A New Inosine Disaccharide from the Crustacean *Ligia exotica*: Isolation and Structure Elucidation by Total Synthesis

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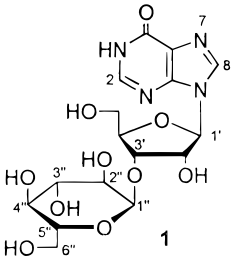
Received February 14, 2000

Abstract: A novel nucleoside has been isolated from the crustacean *Ligia exotica*, and the structure was elucidated as 3′-*O*-(α-D-glucosyl)inosine, **1**, by analysis of spectroscopic data and by total synthesis.

Numerous bioactive nucleoside analogues have been isolated from marine organisms such as nudibranches,[1] sponges,[2] a tunicate,[3] and a seaweed.[4] Tubercidin 5′-α-D-glucopyranose was isolated from a Hawaiian blue green algae.[5] Suzuki and his co-worker reported a mixture of 2′-*O*- and 3′-*O*-substituted inosine, and their structures were thought to be 2′-*O*- and 3′-*O*-(β-D-galactosyl)inosine but without any detailed experimental evidence.[6] During our studies on bioactive substances from marine organisms, we recently isolated a novel nucleoside from the crustacean *Ligia exotica* (Ligiidae). In this paper we describe the isolation, structure elucidation, and synthesis of 3′-*O*-(α-D-glucosyl)inosine (**1**).

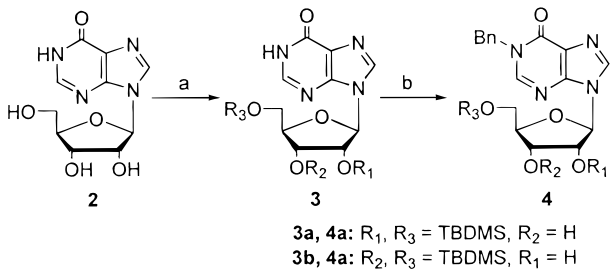
The water-soluble extract of the crustacean subjected to gel filtration followed by repeated reversed-phase HPLC yielded **1**. Compound **1** was obtained as a white amorphous solid.



1

The high-resolution FABMS data of **1** established the molecular formula as $C_{16}H_{22}N_4O_{10}$ [*m*/*z* 431.1378 $(M + H)^+$, calcd for $C_{16}H_{23}N_4O_{10}$, 431.1414], which was supported by the ^{13}C NMR spectrum, showing signals for 16 carbon atoms. The IR absorption bands at 3376 and 1686 cm^{-1} suggested the presence of hydroxyl and carbonyl groups, respectively, and the UV spectrum showed an absorption maximum at λ_{max} 248 nm, which may be attributable to the chromophore of a purine base.[7] Extensive analysis of 2D NMR spectra ($^1H-^1H$ COSY, HMQC, HMBC, NOESY) suggested that **1** consisted of a purine base and two sugar units. Since the ^{13}C NMR chemical shifts for the purine moiety [δ_C 147.0 (d, C2), 148.4 (s, C4), 124.4 (s, C5), 158.0 (s, C6), 138.4 (d, C8)] coincided well with those of the purine base of inosine,[8] the purine base of **1** was assigned as hypoxanthine. This was confirmed by the ^1H NMR spectra, showing two singlets at low-field resonances of δ_H 8.04 (s, H-2) and 8.28 (s, H-8) due to the hypoxanthine ring. The comparison of the NMR spectra of inosine with those of **1** suggests that compound **1** is an inosine derivative that contains a hexopyranose sugar unit, which was identified as a glucopyranose ring. The ^1H NMR signals for the two sugar units were firmly assigned with the aid of $^1H-^1H$ COSY and HMBC, and the correlation of ^{13}C NMR signals with ^1H NMR signals was accomplished by performing an HMQC experiment. The ^{13}C NMR chemical shifts for the ribose unit of **1** were coincident with those from the sugar moiety of inosine except for that of C-3′ (**1**, δ_C 77.5; inosine, δ_C 71.2), implying that the second sugar unit is attached to the C-3′ position.[9] The ^{13}C NMR chemical shift for the anomeric carbon C-1″ (δ_C 100.0, lit.;[10a] $\delta_{C-1''} = 100-101$ in α-form, $\delta_{C-1''} = 103-104$ in β-form) and the coupling constant for the anomeric proton of the glucose unit H-1″ ($J_{1''-2''} = 3.4$ Hz, lit.;[10b] $J_{1''-2''} = 3.5$ Hz in α-form, $J_{1''-2''} = 8.0$ Hz in β-form) indicate that the stereochemistry of glucoside is via an α-linkage. Judging from the results of all the spectral data, it is suggested that the structure of **1** is 3′-*O*-(α-D-glucosyl)inosine.

Scheme 1



Reagents and conditions: (a) TBDMS-Cl, pyridine, rt, 48 h, 80%; (b) benzyl bromide, K_2CO_3, 60 °C, 4 h, 90%

3′-*O*-(α-D-Glucosyl)inosine has been neither isolated nor synthesized. Only a mixture of 2′-*O*- and 3′-*O*-(β-D-galactosyl)inosine was isolated from the reaction of *O*-nitrophenyl-β-galactoside and inosine mediated by β-galactosidase prepared from *E. coli*.[6] Thus, it is a challenge to stereospecically synthesize four isomeric 2′-*O*-α- and -β-, and 3′-*O*-α- and -β-disaccharide nucleosides.

