm) of DEAE cellulose DE-52 equilibrated with 0.01 M TEAB. Elution was performed with a linear gradient of TEAB (0.01—0.5 M, total 550 ml). Fractions of 3 ml were collected every 10 min. The main peak contained the product $A^{2'}p^{5'}A^{2'}p^{5'}A$.

can be detected on TLC in the reaction mixture. Work-up and purification by short silica-gel column chromatography afforded the required dinucleotide **6** in 92% yield. Fully protected dinucleotide **6** was treated with 2% *p*-toluenesulfonic acid solution in a mixture of dichloromethan and methanol (7:3 v/v) for 15 min at 0 °C.[6] The reaction mixture was neutralized with phosphate buffer (pH 8) solution and the mixture was worked up and precipitated with hexane and ether (95:5 v/v) to give partially protected dinucleotide **7** in 92% yield. Compound **7** was used for the next coupling reaction without further purification. Phosphodiester intermediate **5** (1.5 molar equiv.) was condensed with **7** (1.0 molar equiv.) in the presence of QS-te (4.5 molar equiv.) in dry pyridine. After 1 h, the reaction was nearly completed as judged by TLC, and the reaction mixture was worked up and chromatograph to give the fully protected trinucleotide ($A^{2'}p^{5'}A^{2}p^{5'}A$) (core) **8** in 93% yield.

In the above reaction, 1-(8-quinolylsulfonyl)-1*H*-tetrazole (QS-te) can be effectively used as the coupling agent for the synthesis of internucleotidic bonds.

The protecting groups of **8** was removed in the following order to obtain **9**. Treatment of **9** with zinc chloride (25 parts) in aqueous pyridine for 24 h at room temperature removed completely 5-chloro-8-quinolyl protective groups. The resulting compound which contains soley phosphodiester bonds was then treated with concentrated aqueous ammonia for 5 h at 50 °C to cleave the benzoyl groups. Finally, the treatment with 80% acetic acid solution for 5 min removed the dimethoxytrityl group. $A^{2'}p^{5'}A^{2'}p^{5'}A$ (core) **9** thus obtained was then purified through a DEAE cellulose DE-52 column with a linear gradient of 0.01—0.5 M† triethylammonium hydrogencarbonate (TEAB, pH 7.5) (Fig. 1), and the yield was *ca.* 73%.

The purity of **9** was checked by TLC, paper chromatography, and paper electrophoresis. The presence of only 2′—5′ internucleotidic bonds in the completely deblocked product thus obtained was established by complete digestion of **9** with snake venom phosphodiesterase to the expected products in the correct ratio, and it was insensitive to nuclease P1 which cleaves only 3′—5′ linkage.

Experimental

UV absorption spectra were measured with a Shimadzu Model UV-200 spectrophotometer. Proton nuclear magnetic resonance spectra at 100 MHz were taken with JEOL JNMPS 100 spectrometer; the shifts are given in ppm (δ) relative to tetramethylsilane as an internal standard. Merck silica gel $60F_{254}$ plates were usually developed in solvents A [CH_2Cl_2–MeOH (9:1 v/v)], and B [C_6H_6–$(CH_3)_2CO$ (1:1 v/v)]. DC-Alufolien cellulose F sheets were used for TLC. Paper chromatography was performed by using the descending technique on Toyo Roshi No. 51A. The solvent systems employed were the following: solvent C [2-propanol–concentrated aqueous ammonia–water (7:1:2 v/v)], solvent D [1-propanol–concentrated aqueous ammonia–water (55:35:10 v/v)], and solvent E [1-butanol–acetic acid–water (5:2:3 v/v)]. Paper electrophoresis was carried out on Toyo Roshi No. 51A paper in 0.05 M triethylammonium hydrogencarbonate (pH 7.5) at 1100 V/40 cm. Kanto Chemical silica gel was used for short column chromatography. Anion exchange chromatography on DEAE cellulose DE-52 was carried out with a linear gradient of triethylammonium hydrogencarbonate (pH 7.5). Compounds containing the dimethoxytrityl group were detected on TLC by spraying the samples with 10% sulfonic acid solution and drying them in a stream of warm air.

8-Quinolylsulfonyl chloride and tetrazole were purchased from the Dojin Chemical Co.

Enzymatic hydrolyses were done as described previously.[8,9]

2′,3′-O-(Dibutylstannylene)-N⁶-benzoyladenosine (2). A suspension of N^6-benzoyladenosine[10] **1** (4.58 g, 12.3 mmol) and dibutyltin oxide (3.07 g, 12.3 mmol) in dry methanol (300 ml) was heated under reflux. After 30 min the solution was evaporated to dryness and the residue was crystallized from ethanol–acetone (1:1 v/v), giving 4.15 g (56%) of **2**: Mp 167—169 °C; UV (MeOH) λ_{max} 278 nm, λ_{min} 247 nm; R_f 0.1 (solvent A); Calcd for $C_{25}H_{21}N_5O_5$: C, 51.94; H, 6.97; N, 12.11%. Found: C, 51.81; H, 7.06; N, 12.17%.

