Studies on Glycosylation of the Mitomycins. Syntheses of 7-*N*-(4-*O*-Glycosylphenyl)-9a-methoxymitosanes[1)]

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Two new derivatives of glycosyl mitomycin C, 7-*N*-{4-*O*-(β-D-glucopyranosyl and α-sialosyl)phenyl}-9a-methoxymitosanes, were synthesized, and their structures were elucidated by analysis of the nuclear magnetic resonance spectra. Field desorption mass spectrometry was successfully used for the confirmation of these structures. The cytotoxic, antibacterial, and antitumor activities of 7-*N*-(4-glycosylphenyl)-9a-methoxymitosanes were also examined.

Keywords mitomycin A; sialic acid; D-glucose; glycosylation; mitomycin C

Mitomycin C is a potent antitumor agent that is currently used clinically for cancer chemotherapy.[2,3)] Numerous analogs of the mitomycins have been prepared in the hope of obtaining derivatives with superior the therapeutic properties.[4,5)]

Glycosyl residues play important roles in various biological processes (masking of cell-surface antigens, mitogenic receptors for lectins in cell-to cell recognition and other behavior).[6-8)] In view of these facts, it is possible that glycoside derivatives of mitomycin may provide a degree of selectivity between normal cells and some cancer cells. In the previous paper,[9)] we reported on the syntheses of 7-*O*-glycosyl-9a-methoxymitosanes, and some of their biological activities. However, there still remains the problem of *O*-deacetylation of their constituent per-*O*-acetylated glycosides, because the glycoside bond of 7-*O*-glycosyl-9a-methoxymitosanes having an *O*-acetylated glycoside moiety is hydrolyzed more easily than their *O*-acetyl groups under mild basic conditions. In this paper, as a part of our program on the syntheses of glycoconjugates of mitomycins, we describe the synthesis of 7-*N*-{4-*O*-(glucopyranosyl and sialosyl)phenyl}-9a-methoxymitosanes having no hydroxy-protection. The structures of the glycosylation products were elucidated on the basis of the field desorption (FD) mass spectra (MS) and the nuclear magnetic resonance (NMR) spectra. Some of the biological activities of these derivatives were also investigated.

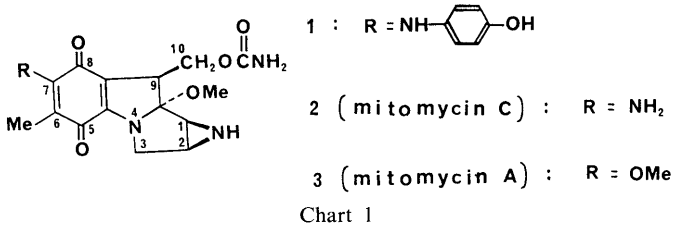

Chart 1

Results and Discussion

Chemistry 7-*N*-(4-Hydroxyphenyl)-9a-methoxymitosane sodium salt (**1**) is one of the most active mitomycin C (**2**) derivatives, showing higher antitumor activity than **2** against several murine tumor models and significantly lower bone marrow toxicity.[10)] Therefore, we investigated the glycosylation of mitomycin A (**3**) using 4-aminophenol as a spacer.

4-Aminophenyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (**4**) was prepared by utilizing the conditions reported by Ekborg *et al.*[11)] Treatment of **3** and **4** in anhydrous methanol gave 7-*N*-{4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)phenyl}-9a-methoxymitosane (**5**) in 69% yield.[12)] *O*-Deacetylation of **5** with sodium methoxide in methanol at 20 °C for 30 min gave two major products.[13)] The reaction mixture was separated by column chromatography on silica gel to give 7-*N*-{4-*O*-(β-D-glucopyranosyl)phenyl}-9a-methoxymitosane (**6**) and its 6-*O*-monoacetyl derivative (**7**). Their structures were elucidated on the basis of the ^{1}H-NMR spectra (Tables I and II). Proton assignments for these compounds were made by two-dimensional (2-D) ^{1}H-^{1}H correlation spectroscopy (COSY). Consistent patterns were noted in the ^{1}H-NMR spectra for **7**, suggesting the presence of 9a-methoxymitosane and a 4-aminophenyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside moiety. The *O*-