To confirm the correct structure of the new inosine disaccharide, the four stereoisomers 2′-*O*-(α-D-glucosyl)- and 2′-*O*-(β-D-glucosyl)inosine and 3′-*O*-(α-D-glucosyl)- and 3′-*O*-(β-D-glucosyl)inosine were synthesized. The synthetic approach to these compounds must include an *O*-glycosylation step to form the disaccharide. Although there are plenty of examples of efficient nucleoside formation from simple, elaborated, or disaccharidal glycosyl donors, there are few cases of successful *O*-glycosylation of a nucleoside.[11] Attachment of a glucopyranosyl subunit at *O*-3′ of inosine would represent a rare example of *O*-glycosylation of a purine nucleoside because it requires overcoming the double obstacle of steric hindrance and competing depurination. Glycosyl acceptor **4a** was prepared through two steps (Scheme 1).

The protection of inosine (**2**) with *tert*-butyldimethylsilyl chloride in pyridine gave a mixture of 2′,5′-di-*O*- and 3′,5′-di-*O*-(*tert*-butyldimethylsilyl)inosines (**3a**, **3b**). *N*-1-Benzylation of the **3a** and **3b** mixture with benzyl bromide yielded the glycosylation acceptor mixture of **4a** and **4b**, which was separated by Si gel column chromatography as

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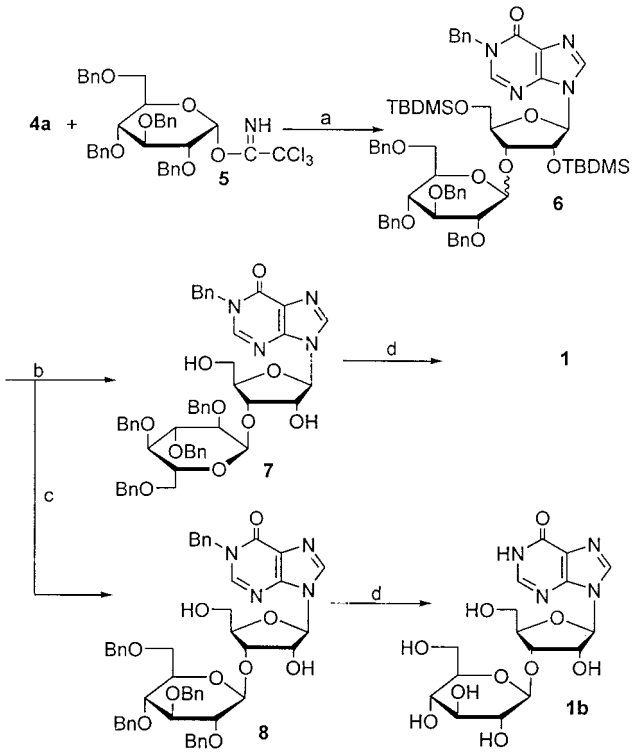
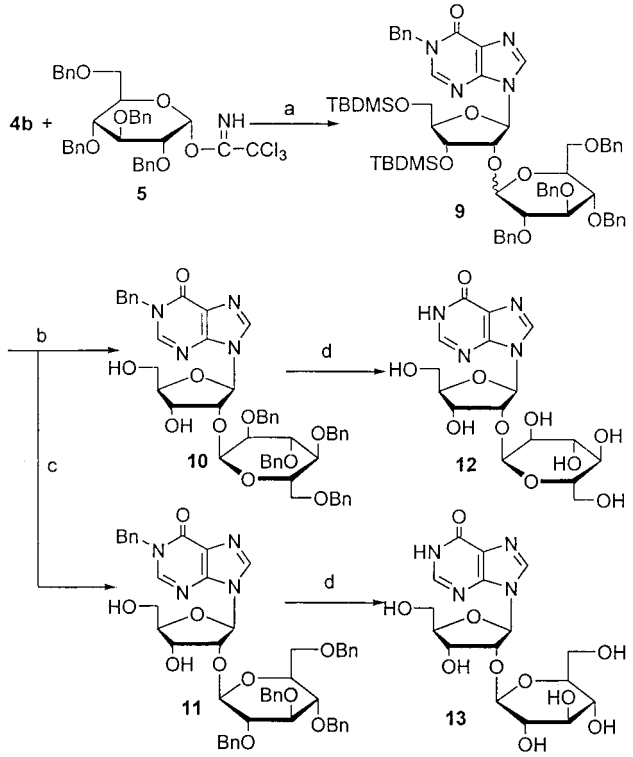
10.1021/np0000724 CCC: $19.00
Published on Web 09/02/2000

Scheme 2



Reagents and conditions (a) BF_3-Et_2O, CH_2Cl_2, -10 °C, 1 h, 56% (α : β = 8 :1); (b) *n*-Bu_4NF, THF, 1 h, 90%; (c) Si gel column separation; (d) $Pd(OH)_2$ / C, cyclohexene, EtOH, reflux, 4 h, 80%

