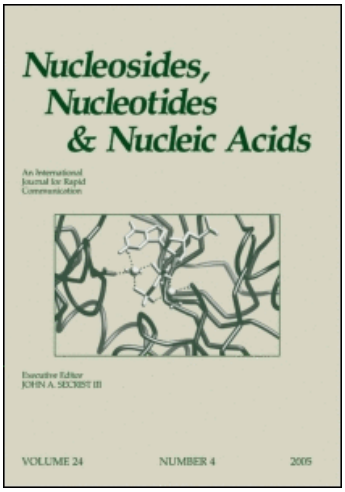

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Partial Protection of Carbohydrate Derivatives. Part 24.[1] Synthesis of Adenylyl-(2'-5')-Adenylyl-(2'-5')-Adenosine Using a 3'-O-(Tetrahydropyran-2-YL) Adenosine Derivative

Kazuo Kamaike[a]; Shun-Ichi Yamakage[a]; Yoshiharu Ishido[a]
[a] Department of Chemistry, Faculty of Science, Tokyo Institute of Technology, Tokyo, Japan

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PARTIAL PROTECTION OF CARBOHYDRATE DERIVATIVES. PART 24.[1] SYNTHESIS OF ADENYLYL-(2' – 5')-ADENYLYL-(2' – 5')-ADENOSINE USING A 3'-O-(TETRAHYDROPYRAN-2-YL)ADENOSINE DERIVATIVE

Kazuo Kamaike, Shun-ichi Yamakage, and Yoshiharu Ishido*

Department of Chemistry, Faculty of Science,
Tokyo Institute of Technology,
O-okayama, Meguro-ku, Tokyo 152, Japan

Abstract: The synthesis of the title compound was performed using a 3'-O-(tetrahydropyran-2-yl)adenosine derivative as the starting material, i.e., a coupling reaction of triethylammonium N^6-benzoyl-5'-O-dimethoxytrityl-3'-O-(tetrahydropyran-2-yl)adenosine 2'-(4-chlorophenyl)phosphate with N^6-benzoyl-2',3'-di-O-benzoyladenosine, followed by a sequence of reactions, O-dedimethoxytritylation, a coupling reaction with the former triethylammonium salt, and complete deblocking of the resultant 2',5'-triadenylic acid derivative.

INTRODUCTION

Regioselective protection of functional groups such as the hydroxyl groups of a sugar moiety and amino or amido groups of a nucleic acid base moiety of nucleosides has been one of the mose basic problems in the synthetic study of compounds related to nucleic acids. Synthesis of RNA-type nucleotide oligomers involving a 3',5'-phosphodiester function requires a ribonucleoside derivative bearing a protecting group at its 2'-position, whose preparation has been the subject of various investigations.[2] On the other hand, the discovery of ppp-$^{5'}A^{2'}p^{5'}A^{2'}p^{5'}A$ and related oligoadenylates[3] stimulated a series of synthetic works[4] because these compounds were induced in cells treated with an interferon and strongly inhibit protein biosynthesis in cells on infection with a virus. In these cases, protection of the 3'-hydroxyl group has been achieved with the tert.-butyldimethylsilyl group,[4a] the 4-methoxytetrahydropyran-4-yl group,[4b] and the tetrahydrofuranyl group,[4c] as well as with the benzoyl[4d] and o-nitrobenzyl groups.[4e]

Oligonucleotide synthesis should ideally involve protecting groups for hydroxyl functions that are not susceptible to migration to a vicinal hydroxyl group, i.e., hemiacetal-type protecting groups[4b,4c] are preferred to others.[4a,4d,4e] We have recently established a simple, efficient preparative procedure for ribonucleoside derivatives bearing a tetrahydropyran-2-yl (THP) protecting group either at the 2' or 3' position arbitrarily.[1] Moreover, the THP group has been shown to be useful also for solid-phase oligonucleotide synthesis because of its appropriate stability under the conditions for removing a 5'-O-dimethoxytrityl group.[5] We now report the synthesis of $A^{2'}p^{5'}A^{2'}p^{5'}A$ starting with N^6-benzoyl-3'-O-(tetrahydropyran-2-yl)adenosine [the less (1a) and the more-polar diastereoisomer (1b) were both used],[1] herein.