N⁶,3′-O-Dibenzoyladenosine (3). Nucleoside derivative **2** (7.50 g, 12.45 mmol) was co-evaporated with dry methanol (2×15 ml) and dissolved in dry methanol (156 ml). To the solution was added benzoyl chloride (7.7 ml, 62.30 mmol) and triethylamine (8.7 ml, 62.30 mmol). After being kept at room temperature for 15 min, the solution was concentrated and the residue was triturated with ether (3×

† 1 M=1 mol dm^{-3}.

150 ml) and water (3×150 ml), and then dried *in vacuo*. The residue was recrystallized from ethanol to give 2.89 g (48%) of **3**: Mp 195—198 °C; R_f 0.50 (solvent A); UV (MeOH) λ_{max} 278, 228 nm, λ_{min} 248 nm; NMR (DMSO-d_6) δ=3.91 (brs, 2H, H-5′), 4.50 (brd, 1H, $J_{3',4'}$=2 Hz, H-4′), 5.22 (dd, 1H, $J_{1',2'}$=7.5 Hz, $J_{2',3'}$=5.5 Hz, H-2′), 5.59 (t, 1H, C_5'-OH), 5.73 (d, 1H, H-3′), 6.18 (d, 1H, C_2'-OH), 6.30 (d, 1H, $J_{1',2'}$=7.5 Hz, H-1′), 7.65 and 8.12 (m, total 10H, Ar), 8.91 (s, 2H, C_2-H and C_8-H), (The signals at δ=5.59 and 6.18 disappeared on addition of D_2O); Calcd for $C_{24}H_{21}N_5O_6$: C, 60.63; H, 4.45; N, 14.73%. Found: C, 60.40; H, 4.59; N, 14.81%.

*5′-O-Dimethoxytrityl-N⁶-3′-O-dibenzoyladenosine (**4**).* N^6-3′-*O*-Dibenzoyladenosine (**3**) (1.35 g, 2.83 mmol) was co-evaporated with dry pyridine and treated with dimethoxytrityl chloride (1.05 g, 3.10 mmol) in dry pyridine (14 ml) at room temperature. After 3 h, an aliquot was analyzed by TLC to confirm the starting material [R_f 0.50 (solvent A)] tritylated [R_f 0.55 (solvent A)]. The mixture was poured into ice-water (30 ml), extracted with CH_2Cl_2 (3× 30 ml), and the extract was washed with water (3 times). The solution was evaporated after being dried over Na_2SO_4 and the residue was co-evaporated with toluene (3 times). The residue was redissolved in CH_2Cl_2 (10 ml) and the solution was poured into hexane (500 ml). The precipitate was collected and dried in a dessicator over P_2O_5 to give **4** (1.67 g, 76%): UV (MeOH) λ_{max} 278, 231 nm, λ_{min} 256 nm; R_f 0.55 (solvent A); NMR ($CDCl_3$) δ=3.79 (s, 6H, OCH_3), 6.27 (d, 1H, $J_{1',2'}$=6.5 Hz, H-1′), 6.65—8.11 (m, 23H, Ar).

*5′-O-Dimethoxytrityl-N⁶-3′-O-dibenzoyladenosine 3′-(5-Chloro-8-quinolyl) Phosphate (**5**).* Nucleoside **4** (2.40 g, 3.09 mmol) and 5-chloro-8-quinolyl phosphate (1.20 g, 4.65 mmol) were co-evaporated with dry pyridine, and then treated with 8-quinolinesulfonyl chloride (QS) (2.13 g, 9.27 mmol) in dry pyridine (30 ml) for 2 h at room temperature. The phosphodiester product was detected by TLC [R_f 0.05 (solvent A)] and the mixture was quenched with ice-water. The product was extracted with CH_2Cl_2 (3×30 ml), and the extract was washed with 0.1 M triethylammonium hydrog ncarbonate (pH 7.5) (3×30 ml), then with water (3× 30 ml), and dried over Na_2SO_4. The CH_2Cl_2 layer was concentrated to 15 ml, and poured into hexane–ether (9:1 v/v) (400 ml). The precipitate was collected and dried in a dessicator over P_2O_5 *in vacuo* to give the triethylammonium salt of **5** (3.17 g, 98%); UV (MeOH) λ_{max} 280, 236 nm, λ_{min} 256 nm; R_f 0.05 (solvent A); ^{31}P NMR (pyridine, 85% H_3PO_4) δ+4.68.