Scheme 3



Reagents and conditions : (a) BF_3-Et_2O, CH_2Cl_2, -10 °C, 1 h, 42% (α ; β = 15 : 1); (b) *n*-Bu_4NF,THF, 1 h, 90%; (c) Si gel column separation; (d) $Pd(OH)_2$ / C, cyclohexene, EtOH, reflux, 4 h, 80%

described in the Experimental Section. The TBDMS moiety is known to migrate to adjacent hydroxyl groups (from **4a** to **4b** and vice versa) under basic conditions.[12] Thus, the second step was performed without separation of **3a** and **3b**. Benzyl-protected glucopyranosyl donor **5** was prepared according to a literature procedure.[13] The nonparticipating benzyloxy group at *C*-2″ was expected to direct α-glycosylation. Scheme 2 shows the synthetic procedure used for the synthesis of **1**.

Activation of the donor **5** with $BF_3{\cdot}Et_2O$ at −10 °C in the presence of acceptor **4a** led to the consumption of both starting materials and formation of glycosylated products as an approximate 8:1 mixture of α- and *β*-anomers. Silica gel column separation of the anomers was readily effected after desilylation to give the α-disaccharide **7** and its *β*-anomer **8**. Compound **7** was treated with $Pd(OH)_2$/C in the presence of cyclohexene and EtOH to give compound **1**, for which spectral data were identical to those of the natural product, and **8** to give 3′-*O*-(*β*-D-glucosyl)inosine (**1b**). 2′-*O*-(α-D-Glucosyl)inosine (**12**) and 2′-*O*-(*β*-D-glucosyl)-inosine (**13**) were respectively prepared by a method similar to that described above using **4b** as glycosyl acceptor (Scheme 3) and identified by ^1H and ^{13}C NMR spectroscopy. Thus the structure of **1** was concluded to be 3′-*O*-(α-D-glucosyl)inosine.

Experimental Section

General Experimental Procedures. All solvents were redistilled. Merck Si gel 60 (230−400 mesh) and Sephadex G-10 were used for chromatography. HPLC was conducted using a UV detector, a JAI GS 320, and a Waters ODS column. UV spectra were obtained on a Beckmann DU-64 instrument and IR spectra on a Bomem MB-100 FT spectrophotometer. Optical rotations were obtained on an Autopoll III automatic polarimeter at room temperature. NMR spectra were obtained on a Bruker AVANCE 400 MHz spectrometer; signals are reported in parts per million (*δ*), referenced to the solvent used. Mass spectra were obtained on a VG Autospec Ultma mass spectrometer.

Animal Material. The crustacean *Ligia exotica* (Ligiidae) was collected off the Haeundae coast, near Busan, Korea. The animal was verified by Prof. S. H. Hur at Bukyung National University. A voucher specimen (PK-9108YHK) was deposited in the Department of Chemistry, Korea Advanced Institute of Science and Technology, Taejon, Korea.

Extraction and Isolation. The crustacean *L. exotica* was immediately stored in methanol (2 L). The crustacean (1 kg wet wt) was separated by decanting the supernatant methanol solution, and the animal tissue was crudely homogenized with distilled water (1.5 L). This homogenate was centrifuged three times with a superspeed centrifuge (5000 rpm, 10 min). The supernatant was freeze-dried to give a gelatinous mixture, which was dialyzed using a cellulose membrane, SPECTAPOR (cylinder vol.; 0.85 mL/min, MW cut off: ca. 3500). Dialysis was performed with water and then a 1% AcOH−water solution. The dialysate was lyophilized to give 38 g of sticky solid. The water extract of *L. exotica* was subjected to gel filtration on Sephadex G-10 (1.9 × 110 cm column; flow rate, 3 mL/min; eluent, H_2O) followed by repeated reversed-phase HPLC [JAI GS-320, 20 × 500 mm, and ODS 20 × 600 mm; flow rate, 3 mL/min; UV detection at 254 nm; eluent, MeOH−H_2O = 70:30, v/v] to give **1** (3.4 mg, 0.0003%).

Compound 1: white amorphous solid; $[\alpha]^{20}_D$ +38° (*c* 0.34, H_2O); UV λ_{max} (H_2O) 248 nm (ϵ 8400); IR (NaCl) ν_{max} 3376, 2926, 1686, 1592, 1312, and 1078 cm^{-1}; ^1H and ^{13}C NMR [Table 1]; HRFABMS *m/z* 431.1378 $(M + H)^+$, calcd for $C_{16}H_{23}N_4O_{10}$, 431.1414.

