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Studies on tRNA and Related Compounds. XXXVII.[1] Synthesis and Physical Properties of 2′- or 3′-*O*-(*o*-Nitrobenzyl)nucleosides: the Use of *o*-Nitrophenyldiazomethane as a Synthetic Reagent

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2′- and 3′-*O*-(*o*-Nitrobenzyl) derivatives of uridine, cytidine, adenosine and guanosine were synthesized by treatment of uridine, *N*-benzoylcytidine, *N*-benzoyladenosine and *N*-isobutyrylguanosine, respectively, with *o*-nitrophenyldiazomethane followed by isolation and deblocking. 3′-*O*-(*o*-Nitrobenzyl)guanosine is a novel compound. By using *N*-acylated nucleosides, separation of the 2′- and 3′-substituted isomers on silica gel became feasible and these compounds were useful intermediates for the synthesis of oligoribonucleotides. Some physical properties of these compounds were studied by ultraviolet, nuclear magnetic resonance, circular dichroism and the 2′-substituted isomers were found to have more stacked structures than the 3′-isomers.

Keywords——NMR; UV; hypochromicity; CD; base stacking

The *o*-nitrobenzyl group[2] is known to be photolabile[3] and has been used as a protecting group for amino and glycosidic groups.[4] We have been using 2′-*O*-(*o*-nitrobenzyl) derivatives of uridine[5] [U2′(nBzl)][6] adenosine,[7a] cytidine[7a] and *N*-isobutyrylguanosine[7b] as starting intermediates in syntheses of oligoribonucleotides.[5-8] These derivatives were prepared by treatment of 2′,3′-*O*-dibutylstannylene uridine[9] or sodium hydride-treated nucleosides[7] with *o*-nitrobenzyl bromide. The use of *o*-nitrophenyldiazomethane in the preparation of 2′-*O*-(*o*-nitrobenzyl) derivatives of adenosine, uridine, cytidine and inosine was reported as a preliminary communication.[10] In this paper, we report a synthesis of 2′- and 3′-*O*-(*o*-nitrobenzyl) derivatives of *N*-isobutyrylguanosine, *N*-benzoyladenosine, *N*-benzoylcytidine and uridine by using *o*-nitrophenyldiazomethane. Further, by using *N*-acylated nucleosides, separation of the 2′- and 3′-isomers became possible by chromatography on silica gel. The proton nuclear magnetic resonance (^{1}HNMR) spectra of these 2′- or 3′-substituted nucleosides showed general features of benzyl ether substitution. The circular dichroism (CD) spectra and ultraviolet (UV) hypochromicities revealed marked differences in stacking structure between the 2′- and 3′-substituted nucleosides.

Preparation of *o*-Nitrophenyldiazomethane and Its Reaction with Nucleosides

For the preparation of *o*-nitrophenyldiazomethane (**2**), *o*-nitrobenzaltosylhydrazone (**1**) was first obtained by treatment of *o*-nitrobenzaldehyde with tosyl hydrazide[11] in a yield of 92%. **1** was converted to **2** by a modification of the published procedures,[12] by treatment with sodium methoxide at room temperature. The product was extracted with chloroform in a yield of about 70%. A four-fold excess of the hydrazone was used for *o*-nitrobenzylation of nucleosides, and freshly prepared *o*-nitrophenyldiazomethane (**2**) was then allowed to react with the nucleosides in DMF in the presence of stannous chloride, as shown in Chart 1. The reaction took 3 to 7 days at room temperature, but at 45° it was completed within 2 days.

