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SYNTHESIS OF 2'- OR 3'-O-(4-METHOXYBENZYL)NUCLEOSIDES AND ITS APPLICATION IN THE 3'-TERMINAL NONANUCLEOTIDE SEQUENCE OF ROUS SARCOMA VIRUS 35S RNA SYNTHESIS[1]

Kazuo Kamaike, Hiromichi Tsuchiya, Kazuaki Imai, and Hiroshi Takaku*

Laboratory of Bioorganic Chemistry, Chiba Institute of Technology, Tsudanuma, Narashino, Chiba 275, Japan

(*Received in Japan 7 May* 1986)

Abstract——2'- or 3'-O-(4-Methoxybenzyl)nucleoside derivatives were synthesized by treatment of uridine, N^4-benzoylcytidine, N^6-benzoyladenosine, and N^2-benzoylguanosine with 4-methoxyphenyldiazomethane. The separation of 2'- or 3'-isomers became possible on a silica gel column chromatography by using N-acylated nucleosides and these compounds could be used as useful starting materials for the synthesis of oligoribonucleotides by the phosphotriester method. The trimer blocks, U-U-Cp, A-C-Cp, and A-C-A were synthesized by the rapid method. The 3'-terminal nonanucleotide, U-U-C-A-C-C-A-C-A, of Rous Sarcoma Virus 35S RNA was obtained by the block condensation of trimers.

The synthesis of oligoribonucleotides by the phosphotriester method is more elaborate and time-consuming than the preparation of oligodeoxyribonucleotides primarily because of the need to protect the 2'-hydroxyl group of ribonucleosides.[2] Recently, we found[3] that the 4-methoxybenzyl group can be introduced to protect the 2'-hydroxyl groups by treatment of 2',3'-O-(dibutylstanylene)uridine[4] and sodium hydride-treated nucleosides with 4-methoxybenzyl bromide. It was converted, under mild conditions by triphenylmethyl fluoroborate[5], into completely unblocked products. However, 3'-O-(4-methoxybenzyl)nucleoside derivatives could not be isolated in the above reactions. Consequently, we examined the separation of 2'- and 3'-isomers and a combination of N-acylated nucleosides and 4-methoxyphenyldiazomethane was found to be more effective for the separation of 2'- or 3'-isomers by a silica gel column chromatography. The utility of 2'-O-(methoxybenzyl)nucleoside derivatives (4) can be demonstrated in the following synthesis of 3'-terminal nonanucleotide sequence of Rous Sarcoma Virus 35S RNA[6].

RESULT AND DISCUSSION

Reaction of N-acylated nucleosides with 4-methoxyphenyldiazomethane (2)

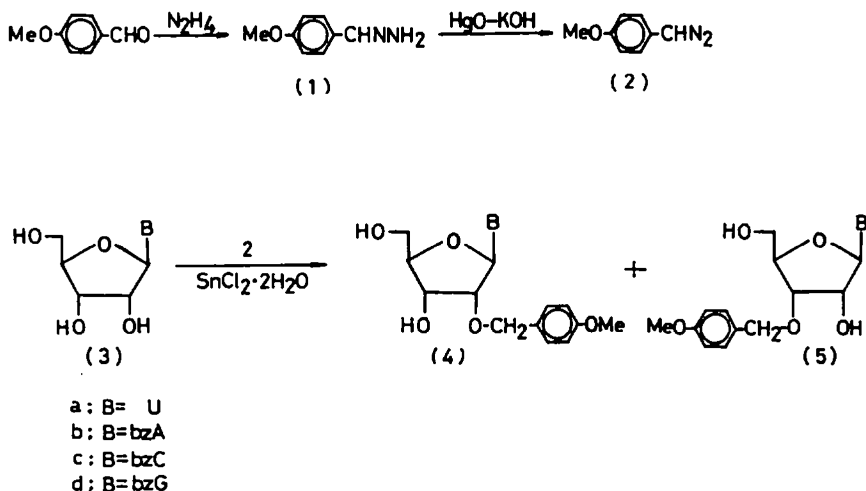
4-Methoxyphenyldiazomethane (2) was prepared according to the procedure reported by Closs[7]. Reaction of 4-methoxybenzaldehyde and anhydrous hydrazine gives the intermediate 1, which is then reacted with yellow mercury (II) oxide under alkaline conditions to give 4-methoxyphenyldiazomethane (2) in 40-50% yield.

The N-benzoylated nucleosides (3b-d) were treated with a three fold excess of 2 in dry DMF in the presence of $SnCl_2$.[8] The reaction was performed at room temperature for 1 day or at 45 °C for 2 h. The 2'- and 3'-isomers of N^6-benzoyladenosine, N^4-benzoylcytidine, and N^2-benzoylguanosine were separated by a silica gel column chromatography. On the other hand, in the case of uridine, the 2'-isomer 4a was recrystallized from ethanol without a silica gel column chromatography.

