Structure-antitumor Activity Relationship of Semi-synthetic Spicamycin Derivatives

TERUYUKI SAKAI*, HIROYUKI KAWAI, MASARU KAMISHOHARA, ATSUO ODAGAWA, AKASHI SUZUKI, TAKESHI UCHIDA, TOMIKO KAWASAKI, TAKASHI TSURUO† and NOBORU ŌTAKE††

Pharmaceutical Research Laboratory, Kirin Brewery Co., Ltd., 3 Miyahara-cho, Takasaki-shi, Gunma 370-12, Japan
†Institute of Molecular and Cellular Biosciences, University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113, Japan
††Department of Biosciences, Teikyo University, 1-1-1 Toyosatodai, Utsunomiya-shi, Tochigi 320, Japan

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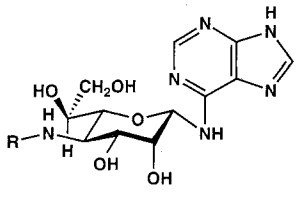
New derivatives of spicamycin modified at the fatty acid moieties of the molecule were synthesized and their structure-activity relationships were examined. The antitumor activity was greatly influenced by modification of the fatty acid moieties to tetradecadienoyl or dodecadienoyl analogues exhibiting better antitumor activity against COL-1 human colon cancer xenograft than SPM VIII.

Spicamycin (**1**)[1,2] is an antitumor antibiotic produced by *Streptomyces alanosinicus* 879-MT_3, and is comprised of four moieties; fatty acid, glycine, amino sugar and adenine unit (the structures are given in Fig. 1). Spicamycin is a mixture of compounds consisting of $C_{12\sim20}$ straight and branched fatty acid moieties. The amino sugar moiety was determined to be a heptose, 4-amino-4-deoxy-L-*glycero*-L-*manno*-heptopyranose[3], and is linked glycosidically to the 6-amino group of adenine.

Previously, we reported the preparation of several semi-synthetic spicamycin analogues which differed in the length of the fatty acid moieties and examined their *in vitro* and *in vivo* structure-activity relationships.[4] SPM VIII (**2**)[4], which is a dodecanoyl analogue, showed a potent antitumor activity against human breast cancer MX-1 and human stomach cancer SC-9 in the human tumor xenograft model, and the therapeutic index (T.I.) of SPM VIII against SC-9 was 4-fold higher (T.I. = 4) than that of spicamycin (T.I. = 1). SPM VIII also showed a good antitumor activity against several other human tumor xenografts.[5]

In this paper, we report the preparation of several new spicamycin analogues and describe their *in vivo* antitumor activities.

Fig. 1. Structures of spicamycin (**1**) and SPM VIII (**2**).



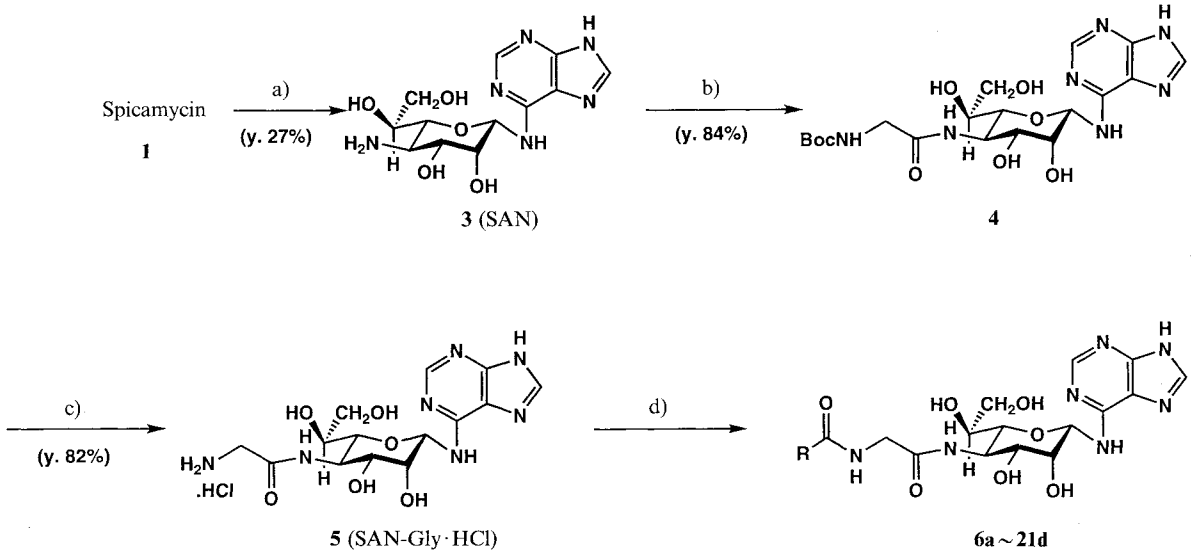
R; $(CH_3)_2CH(CH_2)_nCONHCH_2CO$-
$CH_3CH_2CH_2(CH_2)_nCONHCH_2CO$-, n=8-14
(Spicamycin, **1**)
$CH_3(CH_2)_{10}CONHCH_2CO$- (SPM VIII, **2**)

Chemistry

Spicamycin (**1**) was obtained from the culture broth of *Streptomyces alanosinicus* 879-MT_3 by the method of HAYAKAWA *et al.*[1,2] The amino nucleoside moiety of spicamycin (SAN; **3**) was prepared by hydrolysis of spicamycin with 10% HCl-MeOH.[4] The 4′-N-glycylated SAN (SAN-Gly; **5**) was prepared by removing the BOC group after coupling N-(*tert*-butoxycarbonyl)glycine to SAN (**3**). Condensation of appropriate fatty acids with SAN-Gly (**5**) by the active ester method[6] yielded spicamycin derivatives except **22a**, **22b**, and **22c** (Scheme 1). Except for those which were available commercially, fatty acids for the preparation of analogues were synthesized as described in detail in the experimental section. The carbamate analogues (**22a**, **22b**, **22c**) were synthesized from **5** and appropriate N-alkoxy carbonyloxy succinimide, which was prepared from appropriate alcohol and N,N′-disuccinimidyl carbonate[7] (Scheme 2). The alkylglycylated analogue (**23**) was synthesized from **5** and dodecanal under reductive amination conditions with $NaBH_3CN$[8] (Scheme 3).

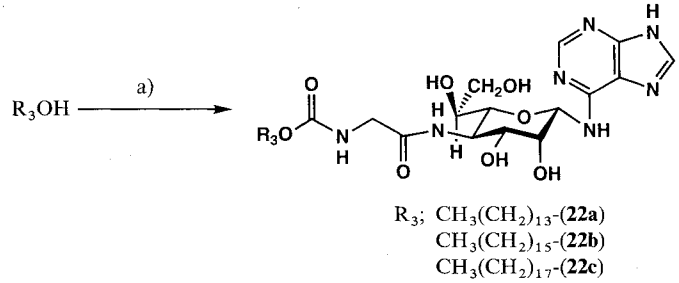
Scheme 1.

a) HCl, MeOH, reflux; b) N-(*t*-butoxycarbonyl) glycine, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, N,N-dimethylformamide, rt; c) HCl, MeOH, rt; d) R-CO_2p-NO_2Ph, triethylamine, N,N-dimethylformamide rt.



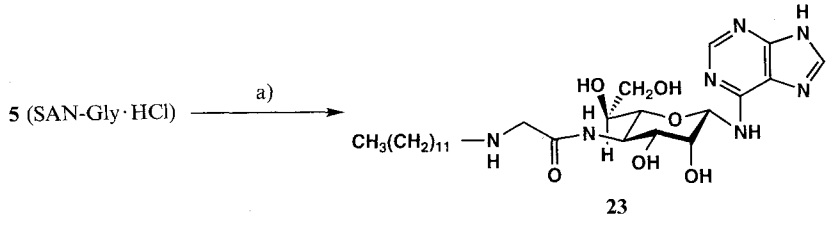
Scheme 2.

a) N',N-disuccinimidyl carbonate, N,N-dimethylformamide rt then **5**, ethanolamine.



Scheme 3.

a) $CH_3(CH_2)_{10}CHO$, $NaBH_3CN$, EtOH, rt.



Results and Discussion

Spicamycin analogues were evaluated for *in vivo* antitumor activities in a human tumor xenograft model,[9] in which fragments of tumor (COL-1) were implanted s.c. into athymic nude mice (BALB/c nu/nu Slc, Japan SLC Inc., Shizuoka, Japan). When the tumors had grown to a palpable size ($100 \sim 300\,mm^3$), spicamycin analogues at each dose were given intravenously by daily injection for five days.

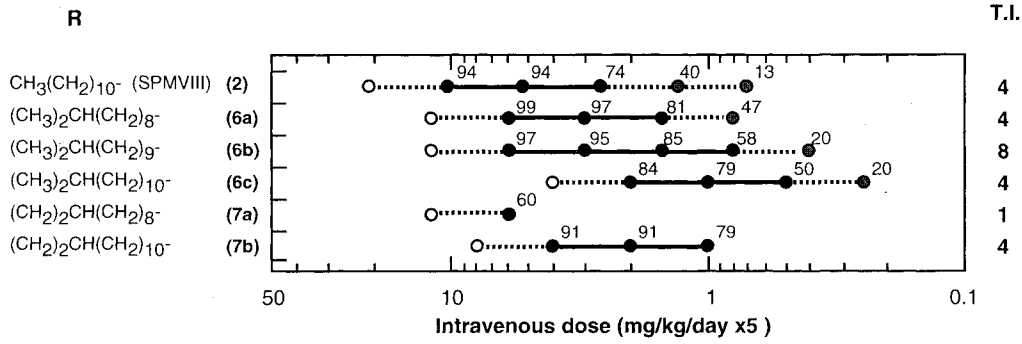
The antitumor activity of spicamycin analogues against COL-1 was evaluated by tumor growth inhibition rate (T.G.I.R.) at each dose. The T.I.[10] which gives a more realistic representation of the margin between antitumor activity and toxicity of each spicamycin

analogues was calculated.

The results are summarized in Figures 2~6 in comparison with those of SPM VIII[4] (T.I.=4). Among the derivatives with the modified fatty acid moiety, ω-methyl substituted (**6a~6c**) and ω-cyclopropyl (**7a, 7b**) substituted analogues showed almost the same activity as the corresponding linear and saturated fatty acid analogues as shown in our previous report[4] (Fig. 2). The ω-halogenated fatty acid analogues **8a** exhibited the same T. I. value that of SPM VIII, $C_{12\sim14}$ acids (**8b, 8c, 8d, 9b, 9c**) derivatives exhibited double T.I. values than that of SPM VIII. (Fig. 3). The α and ω-fluorinated analogues (**10, 11b**) showed a similar activity.

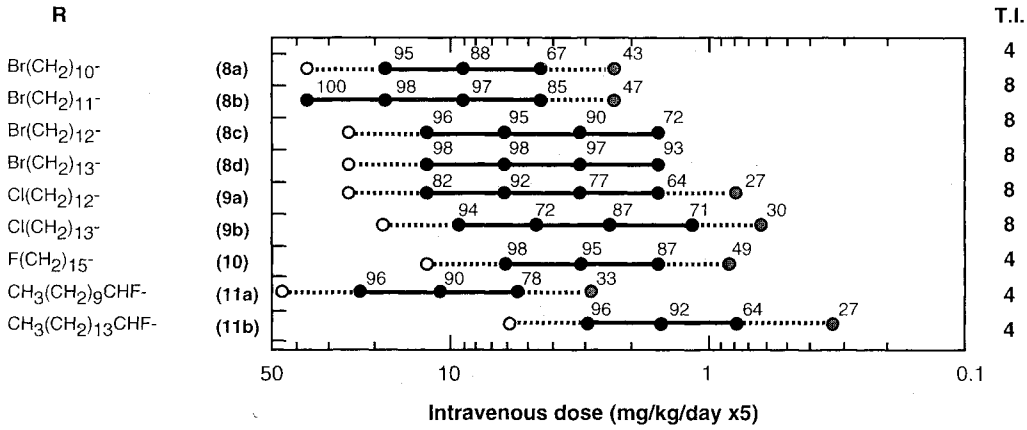
The analogues with one hydroxyl group at α, β or ω position exhibited unique structure-activity relationships (Fig. 4). The mixture of 2-hydroxy diastreoisomers (**12a**) displayed moderate activity, but hydroxylation of the ω-position (**14a, 14b**) led to a significant reduction in activity. To further investigate the influence of the chirality at the α-position, conformational isomers (**12b, 12c**) were prepared. The chiral (2*S*)-hydroxy analogue (**12c**) exhibited higher therapeutic index (T.I.=8) than (2*R*) analogue (**12b**, T.I.=2). In contrast, (3*S*)-hydroxy (**13b**) and (3*R*)-hydroxy (**13a**) analogues had a similar therapeutic index (T.I.=4). These findings suggested that chirality of the α-position is important for high therapeutic index, but that of the β-position does not affect activity. The two carbon elongated 3-hydroxy analogue (**13c**) had the same activity as that of SPM VIII. On the other hand, some of the ω-aryloxy analogues such as those with phenoxy (**15a**), benzyloxy (**15b**) and diphenylmethyloxy (**15g**) at the ω-position exhibited a similar potency as SPM VIII, but other analogues possesing naphthyloxy (**15i**), p-toluenesul-

Fig. 2. Antitumor activities of spicamycin derivatives against COL-1 human colon cancer (**2**), (**6a**)~(**7b**).