In the case of uridine, U2′ (nBzl) (**4a**) and U3′ (nBzl) (**5a**) were found by thin-layer chromatography (TLC) (Table I) in a ratio of 1 : 1. Since these derivatives were insoluble in chloroform, **4a** was recrystallized from ethanol[5] without silica gel chromatography. The 3′-isomer

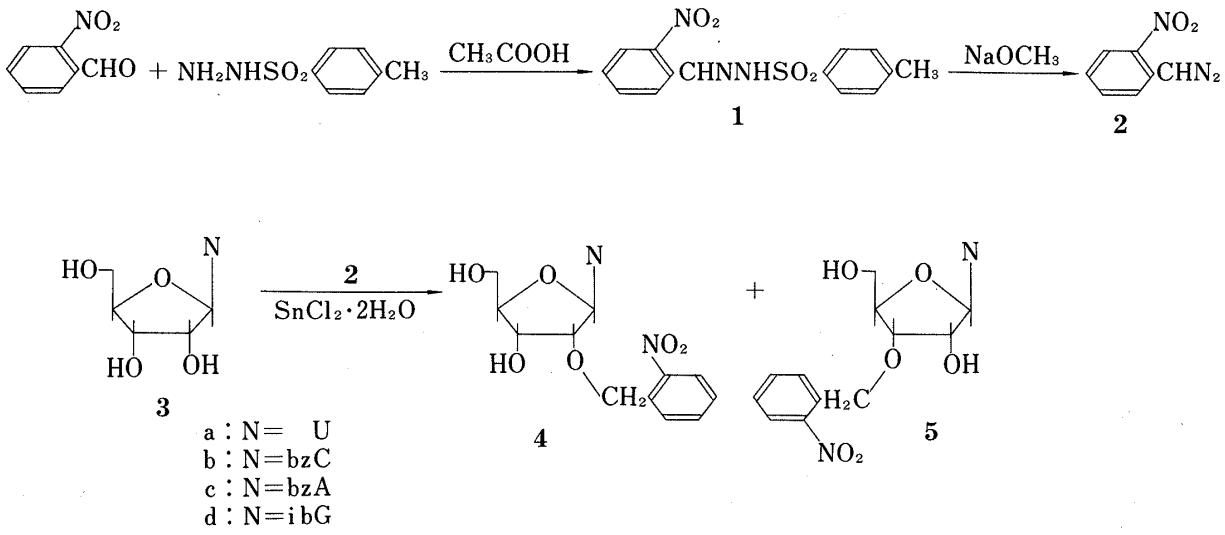

Chart 1

(**5a**) could be isolated from the mother liquor by chromatography on silica gel. Cytidine, adenosine and guanosine were converted to *N*-benzoylcytidine,[13] *N*-benzoyladenosine[14] and *N*-isobutyrylguanosine.[15] bzC2′(nBzl) (**4a**) and bzC3′(nBzl) (**5b**) were separated on a silica gel column after the non-volatile materials of the reaction mixture had been isolated by precipitation with hexane. The melting points and *Rf* values (on TLC) of isomers are given in Table I. The slower moving material was identified by direct comparison with C2′(nBzl)[7a] after deacylation. The faster moving derivative was identified as the 3′-isomer. The ^{1}H-NMR spectra showed a high-field-shifted H-1′ signal compared with that of the 2′-isomer, and the H-2′ signal was shifted slightly to low field. These data are summarized in Table II.

bzA (**3c**) was *o*-nitrobenzylated similarly and the 3′-isomer (**5c**) was recrystallized from aqueous ethanol in a yield of 20%. The 2′- and 3′-isomers in the mother liquor were separated

Table I. Melting Points and *Rf* Values

	Rf		mp (°C)
	CH_3Cl: EtOH 5: 1 (v/v)	CH_3Cl: EtOH 10: 1 (v/v)	
U	0.20	0.05	
U2′(nBzl)	0.54	0.23	204—205[5]
U3′(nBzl)	0.58	0.26	151—153
C2′(nBzl)			234—235[6]
C3′(nBzl)			108—110
bzC		0.13	
bzC2′(nBzl)		0.40	187—188
bzC3′(nBzl)		0.44	186—188
A2′(nBzl)			250[6] (230° colored)
A3′(nBzl)			255 (240° colored)
bzA		0.18	
bzA(2′nBzl)		0.43	99—101
bzA(3′nBzl)		0.46	224—227
G2′(nBzl)			260 dec.[7]
G3′(nBzl)			270
ibG	0.25	0.09	
ibG(2′nBzl)	0.50	0.18	163—164
ibG(3′nBzl)	0.54	0.21	127—129