RESULTS AND DISCUSSION

Compounds 1a and 1b were respectively converted into triethylammonium N^6-benzoyl-5'-O-dimethoxytrityl-3'-O-(tetrahydropyran-2-yl)adenosine 2'-(4-chlorophenyl)phosphate (3a and 3b), by their dimethoxytritylation and subsequent (4-chlorophenyl)phosphorylation,[6] in 66% and 79% yields. The diester 3b (1.25 mol. equiv.) was subjected to a coupling reaction with N^6-benzoyl-2',3'-di-O-benzoyladenosine (1.0 mol. equiv.)[7] in the presence of 8-quinolinesulfonyl chloride (3.125 mol. equiv.) and 1-methylimidazole (6.25 mol. equiv.).[8] Chromatographic separation of the resulting dimers on a column of silica gel afforded the less (4a) and more polar phosphotriester diastereoisomer (4b) in 40% and 45% (85% totally) yields, respectively.

The dinucleotide 4b was then subjected to 5'-O-dedimethoxytritylation with 2% p-toluenesulfonic acid monohydrate in 7:3 chloroform – methanol, giving the dinucleotide unit bearing a free hydroxyl group at its 5'-terminus (5) in 85% yield. The dinucleotide unit 5 (1 mol. equiv.) was finally coupled with 3a (1.25 mol. equiv.) in the presence of 8-quinolinesulfonyl chloride (2.5 mol. equiv.) and 1-methylimidazole (5 mol. equiv.), giving the protected triadenylate derivative (6) in 89% yield after chromatographic purification.

Unmasking of 6 was performed by treating it with 0.3 M N^1,N^1,N^3,N^3-tetramethylguanidinium (E)-2-pyridinealdoximate (TGM – PAO)[9] at room temperature for 24 h, with 28% aqueous ammoniacal solution at 50°C for 5 h, and with hydrochloric acid (pH 2)[10] at room temperature for 2 days.

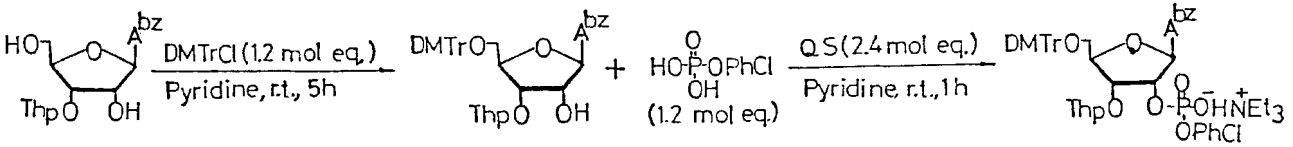
2',5'-Triadenylic acid (7) thus obtained was purified by pater chromatography on Toyo Roshi No. 51 (83% yield; ε 37200[4e]) and, then, its purity was reconfirmed by reversed-phase HPLC.

Compound 7 was decomposed by neither RNase T2 nor Nuclease P1, but by snake venom phosphodiesterase (SVP) completely to give adenosine and 5'-adenylic acid in a proportion of 1.00 : 1.96. It was thus confirmed to be the triadenylic acid involving 2',5'-phosphodiester linkages.