Fragments of COL-1 human colon cancer were implanted subcutaneously into athymic nude mice. When the tumor size reached 100~300 mm^3, spicamycin derivatives were administered iv daily for five days. Results are expressed as the value of maximum tumor growth inhibition rate (T.G.I.R.) at each dose during the experimental period; five mice per group. Terapeutic index (T.I.)=maximum tolerated dose/minimum effective dose. Open circles; toxic (one or more animals died), closed circles; effective (T.G.I.R. $\geq$ 50), gray circles; ineffective (T.G.I.R. $<$ 50).

Fig. 3. Antitumor activities of spicamycin derivatives against COL-1 human colon cancer (**8a**)~(**11b**).

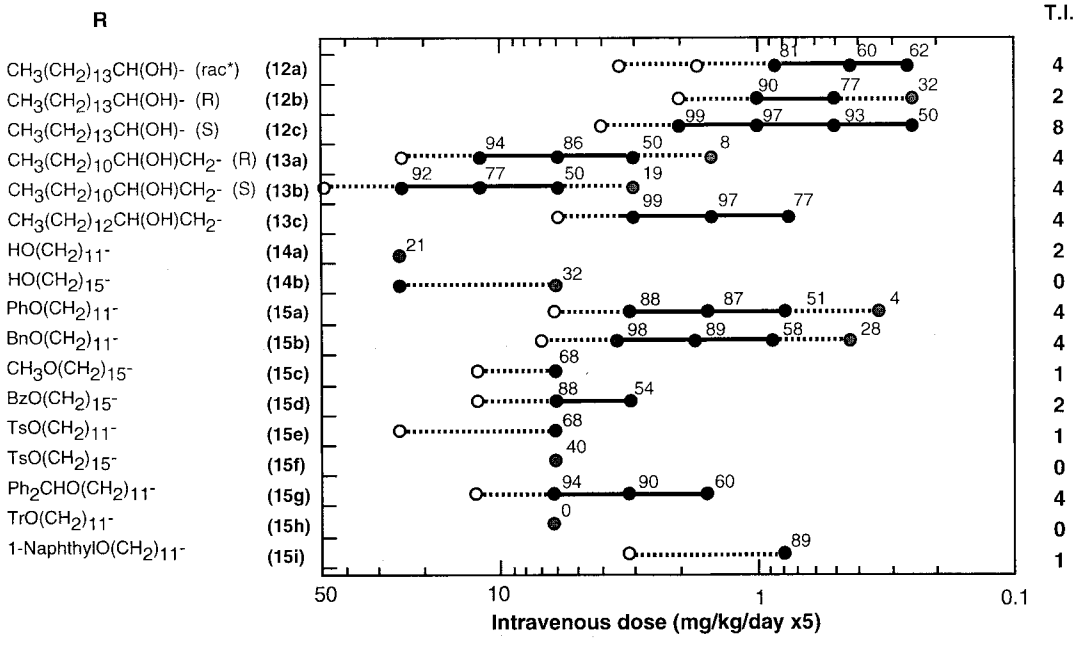


Details are the same as in the captions for Fig. 2.

fonyloxy (**15e, 15f**), and methoxy (**15c**) moieties at the ω-position were less active. The benzoyloxy analogue (**15b**) showed a moderate activity. Replacement of the hydroxyl group at the ω-position with a hydrophobic subsituent resulted in the retention of the activity, but the bulky and hydrophobic analogue (**15h**) did not show a sufficient activity (Fig. 4).

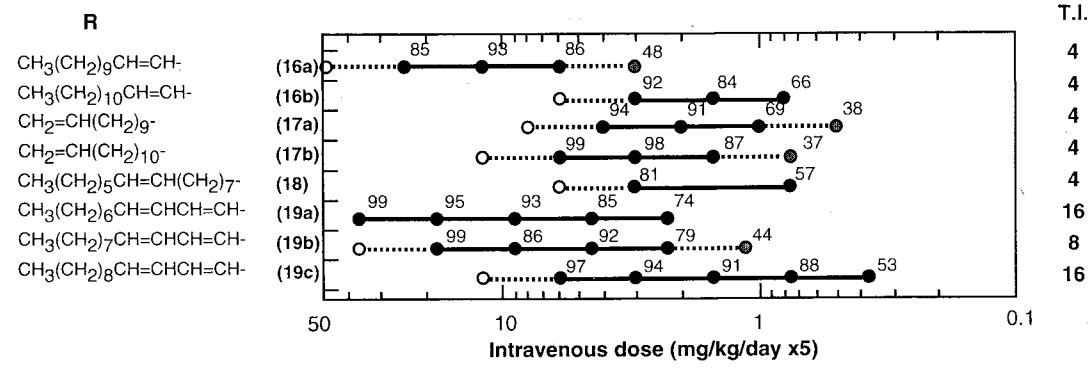
In general, the fatty acid analogues with double bonds exhibited good activities (Fig. 5). The activities of the analogues with one double bond at the $\alpha\beta$ (**16a, 16b**) or ω (**17a,17b**) position were nearly equal to those of saturated analogues with the corresponding carbon chain length in the fatty acid moiety. The maximum tolerated doses of the unsaturated analogues were generally higher than those of the saturated analogues due to the reduction in the toxicity. The introduction of one triple bond at the $\alpha\beta$ (**21a, 21b, 21c, 21d**) or ω (**20a, 20b**) position did not appear to alter the activity except in compound **21d** (Fig. 6). The analogue consisting of C_{16} fatty acids with one double bond at 9-position (**18**) was moderately active (Fig. 5). In contrast, the saturated C_{16} fatty acid analogue showed no activities but high toxicity.[4] These results indicated that the existence of a double bond at 9-position affected on the conformation of the fatty acid moiety resulting in the appearance of antitumor activity. Among the unsaturated fatty acid analogues, the $\alpha\beta,\gamma\delta$-dienoyl derivatives (**19a, 19c**) showed excellent activities; their therapeutic indices of them (T.I. = 16)

Fig. 4. Antitumor activities of spicamycin derivatives against COL-1 human colon cancer (**12a**)~(**15i**).



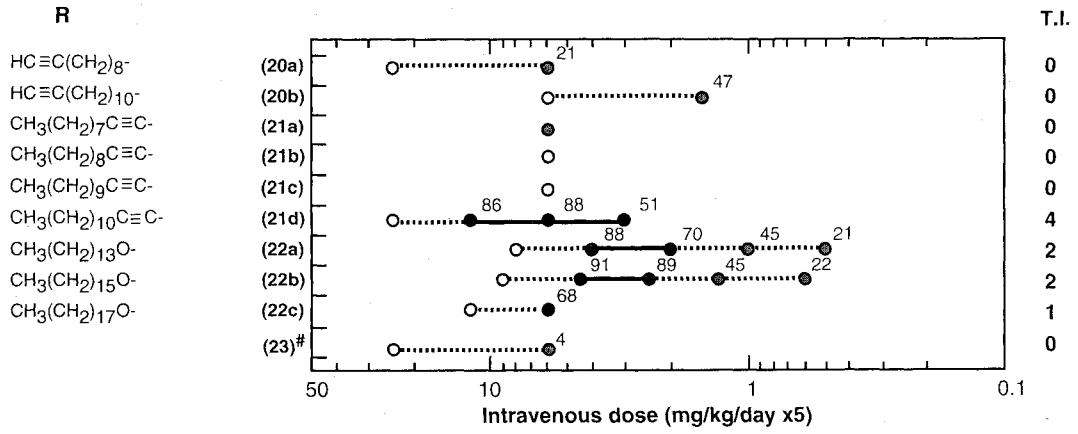
Details are the same as in the captions for Fig. 2.
* racemic

Fig. 5. Antitumor activities of spicamycin derivatives against COL-1 human colon cancer (**16a**)~(**19c**).



Details are the same as in the captions for Fig. 2.

Fig. 6. Antitumor activities of spicamycin derivatives against COL-1 human colon cancer (**20a**)~(**23**).



Details are the same as in the captions for Fig. 2.
Structure was given in Scheme 3.

were 4-fold higher than that of SPM VIII (Fig. 5). As a result of their potent antitumor activities and broad dose ranges of therapeutic effect, these compounds show great potential for further development as useful antitumor agents.

To clarify the influence of the amide bond in spicamycin analogues on the antitumor activities, we synthesized the carbamate analogues (**22a, 22b,22c**) and evaluated their activities (Fig. 6). Interestingly, these analogues did not lose their activities, suggesting that the amide bond is not essential for activity and can be replaced by a carbamate bond. On the other hand, the alkyl analogues of the amino group in the glycine moiety (**23**) showed no activity. From the above results it was suggested that the glycine moiety has an important role in the expression of the antitumor activity. Previously,[11] we speculated the spicamycin is metabolized by an intracellular enzyme to SAN-Gly (**5**) which inhibits the protein synthesis in tumor cells. The importance of the glycine moiety substantiated in this structure-activity relationship study is consistent with the evidence from the biochemical experiments concerning SAN-Gly. In this study, the hydrophobicity of the fatty acid moiety had a large effect on *in vivo* antitumor activity, which could be explained by the permeability of spicamycin derivatives into tumor cells. Similar results were reported previously for N-acyl derivatives of Ara-C.[12] The fatty acid moiety of N-acyl derivatives of Ara-C played an important role in the introduction of the compound into cancer cells. Furthermore, we are interested in the substrate specificity of the intracellular enzyme, since 2-chiral hydroxyl analogues (**12b, 12c**) exhibited different activities despite having the same hydrophobicity.

Further studies regarding the mode of action of spicamycin derivatives are now in progress in our laboratory.

Experimental

General

All Mp's were obtained with Yanagimoto melting point apparatus and uncorrected. IR spectra were measured on a Jasco A-3 spectrophotometer. ^{1}H NMR spectra were recorded with a JEOL GX-500 spectrometer with tetramethylsilane as an internal standard. The FD mass spectra were obtained with a Hitachi M80 mass spectrometer. Optical rotations were measured on a JASCO DIP-140 spectropolarimeter. Silica gel column chromatography was performed on Wako gel C-200 manufactured by Wako Pure Chemical Industries, Ltd.

6-(4-Amino-4-deoxy-L-*glycero*-β-L-*manno*-heptopyranosylamino)-9*H*-purine (3)

A solution of 4.00 g of **1** in 36 ml of 10% HCl-MeOH was refluxed for 4 hours and cooled to 20°C. EtOAc (50 ml) was added to the reaction mixture, the resulting precipitate was collected by filtration, and washed with EtOAc (20 ml). This precipitate was dissloved in water, and bacified with ammonia water to pH 7.5. The resulting precipitate was filtered and dried under reduced pressure. This white powder was suspended in 4 ml water, and acidified with 1 N HCl solution to pH 1. Activated charcol (4 g) was added to the mixture. After filtration of the mixture, and filter cake was washed with diluted HCl solution (3 ml, pH 3). The filtrate was basified with ammonia water to pH 7.5, and stirred for 30 minutes at 25°C. The resulting precipitate was collected by filtration, and washed with water (2 ml). After drying, these pricipitates was suspended with MeOH (10 ml), and stirred for 3 hours at 25°C. This mixture was filtered, and filter cake was dried under reduced pressure to give

(**2**) (0.55g; 27%) as a white powder. IR ν_{max} (KBr): 3300, 1640 cm^{-1}. ^1H NMR (CD_3OD) δ_H 3.04 (1H, dd, J= 10.0, 10.0 Hz), 3.43 (1H, dd, J=6.3, 10.0 Hz), 3.53 (1H, dd, J=3.1, 10.0 Hz), 3.61 (2H, br s), 3.78 (1H, m), 3.95 (1H, dd, J=3.1, <1 Hz), 5.64 (1H, br s), 8.13 (1H, s), 8.30 (1H, s), ^1H NMR (CD_3OD+DCl) δ_H 3.43 (1H, dd, J=10.0, 10.0 Hz), 3.62 (2H, m), 3.72 (1H, dd, J=6.3, 10.0 Hz), 3.62 (2H, m), 3.72 (1H, dd, J=6.3, 10.0 Hz), 3.80 (1H, m), 3.89 (1H, dd, J=3.1, 10.0 Hz), 4.07 (1H, dd, J=<1, 3.1 Hz), 5.72 (1H, br s), 8.20 (1H, s), 8.40 (1H, s). FD-MS 327 (M^++H). $[\alpha]_D^{25}$=+1.2° (*c* 0.25, MeOH). mp 180~183°C.