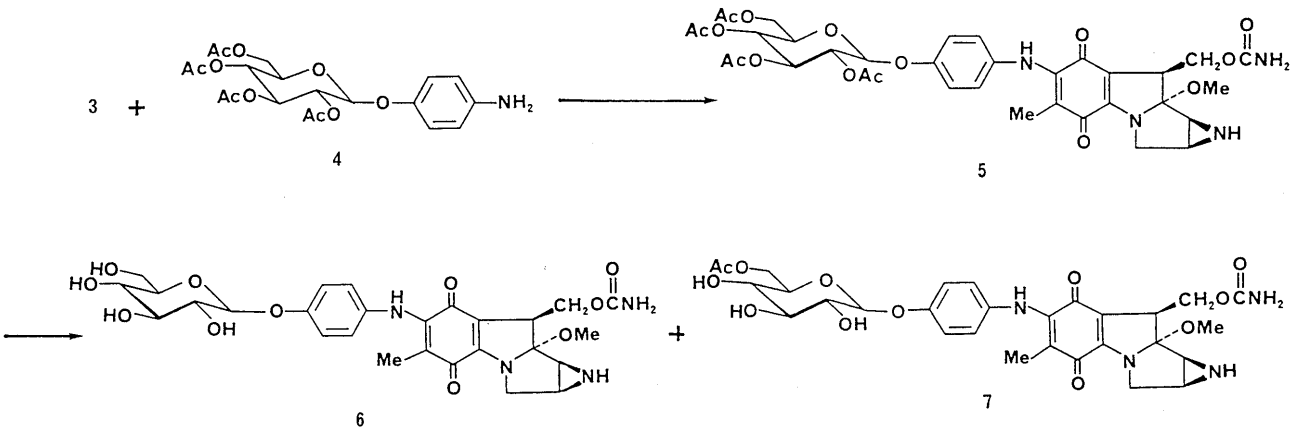

Chart 2

TABLE I. ^{1}H-NMR Data for the Glucosyl Moiety in **5**, **6**, and **7**

Hydrogen number	Compound		
	5	**6**	**7**
1-H	5.03 d (8.0)	5.50 d (7.0)	5.44 d (7.2)
2-H	5.15 t (9.5)		4.15—4.26 (2H)
3-H	5.24 t (9.5)	4.15—4.30 (3H)	
4-H	5.29 t (9.5)		3.98—4.14 (2H)
5-H	3.86 ddd (2.5, 5.5, 10.0)	4.02 m	
6-H	4.36 dd (2.5, 10.0)	4.26 dd (5.0, 12.0)	4.46 dd (6.0, 11.5)
6′-H	4.27 dd (5.0, 12.5)	4.44 dd (2.0, 12.0)	4.83 dd (1.5, 11.5)
OAc	2.02 s, 2.04 s, 2.06 s, 2.07 s		1.83 s
Phenyl group	6.90 d (9.0) 6.95 d (9.0)	6.87 d (9.0) 7.20 d (9.0)	6.96 d (9.0) 7.19 d (9.0)

Spectra were measured in $CDCl_3$ for **5** and in C_5D_5N for **6** and **7**. Chemical shifts are given in δ (ppm). Coupling constans (Hz) are given in parentheses.

TABLE II. ^{1}H-NMR Data for the Mitosane Moiety in **5**, **6**, and **7**

Hydrogen number	Compound		
	5	**6**	**7**
1-H	2.91 br s	3.04 br s	3.04 br d (4.0)
2-H	2.83 br s	2.65 br s	2.65 br d (2.5)
3-H	3.51 br d (13.5)	3.48 br d (13.0)	3.49 br d (12.0)
3-H′	4.24 d (13.5)	4.39 d (13.0)	4.38 d (12.5)
6-Me	1.42 s	1.40 s	1.46 s
7-NH	7.70 s	8.10 s	8.94 s
9-H	3.63 dd (4.7, 10.5)	3.93 dd (4.5, 11.0)	3.93 dd (4.5, 11.0)
9a-OMe	3.22 s	3.16 s	3.12 s
10-H	4.53 br t (10.5)	5.02 br t (11.0)	4.99 br t (10.5)
10-H′	4.70 dd (4.5, 10.5)	5.28 dd (4.5, 10.5)	5.28 dd (4.5, 10.5)
NH_2	4.89 br s		