Table II. NMR Spectral Data for 2′(3′)-*O*-(*o*-Nitrobenzyl)nucleosides

	Chemical shift (δ)[a]					($\Delta\delta$)[b]			
	$H_{1'}$	$J_{1'2'}$(Hz)	$H_{2'}$	$H_{3'}$	$H_{4'}$	$H_{1'}$	$H_{2'}$	$H_{3'}$	$H_{4'}$
U2′(nBzl)[5]	5.95	5		3.90—4.30		−0.17			
U3′(nBzl)	5.82	6	4.29	3.89—4.12		−0.04			
C2′(nBzl)[7a]	5.93	3	3.94	4.10	3.94	−0.15			
C3′(nBzl)	5.83	4	4.25	3.87—4.09		−0.05			
bzC2′(nBzl)	5.99	2	4.29	3.98—4.28					
bzC3′(nBzl)	5.88	4	4.29	4.02—4.20					
A2′(nBzl)[7a]	6.08	6	4.58	4.42—4.05		−0.16	0.05	−0.20	0.02
A3′(nBzl)	5.99	7	4.90	4.23		−0.07	−0.27	−0.01	−0.15
bzA2′(nBzl)	6.25	6	4.67	4.46	4.09				
bzA3′(nBzl)	6.12	6	4.92						
G2′(nBzl)[7b]	5.95	6	4.42		4.02	−0.23	0.02	−0.29	−0.10
G3′(nBzl)	5.75	6	4.66	4.08		−0.03	−0.22	−0.05	−0.16
ibG2′(nBzl)	6.03	6	4.44—4.56		4.05				
ibG3′(nBzl)	5.91	6	4.70	4.10—4.16					

a) DMSO-d_6.
b) $\Delta\delta$=δ(parent nucleoside)−δ(substituted nucleoside). A negative value represents a downfield shift.

on a silica gel column. The 2′-isomer (**4c**) was isolated in a yield of 17%. The products were characterized by elemental analysis and NMR spectroscopy (Table II).

o-Nitrobenzylation of ibG (**3d**) with **2** afforded the 2′- and 3′-isomers in a ratio of 1 : 1 (20% each) after column chromatography on silica gel. The melting points and *Rf* values (on TLC) are shown in Table I. The slower moving compound was identified as the previously obtained 2′-isomer.[7b] The NMR spectra of the faster travelling product showed a high-field-shifted H-1′ signal and a low-field-shifted H-2′ signal compared with those of the 2′-isomer. This compound was identified as ibG3′(nBzl) (**5d**); it was not isolated in reactions with *o*-nitrobenzyl bromide.[7b]

In the reaction with *o*-nitrophenyldiazomethane, the 2′- and 3′-(*o*-nitrobenzyl) derivatives of all four major nucleosides were obtained in a ratio of about 1 : 1 in each case. In the reaction of diazomethane with nucleosides, the 2′-isomers were reported to be the major products.[16] The present method seems to be suitable for the preparation of 3′-(*o*-nitrobenzyl)nucleosides. 3′-*O*-(*o*-Nitrobenzyl)-*N*-benzoyladenosine (**5c**) has been used for the synthesis of 2′-5′ linked oligoadenylates,[17] which were found in intereferon-treated cells as protein synthesis inhibitors.[18]

Physical Properties of 2′- and 3′-(*o*-Nitrobenzyl)nucleosides

NMR data for *o*-nitrobenzyl-substituted nucleosides are summarized in Table II. The chemical shifts of the 2′- and 3′- derivatives were compared with those of the parent nucleosides. For H-1′ of these isomers, the data were consistent with the rules proposed by Reese and his co-workers;[19] namely the 3′-substituted compounds had larger $J_{1',2'}$ coupling constants and their H-1′ signals showed high field shifts compared to those of the 2′-isomers. On substitution at the 2′-position, there was a downfield shift of the H-3′ signal, while each of the 3′-isomers showed a distinct downfield shift of the H-2′ signal. This may be due to an anisotropy effect of the *o*-nitrobenzyl group. Changes in sugar puckering induced by substitution may also affect the chemical shifts of sugar protons.