The 3'-isomer 5a could be isolated after separation by a silica gel column of

mother liquor. The 2'- and 3'-isomers were isolated in 23% and 20% yields, respectively. The locations of 4-methoxybenzyl group were determined by NMR analysis (Table 1). The 2'-isomer 4a (δ 5.88) was at lower field than 3'-isomer 5a (δ5.77). The results were consistent with the rules proposed by Reese and his coworkers[9], namely the 3'-substituted compounds had larger $J_{1',2'}$ coupling constants and their 1'-H signal showed high field shifts compared with the 2'-substituted isomers. The UV spectral data, melting points, and Rf values of the 2'- and 3'-isomers are summarized in Table 2. The compound of lower Rf value was identified as a previously obtained 2'-O-(4-methoxybenzyl)uridine (4a)[3]. The NMR spectra of higher compound of Rf value showed a high-field-shifted 1'-H signal compared with the 2'-isomer 4a and their $J_{1',2'}$ coupling constant showed large value compared with those 2'-isomer 4a. This compound was identified as the 3'-O-(4-methoxybenzyl)uridine (5a), and it could not be isolated from the reaction with 4-methoxybenzyl bromide.[3]

In a similar manner, N^6-benzoyladenosine (3b), N^4-benzoylcytidine (3c), and N^2-benzoylguanosine (3d) were 4-methoxybenzylated and the 2'- and 3'-isomers were



Scheme 1.

Table 1. NMR Spectra data for 2'(3')-O-(4-methoxybenzyl)nucleoside derivatives

			Chemical shift (δ)[a]			
Nucleoside	1'-H	$J_{1',2'}$(Hz)	2'-H	3'-H	4'-H	5'-H
U2'(MBn)	5.88	5	3.80——4.38			3.60
U3'(MBn)	5.77	6	4.17	3.76——4.00		3.54
bzA2'(MBn)	6.17	6	4.30——4.66		4.08	3.63
bzA3'(MBn)	6.04	6	4.83	4.00——4.20		3.59
bzC2'(MBn)	6.03	2	4.24	3.98——4.21		3.68
bzC3'(MBn)	5.94	3	4.35,	4.05	3.98	3.63
bzG2'(MBn)	6.06	6	4.61	4.36	4.04	3.50
bzG3'(MBn)	5.80	6	4.72	4.10	3.90	3.58

[a]DMSO-d_6.

isolated by a silica gel column chromatography. The locations of 4-methoxybenzyl group were determined by NMR analysis (Table 1). Unfortunately, in the case of guanosine, 2',3'-O-di(4-methoxybenzyl)-N^2-benzoylguanosine was formed as a side product in 8% yield along with 4d (24%) and 5d (21%).

The 2'-substituted compounds can be used as starting materials for forming 3'-5' internucletidic bonds, whereas the 3'-substituted compound 4b seems to be suitable for the synthesis of 2'-5' linked oligoadenylates, which were found in extracts of interferon-treated cells.[10]

Table 2. Melting points, Rf values, and UV spectra data for 2'(3')-O-(4-methoxybenzyl)nucleoside derivatives

Nucleoside	M.P. (°C)	Rf value[a] A	B	C	UV (MeOH) λ max	λ min
U2'(MBn)	145-146	0.28	0.23	0.10	261, 225	239
U3'(MBn)	168-170	0.28	0.25	0.11	261, 224	238
bzA2'(MBn)	186-188	0.44	0.33	0.08	278, 225	245
bzA3'(MBn)	186-187	0.47	0.38	1.11	278, 225	245
bzC2'(MBn)	160-162	0.37	0.26	0.20	305, 262 228	289, 241
bzC3'(MBn)	168-170	0.37	0.30	0.22	302, 258 223	286, 238
bzG2'(MBn)	124-127	0.35	0.23	0.21	292, 263, 227	272, 249
bzG3'(MBn)	78-82	0.37	0.29	0.25	289, 260 225	269, 247

[a]A: CH_2Cl_2-MeOH (9:1, v/v), B: CH_2Cl_2-EtOH (10:1, v/v), B: C_6H_6-$(CH_3)_2CO$ (2:1, v/v)