6-[4-(*tert*-Butoxycarbonyl)glycylamino-4-deoxy-L-*glycero*-β-L-*manno*-heptopyranosylamino]-9*H*-purine (**4**)

To a stirred solution of N-(*tert*-butoxycarbonyl)glycine (1.61 g, 9.20 mmol) and **3** (3.00 g, 9.20 mmol) in N,N-dimethylformamide (DMF) 30 ml was added N-hydroxysuccinimide (HOSu, 0.53 g, 4.60 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (WSC·HCl, 2.64 g, 13.8 mmol), and the mixture was stirred for 12 hours at room temperature. Removal of the solvent and purification by silica gel column chromatography ($CHCl_3$-MeOH, 10:1) gave **4** (3.72 g, 84%) as a white powder. IR ν_{max} (KBr) 3300, 1640 cm^{-1}. ^1H NMR (CD_3OD) δ_H 1.46 (9H, s), 3.58~3.80 (7H, m), 4.00 (1H, s), 4.14 (1H, dd, J=10.3, 10.3 Hz), 5.67 (1H, br s), 8.14 (1H, s), 8.30 (1H, s). FD-MS 484 (M^++H). $[\alpha]_D^{25}$=+20.4° (*c* 0.1, MeOH). mp 189~190°C.

6-(4-Deoxy-4-glycylamino-L-*glycero*-β-L-*manno*-heptopyranosyl amino)-9*H*-purine hydrochloride (**5**)

A solution of **4** (1.67 g, 3.46 mmol) in 5% HCl-MeOH was stirred at room temperature for 40 minutes. Removal of the solvent, and then ether was added. The resulting precipitate was filtered and washed with ether to give **5** (1.08 g, 82%) as a white powder. IR ν_{max} (KBr) 3300, 1660 cm^{-1}. ^1H NMR (CD_3OD+DCl) δ_H 3.60~3.90 (7H, m), 4.16 (1H, dd, J=10.0, 10.0 Hz), 5.66 (1H, s), 8.08 (1H, s), 8.28 (1H, s). FD-MS 384 (M^++H). $[\alpha]_D^{25}$=+3.6° (*c* 0.25, MeOH-H_2O). mp 195~198°C.

The typical procedure for the introduction of side chains; the synthesis

6-[4-Deoxy-4-(10-methylundecanoyl)glycylamino-L-*glycero*-β-L-*manno*-heptopyranosylamino]-9*H*-purine (**6a**)

To a mixture of 10-methylundecanoic acid (240 mg, 1.20 mmol) and p-nitrophenol (167 mg, 1.20 mmol) in DMF (30 ml) was added N,N-dicyclohexylcarbodiimide (DCC) (247 mg, 1.20 mmol) at room temperature. The reaction mixture was stirred at the same temperature for 12 hours and filtered. To the filtrate were added **5** (460 mg, 1.10 mmol) and triethylamine (2.0 ml), and the mixture was stirred at the room temperature for 12 hours. After evaporation, the residue was purified by silica gel column chromatography ($CHCl_3$-MeOH, 7:1~5:1) afforded **6a** (121 mg, 19.5%) as a white powder. IR ν_{max} (KBr) 3400, 1630 cm^{-1}. ^1H NMR (CD_3OD) δ_H 0.87 (6H, d, J=6.4 Hz), 1.10~1.70 (15H, m), 2.28 (2H, t, J=7.0 Hz), 3.60~3.90 (5H, m), 3.85 (1H, d, J=15.6 Hz), 3.89 (1H, d, J=15.6 Hz), 4.02 (1H, dd, J=2.1, <1 Hz), 4.15 (1H, dd, J=10.3, 10.3 Hz), 5.68 (1H, br s), 8.12 (1H, s), 8.27 (1H, s). FD-MS 589 (M^++Na+H). $[\alpha]_D^{25}$=+6.2° (*c* 0.1, MeOH). mp 192~195°C. Other spicamycin analogues (**6b**)~(**21d**) were also synthesized from **5** in the same sequence of reaction for the synthesis of **6a** using the corresponding carboxylic acid.

6-[4-Deoxy-4-(11-methyldodecanoyl)glycylamino-L-*glycero*-β-L-*manno*-heptopyranosylamino]-9*H*-purine (**6b**)

This compound was obtained from **5** and 11-methyldodecanoic acid in the typical procedure. Yield 24.0%. IR ν_{max} (KBr) 3300, 1620 cm^{-1}. ^1H NMR (CD_3OD) δ_H 0.89 (6H, d, J=6.4 Hz), 1.10~1.70 (17H, m), 2.28 (2H, t, J=7.0 Hz), 3.65~3.80 (5H, m), 3.85 (1H, d, J=15.6 Hz), 4.01 (1H, dd, J=2.1, <1 Hz), 4.02 (1H, d, J=15.6 Hz), 4.15 (1H, dd, J=10.3, 10.3 Hz), 5.68 (1H, br s), 8.12 (1H, s), 8.27 (1H, s). FD-MS 580 (M^++H). $[\alpha]_D^{25}$=+2.8° (*c* 0.1, MeOH). mp 170~171°C.

6-[4-Deoxy-4-(12-methyltridecanoyl)glycylamino-L-*glycero*-β-L-*manno*-heptopyranosylamino]-9*H*-purine (**6c**)

This compound was obtained from **5** and 12-methyltridecanoic acid in the typical procedure. Yield 28.6%. IR ν_{max} (KBr) 3400, 1630 cm^{-1}. ^1H NMR (CD_3OD) δ_H 0.87 (6H, d, J=6.6 Hz), 1.10~1.70 (19H, m), 2.29 (2H, t, J=7.0 Hz), 3.60~3.80 (5H, m), 3.85 (1H, d, J=15.0 Hz), 3.89 (1H, d, J=15.0 Hz), 4.00 (1H, dd, J=2.1, <1 Hz), 4.13 (1H, dd, J=10.3, 10.3 Hz), 5.65 (1H, br s), 8.15 (1H, s), 8.30 (1H, s). FD-MS 616 (M^++Na). $[\alpha]_D^{25}$=+9.1° (*c* 0.1, MeOH). mp 225~226°C.

6-[4-(9-Cyclopropylnonanoyl)glycylamino-4-deoxy-L-*glycero*-β-L-*manno*-heptopyranosylamino]-9*H*-purine (**7a**)

To a solution of 10-undecenoic acid (1.00 g, 5.43 mmol) in ether (20 ml) was added large excess diazomethane in ether at room temparature. After stirring at room temparature for 20 minutes, $Pd(OAc)_2$ (100 mg, 0.44 mmol) was added to the mixture. After stirring at room temparature for 20 minutes, the reaction mixture was quenched by the addition of acetic acid (2.0 ml) at room temparature, and diluted with ether. The mixture was washed with saturate aqeous $NaHCO_3$ and brine, and dried over $MgSO_4$. Removal of the solvent and purification by silica gel column chromatography (EtOAc-hexane, 1:30) gave the methyl 9-cyclopropylnonanoate (0.58 g, 50.4%). To a solution of methyl 9-cyclopropylnonanoate (0.58 g, 2.74 mmol) in MeOH was added 10% aquous NaOH solution at room tem-

perature. After stirring under reflux for 1 hour, then the mixture was cooled. It was adjusted to the weak acidic range of pH with citric acid and extracted with ether. The organic layer was washed with brine and dried over $MgSO_4$ and concentrated to give 9-cyclopropylnonanoic acid (0.40 g, 73.8%). Compound (**7a**) was obtained from **5** and 9-cyclopropylnonanoic acid in the typical procedure. Yield 38.6%. IR ν_{max} (KBr) 3300, 1630 cm^{-1}. ^1H NMR (CD_3OD) δ_H −0.02~0.00 (2H, m), 0.36~0.41 (2H, m), 0.60~0.70 (1H, m), 1.17~1.22 (2H, m), 1.28~1.45 (10H, m), 1.60~1.67 (2H, m), 2.28 (2H, t, J=7.6 Hz), 3.60~3.80 (5H, m), 3.85 (1H, d, J=16.5 Hz), 3.89 (1H, d, J=16.5 Hz), 4.00 (1H, dd, J=2.5, <1 Hz), 4.14 (1H, dd, J=10.0, 10.0 Hz), 5.68 (1H, br s), 8.13 (1H, s), 8.32 (1H, s). FD-MS 564 (M^++H). $[\alpha]_D^{25}$=+9.1° (*c* 0.1, MeOH). mp 225~226°C.

6-[4-(11-Cyclopropylundecanoyl)glycylamino-4-deoxy-L-*glycero*-β-L-*manno*-heptopyranosylamino]-9*H*-purine (**7b**)

11-Cyclopropylundecanoic acid was obtained from 12-tridecenoic acid in the above procedure. And the compound (**8b**) was obtained from **5** and 11-cyclopropylundecanoic acid in the typical procedure. Yield 51.8%. IR ν_{max} (KBr) 3300, 1630 cm^{-1}. ^1H NMR (CD_3OD) δ_H −0.02~0.00 (2H, m), 0.37~0.42 (2H, m), 0.60~0.70 (1H, m), 1.15~1.22 (2H, m), 1.25~1.42 (14H, m), 1.60~1.70 (2H, m), 2.28 (2H, t, J=8.0 Hz), 3.60~3.80 (5H, m), 3.85 (1H, d, J=16.3 Hz), 3.89 (1H, d, J=16.3 Hz), 4.00 (1H, dd, J=2.5, <1 Hz), 4.14 (1H, dd, J=10.0, 10.0 Hz), 5.68 (1H, br s), 8.16 (1H, s), 8.32 (1H, s). FD-MS 592 (M^++H). $[\alpha]_D^{25}$=+13.2° (*c* 0.1, MeOH). mp 198~199°C.

6-[4-(11-Bromoundecanoyl)glycylamino-4-deoxy-L-*glycero*-β-L-*manno*-heptopyranosylamino]-9*H*-purine (**8a**)

This compound was obtained from **5** and 11-bromoundecanoic acid in the typical procedure. Yield 31.1%. IR ν_{max} (KBr) 3400, 1630 cm^{-1}. ^1H NMR (CD_3OD) δ_H 1.30~1.90 (16H, m), 2.29 (2H, t, J=7.0 Hz), 3.44 (2H, t, J=7.2 Hz), 3.60~3.80 (5H, m), 3.86 (1H, d, J=17.0 Hz), 3.90 (1H, d, J=17.0 Hz), 4.01 (1H, dd, J=2.1, <1 Hz), 4.15(1H, dd, J=10.9, 10.9 Hz), 5.67 (1H, br s), 8.15 (1H, s), 8.30 (1H, s). FD-MS 630, 632 (M^++H). $[\alpha]_D^{25}$=+17.3° (*c* 0.1, MeOH). mp 175~178°C.

6-[4-(12-Bromododecanoyl)glycylamino-4-deoxy-L-*glycero*-β-L-*manno*-heptopyranosylamino]-9*H*-purine (**8b**)

This compound was obtained from **5** and 12-bromododecanoic acid in the typical procedure. Yield 22.7%. IR ν_{max} (KBr) 3400, 1630 cm^{-1}. ^1H NMR (CD_3OD) δ_H 1.20~1.70 (16H, m), 1.82 (2H, m), 2.28 (2H, t, J=7.0 Hz), 3.43 (2H, t, J=7.1 Hz), 3.60~3.80 (5H, m), 3.86 (1H, d, J=15.1 Hz), 3.89 (1H, d, J=15.1 Hz), 4.00 (1H, dd, J=2.1, <1 Hz), 4.14 (1H, dd, J=10.1, 10.1 Hz), 5.68 (1H, br s), 8.15 (1H, s), 8.30 (1H, s). FD-MS 644, 646 (M^++H). $[\alpha]_D^{25}$=0.0° (*c* 0.1, MeOH). mp 164~165°C.