The UV spectra of these compounds were compared in aqueous and in ethanol media. The results are listed in Table III. The 2′-(nBzl)nucleosides showed a distinct increase in ε values in 95% ethanol compared with those in aqueous solution. On the other hand, the 3′-(nBzl)nucleosides showed little difference in the two media. This may well indicated that the *o*-nitrobenzyl moiety on the 2′-hydroxyl group interacts with the base plane, whereas the 3′-nBzl may not be able to approach the base moiety. In solution in ethanol, the ε values

for 2′-(nBzl)nucleosides correspond to additive values for the nucleosides and *o*-nitrobenzyl alcohol. The hypochromicities of these compounds were calculated as shown in Table III, and various degrees of hypochromicity were observed in the 2′-isomers.

The CD spectra of *o*-nitrobenzyl nucleosides were measured and the results are summarized in Table IV. Fig. 1 shows the CD spectra of the uridine derivatives in aqueous and alcoholic media. The CD spectrum of the 2′-isomer changes shape when the medium was changed

Table III. UV Spectral Data for 2′(3′)-*O*-nBzl Nucleosides

Nucleoside	λmax in nm (ε)					Hypo.[b]
	0.01 N HCl	H_2O	0.01 N NaOH	95% EtOH	Calc.in 95% EtOH[a]	
A2′(nBzl)	258.5 (18200)	260 (17800)	260 (18000)	260 (20000)	21100	11%
A3′(nBzl)	257 (19400)	259 (20100)	259 (19600)	260 (20600)	21100	—
G2′(nBzl)	255 (16300)	254 (16900)	258 (15200)	255 (19800)	19000	15%
G3′(nBzl)	255 (18100)	253 (18400)	258 (17200)	254 (18800)	19000	—
U2′(nBzl)	263 (13500)	263 (13700)	263 (11300)	263 (15100)	15700	9%
U3′(nBzl)	262.5 (15300)	262.5 (15500)	262.5 (12500)	262.5 (15800)	15700	—
C2′(nBzl)	277 (17000)	268 (13400)	268 (13500)	268 (14200)	13400	6%
C3′(nBzl)	278 (17500)	270 (14000)	270 (13700)	269 (13700)	13400	—

a) Parent nucleoside (ε)+*o*-nitrobenzyl alcohol (ε) in 95% EtOH.

b) Hypochromicity $= \frac{(95\%\ \text{EtOH}) - (H_2O)}{(95\%\ \text{EtOH})} \times 100.$

Table IV. CD Properties of 2′(3′)-*O*-nBzl Nucleosides

	H_2O		95% EtOH	
	λ (nm)	$[\theta] \times 10^{-4}$	λ (nm)	$[\theta] \times 10^{-4}$
U2′(nBzl)	330[a]	−0.38		
	267[a]	1.27	267	1.63
	225[a]	−0.74	239	−0.49
U3′(nBzl)	325	−0.29	340	−0.31
	268	1.30	270	1.19
C2′(nBzl)	325[b]	−0.15		
	265	1.62	272	2.18
C3′(nBzl)	330	−0.20	340	−2.06
	268	1.30	270	1.19
A2′(nBzl)	334[c]	−1.02		
	275	1.49	279	0.62
	240	0.31	251	−1.02
	230	−0.62		
A3′(nBzl)	—	—	—	—
G2′(nBzl)	325	−1.10		
	262	1.54	285	0.03
	236	−0.72	241	−0.09
G3′(nBzl)	325	−0.19	335	−0.34

a) 0.01 M potassium phosphate, pH 7.0.
b) 0.01 M sodium cacodylate, pH 7.0,
c) 0.01 M potassium phosphate, pH 7.0 plus 0.01 M KCl.