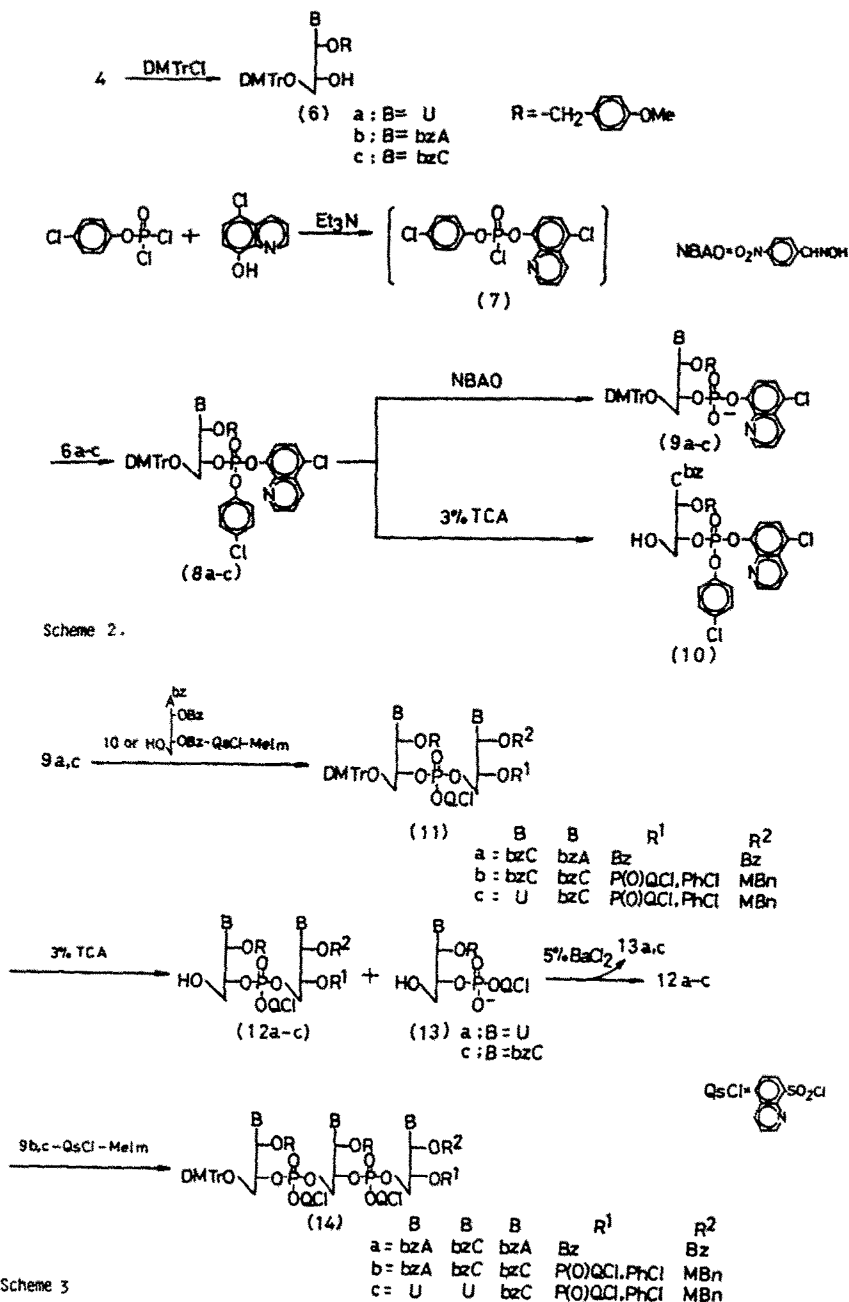
Rapid synthesis of trimer blocks (14)

Next, we examined the synthesis of the 3'-terminal nonanucleotide U-U-C-A-C-C-A-C-A of Rous Sarcoma Virus 35S RNA by using 4. Treatment of 4a-c with dimethoxytrityl chloride in dry pyridine gave the corresponding 5'-O-dimethoxytrityl-2'-O-(4-methoxybenzyl)-N-protected nucleosides (6a-c) in good yields.

The nucleoside 3'-phosphotriester units (8) are the important starting material in oligoribonucleotide synthesis by the phosphotriester method were prepared as follows: Reaction of 4-chlorophenyl phosphorodichloride and 5-chloro-8-hydroxyquinoline gave the intermediate 7[11], which can be further activated in the presence of N-methylimidazole (MeIm). It was further react with nucleoside derivatives (6) to give the fully protected mononucleoside 3'-phosphotriester units (8a-c) in 80-88% yields.

For the rapid synthesis of trimer blocks (14), the terminal units (10) and the internal units (9a-c) were synthesized as shown in Scheme 2. The phosphotriester unit 8c was treated with 3% Cl_3CCOOH in a CH_3NO_2-MeOH (95:5, v/v) mixture at room temperature for 5 min[12] and the 3'-terminal unit (10) was isolated by precipitation from a hexane-ether (9:1, v/v) mixture, and then used for the next coupling reaction without further purification. On the other hand, the internal units (9a-c) was obtained in good yields by treatment of 8a-c with 4-nitrobenzaldoximate (NBAO) in a dioxane-water-triethylamine (1:1:1, v/v) mixture at room temperature for 8 h.[13] The purity of phosphodiesters (9a-c) were confirmed by ^{31}P-NMR spectra and TLC analysis.

The compound 10 (1.0 molar equiv.) was treated with an excess of 9c (1.5 molar



Scheme 2.