Spectra were measured in $CDCl_3$ for **5** and in C_5D_5N for **6** and **7**. Chemical shifts are given in δ (ppm). Coupling constans (Hz) are given in parentheses.

acetylated position of **7** was elucidated to be at C-6 on the basis of the fact that two deshielded signals for 6-H and 6-H′ appeared at δ 4.46 and δ 4.83 in the ^{1}H-NMR spectrum. The structure of the deacetylation product **6** was also in full agreement with the ^{1}H-NMR data.

Recently, many kinds of biological functions of sialosyl-glycoconjugates have been reported.[14-16] As a further step in these investigations, we have synthesized 7-*N*-(4-*O*-sialosylphenyl)-9a-methoxymitosanes.

Methyl (4-nitrophenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)-onate (**8**) was prepared by glycosylation of 4-nitrophenol with methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosyl chlorid)onate as described by Eschenfelder and Brossmer.[17] The nitro group of **8** was reduced to an amino group by catalytic hydrogenation to give the intermediate **9**, whose ^{1}H-NMR spectrum was consistent with the assigned structure.

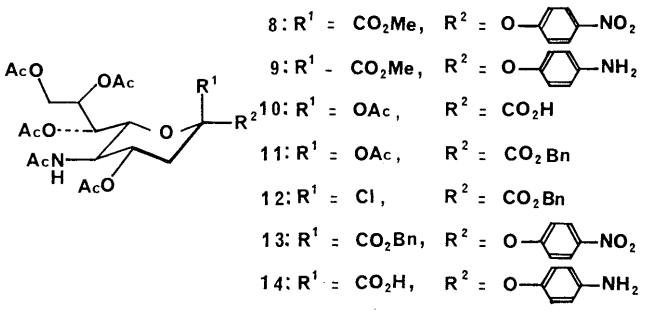

Chart 3

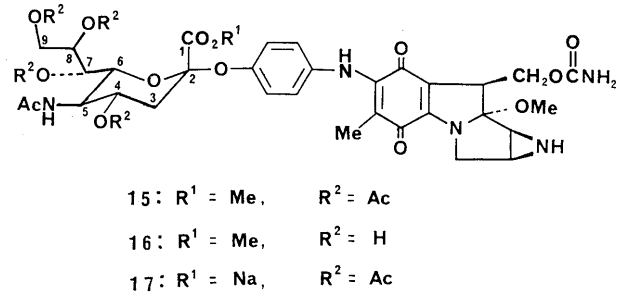

Chart 4

The intermediate 4-aminophenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosidonic acid (**14**) was obtained by the following route.[18] Esterification[19] of the cesium salt **10** with benzyl bromide (BnBr) in *N*,*N*-dimethylformamide (DMF) followed by treatment of the resulting benzyl ester **11** with excess hydrogen chloride gas gave the chloride **12**, which was submitted to the next glycosylation step. Glycosylation of **12** with 4-nitrophenol in DMF afforded benzyl (4-nitrophenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (**13**) as pale-yellow crystals in 20% yield. The structure of **13** was unambiguously assigned from the ^{1}H-NMR spectrum. The anomeric configuration of **13** was also elucidated from the ^{1}H-NMR spectrum. The observed chemical shifts of δ 2.75 for 3-H and δ 4.96 for 4-H of **13** are characteristic[20] of the α-glycosidic linkage of *N*-acetylneuraminic acid. The nitro group was reduced to an amino group and the benzyl group was hydrogenolyzed by with a PtO_2 catalyst to give crystalline **14**.

In the same manner as described for the preparation of **5**, the treatment of **3** in methanol with **9** gave 7-*N*-{4-*O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)phenyl}-9a-methoxymitosane (**15**) in good yield. Removal of the *O*-acetyl group of **15** was carried out by treatment of **15** with sodium methoxide in methanol to give 7-*N*-{4-*O*-(methyl 5-acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)phenyl}-9a-methoxymitosane (**16**) in 75% yield.