2′,5′-Di-*O*-(*tert*-butyldimethylsilyl)inosine (3a) and 3′,5′-Di-*O*-(*tert*-butyldimethylsilyl)inosine (3b). To a stirred dispersion of inosine (5.36 g, 20 mmol) in pyridine was added TBDMSCl (4.50 g, 30 mmol). The mixture was stirred at room temperature for 48 h. After evaporation under reduced pressure, the residue was dissolved in CH_2Cl_2 (100 mL). The

Table 1. ^{1}H and ^{13}C NMR Data and Heteronuclear Multiple Bond Correlations (HMBC) of **1** in DMSO-d_6[a]

position	^{1}H	J (Hz)	^{13}C (mult.)[b]	HMBC (^{1}H)
2	8.04s		147.0 (d)	
4			148.4 (s)	2, 1′
5			124.4 (s)	8
6			158.0 (s)	2
8	8.28 (s)		138.4 (d)	1′
1′	5.87 (d)	6.1	87.5	2′, 3′
2′	4.63 (dd)	5.4, 6.1	73.2 (d)	1″
3′	4.26 (dd)	3.0, 5.4	77.5 (d)	1″
4′	4.11 (ddd)	3.0	84.2 (d)	
5′	3.62 (m)		61.3 (t)	
1″	4.93 (d)	3.4	100.0 (d)	3′
2″	3.23 (dd)	3.4, 9.4	72.0 (d)	1″, 3″, 5″, 6″
3″	3.47 (dd)	8.7, 9.4	73.8 (d)	4″
4″	3.11 (t)	8.7	70.0 (d)	3″, 5″, 6″
5″	3.47 (m)		73.6 (d)	4″, 6″
6″	3.41−3.63 (m)		60.7 (t)	4″

[a] Taken in 400 MHz for ^{1}H and 100 MHz for ^{13}C at 298 K. [b] Multiplicities inferred from a DEPT experiment.

solution was washed consecutively with 1 N HCl (2 × 30 mL) and water (2 × 30 mL) and then dried over $MgSO_4$. Evaporation of the solvent gave the crude product, which was chromatographed on a Si gel column (eluent, CH_2Cl_2−MeOH = 95:5, v/v; flow rate, 10 mL/min) to give a mixture of **3a** and **3b** (7.94 g, 80%). **3a**: mp 216−218 °C; IR (NaCl) ν_{max} 3411, 1697, 1123 and 836 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.20 (1H, s, N*H*), 8.23 (1H, s, H-8), 8.22 (1H, s, H-2), 6.04 (1H, d, J = 4.8 Hz, H-1′), 4.54 (1H, dd, J = 4.8, 4.9 Hz, H-2′), 4.26 (1H, ddd, J = 4.0, 4.4, 4.4 Hz, H-3′), 4.17 (1H, m, H-4′), 3.97 (1H, dd, J = 2.4, 11.6, H_a-5′), 3.83 (1H, dd, J = 2.4, 11.6, H_b-5′), 2.69 (1H, d, J = 4.4 Hz, 3′-OH), 0.92 (9H, s, C(C*H*$_3$)$_3$), 0.12 (3H, s, C*H*$_3$), 0.10 (3H, s, C*H*$_3$), −0.05 (3H, s, C*H*$_3$), −0.12 (3H, s, C*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.3 (C-6), 148.9 (C-4), 145.3 (C-2), 138.5 (C-8), 124.8 (C-5), 88.2 (C-1′), 85.3 (C-4′), 77.4 (C-2′), 71.1 (C-3′), 63.0 (C-5′), 25.9, 25.5, 18.4, 17.8, −5.1, −5.2, −5.4, −5.5; HREIMS m/z 496.2546 (M)$^+$, calcd for $C_{22}H_{40}N_2O_5Si_2$, 496.2535.

3b: mp 208−210 °C; IR (NaCl) ν_{max} 3369, 1698, 1139 and 837 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.11 (1H, s, N*H*), 8.16 (1H, s, H-8), 8.10 (1H, s, H-2), 5.98 (1H, d, J = 4.4 Hz, H-1′), 4.52 (1H, dd, J = 4.5, 5.0 Hz, H-3′), 4.44 (1H, ddd, J = 5.0, 5.2, 5.7 Hz, H-2′), 4.08 (1H, m, H-4′), 3.91 (1H, dd, J = 3.4, 11.5, H_a-5′), 3.75 (1H, dd, J = 3.4, 11.5, H_b-5′), 3.06 (1H, d, J = 5.7, 2′-OH), 0.92 (9H, s, C(C*H*$_3$)$_3$), 0.88 (9H, s, C(C*H*$_3$)$_3$), 0.14 (3H, s, C*H*$_3$), 0.13 (3H, s, C*H*$_3$), 0.05 (3H, s, C*H*$_3$), 0.03 (3H, s, C*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.3 (C-6), 148.7 (C-4), 145.0 (C-2), 138.7 (C-8), 125.1 (C-5), 88.9 (C-1′), 85.3 (C-4′), 77.0 (C-3′), 71.3 (C-2′), 62.1 (C-5′), 25.9, 25.7, 18.3, 18.0, −4.6, −4.8, −5.4, −5.2; HREIMS m/z 496.2509 (M)$^+$, calcd for $C_{22}H_{40}N_2O_5Si_2$, 496.2535.