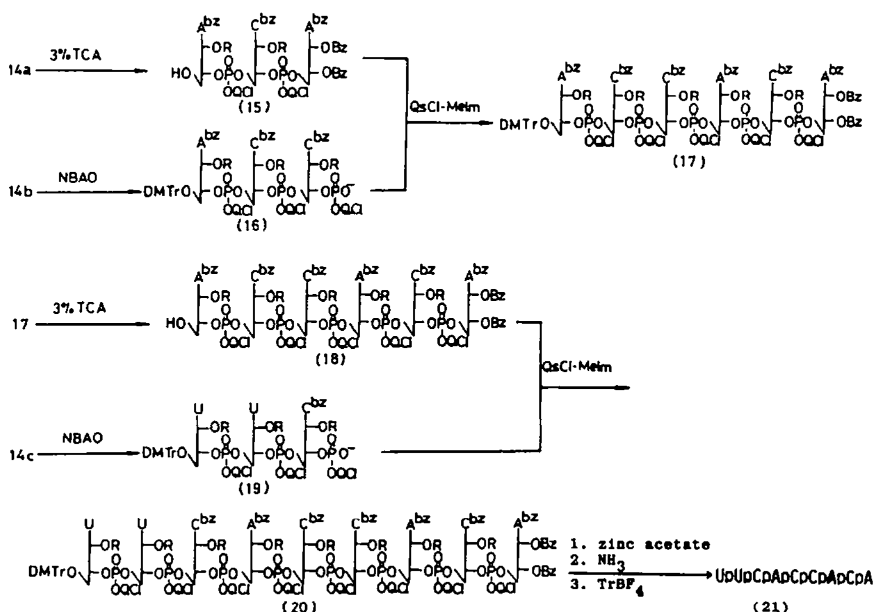
Scheme 3

equiv.) in the presence of 8-quinolinesulfonyl chloride (QsCl)[14] (3.75 molar equiv.) and MeIm[15] (3.75 molar equiv.) in dry pyridine. After 1 h, TLC analysis showed the reaction was complete. The reaction mixture was treated with 3% Cl_3CCOOH in a CH_3NO_2-MeOH (95:5, v/v) mixture at 25 °C for 5 min to give 12b and 13c. Since the phosphodiester 13c is acidic, it can be removed easily from the reaction mixture by extraction by addition of 5% $BaCl_2$ solution.[16] The 5'-hydroxyl dimer (12b) was precipitated from a hexane-ether (9:1, v/v) mixture and then used for the next coupling reaction without further purification. The condensation of 12b and the phosphodiester 9b (1.5 molar equiv.) was carried out in the presence of QsCl (3.75 molar equiv.) and MeIm (3.75 molar equiv.). The condensation reaction was completed within 1 h. Thus, the usual workup gave the fully protected trimer block, A-C-Cp (14b) in 51% yield. By this method, the trimer blocks, A-C-A (14a)

and U-U-Cp (14c) were isolated in 61% and 53% yields, respectively, after the separation by a silica gel column chromatography. The trimer blocks after the removal of all protecting groups showed a single peak with high-pressure liquid chromatography (HPLC) on reversed-phase column (Finepak C-18). The trimer blocks (14) were uncontaminated with the shorter fragments.

Synthesis of A-C-C-A-C-A (17) and U-U-C-A-C-C-A-C-A (20)

The dimethoxytrityl group of 14a was selectively removed by treatment with 3% Cl_3CCOOH in a CH_3NO_2-MeOH (95:5, v/v) mixture to afford the 5'-hydroxyl component (15) in 94% yield. On the other hand, the trimer block (14b) was then converted to the 3'-phosphodiester component (16) by the removal of 3'-terminal protecting group, 4-chlorophenol with NBAO in a dioxane-water-triethylamine (1:1:1, v/v) mixture at room temperature for 8 h. Thus, the phosphodiester 16 was isolated in 85% yield after the separation by a short silica gel column chromatography using a ethyl acetate -CH_2Cl_2-triethylamine (45:45:10, v/v) mixture in the mobil phase. The phosphodiester 16 (1.5 molar equiv.) thus obtained was dissolved in dry pyridine, and 15 (1.0 molar equiv.), QsCl (3.75 mola equiv.), and MeIm (3.75 molar equiv.) were added. The reaction procceded rapidly and was complete within 1 h. After usual workup, the chromatography afforded the fully protected hexamer (17) in 73% yield.



Scheme 4.

The nonamer (20) was obtained starting from 17 and 14c: Treatment of 17 with 3% Cl_3CCOOH in a CH_3NO_2-MeOH (95:5, v/v) mixture afforded the 5'-hydroxyl component 18 in 88% yield, whereas treatment of 14c with NBAO to remove the 4-chlorophenyl group afforded the 3'-phosphodiester component 19. The condensation reaction of 19 (2.0 molar equiv.) and 18 (1.0 molar equiv.) was performed in the presence of 3.1 molar equiv. of QsCl and MeIm. The fully protected nonamer (20) was obtained by chromatography on silica gel column in yield of 85%.

The above condensation reaction proceeds very smoothly because of the sequence does not contain the guanosine unit.

Deprotection of the protecting groups from 20

There are several methods for removal of the 5-chloro-8-quinolyl group. It can

be removed by the zinc ion catalyzed hydrolysis[17] or by oximate promoted hydrolysis[18]. However, the oximate treatment for nonamer was unsuitable for removal of the 5-chloro-8-quinolyl group. Because, the deprotection of the 5-chloro-8-quinolyl group from oligomers by oximate seemed to be depend on the chain length. From this result, the deprotection of the fully protected nonamer (20) was performed as follows: Treatment with a large excess of zinc acetate in aqueous pyridine for 3 days at room temperature resulted in the removal of the 5-chloro-8-quinolyl groups. The resulting phosphodiester compound was further treated with concentrated ammonia at 60 °C for 10 h to remove the acyl groups, and finally with triphenylmethyl fluoroborate ($Tr^{+}BF_4^{-}$) in a CH_3CN-H_2O (4:1, v/v) mixture at room temperature for 10 h to cleave the 4-methoxybenzyl and DMTr groups. The nonamer (21) was purifited by ion-exchange chromatography on DEAE-Shephadex A-25 in the presence of 7 M urea (Fig 1). The main fraction was further purified by HPLC on a C-18 silica gel column[19] (Fig. 2). The purified nonamer (21) was digested with nuclease P1[20] to give the expected products in the correct ratios. The chain lengths of nonamer (21) was confirmed by electrophoresis on 20% polyacrylamide gel (Fig. 3). Thus, the ribononamer (21) was obtained by condensation of purified relatively larger blocks such as 18 and 19. The yield of the fully protected product (20) was 85% and the practical yield of 21 from 20 after purification was ca. 8%.