Moreover, treatment of **3** with **14** in methanol in the presence of triethylamine gave 7-*N*-{4-*O*-(5-acetamido-

TABLE III. ^{1}H-NMR Data for the Sialosyl Moiety in **15**, **16**, and **17**

Hydrogen number	Compound		
	15	**16**	**17**
3-H_{ax}	2.21 t (12.5)	2.46 br t (12.5)	1.91 t (12.5)
3-H_{eq}	2.72 dd (4.5, 13.0)	3.20 dd (4.5, 12.5)	2.65 dd (4.5, 12.5)
4-H	4.93 ddd (4.5, 10.5, 12.5)	4.18—4.50 m	5.02 dd (4.5, 10.5)
5-H	4.10 br q (10.0)	3.37—3.45 m	3.91 t (10.0)
6-H	4.38 dd (1.0, 10.5)	4.18—4.50 m	4.55—4.65 m
7-H	5.34—5.37 (2H)	4.54—4.81 (2H)	5.27 br d (6.0)
8-H			5.33 m
9-H	4.13 dd (1.0, 12.0)	4.18—4.50 (2H)	4.11 dd (6.5, 13.0)
9-H′	4.28 dd (2.0, 12.0)		4.15 d (13.0)
COOMe	3.60 s	3.42 s	—
NAc	1.90 s	1.85 s	1.85 s
OAc	2.04, 2, 2.12 2.14		1.97, 1.98 2.01, 2.08
NH	5.32 d (10.0)		
Phenyl group	6.90 d (9.0) 7.20 d (9.0)	6.62 d (9.0) 7.31 d (9.0)	6.82 d (9.0) 7.05 d (9.0)

Spectra were measured in $CDCl_3$ for **5** and in C_5D_5N for **6** and **7**. Chemical shifts are given in δ (ppm). Coupling constants (Hz) are given in parentheses.

TABLE IV. ^{1}H-NMR Data for the Mitosane Moiety in **15**, **16**, and **17**

Hydrogen number	Compound		
	15	**16**	**17**
1-H	2.92 br s	3.04 d (4.5)	2.86 d (4.5)
2-H	2.83 br s	2.65 br d (4.5)	2.78 br d (4.5)
3-H	3.52 br d (13.0)	3.50 br d (13.0)	3.46 br d (12.0)
3-H′	4.24 d (13.0)	4.36 d (13.0)	4.33 br d (12.0)
6-Me	1.41 s	1.37 s	1.37 s
7-NH	7.68 br s	7.50 br s	
9-H	3.56 dd (4.5, 10.5)	3.94 dd (4.5, 10.5)	3.56 dd (4.5, 10.5)
9a-OMe	3.23 s	3.14 s	3.18 s
10-H	4.56 br t (10.5)	4.99 br t (10.5)	4.38 br t (10.5)
10-H′	4.73 dd (4.5, 10.5)	5.29 dd (4.5, 10.5)	4.55—4.65
NH_2	4.80 br s	5.70 br s	

Spectra were measured in $CDCl_3$ for **5** and in C_5D_5N for **6** and **7**. Chemical shifts are given in δ (ppm). Coupling constants (Hz) are given in parentheses.

4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid)phenyl}-9a-methoxymitosane, and its sodium salt (**17**) was prepared by treatment with an equimolar amount of sodium hydrogen carbonate in 60% yield.

Biological Activity These derivatives of **2**, 7-*N*-(4-*O*-glycosylphenyl)-9a-methoxymitosanes were examined for cytotoxic, anti-*Escherichia coli* (*E. coli*) and antitumor activities.

TABLE V. Growth Inhibitory Concentration of Derivatives of **2** against Tumor Cells and *E. coli in Vitro*

Compound number	IC_{50} value (μg/ml)[a]		
	P388/ADM	P388	*E. coli*[b]
1	1.01	0.108	18.6
2	0.92	0.105	40.1
5	0.48	0.084	16.3
6	1.02	0.108	±
15	0.78	0.112	13.9
16	2.21	0.740	10.0
17	5.63	5.01	—

a) Cells were exposed to the agents for 72 h. *b*) Inhibitory zone (mm) obtained by the paper disc method at a dose of 10 μg/ml.