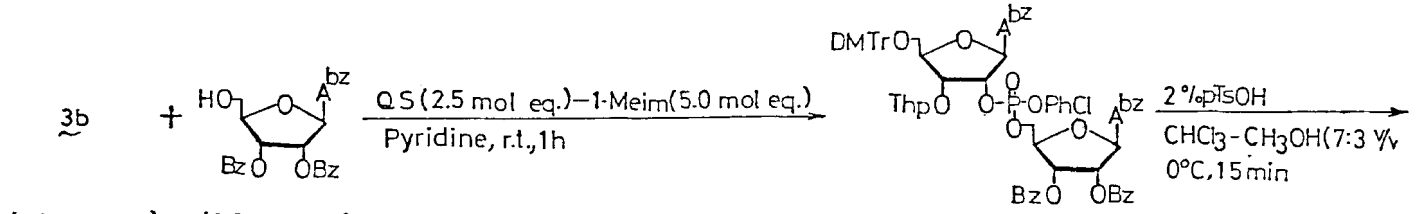
EXPERIMENTAL

Melting points were determined by a Yanagimoto Micro-Melting-Point apparatus, and are uncorrected. T.l.c. was conducted on Merck silica gel F_{254} by developing with 9:1 chloroform – methanol (Solvent A), and reversed-phase t.l.c. was on Merck silanized silica gel RP18 $F_{254}S$ with 6:4 acetone – water (Solvent B). Column chromatography was performed on silica gel (Wakogel C-300, purchased from Wako Pure Chemicals, Co. Ltd.) by the use of chloroform – methanol or methylene chloride – methanol. Paper chromatography on Toyo Roshi No. 51 and high performance liquid chromatography on Lichrosorb RP18 (5 μ; 150 mm, length x 4.6 mm, diameter; purchased from Nippon Seimitsu Kikai Co., Ltd.) were performed by the use of 55:10:35 propyl alcohol – 28% aqueous ammoniacal solution – water (Solvent C) as the eluant. ^{1}H-N.m.r. spectra were recorded on a JEOL JNM FX-200 apparatus with tetramethylsilane as the internal standard. ^{31}P-N.m.r. spectra were recorded on a JEOL JNM FX-100 apparatus with 85% phosphoric acid as the external standard. U.v. spectra were determined with a Varian Techtron Uv-Vis spectrophotometer Model 635. Elemental analyses were achieved with a Perkin-Elmer 240-002 apparatus.

N^6-Benzoyl-5'-O-dimethoxytrityl-3'-O-(tetrahydropyran-2-yl)adenosine (2): Compound 1a (R_F 0.44, Solvent A; 0.6832 g, 1.5 mmol) was dissolved in pyridine (7.5 mL), to which dimethoxytrityl chloride (0.6099 g, 1.8 mmol) was added; the resulting solution was allowed to stir for 5 h at room temperature and, then, for 30 min at room temperature after the addition of chilled water (3 mL). The resulting mixture was extracted with methylene chloride (20 mL), and the organic layer was washed with water (10 mL x 2). After drying over anhydrous magnesium sulfate and filtering it off, the organic solution was evaporated and the residue was subjected to column (5 cm, length x 2.5 cm, dia-

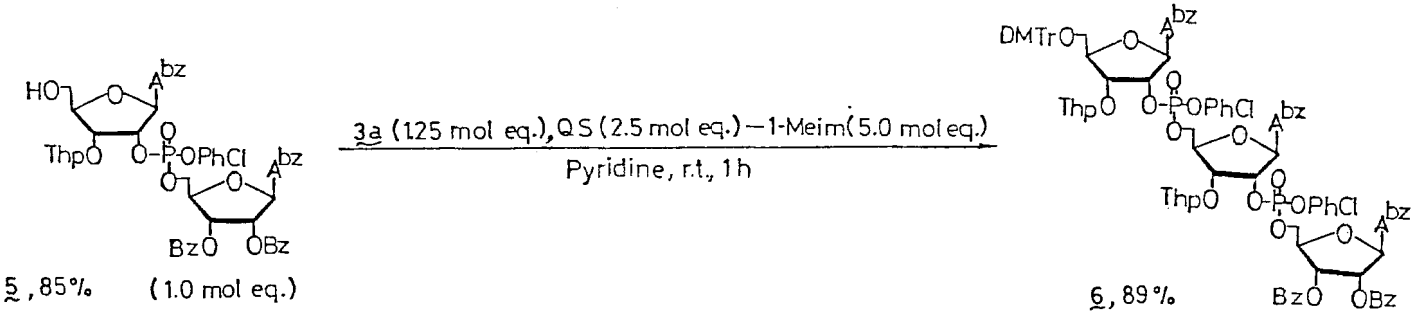


1 a : less polar isomer
b : more polar isomer



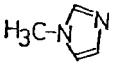
(1.0 mol eq.) (0.8 mol eq.)