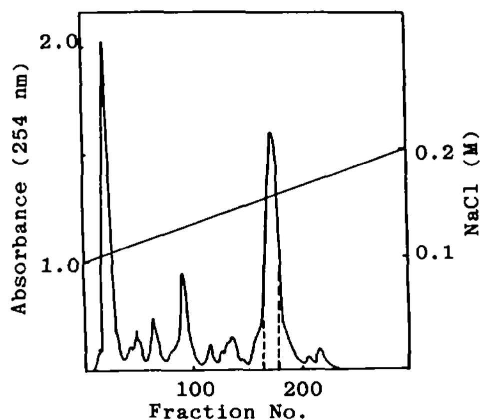


Fig. 1. Chromatography of the nonamer 21 on a column (2 X 45 cm) of DEAE-Shephadex A-25 (chloride). The elution was performed with a lihear gradient of NaCl (0.1-0.25 M, total 2L) in 7 M urea and 0.02 M Tris-HCl (pH 7.5).

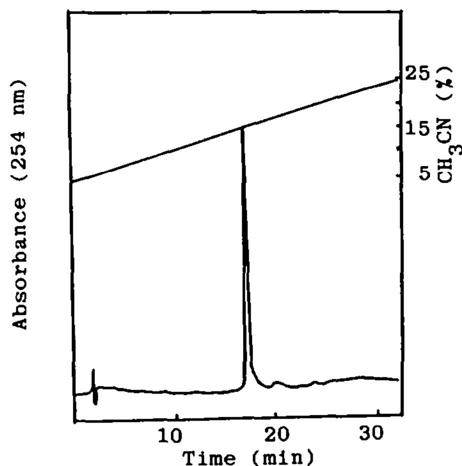


Fig. 2. Reversed-phase HPLC of the nonamer 21 on a column (4.6 X 250 mm) of Finepak SIL C-18. The elution was performed with a linear gradient of acetonitrile (2-25%) in 0.1 M triethylammonium acetate (pH 7.0).

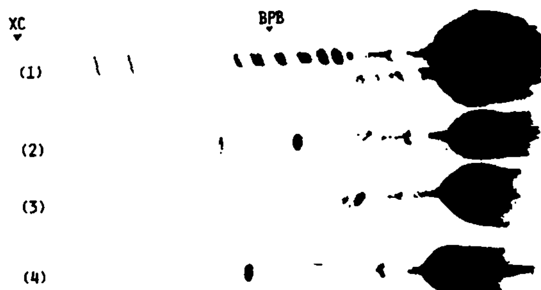


Fig. 3. 20% Acrylamide gel electrophoeresis in 7 M urea of 5'-32P-labeled oligomers [A-C-A-C-A (2), A-C-A (3), U-U-C-A-C-C-A-C-A (4)]. Lane (1): RNA chain length standard.

EXPERIMENTAL

Ultraviolet spectra were recorded on a Shimazu UV-200 spectrometer. ^{1}H-NMR spectra were recorded on a JEOL JNMPS 100 spectrometer with TMS as an internal standard. Thin layer chromatography (TLC) was performed on plates of silica gel 60 F-254 (Merck Art. No. 5715) using solvent A (CH_2Cl_2-MeOH, 9:1, v/v), solvent B (CH_2Cl_2-MeOH, 95:5, v/v), and solvent C (CH_2Cl_2-EtOH, 10:1, v/v). For reversed phase thin layer chromatography (RTLC) using silanized silica gel, RP-8 F-254 (Merck) was performed by a mixture of acetone and water . Column chromatography was performed with silica gel (300 mesh) from Kanto Chem. Co. Ltd.. For reversed phase column chromatography using silanized silica gel (Merck, 70-230 mesh) was equilibrated with 60-70% acetone and compounds in acetone were applied with addition of water until slight turbidity. Elution was performed with 60-80% aqueous acetone. HPLC was performed on Finepak SIL C-18 reversed phase column and the elution was performed by a linear gradient of 5-25% CH_3CN in 0.1 M triethylammonium acetate (pH 7.0) over 32 min at flow rate of 2.0 ml/min. Pyridine was distilled twice from p-toluenesulfonyl chloride and CaH_2 and then stored over molecular sieves 3A. DMF was distilled from CaH_2 and then stored over molecular sieves 3A. 8-Quinolinesulfonyl chloride was purchased from Aldrich Chemical Co.. Nuclease P1 was purchased from Yamasa Shoyu Co. Ltd.. DMTrCl was gifts from Yukigousei Yakuhin Co. Ltd..

4-Methoxyphenyldiazomethane (2)

To a dry ether (80 ml) of 4-methoxybenzaldehyde (12.2 ml, 100 mmol) was added anhydrous hydrazine (6.4 ml, 200 mmol), and the reaction mixture was allowed to stand for overnight at 0-10 °C. After an additional hour of stirring at room temperature, the aqueous base was removed, the ether layer was washed with water (2 X 15 ml) and dried over Na_2SO_4. After removal of Na_2SO_4, the solution was treated with yellow mercuric oxide (32 g, 148 mmol) followed by a solution of KOH (160 mg, 3.0 mmol) in MeOH (4.8 ml). After 1 h, the red solution was filtered twice by suction in order to remove all solid and the dired over Na_2SO_4. Ether was evaporated at 0 °C and the residue was extracted with n-pentane (4 X 20 ml). The combined n-pentane extracts were cooled to -50 °C in a dry ice-acetone bath for 20 min and then quickly filtered under suction. n-Pentane was evaporated at 0 °C and the residue was dissolved in dry CH_2Cl_2. The amount of the product was estimated by esterification of benzoic acid.[20] The yield was 40-50%. 4-Methoxyphenyldiazomethane (2) was very unstable, it should be stored at -80 °C.