TABLE VI. Antitumor Activity of Derivatives of **2** against P388/ADM Leukemia

Total dose (mg/kg)	Percent in increase of life span						
	1	**2**	**5**	**6**	**15**	**16**	**17**
160	Toxic	—	Toxic	—	58.3	Toxic	0
40	91.6	Toxic	16.1	Toxic	25.0	25.0	0
10	25.0	75.0	16.6	0	0	8.3	0

Tumor: P388/ADM 1×10^5 cell/mouse, i.p. Treatment: Days 1, 5, 9, i.p. Toxic: A percent ILS value of −15% or a larger negative value was taken to indicate toxicity.

The cytotoxic effect of the derivatives of **2** was examined against P388 and P388/ADM leukemic cells cultured *in vitro*. As shown in Table V, cytotoxic activities of **5**, **6**, and **15** were similar to that of **2**. In contrast, the derivatives showed weaker antibacterial activity than that of **2**.

The antitumor activity of the derivatives of **2** was examined against P388/ADM as shown in Table VI. Though **15** showed 58.3% ILS which is the highest activity among the derivatives, this activity is still less than that of **1** or **2**.

Experimental

Melting points were measured with a Yamato melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-181 digital polarimeter. Thin layer chromatography (TLC) was performed on silica gel GF-254 (Merck) plates. FD-MS, ultraviolet (UV), and infrared (IR) spectra were measured with JEOL JMA-3100, Hitachi 340, and JASCO IR-A2 instruments, respectively. The NMR spectra were measured in chloroform-d_3 ($CDCl_3$) or pyridine-d_5 (C_5D_5N) with tetramethylsilane (TMS) as an internal standard, with a Varian VXR-300 spectrometer. Column chromatography was conducted on Silica gel 60 (70—230 mesh, Merck).

7-*N*-{4-*O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)phenyl}-9a-methoxymitosane (5) A solution of **3** (100 mg, 0.29 mmol) in methanol was treated with **4** (660 mg, 1.37 mmol). The reaction mixture was stirred for 5 h at room temperature, and evaporated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel with chloroform–methanol (20 : 1) to give **5** (150 mg, yield 69%) as a dark green amorphous powder. *Anal.* Calcd for, $C_{35}H_{40}N_4O_{15}$: C, 55.55; H, 5.32; N, 7.40. Found: C, 55.21; H, 5.46; N, 7.11. FD-MS *m/z*: 758 ($M^+ + 1$), 779 ($M^+ + Na$). IR ν_{max}^{KBr} cm^{-1}: 1750, 1635, 1560, 1505. UV λ_{max}^{MeOH} nm (log ε): 375 (4.23), 255 (4.32), 215 (4.44). ^{1}H-NMR data are given in Tables I and II.

7-*N*-{4-*O*-(β-D-Glycopyranosyl and 6-*O*-Acetyl-β-D-glucopyranosyl)-phenyl}-9a-methoxymitosane (6 and 7) A solution of **5** (80 mg, 0.11 mmol) in methanol (15 ml) was treated with 28% sodium methoxide–methanol (20 mg). The reaction mixture was stirred for 30 min at 20 °C. Sodium methoxide in the reaction mixture was removed by using a small amount of Dowex 50 (H^+, 80 mg) in methanol and the solvent was evaporated off under reduced pressure. The residue was chromatographed on a column

of silica gel with chloroform–methanol (5 : 1) to give two fractions. The faster-eluting fraction gave 6 (46 mg, yield 75%) as a dark green amorphous powder. The second fraction gave **7** (7 mg, yield 10%) as a dark green amorphous powder.

6: *Anal.* Calcd for $C_{27}H_{32}N_4O_{11}$: C, 55.09; H, 5.47; N, 9.51. Found: C, 54.87; H, 5.45; N, 9.32. FD-MS *m/z*: 589 ($M^+ + 1$), 612 ($M^+ + Na$). IR ν_{max}^{KBr} cm^{-1}: 1710, 1630, 1560, 1505. UV λ_{max}^{MeOH} (log ε): 375 (3.85), 355 (3.99), 215 (4.13). ^{1}H-NMR data are given in Tables I and II.