6-[4-(13-Bromotridecanoyl)glycylamino-4-deoxy-L-*glycero*-β-L-*manno*-heptopyranosylamino]-9*H*-purine (**8c**)

This compound was obtained from **5** and 13-bromotridecanoic acid in the typical procedure. Yield 28.9%. IR ν_{max} (KBr) 3400, 1630 cm^{-1}. ^1H NMR (CD_3OD) δ_H 1.20~1.70 (18H, m), 1.82 (2H, m), 2.28 (2H, t, J=7.2 Hz), 3.42 (2H, t, J=7.3 Hz), 3.60~3.80 (5H, m), 3.87 (1H, d, J=16.1 Hz), 3.89 (1H, d, J=16.1 Hz), 4.01 (1H, dd, J=2.1, <1 Hz), 4.14 (1H, dd, J=10.4, 10.4 Hz), 5.68 (1H, br s), 8.12 (1H, s), 8.30 (1H, s). FD-MS 658, 660 (M^++H). $[\alpha]_D^{25}$=+5.5° (*c* 0.1, MeOH). mp 152~153°C.

6-[4-(14-Bromotetradecanoyl)glycylamino-4-deoxy-L-*glycero*-β-L-*manno*-heptopyranosylamino]-9*H*-purine (**8d**)

This compound was obtained from **5** and 14-bromotetradecanoic acid in the typical procedure. Yield 27.3%. IR ν_{max} (KBr) 3400, 1630 cm^{-1}. ^1H NMR (CD_3OD) δ_H 1.30~1.70 (20H, m), 1.84 (2H, m), 2.28 (2H, t, J=7.1 Hz), 3.43 (2H, t, J=7.1 Hz), 3.60~3.80 (5H, m), 3.87 (1H, d, J=16.1 Hz), 3.89 (1H, d, J=16.1 Hz), 4.01 (1H, dd, J=2.1, <1 Hz), 4.14 (1H, dd, J=10.4, 10.4 Hz), 5.68 (1H, br s), 8.15 (1H, br s), 8.31 (1H, s). FD-MS 672, 674 (M^++H). $[\alpha]_D^{25}$=−4.4° (*c* 0.1, MeOH). mp 161~163°C.

6-[4-(13-Chlorotridecanoyl)glycylamino-4-deoxy-L-*glycero*-β-L-*manno*-heptopyranosylamino]-9*H*-purine (**9a**)

This compound was obtained from **5** and 13-chlorotridecanoic acid in the typical procedure. Yield 30.3%. IR ν_{max} (KBr) 3300, 1630 cm^{-1}. ^1H NMR (CD_3OD) δ_H 1.25~1.50 (16H, m), 1.64 (2H, m), 1.75 (2H, m), 2.28 (2H, t, J=7.1 Hz), 3.53 (2H, t, J=7.1 Hz), 3.60~3.80 (5H, m), 3.87 (1H, d, J=16.1 Hz), 3.89 (1H, d, J=16.1 Hz), 4.02 (1H, dd, J=2.0, <1 Hz), 4.15 (1H, dd, J=10.4, 10.4 Hz), 5.69 (1H, br s), 8.13 (1H, br s), 8.30 (1H, s). FD-MS 614, 616 (M^+). $[\alpha]_D^{25}$=+34.3° (*c* 0.1, MeOH). mp 216~220°C.

6-[4-(14-Chlorotetradecanoyl)glycylamino-4-deoxy-L-*glycero*-β-L-*manno*-heptopyranosylamino]-9*H*-purine (**9b**)

This compound was obtained from **5** and 14-chlorotetradecanoic acid in the typical procedure. Yield 33.0%. IR ν_{max} (KBr) 3300, 1630 cm^{-1}. ^1H NMR (CD_3OD) δ_H 1.25~1.50 (18H, m), 1.63 (2H, m), 1.75 (2H, m), 2.28 (2H, t, J=7.1 Hz), 3.54 (2H, t, J=7.1 Hz), 3.60~3.80 (5H, m), 3.87 (1H, d, J=16.1 Hz), 3.89 (1H, d, J=16.1 Hz), 4.02 (1H, dd, J=2.1, <1 Hz), 4.14 (1H, dd, J=10.4, 10.4 Hz), 5.68 (1H, br s), 8.12 (1H, br s), 8.32 (1H, s). FD-MS 628, 630 (M^+). $[\alpha]_D^{25}$=−3.6° (*c* 0.1, MeOH). mp 166~168°C.

6-[4-Deoxy-4-(16-fluorohexadecanoyl)glycylamino-L-*glycero*-β-L-*manno*-heptopyranosylamino]-9*H*-purine (**10**)

This compound was obtained from **5** and 16-fluorohexadecanoic acid in the typical procedure. Yield 29.5%. IR ν_{max} (KBr) 3400, 1630 cm^{-1}. ^{1}H NMR (CD_3OD) δ_H 1.20~1.50 (26H, m), 2.28 (2H, t, J=7.1 Hz), 3.60~3.80 (5H, m), 3.85 (1H, d, J=16.4 Hz), 3.90 (1H, d, J=16.4 Hz), 4.01 (1H, dd, J=2.1, <1 Hz), 4.15 (1H, dd, J=10.4, 10.4 Hz), 4.40 (2H, dt, J=7.1, 42.0 Hz), 5.65 (1H, br s), 8.13 (1H, br s), 8.32 (1H, s). FD-MS 640 (M^++H). $[\alpha]_D^{25}$=+6.4° (*c* 0.1, MeOH). mp 175~176°C.

6-{4-Deoxy-4-[(2*RS*)-fluorododecanoyl]glycylamino-L-*glycero*-β-L-*manno*-heptopyranosylamino}-9*H*-purine (**11a**)

This compound was obtained from **5** and (2*RS*)-fluorododecanoic acid in the typical procedure. Yield 35.4%. IR ν_{max} (KBr) 3300, 1620 cm^{-1}. ^{1}H NMR (CD_3OD) δ_H 0.89 (3H, t, J=7.0 Hz), 1.20~1.53 (16H, m), 1.80~2.00 (2H, m), 3.60~4.05 (8H, m), 4.15 (1H, t, J=10.2 Hz), 4.95 (1H, dm, J=50.0 Hz), 5.65 (1H, s), 8.15 (1H, s), 8.31 (1H, s). FD-MS 607 (M^++Na+H). mp 187~189°C.

6-{4-Deoxy-4-[(2*RS*)-fluorohexadecanoyl]glycylamino-L-*glycero*-β-L-*manno*-heptopyranosylamino}-9*H*-purine (**11b**)

This compound was obtained from **5** and (2*RS*)-fluorohexadecanoic acid in the typical procedure. Yield 16.4%. IR ν_{max} (KBr) 3400, 1630 cm^{-1}. ^{1}H NMR (CD_3OD) δ_H 0.90 (3H, t, J=7.1 Hz), 1.20~2.10 (26H, m), 3.60~3.80 (5H, m), 3.85~4.05 (3H, m), 4.15 (1H, dd, J=10.4, 10.4 Hz), 4.95 (1H, dm, J=50.0 Hz), 5.68 (1H, s), 8.15 (1H, s), 8.32(1H, s). FD-MS 640 (M^++H). mp 174~175°C.

6-{4-Deoxy-4-[(2*RS*)-hydroxyhexadecanoyl]glycylamino-L-*glycero*-β-L-*manno*-heptopyranosylamino}-9*H*-purine (**12a**)

This compound was obtained from **5** and (2*RS*)-hydroxyhexadecanoic acid in the typical procedure. Yield 34.8%. IR ν_{max} (KBr) 3400, 1630 cm^{-1}. ^{1}H NMR (CD_3OD) δ_H 0.89 (3H, t, J=7.1 Hz), 1.20~1.85 (26H, m), 3.60~4.05 (8H, m), 4.08 (1H, m), 4.17 (1H, dd, J=10.3, 10.3 Hz), 5.65 (1H, br s), 8.09 (1H, s), 8.30 (1H, s). FD-MS 660 (M^++Na). mp 190~192°C.

6-{4-Deoxy-4-[(2*R*)-hydroxyhexadecanoyl]glycylamino-L-*glycero*-β-L-*manno*-heptopyranosylamino}-9*H*-purine (**12b**)

This compound was obtained from **5** and (2*R*)-hydroxyhexadecanoic acid in the typical procedure. Yield 10.0%. IR ν_{max} (KBr) 3300, 1630 cm^{-1}. ^{1}H NMR (CD_3OD) δ_H 0.89 (3H, t, J=7.1 Hz), 1.25~1.55 (24H, m), 1.60~1.90 (2H, m), 3.60~3.82 (5H, m), 3.87 (1H, d, J=16.6 Hz), 3.80~4.10 (4H, m), 4.15 (1H, dd, J=10.4, 10.4 Hz), 5.62 (1H, br s), 8.10 (1H, s), 8.25 (1H, s). FD-MS 638 (M^+). $[\alpha]_D^{25}$=−6.0° (*c* 0.1, MeOH). mp 182~186°C.

6-{4-Deoxy-4-[(2*S*)-hydroxyhexadecanoyl]glycylamino-L-*glycero*-β-L-*manno*-heptopyranosylamino}-9*H*-purine (**12c**)

This compound was obtained from **5** and (2*S*)-hydroxyhexadecanoic acid in the typical procedure. Yield 10.6%. IR ν_{max} (KBr) 3300, 1630 cm^{-1}. ^{1}H NMR (CD_3OD) δ_H 0.89 (3H, t, J=7.1 Hz), 1.25~1.55 (24H, m), 1.55~1.90 (2H, m), 3.60~3.80 (5H, m), 3.90 (1H, d, J=16.6 Hz), 3.97 (1H, d, J=16.6 Hz), 4.01 (1H, d, J=2.1, <1 Hz), 4.06 (1H, dd, J=7.1, 4.0 Hz), 4.14 (1H, dd, J=10.4, 10.4 Hz), 5.70 (1H, br s), 8.14 (1H, br s), 8.32 (1H, s). FD-MS 639 (M^++H). $[\alpha]_D^{25}$=−14.4° (*c* 0.1, MeOH). mp 173~174°C.

6-{4-Deoxy-4-[(3*R*)-hydroxytetradecanoyl]glycylamino-L-*glycero*-β-L-*manno*-heptopyranosylamino}-9*H*-purine (**13a**)

This compound was obtained from **5** and (3*R*)-hydroxytetradecanoic acid in the typical procedure. Yield 9.7%. IR ν_{max} (KBr) 3300, 1630 cm^{-1}. ^{1}H NMR (CD_3OD) δ_H 0.89 (3H, t, J=7.1 Hz), 1.20~1.65 (20H, m), 2.33 (1H, dd, J=8.9, 13.9 Hz), 2.45 (1H, dd, J=4.3, 13.9 Hz), 3.60~3.80 (5H, m), 3.85 (1H, d, J=16.1 Hz), 4.02 (1H, dd, J=2.1, <1 Hz), 4.03 (1H, m), 4.14 (1H, dd, J=10.4, 10.4 Hz), 5.67 (1H, br s), 8.14 (1H, br s), 8.3 (1H, s). FD-MS 610 (M^+). $[\alpha]_D^{25}$=−1.2° (*c* 0.1, MeOH). mp 167~168°C.

6-{4-Deoxy-4-[(3*S*)-hydroxytetradecanoyl]glycylamino-L-*glycero*-β-L-*manno*-heptopyranosylamino}-9*H*-purine (**13b**)

This compound was obtained from **5** and (3*S*)-hydroxytetradecanoic acid in the typical procedure. Yield 9.4%. IR ν_{max} (KBr) 3300, 1630 cm^{-1}. ^{1}H NMR (CD_3OD) δ_H 0.89 (3H, t, J=7.1 Hz), 1.20~1.65 (20H, m), 2.33 (1H, dd, J=8.9, 13.9 Hz), 2.45 (1H, dd, J=4.3, 13.9 Hz), 3.60~3.80 (5H, m), 3.85 (1H, d, J=16.1 Hz), 4.02 (1H, dd, J=2.1, <1 Hz), 4.03 (1H, m), 4.14 (1H, dd, J=10.4, 10.4 Hz), 5.67 (1H, br s), 8.14 (1H, br s), 8.30 (1H, s). FD-MS 610 (M^+). $[\alpha]_D^{25}$=−4.4° (*c* 0.1, MeOH). mp 178~180°C.