4, 85 %

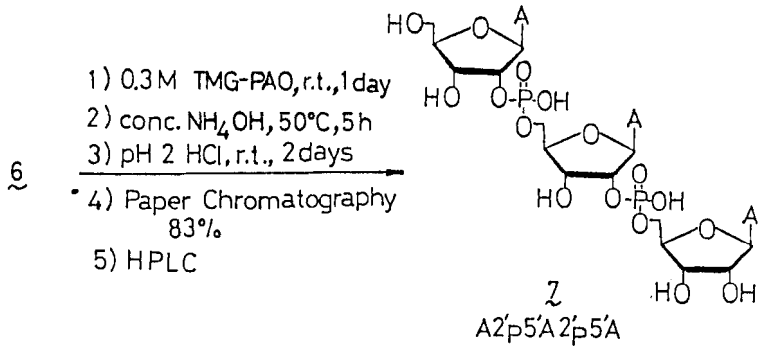


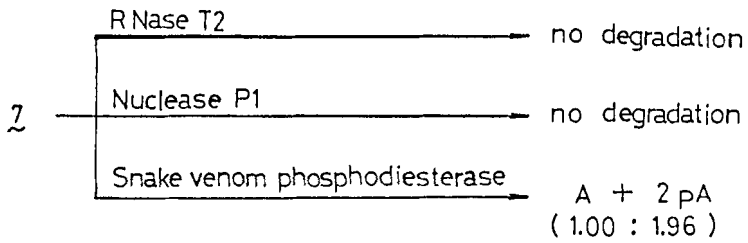
5, 85 % (1.0 mol eq.)

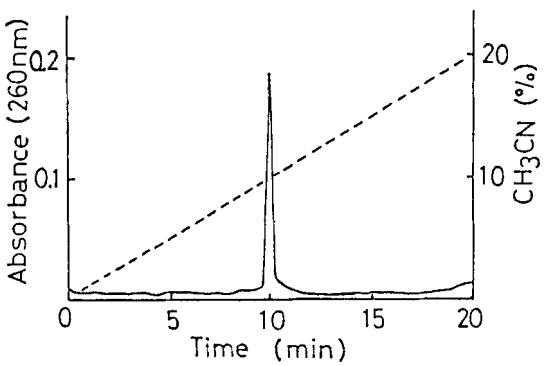
6, 89 %

QS =  1-Meim =  -PhCl = 

COMPOUNDS







Reverse-phase HPLC of A2'p5'A2'p5'A on Lichrosorb RP18 (5 μ, 4.6 x 150 mm)

Elution was performed with a linear gradient of acetonitrile (0–20%) in 0.1M triethylammonium acetate (pH 7.0) in 20 min. The flow rate was 1 ml/min.

COMPOUNDS (continued)

meter) chromatography with methanol – methylene chloride system. Crude product (1.0396 g, 91% yield) thus obtained was further dissolved in methylene chloride (2 mL), and the solution was added dropwise into hexane (50 mL) under vigorous stirring to give the product (0.8827 g, 78% yield) as a white amorphous powder, m.p. 105 – 107°C, R_F 0.58 (Solvent A), ^{1}H-n.m.r. ($CDCl_3$ – TMS): δ 1.42 – 2.32 (6H, m, C-C$\underline{H}_2$-C x 3), 3.33 – 3.58 (2H, m, O-C$\underline{H}_2$), 3.77 (6H, s, OC$\underline{H}_3$ x 2), 3.96 – 4.07 (2H, m, H-5' and 5"), 4.49 – 4.52 (1H, m, O-C$\underline{H}$-O), 4.52 (1H, t, H-3'), 4.80 (1H, q, $J_{2',3'}$ 4.4 Hz, H-2'), 6.12 (1H, d, $J_{1',2'}$ 4.4 Hz, H-1'), 6.71 – 8.06 (18H, m, Ph proton x 18), 8.27 (1H, s, H-8), 8.77 (1H, s, H-2), and 9.35 (1H, br. s, N$\underline{H}$).

Anal. Calcd for $C_{43}H_{43}N_5O_8$: C, 68.15; H, 5.72;N, 9.24. Found: C, 68.32; H, 5.81; N, 9.18.