2'-O-(4-Methoxybenzyl)uridine (4a) and 3'-O-(4-methoxybenzyl)uridine (5a)

Uridine (3a) (860 mg, 3.5 mmol) and $SnCl_2.H_2O$ (73 mg, 0.32 mmol) were dissolved in DMF (35 ml) at 45 °C. 4-Methoxyphenyldiazomethane (2) (14 mmol) in CH_2Cl_2 (5.4 ml) was added to the mixture and the reaction mixture was stirred at 45 °C. After 2 h, 3a was not detected on TLC. The solvent was evaporated and the residue was dissolved in water (20 ml) and washed with ether (4 X 10 ml). Water (20 ml) was added to the aqueous phase and the solution was filtered with heating. The solution was left at room temperature overnight and then cooled in a refrigerator for 1 day. The resulting precipitate was collected by filtration and was recrystallized from aqueous ethanol to give 300 mg (23%) of 4a. (Found: C, 56.16; H, 5.58; N, 7.70; $C_{17}H_{20}N_2O_7$ requires: C, 56.04; H, 5.53; N, 7.69%). The mother liquor was evaporated in vacuo, dissolved in CH_2Cl_2, and applied to a silica gel column. Elution was performed with CH_2Cl_2-MeOH (98:2, v/v) and the appropriate fractions were evaporated. The resulting solid was recrystallized from ethanol to give 280 mg (20%) of 4b. (Found: C, 56.09; H, 5.56; N, 7.43; $C_{17}H_{20}N_2O_7$ requires: C, 56.04; H, 5.53; N, 7.69%).

2'-O-(4-Methoxybenzyl)-N^6-benzoyladensoine (4b) and 3'-O-(4-methoxybenzyl)-N^6-adenosine (5b)

N^6-Benzoyladenosine (3b) (1.45 g, 3.9 mmol) was treated with $SnCl_2.H_2O$ (0.079 g,

0.35 mmol) and 2 (15.7 mmol) in dry DMF (29 ml) at 45 °C. After 2 h, the reaction mixture was evaporated in vacuo and the residue was dissolved in pyridine and poured into hexane. The precipitated product was dissolved in pyridine after removal of hexane and the solution was concentrated and coevaporated three 10 ml portions of toluene. The residue was dissolved in CH_2Cl_2 and applied to a silica gel column (5 X 15 cm). The appropriate fractions [eluted with CH_2Cl_2-MeOH (98:2, v/v)] were evaporated in vacuo. The residue was recrystallized from aqueous ethanol to give 500 mg (27%) of 5b. (Found: C, 60.86; H, 5.09; N, 14.02; $C_{25}H_{25}N_5O_6$ rquires: C, 61.09; H, 5.13; N, 14.25%). Then, the 2'-isomer (4b) was recrystallized from aqueous ethanol. The yield was 540 mg (29%). (Found: C, 61.22; H, 5.10; N, 14.25; $C_{25}H_{25}N_5O_6$ requires: C, 61.09; H, 5.13; N, 14.25%).

2'-O-(4-Methoxybenzyl)-N^4-benzoylcytidine (4c) and 3'-O-(4-methoxybenzyl)-N^4-benzoylcytidine (5c)

N^4-Benzoylcytidine (3c) (1.37 g, 3.9 mmol) was treated with 2 under the condition described in the case of 4b. After the reaction mixture was stirred for 2 h, the same workup as described above, followed by chromatographed to give 4c and 5c. The 3'-isomer (4c) was recrystallized from aqueous ethanol. The yield was 380 mg (21%). (Found: C, 61.35; H, 5.49; N, 8.72; $C_{24}H_{25}N_3O_7$ requires: C, 61.65; H, 5.40; N, 8.89%). The 2'-isomer (5c) was also recrystallized from aqueous ethanol. The yield was 550 mg (30%). (Found: C, 61.50; H, 5.41; N, 8.79; $C_{24}H_{25}N_3O_7$ requires C, 61.65; H, 5.40; N, 8.89%).

2'-O-(4-Methoxybenzyl)-N^2-benzoylguanosine (4d) and 3'-O-(4-methoxybenzyl)-N^2-benzoylguanosine (5d)

N^2-Benzoylguanosine (3d) (3.26 g, 8.42 mmol) was treated with 2 under the conditions described in the case of 4b. After 15 min, the mixture was worked up as described above and the products was applied to a column (5 X 15 cm) of silica gel. The appropriate fractions [eluted with benzene-acetone (65:35, v/v)] were evaporated in vacuo. The 3'-isomer (4d) was obtained in 21% (1.12 g) yield. (Found: C, 59.44; H, 5.09; N, 13.68; $C_{25}H_{25}N_5O_7$ requires: C, 59.22; H, 4.97; N, 13.81%). Then, the 2'-isomer (5d) was obtained in 24% (1.22 g) by recrystallization of the eluted material. (Found: C, 57.11; H, 5.26; N, 13.30; $C_{25}H_{25}N_5O_7 \cdot H_2O$ requires: C, 57.13; H, 5.19; N, 13.33%).

Tritylation of 4a-c

Compounds 4a-c (1 mmol) were treated with DMTrCl (375 mg, 1.1 mmol) in dry pyridine (5 ml) for 1 h. Chromatography, after the usual workup afforded 6a-c in good yields.