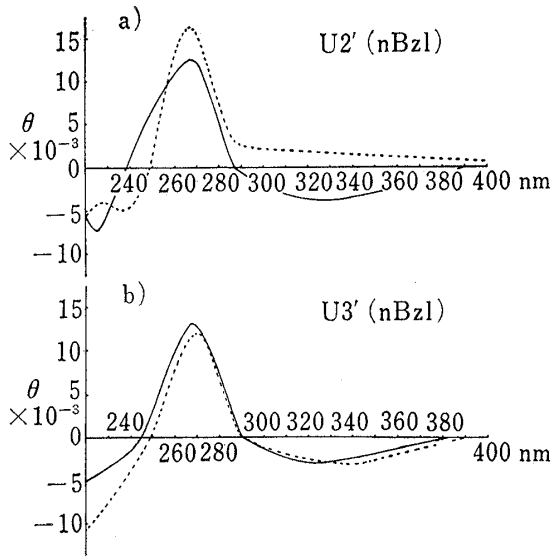

Fig. 1. CD Spectra of U(nBzl): a) the 2′-Isomer; b) the 3′-Isomer in Aqueous Media (——) and 95% Ethanol (------)

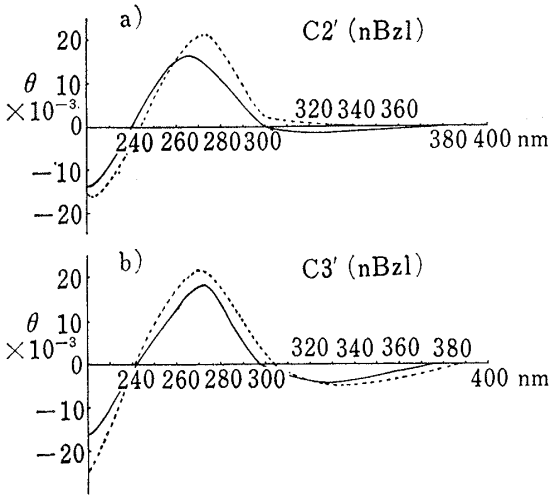

Fig. 2. CD Spectra of C(nBzl): a) the 2′-Isomer; b) the 3′-Isomer in Aqueous Media (——) and 95% Ethanol (------)

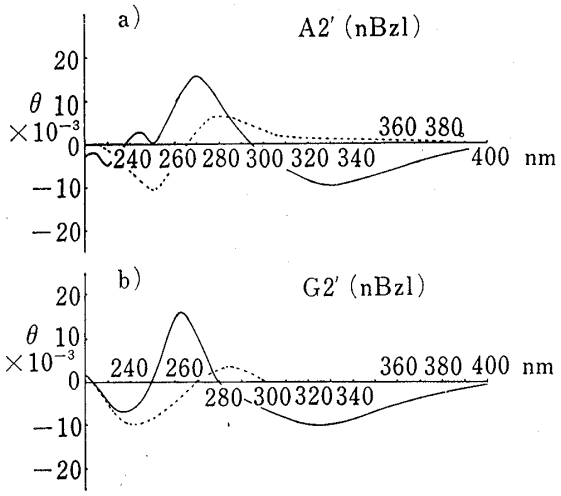

Fig. 3. CD Spectra of the 2′-(nBzl)purine Nucleosides: a) A(nBzl); b) G(nBzl) in Aqueous (——) and 95% Ethanol (------)

from aqueous solution to alcohol, while the 3′-substituted uridine showed almost the same spectrum in both media. This is consistent with the UV hypochromicities. However, in the case of cytidine the isomers showed fairly similar spectra (Fig. 2). The 2′-substituted purine derivatives showed CD spectra with larger θ values (Fig. 3), which were different, from those of the parent nucleosides.[20] However, A3′ (nBzl) and G3′ (nBzl) had very small θ values similar to those of A and G. All 2′-substituted (*o*-nitrobenzyl)nucleosides and C3′ (nBzl) showed a negative band at a wavelength longer than 320 nm. This may be due to n-π* transitions arising from stacking of the two aromatic rings, except for C3′ (nBzl), for which the UV hypochromicity data, do not indicate any stacking.

Experimental

TLC was performed on plates of silica gel (Merck, Kieselgel 60 F_{254}) with a mixture of chloroform–ethanol. For columns, silica gel (Merck, Kieselgel type G) was used. Melting points are uncorrected (Table I). CD spectra were recorded with a Jasco ORD/UV-5 spectropolarimeter equipped with a CD attachment. Calibration of Cotton effect magnitude was effected with *d*-10-camphorsulfonic acid. Solutions of A_{max} *ca.* 1.3 were prepared by using the solvent systems given in the figure legends. Cells of 10 mm path length were used. The UV absorptions of these solutions were measured against appropriate blanks on a Hitachi 124 or 323 spectrophotometer. NMR spectra were recorded on a Hitachi R-22 spectrometer (90 MHz) with TMS as an internal standard.