Compound 1b (R$_F$ 0.33, Solvent A; 0.6832 g, 1.5 mmol) was similarly treated as above to give a crude product (1.1270 g, 99% yield) by chromatography, and a pure sample (0.9661 g, 85% yield) as a white amorphous powder by precipitation, pouring its methylene chroride solution into hexane with vigorous stirring, m.p. 109 – 111°C, R_F 0.50 (Solvent A), ^{1}H-n.m.r. ($CDCl_3$ – TMS): δ 1.40 – 2.40 (6H, m, C-C$\underline{H}_2$-C x 3), 3.28 – 3.54 (2H, m, O-C$\underline{H}_2$), 3.77 (6H, s, OC$\underline{H}_3$ x 2), 3.84 – 3.98 (2H, m, H-5' and 5"), 4.43 – 4.47 (1H, m, H-4'), 4.56 (1H, t, $J_{2',3'} = J_{3',4'}$ 5.6 Hz, H-3'), 4.72 (1H, br. s, O-C$\underline{H}$-O), 4.76 – 4.81 (1H, m, H-2'), 6.11 (1H, d, $J_{1',2'}$ 5.6 Hz, H-1'), 6.77 – 8.04 (18H, m, Ph proton x 18), 8.27 (1H, s, H-8), 8.74 (1H, s, H-2), and 9.31 (1H, br. s, N$\underline{H}$).

Anal. Calcd for $C_{43}H_{43}N_5O_8$: C, 68.15; H, 5.72; N, 9.24. Found: C, 67.95; H, 5.87; N, 9.40.

Triethylammonium N^6-Benzoyl-5'-O-dimethoxytrityl-3'-O-(tetrahydropyran-2-yl)adenosine 2'-(4-Chlorophenyl)phosphate (3): A solution of 2a (2.2588 g, 2.98 mmol) and 4-chlorophenyl dihydrogen phosphate (0.7466 g, 3.58 mmol) in pyridine (5 mL) was evaporated three times in order to remove moisture azeotropically. The residue was dissolved in pyridine (15 mL) and 8-quinolinesulfonyl chloride (1.6278 g, 7.15 mmol) added; the resulting solution was stirred at room temperature for 1 h. White crystals of precipitated 8-quinolinesulfonic acid were filtered off, and chilled water (5 mL) was added to the filtrate. After stirring at room temperature for 30 min, the mixture was extracted with methylene chloride (60 mL) and the extract was washed with 0.5 M aqueous tetra-

ethylammonium bicarbonate (TEAB) solution (30 mL x 2). The organic solution was evaporated and the residue was dissolved in 1:1 pyridine – water (100 mL). The solution was extracted with diethyl ether (40 mL x 3) and then with methylene chloride (100 mL). The organic layer was, after drying over anhydrous magnesium sulfate, evaporated, and the residue was, after dissolving in methylene chloride (16 mL), added dropwise into hexane (300 mL) with vigorous stirring. The resultant white amorphous powder was gathered by filtration to give 3a (2.2458 g, 72% yield), R_F 0.46 (Solvent B), ^{31}P-n.m.r. ($CDCl_3$ – 85% phosphoric acid): δ -6.321, ^{1}H-n.m.r. ($CDCl_3$ – TMS): δ 1.24 – 2.12 (6H, m, C-CH_2-C x 3), 1.27 (9H, t, J 7.32 Hz, CH_3 of N-ethyl group x 3), 3.02 (6H, q, C-CH_2-N x 3), 3.34 – 3.54 (2H, m, O-CH_2-C), 3.77 (6H, s, OCH_3 x 2), 3.86 – 4.02 (2H, m, H-5' and 5"), 4.46 – 4.52 (1H, m, H-4'), 4.64 – 4.70 (1H, m, O-CH-O), 5.00 – 5.10 (1H, m, H-3'), 5.27 (1H, t, H-2'), 6.38 (1H, d, $J_{1',2'}$ 7.08 Hz, H-1'), 6.75 – 8.05 (22H, m, Ph proton x 22), 8.17 (1H, s, H-8), 8.71 (1H, s, H-2), and 9.15 – 9.31 (1H, m, NH).