Phosphorylation of the partially protected nucleosides (8)

A dry THF (3 ml) solution of 5-chloro-8-hydroxyqunoline (395 mg, 2.2 mmol) and triethylamine (0.31 ml, 2.2 mmol) was added to a dry THF (3 ml) solution of 4-chlorophenylphosphorodichloridate (0.33 ml, 2.0 mmol) at -10 °C. The reaction mixture was stirred at 22 °C for 45 min. The precipitated triethylammonium hydrochloride was removed by filtration, and the filtrate was concentrated (to 5 ml) under reduced pressure. To this solution was added nucleoside derivatives (6) (1.5 mmol) and MeIm (0.24 ml, 3.0 mmol), and the reaction mixture was stirred for 30 min. The mixture was quenched with ice-water (2 ml) and repeatedly extracted with CH_2Cl_2 (2 X 25 ml). Combined organic extracts were washed with water (2 X 40 ml), and the CH_2Cl_2 was concentrated. The residue was dissolved in CH_2Cl_2 and chromatographed on a silica gel column (2 X 16 cm). The appropriate fractions [eluted with CH_2Cl_2-MeOH (95:5, v/v)] were evaporated to give the fully protected mononucleotide units (8a-c) in 80-88% yield.

8a: m.p. 97-99 °C; λ_{max}^{MeOH} 264, 232 nm; Rf 0.55 (solvent A). (Found: C, 62.69; H, 4,53; N, 4.20; $C_{53}H_{47}N_3O_{13}PCl_2$ requires: C, 62.48; H, 4.65; N, 4.12%).

8b: m.p. 95-97 °C; λ_{max}^{MeOH} 280, 233 nm; Rf 0.63 (solvent A). (Found C, 63.78; H, 4.85; N, 7.24; $C_{16}H_{52}N_6O_{11}PCl_2$ requires: C, 63.93; H, 4.57; N, 7.33%).

8c: m.p. 98-100 °C; λ_{max}^{MeOH} 301, 260, 232 nm; Rf 0,60 (solvent A). (Found: C, 63.34; H, 4.51; N, 4.75; $C_{61}H_{51}N_4O_{12}PCl_2$ requires: C, 63.21; H, 4.70; N, 4.92%).

Selective deprotection of 4-chlorophenyl group from 8a-c

4-Nitrobenzaldoxime (1.5 g, 9.0 mmol) was dissolved in a mixture of dioxane-water-triethylamine (1:1:1, v/v, 60 ml) and stirred for 20 min. To this solution was added 8a-c (1.0 mmol) and the reaction mixture was stirred for 10 h. The mixtre was evaporated in vacuo and then coevaporated with pyridine (10 ml) and toluene (3 X 10 ml). The residue was dissolved in CH_2Cl_2 and applied to a silica gel column (2 X 10 cm). The appropriate fractions [eluted with CH_2Cl_2-MeOH-Et_3N (95:5:5, v/v, 100 ml)] were evaporated in vacuo and precipitated in hexane. The triethylammonium salt of 9a-c were obtained 89-92% yields as stable colorless solids.

9a: ^{31}P-NMR (C_6D_6, 85% H_3PO_4) δ -5.45; 9b: ^{31}P-NMR (C_6D_6, 85% H_3PO_4) δ -5.76; 9c: ^{31}P-NMR (C_6D_6, 85% H_3PO_4) δ -6.42.

Detritylation from 10, 14, and 17

The fully protected compounds were treated with 3% Cl_3CCOOH in a mixture of CH_3NO_2-MeOH (95:5, v/v) at room temperature for 5-10 min. It was quenched with a mixture of pyridine-water (1:1, v/v) and extracted several times with CH_2Cl_2. The CH_2Cl_2 extracts was washed with water, dried over Na_2SO_4, filtered, and evaporated in vacuo. The residue was precipitated in a mixture of hexane-ether (9:1, v/v), which was used as 5'-hydroxyl components in the subsequent condensation without purification.

Rapid synthesis of trimer blocks (14)

The 5'-hydroxyl component (10) (1.22 g, 1.5 mmol) was combined with the 3'-phosphodiester component (9c) (2.51 g, 2.25 mmol), rendered anhydrous by coevaporation of dry pyridine (three times), and then treated with QsCl (1.27 g, 5.6 mmol) and MeIm (0.46 ml, 5.6 mmol) in dry pyridine (7.5 ml) at room temperature. After 1 h, no 5'-hydroxyl component (10) could be detected on TLC in the reaction mixture. The reaction mixture was quenched with ice-water and extracted with CH_2Cl_2 (50 ml). The CH_2Cl_2 extracts was washed with 0.1 M TEAB solution (pH 7.5, 2 X 30 ml) and wate (30 ml) and then concentrated. The residue was coevaporated with toluene and treated with 3% Cl_3CCOOH in a mixture of CH_3NO_2-MeOH (95:5, v/v, 10 ml) at room temperature for 10 min. It was quenched with 50% aqueous pyridine (5 ml) and extracted with CH_2Cl_2 (2 X 30 ml). The detritylated phosphodiester (13c) was removed from the reaction mixture by simple extraction with 5% $BaCl_2$ solution (4 X 50 ml). The organic layer was dried over Na_2SO_4 and evaporated in vacuo. The residue was dissolved in a small amount of CH_2Cl_2 and poured into a hexane-ether (95:5, v/v, 150 ml). The resulting precipitate was collected by filtration and was used for the next coupling reaction without further purification. The 5'-hydroxyl dimer (12b) was treated with 9b (2.55 g, 2.25 mmol) in the preaence of QsCl (1.26 g, 5.6 mmol) and MeIm (0.46 ml, 5.6 mmol) in dry pyridine (7.5 ml) for 1 h. Then ice-water was added and the product was extracted with CH_2Cl_2 (2 X 40 ml). The combined CH_2Cl_2 extracts were washed with 0.1 M TEAB solution and then with water. The organic layer was dried over Na_2SO_4, filtered, and evaporated in vacuo. The residue was dissolved in a small amount of CH_2Cl_2 and purified by a short silica gel column (2 X 12 cm) chromatography. The appropriate fractions [eluted with CH_2Cl_2-MeOH (98:2, v/v)] were evaporated to give the fully protected trimer (14b) which was isolated as a solid (1.98 g, 51%) by precipitation from hexane; λ_{max}^{MeOH} 312 (sh), 280, 260, 232 nm; Rf 0.37 (solvent A), Rf 0.15 (solvent B).