6-{4-Deoxy-4-[(3*RS*)-hydroxyhexadecanoyl]glycylamino-L-*glycero*-β-L-*manno*-heptopyranosylamino}-9*H*-purine (**13c**)

This compound was obtained from **5** and (3*RS*)-hydroxyhexadecanoic acid in the typical procedure. Yield 26.0%. IR ν_{max} (KBr) 3400, 1630 cm^{-1}. ^{1}H NMR (CD_3OD) δ_H 0.90 (3H, t, J=7.1 Hz), 1.20~1.60 (24H, m), 2.30~2.50 (2H, m), 3.60~4.10 (9H, m), 4.14 (1H, dd, J=10.4, 10.4 Hz), 5.69 (1H, br s), 8.15 (1H, br s), 8.31 (1H, s). FD-MS 642 (M^++Na−H_2O). mp 178~180°C.

6-[4-Deoxy-4-(12-hydroxydodecanoyl)glycylamino-L-*glycero*-β-L-*manno*-heptopyranosylamino]-9*H*-purine (**14a**)

This compound was obtained from **5** and 12-hydroxydodecanoic acid in the typical procedure. Yield 11.2%. IR ν_{max} (KBr) 3330, 1620 cm^{-1}. ^1H NMR (CD_3OD) δ_H 1.25~1.45 (14H, m), 1.47~1.55 (2H, m), 1.58~1.68 (2H, m), 2.28 (2H, t, J=6.2 Hz), 3.53 (2H, t, J=6.2 Hz), 3.60~3.80 (5H, m), 3.86 (1H, d, J=14.0 Hz), 3.89 (1H, d, J=14.0 Hz), 4.00 (1H, dd, J=2.5, <1 Hz), 4.14 (1H, dd, J=8.5, 8.5 Hz), 5.68(1H, br s), 8.15 (1H, br s), 8.30 (1H, s). FD-MS 582 (M^++H). $[\alpha]_D^{25}$ = +33.2° (*c* 0.1, MeOH). mp 174~175°C.

6-[4-Deoxy-4-(16-hydroxyhexadecanoyl)glycylamino-L-*glycero*-β-L-*manno*-heptopyranosylamino]-9*H*-purine (**14b**)

This compound was obtained from **5** and 16-hydroxyhexadecanoic acid in the typical procedure. Yield 33.2%. IR ν_{max} (KBr) 3330, 1630 cm^{-1}. ^1H NMR (CD_3OD) δ_H 1.24~1.40 (22H, m), 1.47~1.55 (2H, m), 1.58~1.64 (2H, m), 2.28 (2H, t, J=7.8 Hz), 3.54 (2H, t, J=6.4 Hz), 3.58~3.78 (5H, m), 3.85 (1H, d, J=16.0 Hz), 3.89 (1H, d, J=16.0 Hz), 4.00 (1H, dd, J=2.5, <1 Hz), 4.13 (1H, dd, J=10.7, 10.7 Hz), 5.66 (1H, br s), 8.16 (1H, br s), 8.32 (1H, s). FD-MS 638 (M^++H). $[\alpha]_D^{25}$ = −6.8° (*c* 0.1, MeOH). mp 186~187°C.

6-[4-Deoxy-4-(12-phenoxydodecanoyl)glycylamino-L-*glycero*-β-L-*manno*-heptopyranosylamino]-9*H*-purine (**15a**)

12-Bromododecanoic acid (5.00 g, 17.9 mmol) was dissolved in 10% HCl-MeOH (30 ml), and the mixture was stirred at room temperature for 1 hour. The reaction mixture was concentrated under reduced pressure. The resulting oil was dissolved in $CHCl_3$ and washed with water and saturated aqueous $NaHCO_3$, and dried over $MgSO_4$. Removal of the solvent afforded methyl 12-bromododecanoate (5.00 g, 95.2%). To a solution of methyl 12-bromododecanoate (1.04 g, 3.55 mmol) in DMF (30 ml) was added NaH (917 mg, 22.9 mmol, 60% dispersion in mineral oil) and phenol (0.72 g, 7.65 mmol) at room temperature, and strirred for 17 hours. The reaction mixture was concentrated under reduced pressure. The resulting oil was diluted with $CHCl_3$, and washed with water and dried over $MgSO_4$. Removal of the solvent and purification by silica gel chromatography (hexane-EtOAc, 10:1) afforded methyl 12-phenoxydodecanoate (1.05 g, 96.3%). Potassium hydroxide (920 mg, 16.4 mmol) was dissoloved in a mixed solvent of EtOH-water (1:1) (20 ml). The methyl 12-phenoxydodecanoate (1.05 g, 3.43 mmol) was added to the mixture, and the reaction mixture was stirred at 60°C for 2 hours. After the reaction mixture was cooled, and it was adjusted to the weak acidic range of pH with citric acid and extracted with EtOAc. The organic layer was dried over $MgSO_4$ and concentrated to give 12-phenoxydodecanoic acid (850 mg, 84.8%). Compound (**15a**) was obtained from **5** and 12-phenoxydodecanoic acid in the typical procedure. Yield 24.5%. IR ν_{max} (KBr) 3300, 1630 cm^{-1}. ^1H NMR (CD_3OD) δ_H 1.30~1.45 (12H, m), 1.48 (2H, m), 1.76 (2H, m), 2.28 (2H, t, J=7.1 Hz), 3.60~3.80 (5H, m), 3.87 (1H, d, J=16.7 Hz), 3.89 (1H, d, J=16.7 Hz), 3.95 (2H, t, J=7.1 Hz), 4.01 (1H, dd, J=2.1, <1 Hz), 4.14 (1H, dd, J=10.4, 10.4 Hz), 5.68 (1H, br s), 6.87 (3H, m), 7.24 (2H, t, J=7.6 Hz), 8.15 (1H, br s), 8.32 (1H, s). FD-MS 658 (M^++H). $[\alpha]_D^{25}$ = −8.4° (*c* 0.1, MeOH). mp 175~177°C.

6-[4-(12-Benzyloxydodecanoyl)glycylamino-4-deoxy-L-*glycero*-β-L-*manno*-heptopyranosylamino]-9*H*-purine (**15b**)

12-Hydroxydodecanoic acid (2.00 g, 9.25 mmol) was dissolved in 10% HCl-MeOH (30 ml), and the mixture was stirred at room temperature for 1 hour. The reaction mixture was concentrated under reduced pressure. The resulting oil was dissolved in $CHCl_3$ and washed with water and saturated aqueous $NaHCO_3$, and dried over $MgSO_4$. Removal of the solvent afforded methyl 12-hydroxydodecanoate (2.00 g, 93.9%). To a solution of methyl 12-hydroxydodecanoate (1.36 g, 5.90 mmol) in DMF (20 ml) was added NaH (480 mg, 12.0 mmol, 60% dispersion in mineral oil) and benzylbromide (1.03 g, 6.00 mmol) at room temperature, and stirred for 24 hours. The reaction was quenched by the addition of water, followed by extraction with EtOAc. The combined organic layer was washed with water and dried over $MgSO_4$. Removal of the solvent afforded crude methyl 12-hydroxydodecanoate. Sodium hydroxide (1.80 g, mmol) was dissoloved in a mixed solvent of EtOH-water (1:1) (20 ml). The crude methyl 12-benzyloxydecanoate was added to the mixture, and the reaction mixture was stirred at 70°C for 1 hour. After the reaction mixture was cooled, and it was adjusted to the weak acidic range of pH with citric acid and extracted with EtOAc. The organic layer was dried over $MgSO_4$ and concentrated to give 12-benzyloxydodecanoic acid (850 mg, 47.0%). And the compound (**15b**) was obtained from **5** and 12-benzyloxydodecanoic acid in the typical procedure. Yield 20.5%. IR ν_{max} (KBr) 3300, 1620 cm^{-1}. ^1H NMR (CD_3OD) δ_H 1.26~1.50 (14H, m), 1.55~1.68 (4H, m), 2.28 (2H, t, J=7.9 Hz), 3.48 (2H, t, J=7.1 Hz), 3.60~3.80 (5H, m), 3.86 (1H, d, J=16.4 Hz), 3.89 (1H, d, J=16.4 Hz), 4.00 (1H, dd, J=2.5, <1 Hz), 4.14 (1H, dd, J=10.4, 10.4 Hz), 4.48 (2H, s), 5.67 (1H, br s), 7.23~7.38 (5H, m), 8.16 (1H, br s), 8.31 (1H, s). FD-MS 672 (M^++H). $[\alpha]_D^{25}$ = +11.6° (*c* 0.1, MeOH). mp 206~207°C.

6-[4-Deoxy-4-(16-methoxyhexadecanoyl)glycylamino-L-*glycero*-β-L-*manno*-heptopyranosylamino]-9*H*-purine (**15c**)

16-Methoxyhexadecanoic acid was obtained from 16-hydroxyhexadecanoic acid and methyl iodide in the above procedure. And the compound (**15c**) was obtained from **5** and 16-methoxyhexadecanoic acid in the typical

procedure. Yield 17.1%. IR ν_{max} (KBr) 3300, 1630 cm^{-1}. ^1H NMR (CD_3OD) δ_H 1.25~1.40 (22H, m), 1.50~1.70 (4H, m), 2.28 (2H, t, J=7.9 Hz), 3.32 (3H, s), 3.39 (2H, t, J=7.9 Hz), 3.60~3.80 (5H, m), 3.85 (1H, d, J=16.5 Hz), 3.90 (1H, d, J=16.5 Hz), 4.01 (1H, dd, J=2.5, <1 Hz), 4.14 (1H, dd, J=10.4, 10.4 Hz), 5.65 (1H, br s), 8.10 (1H, br s), 8.30 (1H, s). FD-MS 652 (M^++H). $[\alpha]_D^{25}$ = +13.2° (*c* 0.1, MeOH). mp 202~204°C.

6-[4-(16-Benzoyloxyhexadecanoyl)glycylamino-4-deoxy-L-*glycero*-β-L-*manno*-heptopyranosylamino]-9*H*-purine (**15d**)

To a solution of 16-hydroxyhexadecanoic acid (1.00 g, 3.67 mmol) and triethylamine (1.10 ml) in CH_2Cl_2 was added benzoylchloride (0.54 g, 3.84 mmol) at 0°C, and the mixture was stirred at room temperature for 30 minutes. The reaction mixture was quenched with water, followed by extraction with EtOAc. The combined organic layer was dried over $MgSO_4$. Removal of the solvent afforded crude 16-benzoyloxyhexadecanoic acid. This was recrystallized from *n*-hexane - EtOAc, (5 : 1) to give 1.20 g of 16-benzoyloxyhexadecanoic acid (87.0%). And the compound (**15d**) was obtained from **5** and 16-benzoyloxyhexadecanoic acid in the typical procedure. Yield 28.0%. IR ν_{max} (KBr) 3250, 1720, 1620 cm^{-1}. ^1H NMR (CD_3OD) δ_H 1.25~1.50 (22H, m), 1.60~1.67 (2H, m), 1.74~1.80 (2H, m), 2.27 (2H, t, J=8.0 Hz), 3.60~3.80 (5H, m), 3.85 (1H, d, J=17.1 Hz), 3.89 (1H, d, J=17.1 Hz), 4.01 (1H, dd, J=2.5, <1 Hz), 4.14 (1H, dd, J=10.4, 10.4 Hz), 4.31 (2H, t, J=6.6 Hz), 5.67 (1H, br s), 7.46 (2H, dd, J=8.0, 8.0 Hz), 7.56 (1H, dd, J=8.0, 8.0 Hz), 8.00 (2H, d, J=8.0 Hz), 8.15 (1H, s), 8.30 (1H, s). FD-MS 742 (M^++H). $[\alpha]_D^{25}$ = +2.0° (*c* 0.1, MeOH). mp 201~203°C.