1-*N*-(Benzyl)-2′,5′-di-*O*-(*tert*-butyldimethylsilyl)inosine (4a) and 1-*N*-(Benzyl)-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)inosine (4b). To a stirred solution of a mixture of **3a** and **3b** (7.45 g, 15 mmol) in THF (75 mL) were added benzyl bromide (3.57 mL, 30 mmol) and potassium carbonate (4.15 g, 30 mmol). The mixture was stirred at 60 °C for 4 h and quenched by aqueous NH_4Cl (5 mL). After evaporation under reduced pressure, the residue was dissolved in EtOAc (200 mL). The solution was washed with water (2 × 100 mL) and then dried over $MgSO_4$. Evaporation of solvent gave the crude product, which was chromatographed on a Si gel column (eluent, EtOAc−*n*-hexane−CH_2Cl_2 = 50:35:15, v/v/v; flow rate, 10 mL/min) to give products [**4a** (4.48 g) and **4b** (3.00 g), 90%]. **4a**: mp 159−160 °C; IR (NaCl) ν_{max} 3494, 1697, 1133 and 837 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (1H, s, H-8), 7.97 (1H, s, H-2), 7.28−7.31 (5H, m, Ph*H*), 5.99 (1H, d, J = 5.2 Hz, H-1′), 5.25 (2H, d, J = 3.4 Hz, N−C*H*$_2$Ph), 4.52 (1H, dd, J = 5.0, 5.2 Hz, H-2′), 4.23 (1H, ddd, J = 4.1, 4.2, 5.0 Hz, H-3′), 4.17 (1H, m, H-4′), 3.95 (1H, dd, J = 2.4, 11.5, H_a-5′), 3.81 (1H, dd, J = 3.4, 11.5, H_b-5′), 2.66 (1H, d, J = 4.2 Hz, 3′-OH), 0.92 (9H, s, C(C*H*$_3$)$_3$), 0.80 (9H, s, C(C*H*$_3$)$_3$), 0.11 (3H, s, C*H*$_3$), 0.10 (3H, s, C*H*$_3$), −0.05 (3H, s, C*H*$_3$), −0.15 (3H, s, C*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.5 (C-6), 147.3 (C-4), 147.0 (C-2), 138.2 (C-8), 135.9 (C_q, Ph), 128.9,128.2,127.9 (*C*H, Ph), 124.6 (C-5), 87.8 (C-1′), 85.4 (C-4′), 77.4 (C-2′), 71.3 (C-3′), 63.1 (C-5′), 48.9 (N-*C*H$_2$Ph), 25.9, 25.5, 18.4, 17.8, −5.1, −5.2, −5.4, −5.5; HREIMS m/z 586.2999 (M)$^+$, calcd for $C_{29}H_{46}N_2O_5Si_2$, 586.3007.

4b: mp 194−196 °C; IR (NaCl) ν_{max} 3369, 1698, 1139 and 836 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (1H, s, H-8), 7.98 (1H, s, H-2), 7.28∼7.31 (5H, m, Ph), 5.93 (1H, d, J = 4.6 Hz, H-1′), 5.16−5.31 (2H, d, J = 29.4 Hz, N−C*H*$_2$Ph), 4.49 (1H, dd, J = 4.4, 4.5 Hz, H-3′), 4.41 (1H, ddd, J = 4.4, 4.5, 4.9 Hz, H-2′), 4.05 (1H, m, H-4′), 3.87 (1H, dd, J = 3.4, 11.6, H_a-5′), 3.76 (1H, dd, J = 3.4, 11.6, H_b-5′), 3.02 (1H, d, J = 4.9 Hz, 2′-OH), 0.92 (9H, s, C(C*H*$_3$)$_3$), 0.86 (9H, s, C(C*H*$_3$)$_3$), 0.14 (3H, s, C*H*$_3$), 0.13 (3H, s, C*H*$_3$), 0.05 (3H, s, C*H*$_3$), 0.04 (3H, s, C*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.5 (C-6), 147.1 (C-4), 147.0 (C-2), 138.4 (C-8), 136.2 (C_q, Ph), 128.9, 128.2, 128.1 (*C*H, Ph), 124.9 (C-5), 88.7 (C-1′), 85.3 (C-4′), 75.4 (C-2′), 71.3 (C-3′), 61.1 (C-5′), 49.0 (N-*C*H$_2$, Ph), 25.9, 25.7, 18.3, 18.0, −4.6, −4.8, −5.4, −5.2; HREIMS m/z 586.3007 (M)$^+$, calcd for $C_{29}H_{46}N_2O_5Si_2$, 586.3007.

1-*N*-(Benzyl)-2′,5′-di-*O*-(*tert*-butyldimethylsilyl)-3′-*O*-(2″,3″,4″,6″-tetra-*O*-benzylglucopyranosyl)inosine (6). To a cooled (−10 °C), stirred mixture of 1-*N*-(benzyl)-2′,5′-di-*O*-(*tert*-buthyldimethylsilyl)inosine (**4a**, 1.16 g, 2.0 mmol) and *O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)trichloroacetimidate (**5**, 2.055 g, 3.0 mmol) in CH_2Cl_2 (20 mL) was added BF_3·Et_2O (1.23 mL, 10 mmol). Stirring was continued for an additional 1 h at −10 °C under an argon atmosphere. The reaction mixture was quenched with saturated aqueous sodium bicarbonate (5 mL) and extracted with CH_2Cl_2 (2 × 20 mL). The combined organic layer was washed water (2 × 20 mL) and then dried over $MgSO_4$. Evaporation of the solvent gave the crude product, which was chromatographed on a Si gel column (eluent, CH_2Cl_2−MeOH = 95:5, v/v; flow rate, 5 mL/min) to give a mixture of the α- and β-form of **6** (1.237 g, 56%). **6**: HRFABMS m/z 1109.5482 (M + H)$^+$, calcd for $C_{63}H_{81}N_4O_{10}Si_2$ 1109.5491.