7: *Anal.* Calcd for $C_{29}H_{34}N_4O_{12}$: C, 55.23; H, 5.43; N, 8.88. Found: C, 55.15; H, 5.42; N, 8.79. FD-MS *m/z*: 631 ($M^+ + 1$), 653 ($M^+ + Na$). IR ν_{max}^{KBr} cm^{-1}: 3400, 1720, 1638, 1560, 1510. UV λ_{max}^{MeOH} (log ε): 378 (4.07), 255 (4.20), 215 (4.32). ^{1}H-NMR data are given in Tables I and II.

Methyl (4-Aminophenyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (9) A solution of **8** (307 mg, 0.50 mmol) in ethyl acetate (20 ml) was treated with hydrogen over PtO_2 (100 mg) for 2 h at room temperature. The solution was filtered through Celite and evaporated to dryness at 20 °C to give **9** (251 mg, yield 84%) as an amorphous powder. $[\alpha]_D^{22}$ +30° (*c* = 1, MeOH). *Anal.* Calcd for $C_{27}H_{34}N_2O_{13}$: C, 54.54; H, 5.76; N, 4.71. Found: C, 54.85; H, 5.65; N, 4.40. IR ν_{max}^{KBr} cm^{-1}: 3360, 1745, 1675, 1510. ^{1}H-NMR ($CDCl_3$): 1.88 (3H, s, NAc), 2.01 (3H, s, OAc), 2.04 (3H, s, OAc), 2.10 (3H, s, OAc), 2.12 (3H, s, OAc), 2.03 (1H, t, *J* = 13.0 Hz, 3-H_{ax}), 2.66 (1H, dd, *J* = 4.8, 13.0 Hz, 3-H_{eq}), 3.66 (3H, s, –COOH), 4.04 (1H, br q, *J* = 10.5 Hz, 5-H), 4.18 (1H, dd, *J* = 5.0, 12.5 Hz, 9-H), 4.20 (1H, br d, *J* = 10.5 Hz, 6-H), 4.34 (1H, dd, *J* = 2.0, 12.5 Hz, 9-H'), 4.92 (1H, ddd, *J* = 4.8, 10.5, 12.0 Hz, 4-H), 5.30 (1H, d, *J* = 10.0 Hz, NH), 5.34 (2H, br s, 7-H and 8-H), 6.57 (2H, d, *J* = 9.0 Hz, phenyl group), 6.87 (2H, d, *J* = 9.0 Hz, phenyl group).

Benzyl (4-Nitrophenyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (13) A suspension of **10** (1.02 g, 1.96 mmol), Cs_2CO_3 (0.5 g, 1.54 mmol), and BnBr (0.5 g, 2.92 mmol) in DMF (5 ml) was stirred for 3 h at room temperature, then poured into water (20 ml) and extracted with chloroform. The extract was washed with water, dried over sodium sulfate, and evaporated to dryness to give **11** as an amorphous material. A solution of **11** in acetic acid (20 ml) and acetyl chloride (5 ml) was cooled in ice bath and saturated with dry hydrogen chloride gas. The reaction mixture was kept at room temperature for 16 h, then evaporated to a syrup. This was dissolved in dry benzene and the solution was evaporated to dryness at 20 °C. This was repeated twice to give **12** as a colorless solid.