6-[4-Deoxy-4-(12-p-toluenesulfonyloxydodecanoyl)-glycylamino-L-*glycero*-β-L-*manno*-heptopyranosyl-amino]-9*H*-purine (**15e**)

12-hydroxydodecanoic acid (1.0 g, 3.77 mmol) was dissolved in a mixed solvent of 10% HCl-MeOH - CH_2Cl_2 (5 : 1) (25 ml), and the mixture was stirred at room temperature for 1 hour. The reaction mixture was concentrated under reduced pressure. The resulting oil was dissolved in $CHCl_3$ and washed with water and saturated aqueous $NaHCO_3$, and dried over $MgSO_4$. Removal of the solvent afforded methyl 12-hydroxydodecanoate (1.04 g, 99.6%). To a solution of methyl 12-hydroxydodecanoate (1.04 g, 3.66 mmol) in CH_2Cl_2 was added pyridine (1 ml) and p-toluenesulfonyl chloride (0.73 g, 3.83 mmol) at room temperature, and stirred for 3 hours. The reaction mixture was diluted with $CHCl_3$, and washed with water and dried over $MgSO_4$. Removal of the solvent and purification by silica gel chromatography (hexane - EtOAc, 100 : 1) afforded methyl 12-p-toluenesulfonyloxydodecanoate (1.58 g, 98.5%). Potassium hydroxide (0.85 g, 15.1 mmol) was dissoloved in a mixed solvent of EtOH - water (1 : 1) (20 ml). The methyl 12-p-toluenesulfonyloxydodecanoate (1.28 g, 3.33 mmol) was added to the mixture, and the reaction mixture was stirred at 70°C for 1 hour. After the reaction mixture was cooled, and it was adjusted to the weak acidic range of pH with citric acid and extracted with EtOAc. The organic layer was dried over $MgSO_4$ and concentrated to give 12-p-toluenesulfonyloxydodecanoic acid (0.85 g, 69.0%). And the compound (**15e**) was obtained from **5** and 12-p-toluenesulfonyloxydodecanoic acid in the typical procedure. Yield 22.5%. IR ν_{max} (KBr) 3300, 1630 cm^{-1}. ^1H NMR (CD_3OD) δ_H 1.18~1.50 (14H, m), 1.55~1.70 (4H, m), 2.28 (2H, t, J=7.5 Hz), 2.46 (3H, s), 3.60~3.80 (5H, m), 3.85 (1H, d, J=16.4 Hz), 3.89 (1H, d, J=16.4 Hz), 3.96~4.05 (3H, m), 4.14 (1H, dd, J=10.0, 10.0 Hz), 5.65 (1H, br s), 7.44 (2H, d, J=8.9 Hz), 7.77 (2H, d, J=8.9 Hz), 8.15 (1H, s), 8.32 (1H, s). FD-MS 736 (M^++H). $[\alpha]_D^{25}$ = −11.2° (*c* 0.1, MeOH). mp 155~157°C.

6-[4-Deoxy-4-(16-p-toluenesulfonyloxyhexadecano-yl)glycylamino-L-*glycero*-β-L-*manno*-heptopyranosyl-amino]-9*H*-purine (**15f**)

16-p-toluenesulfonyloxyhexadecanoic acid was obtained from 16-hydroxyhexadecanoic acid in the above procedure. And the compound (**15f**) was obtained from and 16-p-toluenesulfonyloxyhexadecanoic acid in the typical procedure. Yield 22.1%. IR ν_{max} (KBr) 3300, 1630 cm^{-1}. ^1H NMR (CD_3OD) δ_H 1.17~1.40 (22H, m), 1.55~1.66 (4H, m), 2.27 (2H, t, J=7.3 Hz), 2.45 (3H, s), 3.55~3.80 (5H, m), 3.85 (1H, d, J=16.6 Hz), 3.89 (1H, d, J=16.6 Hz), 3.97~4.05 (3H, m), 4.13 (1H, dd, J=10.0, 10.0 Hz), 5.65 (1H, br s), 7.43 (2H, d, J=8.3 Hz), 7.77 (2H, d, J=8.3 Hz), 8.13 (1H, s), 8.30 (1H, s). FD-MS 792 (M^++H). $[\alpha]_D^{25}$ = −1.2° (*c* 0.1, MeOH). mp 162~164°C.

6-[4-Deoxy-4-(12-diphenylmethyloxydodecanoyl)-glycylamino-L-*glycero*-β-L-*manno*-heptopyranosyl-amino]-9*H*-purine (**15g**)

12-diphenylmethyloxydodecanoic acid was obtained from 12-bromododecanoic acid and benzhydrol in the above procedure for (**15a**). And the compound (**15g**) was obtained from **5** and 12-diphenylmethyloxydodecanoic acid in the typical procedure. Yield 15.2%. IR ν_{max} (KBr) 3300, 1630 cm^{-1}. ^1H NMR (CD_3OD) δ_H 1.25~1.45 (16H, m), 1.50~1.60 (2H, m), 2.28 (2H, t, J=7.8 Hz), 3.44 (2H, t, J=7.1 Hz), 3.60~3.80 (5H, m), 3.85 (1H, d, J=15.7 Hz), 3.89 (1H, d, J=15.7 Hz), 4.00 (1H, dd, J=2.5, <1 Hz), 4.14 (1H, dd, J=10.4, 10.4 Hz), 5.35 (1H, s), 5.68 (1H, br s), 7.20~7.38 (10H, m), 8.10 (1H, br s), 8.31 (1H, s). FD-MS 748 (M^++H). $[\alpha]_D^{25}$ = +7.6° (*c* 0.1, MeOH). mp 194~196°C.

6-[4-Deoxy-4-(12-triphenylmethyloxydodecanoyl)-glycylamino-L-*glycero*-β-L-*manno*-heptopyranosyl-amino]-9*H*-purine (**15h**)

To a solution of 12-hydroxydodecanoic acid (1.50 g, 6.93 mmol) in CH_2Cl_2 (30 ml) was added triphenylmethylchloride (2.12 g, 7.60 mmol), and pyridine (2 ml)

at room temperature. The reaction mixture was stirred at the same temperature for 12 hours. The mixture was diluted with $CHCl_3$ and washed with water. After drying with $MgSO_4$, the solvent was concentrated under reduced pressure to give 2.00 g of 12-triphenylmethyloxydodecanoic acid (62.9%). And the compound (**15h**) was obtained from **5** and 12-triphenylmethyloxydodecanoic acid in the typical procedure. Yield 13.5%. IR ν_{max} (KBr) 3300, 1630 cm^{-1}. ^1H NMR (CD_3OD) δ_H 1.20~1.70 (18H, m), 2.27 (2H, t, J=7.7 Hz), 3.54 (2H, t, J=6.3 Hz), 3.60~3.80 (5H, m), 3.85 (1H, d, J=16.3 Hz), 3.90 (1H, d, J=16.3 Hz), 4.00 (1H, dd, J=2.5, <1 Hz), 4.14 (1H, dd, J=10.0, 10.0 Hz), 5.68 (1H, br s), 7.22 (3H, t, J=7.7 Hz), 7.28 (6H, t, J=8.0 Hz), 7.41 (6H, d, J=8.6 Hz), 8.14 (1H, br s), 8.29 (1H, s). FD-MS 825 (M^++H). $[\alpha]_D^{25}$=0.0° (c 0.1, MeOH). mp 203~205°C.

6-{4-Deoxy-4-[12-(1-naphthyloxy)dodecanoyl]glycylamino-L-*glycero*-β-L-*manno*-heptopyranosylamino}-9*H*-purine (**15i**)

12-(1-Naphthyloxy)dodecanoic acid was obtained from 12-bromododecanoic acid and 1-naphthol in the above procedure for (**15a**). And the compound (**15i**) was obtained from **5** and 12-(1-naphthyloxy)dodecanoic acid in the typical procedure. Yield 14.3%. IR ν_{max} (KBr) 3300, 1630 cm^{-1}. ^1H NMR (CD_3OD) δ_H 1.30~1.50 (14H, m), 1.56~1.68 (2H, m), 1.90~1.97 (2H, m), 2.28 (2H, t, J=8.2 Hz), 3.65~3.80 (5H, m), 3.86 (1H, d, J=16.1 Hz), 3.91 (1H, d, J=16.1 Hz), 4.02 (1H, dd, J=2.5, <1 Hz), 4.13 (2H, t, J=7.2 Hz), 4.14 (1H, dd, J=10.0, 10.0 Hz), 5.65 (1H, br s), 6.85 (1H, d, J=7.1 Hz), 7.32~7.48 (4H, m), 7.77 (1H, d, J=7.9 Hz), 8.10 (1H, br s), 8.22 (1H, d, J=7.9 Hz), 8.31 (1H, s). FD-MS 748 (M^++H). $[\alpha]_D^{25}$=−1.2° (c 0.1, MeOH). mp 182~184°C.

6-{4-Deoxy-4-[(2*E*)-tridecenoyl]glycylamino-L-*glycero*-β-L-*manno*-heptopyranosylamino}-9*H*-purine (**16a**)

This compound was obtained from **5** and (2*E*)-tridecenoic acid in the typical procedure. Yield 30.2%. IR ν_{max} (KBr) 3400, 1620 cm^{-1}. ^1H NMR (CD_3OD) δ_H 0.90 (3H, t, J=7.1 Hz), 1.20~1.50 (16H, m), 2.22 (2H, m), 3.60~3.80 (5H, m), 3.95 (1H, d, J=16.0 Hz), 3.98 (1H, d, J=16.0 Hz), 4.02 (1H, dd, J=2.1, <1 Hz), 4.17 (1H, dd, J=10.5, 10.5 Hz), 5.68 (1H, br s), 6.00 (1H, d, J=15.2 Hz), 6.85 (1H, dt, J=6.8, 15.2 Hz), 8.12 (1H, br s), 8.32 (1H, s). FD-MS 578 (M^++H). $[\alpha]_D^{25}$=+8.8° (c 0.1, MeOH). mp 215~217°C.

6-{4-Deoxy-4-[(2*E*)-tetradecenoyl]glycylamino-L-*glycero*-β-L-*manno*-heptopyranosylamino}-9*H*-purine (**16b**)

This compound was obtained from **5** and (2*E*)-tetradecenoic acid in the typical procedure. Yield 23.1%. IR ν_{max} (KBr) 3400, 1630 cm^{-1}. ^1H NMR (CD_3OD) δ_H 0.90 (3H, t, J=7.0 Hz), 1.20~1.60 (18H, m), 2.26 (2H, t, J=7.1 Hz), 3.30~3.80 (5H, m), 3.94 (1H, d, J=16.4 Hz), 3.98 (1H, d, J=16.4 Hz), 4.00 (1H, dd, J=2.1, <1 Hz), 4.16 (1H, dd, J=10.1, 10.1 Hz), 5.65 (1H, br s), 6.00 (1H, d, J=15.0 Hz), 6.83 (1H, dt, J=6.4, 15.0 Hz), 8.18 (1H, s), 8.32 (1H, s). FD-MS 592 (M^++H).$[\alpha]_D^{25}$=+5.6° (c 0.1, MeOH). mp 171~172°C.

6-[4-Deoxy-4-(11-dodecenoyl)glycylamino-L-*glycero*-β-L-*manno*-heptopyranosylamino]-9*H*-purine (**17a**)

This compound was obtained from **5** and 11-dodecenoic acid in the typical procedure. Yield 33.0%. IR ν_{max} (KBr) 3400, 1630 cm^{-1}. ^1H NMR (CD_3OD) δ_H 1.20~1.70 (14H, m), 2.03 (2H, m), 2.28 (2H, t, J=7.6 Hz), 3.65~3.85 (5H, m), 3.88 (1H, d, J=16.0 Hz), 3.91 (1H, d, J=16.0 Hz), 4.02 (1H, dd, J=2.1, <1 Hz), 4.17 (1H, dd, J=10.3, 10.3 Hz), 4.92 (1H, d, J=10.9 Hz), 4.98 (1H, d, J=17.0 Hz), 5.67 (1H, br s), 5.79 (1H, m), 8.12 (1H, s), 8.26 (1H, s). FD-MS 564 (M^++H). $[\alpha]_D^{25}$=+21.5° (c 0.1, MeOH). mp 222~224°C.

6-[4-Deoxy-4-(12-tridecenoyl)glycylamino-L-*glycero*-β-L-*manno*-heptopyranosylamino]-9*H*-purine (**17b**)

This compound was obtained from **5** and 12-tridecenoic acid in the typical procedure. Yield 16.6%. IR ν_{max} (KBr) 3400, 1630 cm^{-1}. ^1H NMR (CD_3OD) δ_H 1.20~1.70 (16H, m), 2.03 (2H, m), 2.28 (2H, t, J=7.2 Hz), 3.60~3.80 (5H, m), 3.86 (1H, d, J=15.0 Hz), 3.89 (1H, d, J=15.0 Hz), 4.00 (1H, dd, J=2.0, <1 Hz), 4.14 (1H, dd, J=10.0, 10.0 Hz), 4.92 (1H, d, J=10.9 Hz), 4.98 (1H, d, J=17.0 Hz), 5.67 (1H, br s), 5.81 (1H, m), 8.15 (1H, s), 8.32 (1H, s). FD-MS 577 (M^++H). $[\alpha]_D^{25}$=+13.3° (c 0.1, MeOH). mp 182~184°C.