A similar treatment of the diastereomer 2b (12.6524 g, 3.5 mmol) with 4-chlorophenyl dihydrogen phosphate (0.8757 g, 4.2 mmol) in the presence of 8-quinolinesulfonyl chloride (1.9124 g, 4.2 mmol), followed by the work-up described above, gave 3b (2.9569 g, 80% yield), R_F 0.50 (Solvent B), ^{31}P-n.m.r. ($CDCl_3$ – 85% phosphoric acid): δ -6.442, ^{1}H-n.m.r. ($CDCl_3$ – TMS): δ 1.24 – 1.98 (6H, m, C-CH_2-C x 3), 1.27 (9H, t, J 7.32 Hz, C-CH_3 of N-ethyl group x 3), 3.02 (6H, q, C-CH_2-N x 3), 3.72 – 4.04 (4H, m, H-5',5", and O-CH_2-C), 3.78 (6H, s, OCH_3 x 2), 4.64 – 4.76 (2H, m, H-4' and O-CH-O), 4.92 – 5.02 (1H, m, H-3'), 5.09 – 5.19 (1H, m, H-2'), 6.12 (1H, d, $J_{1',2'}$ 5.85 Hz, H-1'), 6.79 – 8.02 (22H, m, Ph proton x 22), 8.26 (1H, s, H-8), and 8.71 – 8.73 (2H, m, H-2 and NH).

Fully Protected 2',5'-Diadenylic Acid (4): A mixture of 3b (2.9388 g, 2.8 mmol) and N^6-benzoyl-2',3'-di-O-benzoyladenosine[7] (1.2982 g, 2.8 mmol) was evaporated from pyridine (5 mL) three times, and dissolved in pyridine (11 mL). To the resultant solution, were added 8-quinolinesulfonyl chloride (1.5936 g, 7 mmol) and 1-methylimidazole (1.11 mL, 14 mmol), and the mixture was stirred at room temperature for 1 h. After quenching the reaction by the addition of water (3 mL), followed by stirring for 30 min, the resulting mixture was extracted with methylene chloride (60 mL). The organic solution was successively washed with 5% aqueous sodium hydrogen carbonate solution (20 mL x 2) and water (20 mL).

The organic solution was, after drying over anhydrous magnesium sulfate, evaporated and the residue was then subjected to column (8 cm, length x 4.5 cm, diameter) chromatography with the nethanol – methylene chloride system to give a less polar (4a)(1.3259 g, 40% yield) and a more polar triester diastereomer (4b)(1.5192 g, 45% yield).

Compound 4a was a glass, R_F 0.55 (Solvent A), ^{1}H-n.m.r. ($CDCl_3$ – TMS): δ Signals assigned to the 5'-terminal moiety; 1.28 – 2.54 (6H, m, C-C$\underline{H}_2$-C x 3), 3.12 – 3.68 (4H, m, H-5', 5", and O-C$\underline{H}_2$), 3.74 (6H, s, O-C$\underline{H}_3$ x 2), 4.40 – 4.46 (1H, m, H-4'), 4.78 – 4.82 (2H, m, H-3' and O-C$\underline{H}$-O), 5.71 – 5.79 (1H, m, H-2'), 6.34 (1H, d, $J_{1',2'}$ 5.62 Hz, H-1'), those assigned to the 3'-terminal moiety; 4.61 – 4.64 (3H, m, H-4', 5', and 5"), 6.00 – 6.04 (1H, m, H-3'), 6.19 (1H, t, H-2'), 6.52 (1H, d, $J_{1',2'}$ 6.52 Hz, H-1'), those impossible to discriminate; 6.76 – 8.02 (37H, m, Ph proton x 37), 8.28 and 8.36 (2H, s x 2, H-8 x 2), 8.66 and 8.79 (2H, s x 2, H-2 x 2), and 9.28 and 9.34 (2H, br. s x 2, N$\underline{H}$ x 2).