Other general methods for the characterization of nucleosides by deacylation or removal of the *o*-nitrobenzyl group were as described previously.[7a,8a]

o-Nitrobenzaltosylhydrazone (1)——Tosyl hydrazide (74.5 g, 0.4 mol) was dissolved in hot acetic acid (100 ml) and mixed with a heated solution of *o*-nitrobenzaldehyde (0.4 mol) in acetic acid (100 ml). The mixture was heated to the boiling point, cooled to room temperature and kept at 4°. The resulting yellow

crystals were collected and washed with cold acetic acid (100 ml), 50% aqueous acetic acid (100 ml) and water (200 ml). The yield was 117.9 g (92%) mp 149—152°. NMR (d_6-DMSO) δ: 2.4 (s, 3H, $-CH_3$), 8.3 (s, 1H, -CH=N-), 12.5 (s, 1H, -NH-).

***o*-Nitrophenyldiazomethane (2)**——Compound **1** (40 mmol) was added to a mixture of sodium methoxide (80 mmol in 120 ml of methanol) and triethylene glycol (40 ml). Methanol was removed by evaporation in the dark. The residue was kept at room temperature for 30—60 min, then ice-water (40 ml) was added. The product was extracted twice with chloroform (30 ml), and washed with water until the organic phase became orange in color. The aqueous phase was kept at room temperature in the dark overnight. The chloroform solution was kept below −20° for 10 min and insoluble materials were removed by rapid filtration. The amount of the product was estimated by esterification of benzoic acid. The yield was 60—70%. Some of the product was recovered from the aqueous phase by extraction with chloroform.

2′-*O*-(*o*-Nitrobenzyl)uridine (4a) and 3′-*O*-(*o*-Nitrobenzyl)uridine (5a)——Uridine (**3a**) (2.44 g, 10 mmol) and $SnCl \cdot 2H_2O$ (200 mg) were dissolved in DMF at 45°. *o*-Nitrophenyldiazomethane (**2**) [obtained from 40 mmol of **1**] in chloroform (10 ml) was added to the mixture and the whole was kept at 45°. After 24 hr, a small amount of uridine was detected besides products (**4a, 5a**) on TLC (Table I). The solvent was removed after 2 days and the residue was mixed vigorously with water (50 ml) and ether. The ether layer was decanted off, and the procedure was repeated with added ether. Water (50 ml) was added to the aqueous phase and the solution was filtered with heating. The product (**4a**) crystallized out upon cooling the solution to 4° and was recrystallized twice from aqueous ethanol. The yield was 738.2 mg (20%). The mother liquor was concentrated by evaporation with pyridine, dissolved in chloroform and applied to a silica gel column to obtain **5a**.

2′-*O*-(*o*-Nitrobenzyl)-*N*-benzoylcytidine (4b) and 3′-*O*-(*o*-Nitrobenzyl)-*N*-benzoylcytidine (5b)——*N*-Benzoylcytidine (**3b**) (10 ml) was treated with **2** under the conditions described for the synthesis of **4a**. After 2 days, TLC (10: 1) showed two new spots (**4a, 5b**, see Table I) and the starting material (**3b**) was not detected. The volatile materials were removed by evaporation and the residue was precipitated with hexane from its solution in pyridine. The precipitated syrup was dissolved in pyridine after the removal of hexane by decantation, and the solution was evaporated to dryness. The residue was coevaporated with toluene, dissolved in chloroform and applied to a column (3.5 × 29 cm) of silica gel (150 g). Elution was performed with chloroform–ethanol (45: 1) and eluted materials were identified by TLC. The 3′-isomer (**5b**) (482.9 mg) was obtained in a yield of 10% UV: λ_{max} 261, 300 nm (sh) in 95% ethanol. *Anal.* Calcd for $C_{23}H_{22}N_4O_8$ (482.46): C, 57.26; H, 4.60; N, 11.61. Found: C, 56.93; H, 4.56; N, 11.43. The 2′-isomer (**4b**) was recrystallized from aqueous ethanol. The yield was 1.25 g (20%). UV: λ_{max} 261, 300 nm (sh) in 95% ethanol.