6-{4-Deoxy-4-[(9*Z*)-hexadecenoyl]glycylamino-L-*glycero*-β-L-*manno*-heptopyranosylamino}-9*H*-purine (**18**)

This compound was obtained from **5** and (9*Z*)-hexadecenoic acid in the typical procedure. Yield 36.0%. IR ν_{max} (KBr) 3400, 1630 cm^{-1}. ^1H NMR (CD_3OD) δ_H 0.90 (3H, t, J=7.0 Hz), 1.25~1.70 (20H, m), 2.05 (4H, m), 2.28 (2H, t, J=7.1 Hz), 3.60~3.80 (5H, m), 3.86 (1H, d, J=16.1 Hz), 3.89 (1H, d, J=16.1 Hz), 4.01 (1H, dd, J=2.1, <1 Hz), 4.14 (1H, dd, J=10.1, 10.1 Hz), 5.34 (2H, m), 5.68 (1H, br s), 8.15 (1H, s), 8.30 (1H, s). FD-MS 621 (M^++H). $[\alpha]_D^{25}$=+18.0° (c 0.1, MeOH). mp 174~175°C.

6-{4-Deoxy-4-[(2*E*,4*E*)-dodecadienoyl]glycylamino-L-*glycero*-β-L-*manno*-heptopyranosylamino}-9*H*-purine (**19a**)

To a solution of (2*E*)-decenal (5.0 g, 32.0 mmol) in methylenechloride (40 ml) was added methyl (triphenylphosphoranylidene)acetate (11.99 g, 35.9 mmol) at room temperature, and the mixture was stirred at the same temperature for 2 hours. Removal of the solvent afforded an oily residue, which was purified by silica gel column chromatography (hexane - EtOAc, 100 : 1~20 : 1) to afford the methyl (2*E*,4*E*)-dodecadienoate (6.1 g, 90.8%). Potassium hydroxide (8.1 g, 144.6 mmol) was dissolved in a mixed solvent of EtOH - water (1 : 1)

(100 ml). The methyl (2*E*,4*E*)-dodecadienoate (6.1 g, 29.0 mmol) was added to the mixture, and the reaction mixture was stirred at 60°C for 40 minutes. After the reaction mixture was cooled, and it was adjusted to the weak acidic range of pH with citric acid and extracted with EtOAc. The organic layer was dried over $MgSO_4$ and concentrated to give (2*E*,4*E*)-dodecadienoic acid (5.4 g, 94.9%). Compound (**19a**) was obtained from **5** and (2*E*,4*E*)-dodecadienoic acid in the typical procedure. Yield 29.0%. IR ν_{max} (KBr) 3400, 1630 cm^{-1}. ^1H NMR (CD_3OD) δ_H 0.90 (3H, t, J=7.3 Hz), 1.20~1.50 (10H, m), 2.18 (2H, dt, J=7.3, 7.3 Hz), 3.60~3.80 (5H, m), 3.95 (1H, d, J=16.3 Hz), 3.98 (1H, d, J=16.3 Hz), 4.00 (1H, dd, J=2.9, <1 Hz), 4.15 (1H, dd, J=10.8, 10.8 Hz), 5.68 (1H, br s), 6.00 (1H, d, J=15.7 Hz), 6.13 (1H, dt, J=7.3, 15.7 Hz), 6.22 (1H, dd, J=10.0, 15.7 Hz), 7.17 (1H, dd J=10.0, 15.7 Hz), 8.15 (1H, s), 8.30 (1H, s). FD-MS 562 (M^++H). $[\alpha]_D^{25}$=+7.6° (*c* 0.1, MeOH). mp 168~169°C.

6-{4-Deoxy-4-[(2*E*,4*E*)-tridecadienoyl]glycylamino-L-*glycero*-β-L-*manno*-heptopyranosylamino}-9*H*-purine (**19b**)

(2*E*,4*E*)-Tridecadienoic acid was obtained from (2*E*)-undecenal in the above procedure. And the compound (**19b**) was obtained from **5** and (2*E*,4*E*)-tridecadienoic acid in the typical procedure. Yield 34.7%. IR ν_{max} (KBr) 3250, 1650, 1620 cm^{-1}. ^1H NMR (CD_3OD) δ_H 0.90 (3H, t, J=7.3 Hz), 1.20~1.50 (12H, m), 2.18 (2H, dt, J=7.3, 7.3 Hz), 3.60~3.80 (5H, m), 3.95 (1H, d, J=16.3 Hz), 3.98 (1H, d, J=16.3 Hz), 4.00 (1H, dd, J=2.9, <1 Hz), 4.15 (1H, dd, J=10.8, 10.8 Hz), 5.68 (1H, br s), 6.00 (1H, d, J=15.7 Hz), 6.13 (1H, dt, J=7.3, 15.7 Hz), 6.22 (1H, dd, J=10.0, 15.7 Hz), 7.17 (1H, dd J=10.0, 15.7 Hz), 8.15 (1H, s), 8.30 (1H, s). FD-MS 598 (M^++Na). $[\alpha]^{25}$=+6.8° (*c* 0.1, MeOH). mp 177~179°C.

6-{4-Deoxy-4-[(2*E*,4*E*)-tetradecadienoyl]glycylamino-L-*glycero*-β-L-*manno*-heptopyranosylamino}-9*H*-purine (**19c**)

(2*E*,4*E*)-Tetradecadienoic acid was obtained from (2*E*)-dodecenal in the above procedure. And the compound (**19c**) was obtained from **5** and (2*E*, 4*E*)-tetradecadienoic acid in the typical procedure. Yield 51.0%. IR ν_{max} (KBr) 3250, 1650, 1620 cm^{-1}. ^1H NMR (CD_3OD) δ_H 0.89 (3H, t, J=7.3 Hz), 1.20~1.50 (14H, m), 2.18 (2H, dt, J=7.3, 7.3 Hz), 3.60~3.80 (5H, m), 3.95 (1H, d, J=16.3 Hz), 3.98 (1H, d, J=16.3 Hz), 4.00 (1H, dd, J=2.9, <1 Hz), 4.15 (1H, dd, J=10.8, 10.8 Hz), 5.66 (1H, br s), 5.98 (1H, d, J=15.7 Hz), 6.12 (1H, dt, J=7.3, 15.7 Hz), 6.22 (1H, dd, J=10.0, 15.7 Hz), 7.17 (1H, dd J=10.0, 15.7 Hz), 8.15 (1H, s), 8.30 (1H, s). FD-MS 590 (M^++H). $[\alpha]_D^{25}$=+0.0° (*c* 0.1, MeOH). mp 182~183°C.

6-[4-Deoxy-4-(10-undecynoyl)glycylamino-L-*glycero*-β-L-*manno*-heptopyranosylamino]-9*H*-purine (**20a**)

This compound was obtained from **5** and 10-undecynoic acid in the typical procedure. Yield 40.9%. IR ν_{max} (KBr) 3250, 1620 cm^{-1}. ^1H NMR (CD_3OD) δ_H 1.26~1.60 (12H, m), 2.12~2.18 (2H, m), 2.28 (2H, t, J=7.2 Hz), 3.60~3.80 (5H, m), 3.85 (1H, d, J=15.0 Hz), 3.90 (1H, d, J=15.0 Hz), 4.01 (1H, dd, J=2.1, <1 Hz), 4.14 (1H, dd, J=10.5, 10.5 Hz), 5.67 (1H, br s), 8.16 (1H, s), 8.32 (1H, s). FD-MS 548 (M^++H). $[\alpha]_D^{25}$=+13.6° (*c* 0.1, MeOH). mp 158~189°C.

6-[4-Deoxy-4-(12-tridecynoyl)glycylamino-L-*glycero*-β-L-*manno*-heptopyranosylamino]-9*H*-purine (**20b**)

This compound was obtained from **5** and 12-tridecynoic acid in the typical procedure. Yield 34.5%. IR ν_{max} (KBr) 3400, 1650 cm^{-1}. ^1H NMR (CD_3OD) δ_H 1.26~1.60 (16H, m), 2.12~2.18 (2H, m), 2.28 (2H, t, J=7.2 Hz), 3.60~3.80 (5H, m), 3.86 (1H, d, J=15.0 Hz), 3.90 (1H, d, J=15.0 Hz), 4.01 (1H, dd, J=2.5, <1 Hz), 4.15 (1H, dd, J=10.5, 10.5 Hz), 5.67 (1H, br s), 8.14 (1H, s), 8.30 (1H, s). FD-MS 576 (M^++H). $[\alpha]_D^{25}$=+17.2° (*c* 0.1, MeOH). mp 198~199°C.

6-[4-Deoxy-4-(2-undecynoyl)glycylamino-L-*glycero*-β-L-*manno*-heptopyranosylamino]-9*H*-purine (**21a**)

A solution of *n*-butyllithiun in *n*-hexane (2.4 M, 9.0 ml, 26.7 mmol) was added to a stirred solution of 1-decyne (2.76 g, 20.0 mmol) in THF (40 ml) at −10°C under an argon atmosphere, and stirred at the same temperature for 30 minutes. Then a solution of ethyl chloroformate (2.39 g, 22.0 mmol) in THF (10 ml) was added to the reaction mixture at the same temperature. The mixture was stirred at 0°C for 1 hour. The reaction mixture was quenched by addition of saturated aquous NH_4Cl. After extraction with ether, the combined organic layer was washed with water and brine, and dried over $MgSO_4$. Removal of the solvent, the residue was purified by silica gel chromatography (*n*-hexane - EtOAc, 100 : 1) to afford ethyl 2-undecynoate (2.33 g, 55.6%) as a colorless oil. To a solution of ethyl 2-undecynoate (0.60 g, 2.86 mmol) in MeOH (5 ml) was added 10% aquous LiOH (5 ml) at room temperature. The reaction mixture was stirred at the same temperature for 12 hours. The reaction mixture was acidifed with 2 N HCl, followed by extraction with ether. The organic layer was dried over $MgSO_4$. Removal of the solvent afforded 2-undecynoic acid (0.45 g, 86.5%). Compound (**21a**) was obtained from **5** and 2-undecynoic acid in the typical procedure. Yield 9.2%. IR ν_{max} (KBr) 3400, 1650 cm^{-1}. ^1H NMR (CD_3OD) δ_H 0.90 (3H, t, J=7.1 Hz), 1.25~1.40 (8H, m), 1.40~1.50 (2H, m), 1.55~1.62 (2H, m), 2.36 (2H, t, J=7.1 Hz), 3.60~3.80 (5H, m), 3.89 (1H, d, J=17.1 Hz), 3.93 (1H, d, J=17.1 Hz), 4.00 (1H, dd, J=2.5, <1 Hz), 4.14 (1H, dd, J=10.7, 10.7 Hz), 5.66 (1H, br s), 8.15 (1H, s), 8.30 (1H, s). FD-MS 548 (M^++H). $[\alpha]_D^{25}$=+19.6° (*c* 0.1, MeOH). mp 172~173°C.

6-[4-Deoxy-4-(2-dodecynoyl)glycylamino-L-*glycero*-β-L-*manno*-heptopyranosylamino]-9*H*-purine (**21b**)

2-Dodecynoic acid was obtained from 1-undecyne in

the above procedure. And the compound (**21b**) was obtained from **5** and 2-dodecynoic acid in the typical procedure. Yield 8.1%. IR ν_{max} (KBr) 3400, 2200, 1650 cm^{-1}. ^1H NMR (CD_3OD) δ_H 0.90 (3H, t, J=7.1 Hz), 1.25~1.38 (10H, m), 1.40~1.48 (2H, m), 1.54~1.62 (2H, m), 2.27 (2H, t, J=7.1 Hz), 3.60~3.80 (5H, m), 3.90 (1H, d, J=17.1 Hz), 3.94 (1H, d, J=17.1 Hz), 4.00 (1H, dd, J=2.5, <1 Hz), 4.15 (1H, dd, J=10.7, 10.7 Hz), 5.67 (1H, br s), 8.15 (1H, s), 8.30 (1H, s). FD-MS 584 (M^++Na). $[\alpha]_D^{25}$=+22.8° (*c* 0.1, MeOH). mp 177~178°C.