Compound 4b was a glass, R_F 0.52 (Solvent A), ^{1}H-n.m.r. ($CDCl_3$ – TMS): δ Signals assigned to the 5'-terminal moiety; 1.49 – 2.65 (6H, m, C-C$\underline{H}_2$-C x 3), 3.25 – 3.81 (4H, m, H-5', 5", and O-C$\underline{H}_2$), 3.75 (6H, s, O-C$\underline{H}_3$ x 2), 4.37 – 4.45 (1H, m, H-4'), 4.71 (1H, br. s, O-C$\underline{H}$-O), 4.79 (1H, t, $J_{2',3'}$ 6.84 Hz, H-3'), 5.68 – 5.77 (1H, m, H-2'), 6.44 (1H, d, $J_{1',2'}$ 4.4 Hz, H-1'), those assigned to the 3'-terminal moiety; 4.58 – 4.66 (3H, m, H-4', 5', and 5"), 5.98 – 6.04 (1H, m, H-3'), 6.15 (1H, m, H-2'), 6.49 (1H, d, $J_{1',2'}$ 5.61 Hz, H-1'), those impossible to discriminate; 6.77 – 8.04 (37H, m, Ph proton x 37), 8.31 and 8.32 (2H, s x 2, H-8 x 2), 8.66 – 8.75 (2H, s x 2, H-2 x 2), and 9.34 and 9.38 (2H, br. s x 2, N$\underline{H}$ x 2).

Diadenylic Acid Derivative Bearing a Free Alcoholic Function at the 5'-Position (5): To a solution of 4b (0.7550 g, 0.5 mmol) in 7:3 chloroform – methanol (30 mL), was added a solution of p-toluenesulfonic acid monohydrate (1.2814 g) in the same solvent (20 mL) with stirring at 0°C and the mixture was stirred for 10 min; in t.l.c (Solvent A), the spot of 4b (R_F 0.58) disappeared and a new spot (R_F 0.46), which showed no color peculiar to the dimethoxytrityl group on spraying a 5% methanolic sulfuric acid solution, appeared. The resulting mixture was neutralized with a 5% aqueous sodium bicarbonate solution with stirring, and extracted with chloroform (50 mL). The organic layer was washed with water (50 mL x 2) and, after drying over anhydrous magnesium sulfate,

evaporated. The residue was subjected to column (5 cm, length x 2.5 cm, diameter) chromatography with methanol – methylene chloride system to give 5 (0.5146 g, 86% yeild), a glass, R_F 0.42 (Solvent A), ^{1}H-n.m.r. ($CDCl_3$ – TMS): δ , Signals assigned to the 5'-terminal moiety; 1.38 – 2.50 (6H, m, C-C<u>H</u>$_2$-C x 3), 3.42 – 3.98 (4H, m, H-5', 5", and O-C<u>H</u>$_2$), 4.38 – 4.50 – (1H, m, H-4'), 4.65 – 4.71 (2H, m, H-3' and O-C<u>H</u>-O), 5.34 – 5.54 (1H, m, O<u>H</u>-5'), 5.68 (1H, q, H-2'), 6.23 (1H, d, $J_{1',2'}$ 6.59 Hz, H-1'), those assigned to the 3'-terminal moiety; 4.39 – 4.50 (3H, m, H-4', 5', and 5"), 5.95 – 6.01 (1H, m, H-3'), 6.10 (1H, t, H-2'), 6.52 (1H, d, $J_{1',2'}$ 5.61 Hz, H-1'), and those impossible to discriminate; 6.91 – 8.07 (24H, m, Ph proton x 24), 8.91 and 8.45 (2H, s x 2, H-8 x 2), 8.74 and 8.08 (2H, s x 2, H-2 x 2), and 9.27 and 9.42 (2H, br. s, s x 2, N<u>H</u> x 2).

<u>Fully Protected Triadenylic Acid</u> (6): A mixture of 5 (0.4226 g, 0.35 mmol) and 3a (0.4592 g, 0.438 mmol) was evaporated from pyridine (3 mL) three times in order to remove moisture azeotropically. To a solution of the resulting residue in pyridine (1.75 mL), were added 8-quinolinesulfonyl chloride (0.1992 g, 0.875 mmoL) and 1-methylimidazole (0.14 mL, 1.75 mmol), and the solution was stirred at room temperature for 1 h. The resulting mixture was, after quenching with chilled water (1 mL), further stirred for 30 min and extracted with methylene chloride (20 mL). The extract was washed with a 5% aqueous sodium bicarbonate solution (10 mL x 2) and then with water (10 mL), and dried over anhydrous magnesium sulfate. After filtering off the desiccant, the filtrate was evaporated and the residue was subjected to colum (5 cm, length x 2.5 cm, diameter) chromatography with methanol – methylene chloride system to give 6 (0.6665 g, 89% yield) as a diastereomer mixture, a glass, R_F 0.49 (Solvent A).