In a similar manner, the fully protected trimer (14a,c) were isolated as

solids. The yield of 14a was 1.39 g (61%); λ_{max}^{MeOH} 312 (sh), 275, 260, 232 nm; Rf 0.35 (solvent A), Rf 0.14 (solbent B). The yield of 14b was 480 mg (53%); λ_{max}^{MeOH} 305 (sh) 260, 230 nm; Rf 0.38 (solvent A), Rf 0.16 (solvent B).

Synthesis of hexamer (17)

The 3'-phosphodiester component (16) (1.32 g, 0.53 mmol) obtained from 14b (1.42 g, 0.56 mmol) by treatment with NBAO as described for 9 was combined with 15 (0.66 g, 0.32 mmol), rendered anhydrous by coevaporation of dry pyridine three times, and then treated with QsCl (0.42 g, 1.57 mmol) and MeIm (0.12 ml, 1.57 mmol) in dry pyridine (3 ml) for 1.5 h. The mixture was worked up as described for the preparation of 14 and purified by a silica gel column (4 X 20 cm) chromatography. The appropriate fractions [eluted with a stepwise gradient of MeOH (0-5%) in CH_2Cl_2] were evaporated to ca. 3-4 ml and poured into hexane (300 ml). A white prepcipitate was collected to give 17 (1.04 g, 73%); λ_{max}^{MeOH} 315 (sh), 275, 260, 232 nm; Rf 0.35 (solvent A), Rf 0.13 (solvent B).

Synthesis of nonamer (20)

The 3'-phosphodiester component (19) (89.2 mg, 0.039 mmol) obtained from 17 (96.5 mg, 0.042 mmol) by treatment with NBAO as described for 9 was combined with 18 (77,9 mg, 0.019 mmol), rendered anhydrous by coevaporation of pyridine three times and then treated with QsCl (32 mg, 0.12 mmol) and MeIm (0.01 ml, 0.12 mmol) in dry pyridine (0.3 ml) for 2 h. The mixture was worked up as a described for the preparation of 16 and purified by a C-18 silica gel (2 X 30 cm) column chromatography. The appropriate fractions [eluted with acetone (50-80%) in 0.2% aqueous pyridine] were evaporated to give the fully protected nonamer (20) which was isolated as a solid (103 mg, 85%) by precipitation from hexane; λ_{max}^{MeOH} 312 (sh), 280 (sh), 260, 232 nm; Rf 0.35 (solvent A), Rf 0.12 (solvent B).

Deprotection of the fully protected nonamer (20)

The nonamer 20 (103 mg, 16.2 umol) was treated with zinc acetate (567 mg, 2.59 mmol) in aqueous pyridine (90%, 10 ml) at room temperature for 3 days. The solution was passed through a column (1 X 20 cm) of Dowex 50W-X2 (pyridinium form) in aqueous pyridine (30%). The combined washings were concentrated. The residue was treated with $Tr^+BF_4^-$ (320 mg, 1.48 mmol) in a mixture of CH_3CN-H_2O (4:1,v/v, 12 ml) at room temperature for 8 h. Pyridine was added to the mixture, and the solution was evaporated in vacuo. The residue was dissolved in pyridine (3 ml) and treated with concentrated ammonia (27 ml) at 60 °C for 6 h. Ammonia was removed and the residue was dissolved in aqueous pyridine and washed with ethyl acetate. The solution was conecntrated to ca. 10 ml and applied to a column (2 X 45 cm) of DEAE-cellulose in 7 M urea, 0.02 M Tris-HCl (pH 7.5). Elution was performed with a linear gradient of NaCl and the nonamer (21) was eluted with 0.2 M NaCl (Fig. 1.). The product was further purified by HPLC (Finepak SIL C-18) (Fig. 2.). The fractionated 21 (39 OD) was checked by HPLC and characterized by base composition analysis by anion exchange HPLC after complete digestion with nuclease P1. The ratio of pU/pC/pA was 1.00:3.84:3.22. The chain lengths of the nonamer (21) was analyzed by electrophoresis of 20% polyacrylamide gel (Fig. 3.). $\lambda_{max}^{H_2O}$ (pH 7.0) 259 nm; Rf 0.23 (cellulose TLC, devepoment with n-PrOH-conc. NH_4OH-H_2O (55:35:10, v/v).

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