1-*N*-Benzyl-3′-*O*-α- and 1-*N*-Benzyl-3′-*O*-β-D-(2″,3″,4″,6″-tetra-*O*-benzylglucopyranosyl)inosines (7, 8). To a cooled (0 °C), stirred solution of a mixture of **6** (1.105 g, 1.0 mmol) in THF (10 mL) was added 1 M tetrabutylammonium fluoride in THF (2.2 mL, 2.2 mmol). After being stirred for 1 h at room temperature, the reaction mixture was evaporated to give a residue, which was dissolved in CH_2Cl_2 (20 mL). The organic layer was washed with water (10 mL) and then dried over $MgSO_4$. Evaporation of solvent gave the crude product, which was chromatographed on a Si gel column (eluent $CHCl_3$−MeOH = 30:1, v/v; flow rate, 0.1 mL/min) to give products [**7** (704 mg) and **8** (88 mg), 90%]. **7**: IR (NaCl) ν_{max} 3419, 1702, and 1590 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (1H, s, H-8), 7.79 (1H, s, H-2), 7.12 ∼ 7.35 (25H, m, Ph*H*), 5.61 (1H, d, J = 7.3 Hz, H-1′), 5.22 (2H, s, N-C*H*$_2$Ph), 4.90 (1H, d, J = 3.9 Hz, H-1″), 4.44 ∼ 4.94 (8H, m, O−C*H*$_2$Ph), 4.61 (1H, t like, H-2′), 4.33 (1H, m, H-3′), 4.23 (1H, m, H-4′), 4.01 (1H, m, H-3″), 3.78 (1H, m, H-5″), 3.59−3.68 (6H, m, H-5′, H-2″, H-4″, H-6″); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.1 (C-6), 147.0 (C-4), 145.9 (C-2), 140.3 (C-8), 138.3, 137.7, 137.6, 136.9, 135.4 (5 x C_q, Ph), 127.7−129.1 (*C*H, Ph), 124.1 (C-5), 100.1 (C-1″), 91.7 (C-1′), 86.3, 85.9, 82.1, 80.1, 74.6, 73.6, 71.6, 68.2, 62.7 (C-2′, C-3′, C-4′, C-5′, C-2″, C-3″, C-4″, C-5″, C-6″), 79.0, 77.7, 75.2, 74.6 (4 × O-*C*H$_2$Ph), 49.3 (N-*C*H$_2$Ph); HREIMS m/z 880.3555 (M)$^+$, calcd for $C_{51}H_{52}N_4O_{10}$, 880.3683.

8: IR (NaCl) ν_{max} 3411, 1701, and 1587 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (1H, s, H-8), 7.87 (1H, s, H-2), 7.12−7.33 (25H, m, Ph*H*), 5.71 (1H, d, J = 6.7 Hz, H-1′), 5.24 (2H, s, N−C*H*$_2$Ph), 4.41−4.90 (8H, m, O−C*H*$_2$Ph), 4.69 (1H, t like, H-2′), 4.48 (1H, d, J = 7.2 Hz, H-1"), 4.46 (1H, m, H-3′), 4.21 (1H, m, H-4′), 3.50−3.84 (8H, m, H-5′, H-2″, H-3″, H-4″, H-5″, H-6″); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.1 (C-6), 146.9 (C-4), 146.0 (C-2), 140.2 (C-8), 138.1, 137.9, 137.6, 137.4, 135.5 (5 × C_q, Ph), 127.7−129.1 (*C*H, Ph), 124.1 (C-5), 103.8 (C-1″), 91.4 (C-1′), 85.4, 84.3, 82.1, 81.8, 74.8, 73.9, 73.4, 68.2, 62.4 (C-2′, C-3′, C-4′, C-5′, C-2″, C-3″, C-4″, C-5″, C-6″), 77.1, 75.7, 75.2,

75.1 (4 × O-*C*H_2Ph), 49.3 (N-*C*H_2Ph); HREIMS *m/z* 880.3565 $(M)^+$, calcd for $C_{51}H_{52}N_4O_{10}$, 880.3683.

3′-*O*-(α-D-glucosyl)inosine (1). To a stirred solution of **7** (438 mg, 0.5 mmol) in EtOH (20 mL) were added 20% $Pd(OH)_2$/C (438 mg) and cyclohexene (14 mL). The mixture was stirred for 4 h under reflux. The catalyst was removed by filtration, and the filtrate was concentrated to dryness. The resulting residue was separated by reversed-phase HPLC (ODS column; eluent, MeOH−H_2O = 70:30, v/v; flow rate, 1 mL/min; detection, UV 254 nm) to give **1** (172 mg, 80%). The spectral data of synthetic compound **1** were identical to those of the natural product. $[\alpha]_D^{20}$ +40° (*c* 1.0, H_2O); IR (NaCl) ν_{max} 3376, 1682 and 1592 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 8.28 (1H, s; H-8), 8.05 (1H, s, H-2), 5.87 (1H, d, *J* = 6.1 Hz, H-1′), 4.94 (1H, d, *J* = 3.4 Hz, H-1″), 4.63 (1H, dd, *J* = 5.4, 6.1 Hz, H-2′), 4.26 (1H, dd, *J* = 3.0, 5.4 Hz, H-3′), 4.12 (1H, ddd, *J* = 3.0 Hz, H-4′), 3.63 (2H, m, H-5′), 3.47 (2H, m, H-3″, H-5″), 3.41−3.62 (2H, m, H-6″), 3.22 (1H, dd, *J* = 3.4, 9.4 Hz, H-2″), 3.11(1H, t, *J* = 3.4, H-4″); ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.1 (C-6), 148.4 (C-4), 147.0 (C-2), 138.3 (C-8), 124.4 (C-5), 100.1 (C-1″), 87.5 (C-1′), 84.3 (C-4′), 77.5 (C-3′), 73.7 (C-3″), 73.6 (C-5″), 73.2 (C-2′), 72.1 (C-2″), 70.0 (C-4″), 61.2 (C-5′), 60.5 (C-6″); HRFABMS *m/z* 453.1233 (M + Na)$^+$, calcd for $C_{16}H_{22}N_4O_{10}Na$ 453.1234.