2′-*O*-(*o*-Nitrobenzyl)-*N*-benzoyladenosine (4c) and 3′-*O*-(*o*-Nitrobenzyl)-*N*-benzoyladenosine (5c)——Compound **3c** (6.32 g, 17 mmol) was *o*-nitrobenzoylated with $SnCl_2 \cdot 2H_2O$ (300 mg) and **2** [prepared from the tosyl hydrazide (**1**, 70 mmol)], as described for the synthesis of **4a**. After 3 days, the reaction mixture was worked up as above. The products were dissolved in chloroform and applied to a column (5.5 × 23 cm) of silica gel. The column was washed with chloroform and then **5c** and **4c** were eluted with chloroform–ethanol (45: 1). bzA3′ (nBzl) (**5c**) was recrystallized from aqueous ethanol. The yield was 1.68 g (20%). UV: λ_{max} 278 nm in ethanol. *Anal.* Calcd for $C_{24}H_{22}N_6O_7$ (506.48): C, 56.91; H, 4.38; N, 16.60. Found: C, 57.07; H, 4.36; N, 16.76. The 2′-isomer (**4c**) was precipitated with ether–hexane from the concentrated column eluate. The yield was 1.44 g (17%). UV: λ_{max} 278 nm in methanol.

3′-*O*-(*o*-Nitrobenzyl)adenosine——Compound **5c** (178.8 mg, 0.35 mmol) was dissolved in pyridine (3 ml), and concentrated ammonia (10 ml), was added. After 16 hr at 30°, deacylation was completed. The volatile materials were removed and the product was recrystallized from 95% ethanol. The yield was 97.2 mg (68.6%). UV: λ_{max} 260 nm in 95% ethanol. *Anal.* Calcd for $C_{17}H_{18}N_6O_6$ (402.36): C, 50.74; H, 4.51; N, 20.89. Found: C, 50.72; H, 4.43; N, 20.67.

2′-*O*-(*o*-Nitrobenzyl)-*N*-isobutyrylguanosine (4d) and 3′-*O*-(*o*-Nitrobenzyl)-*N*-isobutyrylguanosine (5d)——ibG (**3d**) (7.97 g, 20 mmol) and $SnCl_2 \cdot 2H_2O$ (200 mg) were dissolved in DMF (150 ml) and reacted with **2** [prepared from **1** (70 mmol)] in chloroform (20 ml) at 45° for 3 days as described for the synthesis of **4a**. The mixture was concentrated and added to hexane. The whole was kept at room temperature overnight, deacylated, dissolved in pyridine and applied to a column (6.5 × 18 cm) of silica gel (250 g). The column was washed with chloroform and the products were eluted with chloroform–ethanol (25: 1). **5d** (1.96 g) was obtained in a yield of 20%. UV: λ_{max} 259, 278 nm in 95% ethanol. *Anal.* Calcd for $C_{21}H_{24}N_6O_8$ (488.46): C, 51.63; H, 4.95; N, 17.21. Found: C, 51.91; H, 4.85; N, 16.98. The 2′-isomer (**4d**) was obtained by recrystallized of the eluted material in a yield of 32% (3.14 g).

3′-*O*-(*o*-Nitrobenzyl)guanosine——Compound **5d** (200 mg, 0.41 mmol) was treated overnight with methanolic ammonia at room temperature. The mixture was concentrated to dryness and the residue was recrystallized from aqueous methanol. The yield was 133.5 mg (75%). UV: λ_{max} 254, 270 nm (sh) in 95% ethanol. *Anal.* Calcd for $C_{17}H_{18}N_6O_7 \cdot H_2O$ (436.38): C, 46.79; H, 4.62; N, 19.26. Found: C, 46.76; H, 4.79; N, 19.34.

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