A solution of **12** and sodium 4-nitrophenol (1.0 g, 7.19 mmol) in DMF (10 ml) was stirred at room temperature for 16 h. The reaction mixture was poured into water (50 ml) and extracted with chloroform. The extract was dried over sodium sulfate then evaporated to dryness. The residual oil was purified by column chromatography on silica gel. 4-Nitrophenol was eluted with ether and **13** was eluted with ethyl acetate. The fractions containing only **13** were combined and concentrated. The residue was crystallized from ether–hexane to give **13** (270 mg, yield 20%) as pale-yellow needles. mp 88—90 °C. $[\alpha]_D^{22}$ +20° (*c* = 1, MeOH). *Anal.* Calcd for $C_{32}H_{36}N_2O_{15}$: C, 55.81; H, 5.27; N. 4.07. Found: C, 55.50; H, 5.44; N, 3.96. IR ν_{max}^{KBr} cm^{-1}: 1750, 1665, 1615, 1595, 1515. ^{1}H-NMR ($CDCl_3$): 1.91 (3H, s, NAc), 2.03 (3H, s, OAc), 2.04 (3H, s, OAc), 2.10 (3H, s, OAc), 2.18 (3H, s, OAc), 2.30 (1H, br t, *J* = 13.0 Hz, 3-H_{ax}), 2.75 (1H, dd, *J* = 4.5, 13.0 Hz, 3-H_{eq}), 4.08 (1H, dd, *J* = 4.5, 12.0 Hz, 9-H), 4.14 (1H, br q, *J* = 10.5 Hz, 5-H), 4.21 (1H, dd, *J* = 2.5, 12.0 Hz, 9-H'), 4.67 (1H, br d, *J* = 11.0 Hz, 6-H), 4.92 (1H, d, *J* = 11.5 Hz, –CHPh), 4.96 (1H, ddd, *J* = 4.5, 10.0, 12.0 Hz, 4-H), 5.17 (1H, d, *J* = 11.5 Hz, –CHPh), 5.35 (2H, br s, 7-H and 8-H), 5.38 (1H, d, *J* = 10.0 Hz, NH), 7.00 (2H, d, *J* = 9.0 Hz, phenyl group), 7.12—7.25 (4H, m, phenyl group), 7.32—7.38 (1H, m, phenyl group), 7.96 (2H, d, *J* = 9.0 Hz, phenyl group).

4-Aminophenyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosidonic Acid (14) A solution of **13** (230 mg, 0.33 mmol) in ethyl acetate (20 ml) was treated with hydrogen over PtO_2 (100 mg) for 3 h at room temperature. The solution was filtered through Celite and evaporated to dryness at 20 °C. The residue was crystallized from chloroform–methanol to give **14** (163 mg, yield 86%) as colorless needles. mp 128 °C (dec.). $[\alpha]_D^{22}$ +248° (*c* = 1, MeOH). *Anal.* Calcd for $C_{25}H_{32}N_2O_{13}$: C, 53.79; H, 5.56; N, 4.83. Found: C, 53.99; H, 5.74; N, 4.46. IR ν_{max}^{KBr} cm^{-1}: 350, 1740, 1660, 1515. ^{1}H-NMR ($CDCl_3$: CD_3OD = 1 : 1): 1.78 (3H, s, NAc), 1.91 (3H, s, OAc), 1.99 (3H, s, OAc), 2.02 (3H, s, OAc), 1.93 (1H, t, *J* = 12.5 Hz, 3-H_{ax}), 2.58 (1H, dd, *J* = 4.5, 12.5 Hz, 3-H_{eq}), 3.90 (1H, br t, *J* = 10.5 Hz, 5-H), 4.05 (1H, dd, *J* = 4.5, 12.5 Hz, 9-H), 4.25 (1H, dd, *J* = 2.0, 12.5 Hz, 9-H'), 4.28 (1H, br d, *J* = 10.5 Hz, 6-H), 4.92 (1H, ddd, *J* = 4.5, 10.5, 12.0 Hz, 4-H), 5.23 (2H, br s, 7-H and 8-H), 6.61 (2H, d, *J* = 9.0 Hz, phenyl group), 6.84 (2H, d, *J* = 9.0 Hz, phenyl group).

7-*N*-{4-*O*-(Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)phenyl}-9a-methoxymitosane (15) A solution of **3** (100 mg, 0.27 mmol) in methanol (40 ml) was treated with **9** (800 mg, 1.35 mmol). The reaction mixture was stirred at room temperature for 5 d. The residue was purified by column chromatography on silica gel with chloroform–methanol (20 : 1) to give **15** (131 mg, yield 51%) as dark bluish purple amorphous powder. *Anal.* Calcd for $C_{41}H_{49}N_5O_{18}$: C, 54.73; H, 5.49; N, 7.78. Found: C, 54.65; H, 5.49; N, 7.76. FD-MS *m/z*: 900 ($M^+ + 1$), 922 ($M^+ + Na$). IR ν_{max}^{KBr} cm^{-1}: 1735, 1635, 1560, 1500. UV λ_{max}^{MeOH} nm (log ε): 375 (4.08), sh 250 (4.27), 215 (4.34). ^{1}H-NMR data are given in Tables III and IV.