6-[4-Deoxy-4-(2-tridecynoyl)glycylamino-L-*glycero*-β-L-*manno*-heptopyranosylamino]-9*H*-purine (**21c**)

2-Tridecynoic acid was obtained from 1-dodecyne in the above procedure. And the compound (**21c**) was obtained from **5** and 2-tridecynoic acid in the typical procedure. Yield 4.9%. IR ν_{max} (KBr) 3400, 2200, 1650 cm^{-1}. ^1H NMR (CD_3OD) δ_H 0.90 (3H, t, J=7.1 Hz), 1.25~1.40 (12H, m), 1.40~1.50 (2H, m), 1.55~1.62 (2H, m), 2.36 (2H, t, J=7.1 Hz), 3.60~3.80 (5H, m), 3.89 (1H, d, J=17.1 Hz), 3.93 (1H, d, J=17.1 Hz), 4.00 (1H, dd, J=2.5, <1 Hz), 4.14 (1H, dd, J=10.7, 10.7 Hz), 5.66 (1H, br s), 8.15 (1H, s), 8.30 (1H, s). FD-MS 576 (M^++H). $[\alpha]_D^{25}$=+16.4° (*c* 0.1, MeOH). mp 190~191°C.

6-[4-Deoxy-4-(2-tetradecynoyl)glycylamino-L-*glycero*-β-L-*manno*-heptopyranosylamino]-9*H*-purine (**21d**)

2-Tetradecynoic acid was obtained from 1-tridecyne in the above procedure. And the compound (**21d**) was obtained from **5** and 2-tetradecynoic acid in the typical procedure. Yield 10.9%. IR ν_{max} (KBr) 3400, 2200, 1650 cm^{-1}. ^1H NMR (CD_3OD) δ_H 0.90 (3H, t, J=7.1 Hz), 1.25~1.38 (14H, m), 1.39~1.50 (2H, m), 1.55~1.62 (2H, m), 2.37 (2H, t, J=7.1 Hz), 3.65~3.80 (5H, m), 3.91 (1H, d, J=16.8 Hz), 3.95 (1H, d, J=16.8 Hz), 4.02 (1H, dd, J=2.5, <1 Hz), 4.15 (1H, dd, J=10.7, 10.7 Hz), 5.65 (1H, br s), 8.12 (1H, s), 8.30 (1H, s). FD-MS 590 (M^++H). $[\alpha]_D^{25}$=+17.6° (*c* 0.1, MeOH). mp 201~202°C.

6-(4-Deoxy-4-tetradecyloxycarbonylglycylamino-L-*glycero*-β-L-*manno*-heptopyranosylamino)-9*H*-purine (**22a**)

To a solution of 1-tetradecanol (300 mg, 1.40 mmol) in DMF (15 ml) was added N,N′-disuccinimidyl carbonate (317 mg, 1.24 mmol) at room temperature, and the mixture was stirred at the same temperature for 12 hours. Then **5** (479 mg, 1.14 mmol) and ethanolamine (0.5 ml) was added to the reaction mixture at room temperature and the mixture was stirred at the same temperature for 12 hours. After evaporation, the residue was purified by silica gel column chromatography ($CHCl_3$ - MeOH, 5 : 1) afforded (**22a**) (107 mg, 15.0%) as a white powder. IR ν_{max} (KBr) 3250, 1690, 1610 cm^{-1}. ^1H NMR (CD_3OD) δ_H 0.90 (3H, t, J=7.5 Hz), 1.25~1.43 (22H, m), 1.60~1.68 (2H, m), 3.60~3.80 (5H, m), 3.78 (1H, d, J=18.0 Hz), 3.83 (1H, d, J=18.0 Hz), 4.00 (1H, dd, J=2.5, <1 Hz), 4.06 (2H, t, J=7.0 Hz), 4.14 (1H, dd, J=10.3, 10.3 Hz), 5.67 (1H, br s), 8.14 (1H, s), 8.31 (1H, s). FD-MS 624 (M^++H). $[\alpha]_D^{25}$=+10.4° (*c* 0.1, MeOH). mp 194~195°C.

6-(4-Deoxy-4-hexadecyloxycarbonylglycylamino-L-*glycero*-β-L-*manno*-heptopyranosylamino)-9*H*-purine (**22b**)

This compound was obtained from **5** and 1-hexadecanol in the above procedure. Yield 14.2%. IR ν_{max} (KBr) 3330, 1700, 1620 cm^{-1}. ^1H NMR (CD_3OD) δ_H 0.90 (3H, t, J=7.7 Hz), 1.25~1.45 (26H, m), 1.60~1.70 (2H, m), 3.60~3.80 (5H, m), 3.78 (1H, d, J=16.9 Hz), 3.83 (1H, d, J=16.9 Hz), 4.00 (1H, dd, J=2.5, <1 Hz), 4.06 (2H, t, J=7.0 Hz), 4.15 (1H, dd, J=10.0, 10.0 Hz), 5.67 (1H, br s), 8.15 (1H, s), 8.32 (1H, s). FD-MS 652 (M^++H). $[\alpha]_D^{25}$=−0.4° (*c* 0.1, MeOH). mp 203~204°C.

6-(4-Deoxy-4-octadecyloxycarbonylglycylamino-L-*glycero*-β-L-*manno*-heptopyranosylamino)-9*H*-purine (**22c**)

This compound was obtained from **5** and 1-octadecanol in the above procedure. Yield 17.1%. IR ν_{max} (KBr) 3330, 1690, 1620 cm^{-1}. ^1H NMR (CD_3OD) δ_H 0.90 (3H, t, J=6.7 Hz), 1.25~1.40 (30H, m), 1.60~1.70 (2H, m), 3.65~3.80 (5H, m), 3.80 (1H, d, J=16.4 Hz), 3.84 (1H, d, J=16.9 Hz), 4.02 (1H, dd, J=2.5, <1 Hz), 4.07 (2H, t, J=7.1 Hz), 4.15 (1H, dd, J=10.0, 10.0 Hz), 5.65 (1H, br s), 8.14 (1H, s), 8.32 (1H, s). FD-MS 680 (M^++H). $[\alpha]_D^{25}$=−10.8° (*c* 0.1, MeOH). mp 212~213°C.

6-(4-Deoxy-4-dodecylglycylamino-L-*glycero*-β-L-*manno*-heptopyranosylamino)-9*H*-purine (**23**)

To a solution of 1-dodecanal (960 mg, 5.21 mmol), **5** (200 mg, 0.52 mmol) and triethylamine (600 mg, 6.00 mmol) in EtOH (20 ml) was added sodiumcyanoborohydride (377 mg, 6.00 mmol) at room temperature. The reaction mixture was stirred at the same temperature for 12 hours. The mixture was diluted with $CHCl_3$ and washed with water. After drying with $MgSO_4$, the solvent was concentrated under reduced pressure to give crude products. This was purified by silicagel chromatography ($CHCl_3$, methanol=5 : 1) to give **23** (66 mg, 22.9%). IR ν_{max} (KBr) 3300, 1630 cm^{-1}. ^1H NMR (CD_3OD) δ_H 0.89 (3H, t, J=7.0 Hz), 1.20~1.50 (18H, m), 1.55~1.70 (2H, m), 2.70~2.80 (2H, m), 3.20 (2H, dd, J=16.3, 8.0 Hz), 3.70~3.90 (5H, m), 4.05 (1H, dd, J=2.0, <1 Hz), 4.16 (1H, dd, J=10.0, 10.0 Hz), 5.68 (1H, br s), 8.13 (1H, br s), 8.27 (1H, s). FD-MS 552 (M^++H). $[\alpha]_D^{25}$=+13.2° (*c* 0.1, MeOH). mp 203~205°C.

In Vivo Antitumor Activity

For all experiments, 6~8-week-old female, athymic nude mice (BALB/c nu/nu Slc, Japan SLC Inc., Shizuoka,

Japan) were used. The animals were kept under specific pathogen free conditions using laminar air flow racks and were fed sterile food and water *ad libitum*.

Human colon cancer COL-1 were kindly supplied by the Central Institute for Experimental Animals, Kanagawa, Japan, and maintained in athymic nude mice. Chemotherapeutic experiments were performed as described by INABA *et al.* Fragments of xenografts were implanted sc into the right subaxillary region of athymic nude mice. When the tumors had grown to a palpable size ($100 \sim 300\,mm^3$), the mice were randomly allocated to several experimental groups consisting of five animals each and spicamycin derivatives at each dose were given intravenously by daily injection for five days. Control mice were given 10 ml/kg vehicle. From the start of the injections, the tumor volume (V) was calculated once or twice a week for 3 weeks as follows; $V = abc/2$, where a and b are the long and short diameter and c is the height of the tumor mass in mm. Relative tumor volume (RV) is expressed as $RV = V_n/V_0$, where V_n is the tumor volume on day n and V_0 is the initial tumor volume at the time treatment was commenced (day 1). T.G.I.R. was determined as follows; $T.G.I.R. = (1 - T/C) \times 100$ where T is the mean of RV in treated mice and C is the mean of RV in control mice.

Evaluation as "effective" was based on the maximum T.G.I.R. (%) for an experimental span of 50% or more showing ststistical significance as determined by the MANN-WHITNEY's U-test ($P < 0.05$, one sided). A toxic dose was difined as one causing the death of one or more mice in a group. The T.I. was determined as follows; T.I. = maximum tolerated dose/minimum effective dose.

References

1) HAYAKAWA, Y.; M. NAKAGAWA, H. KAWAI, K. TANABE, H. NAKAYAMA, A. SHIMAZU, H. SETO & N. ŌTAKE: Studies on the differentiation inducers of myeloid leukemic cells III. Spicamycin, a new inducer of differentiation of HL-60 human promyelocytic leukemia cells. J. Antibiotics 36: 934~937, 1983
2) HAYAKAWA, Y.; M. NAKAGAWA, H. KAWAI, K. TANABE, H. NAKAYAMA, A. SHIMAZU, H. SETO & N. ŌTAKE: Spicamycin, a new differentiation inducer of mouse myeloid leukemia cells (M1) and human promyelocytic leukemia cells (HL-60). Agric. Biol. Chem. 49: 2685~2691, 1985
3) SAKAI, T.; K. SHINDO, A. ODAGAWA, A. SUZUKI, H. KAWAI, K. KOBAYASHI, Y. HAYAKAWA, H. SETO & N. ŌTAKE: Absolute configuration of spicamycin, an antitumor antibiotic produced by *Streptomyces alanosinicus*. J. Antibiotics 48: 899~900, 1995
4) KAMISHOHARA, M.; H. KAWAI, A. ODAGAWA, T. ISOE, J. MOCHIZUKI, T. UCHIDA, Y. HAYAKAWA, H. SETO, T. TSURUO & N. ŌTAKE: Structure-antitumor activity relationship of semi-synthetic spicamycin analogues. J. Antibiotics 46: 1439~1446, 1993
5) KAMISHOHARA, M.; H. KAWAI, A. ODAGAWA, T. ISOE, J. MOCHIZUKI, T. UCHIDA, Y. HAYAKAWA, H. SETO, T. TSURUO & N. ŌTAKE: Antitumor activity of SPM VIII, a derivative of the nucleoside antibiotic Spicamycin, against human tumor xenografts. J. Antibiotics 47: 1305~1311, 1994
6) ACTON, E. M.; K. J. RYAN & A. E. LUETZOW: Antitumor septacidin analogues. J. Med. Chem. 20: 1362~1371, 1977
7) OGURA, H.; T. KOBAYASHI, K. SHIMIZU, K. KAWABE & K. TAKEDA: A novel active ester synthesis reagent (N,N'-Disuccinimidyl carbonate). Tetrahedron Lett. 4745~4746, 1979
8) BORCH, R. F.: Reductive amination with sodium cyanoborohydride: N,N-Dimethylcyclohexylamine. Organic Syntheses 52: 124~127, 1972
9) INABA, M.; T. KOBAYASHI, T. TASHIRO, Y. SAKURAI, K. MARUO, Y. OHNISHI, Y. UEYAMA & T. NOMURA: Evaluation of antiumor activity in a human breast tumor/nude mouse model with a special emphasis on treatment dose. Cancer 64: 1577~1582, 1989
10) DOUBLE, J. A. & M. C. BIBBY: Therapeutic Index: A Vital Componeny in Selection of Anticancer Agents for Clinical Trial. J. Nat. Cancer. Inst. 81: 988~994, 1989
11) KAMISHOHARA, M.; H. KAWAI, T. SAKAI, T. ISOE, K. HASEGAWA, J. MOCHIZUKI, T. UCHIDA, S. KATAOKA, H. YAMAKI, T. TSURUO & N. ŌTAKE: Antitumor Activity of a Spicamycin Derivative, KRN5500, and its Active Metaborite in Tumor Cells. Oncol. Res. 6: 383~390, 1994
12) AOSHIMA, M.; S. TSUKAGOSHI, Y. SAKURAI, J. OH-ISHI, T. ISHIDA & H. KOBAYASHI: Antitumor activities of newly synthesized N^4-acyl-1-β-D-arabinofuranosylcytosine. Cancer Res 36: 2726~2732, 1976