*Preparation of Fully Protected Dinucleotide (**6**).* Phosphodiester **6** (1.08 g, 1.03 mmol) and N^6,2′,3′-*O*-tribenzoyladenosine (0.39 g, 0.68 mmol) were co-evaporated with dry pyridine, and then treated with 1-(8-quinolylsulfonyl)-1*H*-tetrazole (QS-te) (0.68 g, 2.60 mmol) in dry pyridine (5 ml) for 1 h at room temperature. The reaction mixture was quenched with ice-water, and then extracted with CH_2Cl_2 (3×15 ml). The combined organic extracts were washed with 0.1 M triethylammonium hydrogencarbonate (pH 7.5) (2×20 ml), then with water (2×20 ml), and was dried over Na_2SO_4 and concentrated. The residue was co-evaporated with toluene (3 times), dissolved in CH_2Cl_2, and the solution was applied on a short column of silica gel. Elution was performed with a stepwise gradient of methanol (0—5%) in CH_2Cl_2, and the product was precipitated with hexane–ether (95:5 v/v) (150 ml). The precipitate was collected and dried in a dessicator over P_2O_5 *in vacuo* to give fully protected dinucleotide **6** (1.02 g, 92%): *R* 0.53 (solvent A), 0.81 (solvent B); UV (MeOH) λ_{max} 278, 230 nm, λ_{min} 255 nm.

*Detritylation of Fully Protected Dinucleotide (**6**).* Fully protected dinucleotide **6** (0.79 g, 0.49 mmol) was dissolved in CH_2Cl_2–MeOH (7:3 v/v) (17 ml) containing 2% *p*-toluenesulfonic acid and the solution was kept for 15 min at 0 °C. The detritylated product was detected by TLC [R_f 0.48 (solvent A)] and the reaction mixture was washed with phosphate buffer (pH 8), and then with water (3× 20 ml). The CH_2Cl_2 layer was dried over Na_2SO_4 and evaporated *in vacuo*. The residue was dissolved in CH_2Cl_2 and the solution was poured into hexane–ether (95:5 v/v) (80 ml). The precipitate was collected and dried in a dessicator over P_2O_5 *in vacuo* to give the 5′-hydroxyl dinucleotide **7** (0.59 g, 92%): R_f 0.48 (solvent A), R_f 0.62 (solvent B); UV (MeOH) λ_{max} 278, 232 nm, λ_{min} 255 nm.

*Preparation of Fully Protected Trinucleotide (**8**).* Phosphodiester derivative **5** (0.76 g, 0.72 mmol) and 5′-hydroxyl dinucleotide **7** (0.60 g, 0.40 mmol) were co-evaporated with dry pyridine, and then treated with QS-te (0.47 g, 1.80 mmol) in dry pyridine (4 ml) for 1 h at room temperature. After usual work-up as described in the preparation of **6**, the product was applied on a short column of silica gel. Elution was performed with a stepwise gradient of methanol (0—5%) in CH_2Cl_2 and the desired fully protected trinucleotide was precipitated with hexane–ether (95:5 v/v) (50 ml). The precipitate was collected and dried in a dessicator over P_2O_5 *in vacuo* to give fully protected trinucleotide **8** (0.99 g, 93%): R_f 0.50 (solvent A), R_f 0.75 (solvent B); UV (MeOH) λ_{max} 278, 230 nm, λ_{min} 256 nm.

*Unblocking of Fully Protected Trinucleotide (**8**).* A solution of fully protected trinucleotide **8** (23 mg, 0.01 mmol) and $ZnCl_2$ (1.02 g, 5.0 mmol) in aqueous pyridine (10%) (20 ml) was stirred at room temperature. After 24 h the reaction mixture was treated with Dowex 50W-X2 (pyridinium form), filtered, and the filtrate was concentrated to gum. The gum was redissolved in concentrated aqueous ammonia (20 ml) at 50 °C. After 5 h the solution was evaporated under reduced pressure. The residue was treated with 80% acetic acid (15 ml) for 15 min. The acetic acid was removed by repeated co-evaporation with 1-butanol. The residue was dissolved in 0.01 M triethylammonium hydrogencarbonate (pH 7.5) (50 ml) and the solution was washed with CH_2Cl_2 (2×20 ml). The solution was applied on a column of DEAE cellulose DE-52 (hydrogencarbonate form). The elution profile and conditions are shown in Fig. 1. The appropriate fractions were combined and lyophilized to give the corresponding trinucleotide **9** (252 OD, 73%). The purity of **9** was checked by TLC, paper chromatography, and paper electrophoresis as well as complete hydrolysis by snake venom phosphodiesterase to adenosine (A_{260}=8.44) and adenosine 5′-phosphate (A_{260}=16.80). Ratio of adenosine and adenosine 5′-phosphate was 1.00: 1.94. On the other hand, when it was incubated with nuclease Pl, A²′p⁵′A²′p⁵′A, **9** was recovered in 99.2% yield: R_f 0.21 (solvent D), $R_{f_{pA}}$ 0.71 (solvent D); $R_{f_{pA}}$ 0.78 (0.05 M triethylammonium hydrogencarbonate, pH 7.5); UV (H_2O, pH 7.0) λ_{max} 257 nm.

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