7-*N*-{4-*O*-(Methyl 5-Acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonuropyranosylonate)phenyl}-9a-methoxymitosane (16) A solution of **15** (20 mg, 0.022 mmol) in methanol (15 ml) was treated with 28% sodium methoxide–methanol (20 mg). The reaction mixture was stirred at room temperature for 25 min and the progress of the reaction was monitored by TLC with chloroform–methanol (10 : 1). Then sodium methoxide in the reaction mixture was removed by column chromatography on silica gel with methanol and the eluate was evaporated under reduced pressure. The residue was extracted with ethyl acetate (30 ml) and filtered, and the filtrate was evaporated to dryness to give **16** (12 mg, yield 75%) as a dark green amorphous powder. *Anal.* Calcd for $C_{33}H_{41}N_5O_4$: C, 69.33; H, 7.23; N, 12.25. Found: C, 69.29; H, 7.15; N, 12.22. FD-MS *m/z*: 732 ($M^+ + 1$), 754 ($M^+ + Na$). IR ν_{max}^{KBr} cm^{-1}: 1730, 1635, 1560, 1505. UV λ_{max}^{MeOH} nm (log ε): 375 (4.20), sh 250 (4.25), 215 (4.50). ^{1}H-NMR data are given in Tables III and IV.

7-*N*-{4-*O*-(Sodium 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)phenyl}-9a-methoxymitosane (17) A solution of **3** (100 mg, 0.27 mmol) in pyridine (1 ml) and methanol (30 ml) was treated with **14** (500 mg, 0.88 mmol). The reaction mixture was stirred for 12 h at room temperature and evaporated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel (after quenching with pyridine) with chloroform–methanol (10 : 1) and the eluate was evaporated to dryness under reduced pressure. The resulting powder was dissolved in 3% $NaHCO_3$, and chromatographed on Diaion HP20 with 75% methanol. The eluent was evaporated under reduced pressure. Lyophilization of the residue gave **17** (156 mg, yield 60%) as a dark green amorphous powder. *Anal.* Calcd for $C_{40}H_{46}N_5NaO_{18}$: C, 52.92; H, 5.10; N, 7.71. Found: C, 52.88; H, 5.10; N, 7.67. FD-MS *m/z*: 930 ($M^+ + Na$). IR ν_{max}^{KBr} cm^{-1}: 1730, 1630, 1560, 1505. UV λ_{max}^{MeOH} nm (log ε): 395 (3.98), 230 (4.35). ^{1}H-NMR data are given in Tables III and IV.

Cytotoxicity Tumor cells were maintained in plastic dishes in RPMI 1640 medium supplemented with 10% fetal calf serum and kanamycin (60 μg/ml). For the drug treatment, tumor cells (4×10^3 P388 or P388/ADM) were incubated at 37 °C for 24 h in a 96-well microplate containing 200 μl/ml of growth medium in a humidified atmosphere of 5% CO_2. Drug solution (5 μl) was added to each well and incubation was continued for 72 h. After the incubation, 20 μl of tetrazolium (MTT) stock solution (5 mg/ml) was added to each well and the plate was incubated at 37 °C for 4 h. After aspiration of the medium, dimethyl sulfoxide (100 μl) was added to each well and mixed. The plate was read on a microplate reader, using a test wavelength of 570 nm (reference wavelength at 630 nm) as described by Alley *et al.*[21] The IC_{50} values were determined by plotting the logarithm of the drug concentration versus the growth rate of the treated cells.

Antibacterial Activity *E. coli* NIHJ was cultured in peptone broth (polypeptone 1%, NaCl 0.5%) at 37 °C for 24 h. Nutrient agar (Difco Lab., Michigan) medium was mixed with the culture solution of *E. coli* to produce a final concentration of 0.1%. Anti-*E. coli* activities of derivatives of **2** were determined by the paper disc (6 mm in diameter, thin type) method.

Antitumor Activity For evalution of antitumor activity of derivatives of **2**, P388/ADM (1×10^5) cells were inoculated i.p. into CDF1 mice on day 0, and derivatives were administered i.p. on days 1, 5, and 9 after tumor inoculation. Antitumor activity was expressed as the percent increase in life span (ILS).

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