3′-*O*-(β-D-glucosyl)inosine (1b). This was prepared from **8** (88 mg 0.1 mmol), 20% $Pd(OH)_2$/C (88 mg), and cyclohexene (3 mL) in EtOH by the same method as **1** (34 mg, 80%). $[\alpha]_D^{20}$ −33° (*c* 1.0, H_2O); IR (NaCl) ν_{max} 3380, 1698 and 1585 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 8.32 (1H, s, H-8), 8.05 (1H, s, H-2), 5.89 (1H, d, *J* = 5.2 Hz, H-1′), 4.55 (1H, dd, *J* = 5.1, 5.2 Hz, H-2′), 4.35 (1H, dd, *J* = 3.8, 5.1 Hz, H-3′), 4.33 (1H, d, *J* = 7.4 Hz, H-1″), 4.13 (1H, ddd, *J* = 3.8 Hz, H-4′), 3.67−3.60 (3H, m; H-5′, H-5″), 3.43 (1H, dd, *J* = 5.2, 5.2 Hz, H-4″), 3.17−3.08 (4H, m, H-2″, H-3″, H-6″); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.3 (C-6), 148.2 (C-4), 146.5 (C-2), 138.4 (C-8), 124.4 (C-5), 102.1 (C-1″), 87.6 (C-1′), 83.2 (C-4′), 77.2 (C-3′), 76.9 (C-3″), 76.5 (C-5″), 73.5 (C-2″), 73.1 (C-2′), 69.8 (C-4″), 60.9 (C-5′), 60.8 (C-6″); HRFABMS *m/z* 453.1227 (M + Na)$^+$, calcd for $C_{16}H_{22}N_4O_{10}Na$ 453.1234.

1-*N*-(Benzyl)-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-*O*-(2″,3″,4″,6″-tetra-*O*-benzylglucopyranosyl)inosine (9). This was prepared from **4b** (1.164 g, 2.0 mmol), **5** (2055 mg, 3.0 mmol), and BF_3·Et_2O (1.229 mL, 10 mmol) by the same method as **6** (931 mg, 42%). **9**: HRFABMS *m/z* 1109.5478 (M + H)$^+$, calcd for $C_{63}H_{81}N_4O_{10}Si_2$ 1109.5491.

1-*N*-Benzyl-3′-*O*-α- and 1-*N*-Benzyl-3′-*O*-β-D-(2″,3″,4″,6″-tetra-*O*-benzyl glucopyranosyl)inosines (10, 11) These were prepared from **9** (800 mg, 0.72 mmol) and 1 M tetrabutylammonium fluoride in THF (1.6 mL, 1.6 mmol) by the same method as **7** and **8** [**10** (532 mg) and **11** (38 mg), 90%]. **10**: IR (NaCl) ν_{max} 3405, 1705, and 1585 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.94 (1H, s, H-8), 7.80 (1H, s, H-2), 7.14−7.30 (25H, m, Ph*H*), 5.90 (1H, d, *J* = 7.8 Hz, H-1′), 5.19 (2H, d, *J* = 5.9 Hz, N−CH_2Ph), 4.46−4.80 (8H, m, O-CH_2Ph), 4.70 (1H, t like, H-2′), 4.63 (1H, d, *J* = 4.0 Hz, H-1″), 4.37 (1H, t like, H-3′), 4.20 (1H, m, H-4′), 4.12 (1H, m, H-5″), 3.84 (2H, m, H-5′, H-3″), 3.67(2H, m, H-5′, H-6″), 3.31−3.45 (3H, m, H-2″, H-4″, H-6″); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.1 (C-6), 146.9 (C-4), 145.9 (C-2), 140.7 (C-8), 138.2, 137.7, 137.5, 137.2, 135.4 (5 × C_q, Ph), 127.7−129.1 (*C*H, Ph), 127.1 (C-5), 98.4 (C-1″), 88.8 (C-1′), 88.1, 82.4, 81.5, 79.2, 77.5, 72.8, 71.5, 68.8, 62.9 (C-2′, C-3′, C-4′, C-5′, C-2″, C-3″, C-4″, C-5″, C-6″), 76.8, 76.7, 75.0, 73.5 (4 × O-CH_2Ph), 49.3 (N-CH_2 Ph); HREIMS *m/z* 880.3580 $(M)^+$, calcd for $C_{51}H_{52}N_4O_{10}$, 880.3683.