<u>Complete Unmasking of 6</u>: Compound 6 (0.0641 g, 0.03 mmol) was dissolved in a 0.3 M solution of <u>N</u>^{1},<u>N</u>^{1},<u>N</u>^{3},<u>N</u>^{3}-tetramethylguanidium (<u>E</u>)-2-pyridinealdoximate in 1:1 dioxane – water (4 mL), and the solution was stirred at room temperature for 1 day. The whole of the resulting solution was put on a column (5 cm, length x 2 cm, diameter) of Dowex 50W (pyridinium form) and the column was eluted with 50% aqueous pyridine (100 mL). After evaporating the eluate, the residue was dissolved in 9:1 28% aqueous ammonical solution – pyridine (5 mL), and then stirred at 50 °C for 5 h. The resulting mixture was, after cooling, evaporated, and the residue was treated at pH 2 in hydrochloric acid (10 mL) at room

temperature for 2 days. To the solution was added pyridine (2 mL) and the resulting solution was evaporated. After dissolving the residue in (40 mL), the aqueous solution was washed with diethyl ether (20 mL x 2), and evaporated. The residue was dissolved in water and its total volume was adjustedto 10 mL, whose 0.2 mL-aliquot was subjected to paper chromatography in the descending manner with Solvent C. The band corresponding to R_F 0.38 was extracted to give 2',5'-triadenylic acid (7)(83% yield; 18.5 OD, ε 37200[4e]). Further evaluation of 7 by high performance liquid chromatography confirmed that 7 obtained by paper chromatography was pure enough to give a sharp single peak [R_F 0.38 (Solvent C)], λ_{max} 257 nm and λ_{min} 226 nm.

Structure Confirmation of 7 Through Degradation by Enzymes[11]

Treatment with Ribonuclease T2 (RNase T2): To a solution of 7 (9.750 OD) in water (200 μL), were added 1 M sodium acetate buffer (pH 4.5; 10 μL) and RNase T2 (Sankyo Chemical Co.; 500 units/mL)(8 μL, 4 units), and the solution was allowed to stand at 37°C for 5 h. A paper chromatogram of the resulting mixture showed no bands other than the original one with R_F 0.38. A u.v. determination of 7 at 260 nm after extracting the band with water gave 9.00 OD (92% recovery yield); this result confirmed that 7 structurally involved no 3',5'-phosphodiester linkages.

Treatment of 7 with Penicillium Nuclease P1: To a solution of 7 (9.75 OD) in water (200 L), were added a 0.1 M ammonium acetate buffer (pH 5.7; 12 μL) and the Nuclease P1 (Yamasa Shoyu Co., 1 mg/mL)(10 μL; 10 μg), and the solution was allowed to stand at 37°C for 5 h. A paper chromatogram obtained in a similar manner as above gave a single band of R_F 0.38 (Solvet C), and a u.v. determination at 260 nm gave 7 (8.70 OD; 89% recovery yield).

Treatment of 7 with Snake Venom Phosphodiesterase (SVP): To a solution of 7 (9.00 OD) in water 200 μL), were added 1 M Tris acetate buffer (pH 7.6; 13 μL), 1 M aqueous magnesium sulfate solution (5 μL), and SVP (Worthington Biochemical Corp., 4 mg/mL, 25 units/mg)(8 mL). The solution was allowed to stand at 37°C for 5 h. A paper chromatogram showed two spots of R_F 0.76 and 0.40 corresponding to adenosine and 5'-adenylic acid, respectively. A u.v. determination of each spot, after extracting with water, revealed that the proportion of adenosine (3.03 OD) : 5'-adenylic acid (5.98 OD) was 1.00 : 1.96.

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