11: IR (NaCl) ν_{max} 3415, 1698, and 1579 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.98 (1H, s, H-8), 7.88 (1H, s, H-2), 7.12−7.34 (25H, m, Ph*H*), 5.87 (1H, d, *J* = 6.8 Hz, H-1′), 4.91−5.27 (2H, dd, *J* = Hz, N-CH_2Ph), 4.87 (1H, t like, H-2′), 4.71−4.81 (8H, m, O-CH_2Ph), 4.49 (1H, m, H-3′), 4.46 (1H, d, *J* = 8.5 Hz, H-1″), 4.30 (1H, t like, H-2″), 4.18 (1H, m, H-4′), 3.61−3.88 (2H, m, H-5′), 3.21−3.55 (5H, m, H-3″, H-4″, H-5″, H-6″); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.1 (C-6), 146.9 (C-4), 146.0 (C-2), 140.8 (C-8), 138.1, 137.9, 137.7, 137.5, 135.5 (5 × C_q, Ph), 127.7−129.1 (*C*H, Ph), 126.3 (C-5), 102.1 (C-1″), 89.5 (C-1′), 86.7, 84.9, 81.0, 80.8, 77.4, 73.2, 70.8, 68.0, 62.7 (C-2′, C-3′, C-4′, C-5′, C-2″, C-3″, C-4″, C-5″, C-6″), 75.6, 75.3, 75.1, 75.0 (4 × O-CH_2-Ph), 49.2 (N-CH_2Ph); HREIMS *m/z* 880.3565 $(M)^+$, calcd for $C_{51}H_{52}N_4O_{10}$, 880.3683.

2′-*O*-(α-D-glucosyl)inosine (12). This was prepared from **10** (176 mg 0.2 mmol), 20% $Pd(OH)_2$/C (176 mg), and cyclohexene (10 mL) in EtOH by the same method as **1** (69 mg, 80%). $[\alpha]_D^{20}$ +31° (*c* 1.0, H_2O); IR (NaCl) ν_{max} 3395, 1686, and 1576 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 8.20 (1H, s, H-8), 8.05 (1H, s, H-2), 6.01 (1H, d, *J* = 5.0 Hz; H-1′), 4.67 (1H, d, *J* = 3.2 Hz, H-1″), 4.57 (1H, dd, *J* = 4.6, 5.0 Hz, H-2′), 4.29 (1H, dd, *J* = 3.5, 4.6 Hz, H-3′), 3.99 (1H, ddd, *J* = 3.5 Hz, H-4′), 3.58−3.69 (2H, m, H-5′), 3.36−3.54 (4H, m, H-3″, H-5″, H-6″), 3.12 (1H, dd, *J* = 3.2, 9.5 Hz, H-2″), 3.07 (1H, t like, H-4″); ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.3 (C-6), 148.6 (C-4), 147.0 (C-2), 138.4 (C-8), 124.6 (C-5), 98.8 (C-1″), 86.1 (C-1′), 85.7 (C-4′), 79.1 (C-2′), 72.9 (C-3″), 72.8 (C-5″), 71.7 (C-3′), 69.9 (C-2″), 69.6 (C-4″), 60.9 (C-5′), 60.6 (C-6″); HRFABMS *m/z* 453.1236 (M + Na)$^+$, calcd for $C_{16}H_{22}N_4O_{10}Na$ 453.1234.

2′-*O*-(β-D-glucosyl)inosine (13). This was prepared from **11** (26 mg 0.1 mmol), 20% $Pd(OH)_2$/C (26 mg), and cyclohexene (3 mL) in EtOH by the same method as **1** (10 mg, 80%). $[\alpha]_D^{20}$ −34° (*c* 1.0, H_2O); IR (NaCl) ν_{max} 3386, 1703, and 1579 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 8.32 (1H, s, H-8), 8.05 (1H, s, H-2), 6.07 (1H, d, *J* = 4.6 Hz, H-1′), 4.70 (1H, dd, *J* = 4.2, 4.6 Hz, H-2′), 4.39 (1H, dd, *J* = 3.5, 4.6 Hz, H-3′), 4.30 (1H, d, *J* = 7.7 Hz, H-1″), 3.93 (1H, ddd, *J* = 3.5, Hz, H-4′), 3.48−3.67 (2H, m, H-5′), 3.33−3.47 (3H, m, H-5″, H-6″), 2.98−3.14 (3H, m, H-2″, H-3″, H-4″); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.2 (C-6), 147.5 (C-4), 146.2 (C-2), 138.1 (C-8), 124.3 (C-5), 103.2 (C-1″), 86.2 (C-1′), 85.1 (C-4′), 81.2 (C-2′), 76.8 (C-3″), 75.9 (C-5″), 73.5 (C-2″), 69.5 (C-3′), 69.4 (C-4″), 61.1 (C-5′), 60.5 (C-6″); HRFABMS *m/z* 453.1233 (M + Na)$^+$, calcd for $C_{16}H_{22}N_4O_{10}Na$ 453.1234.

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NP0000724