AMINOGLYCOSIDE ANTIBIOTICS. XIV

SYNTHESIS AND ACTIVITY OF 6-*O*-(3-AMINO-3-DEOXY-α-D-GLUCOPYRANOSYL)- AND 5-*O*-(β-D-RIBOFURANOSYL)APRAMYCINS

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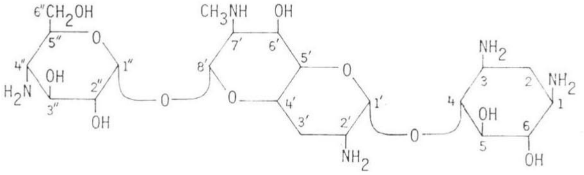
6-*O*-(3-Amino-3-deoxy-α-D-glucopyranosyl)apramycin (**17**) was prepared by glycosidation of a suitably blocked 5,6-dihydroxy derivative (**11**) of apramycin with a blocked 3-aminoglucosyl chloride (**15**). Ribosylation of the 5-hydroxy-6-*O*-tetrahydropyranyl (THP) derivative (**19**) of apramycin gave 5-*O*-(β-D-ribofuranosyl)apramycin (**24**) along with the 6α (**25**) and 6β (**26**) isomers. Similar reaction with the 6-hydroxy-5-*O*-THP derivative (**20**) or **11** gave only **25** and **26**, but not **24**. **17** was at least as active as apramycin against most Gram-positive and Gram-negative bacteria tested and more active than apramycin against strains producing aminoglycoside-modifying enzymes. Strains of *Pseudomonas aeruginosa* were generally less sensitive to **17** than to apramycin. **24** was the most active of the three ribofuranosyl derivatives prepared though it was less active than **17**.

Apramycin[1] is a 2-deoxystreptamine(DOS)-containing aminoglycoside antibiotic possessing moderate activity[2] against a variety of Gram-positive and Gram-negative bacteria including strains which produce aminoglycoside-modifying enzymes. The antibiotic, structurally categorized as a 4-*O*-monosubstituted DOS, is unique in that an unusual 4-aminoglucosyloctadiose moiety is glycosidically linked to the 4-hydroxyl group of DOS while the 5- and 6-hydroxyl groups are unsubstituted. Naturally occurring 4,6- or 4,5-*O*-disubstituted DOS-containing antibiotics are usually more active than the corresponding 4-*O*-monosubstituted DOS congeners, as exemplified by kanamycin B, xylostasin and ribostamycin, which are more active than neamine[3]. In apramycin, therefore, enhancement of activity might be expected by glycosidation of the 6-hydroxyl group with 3-amino-3-deoxyglucose (3-AG) or of the 5-hydroxyl group with ribose on the basis of the above structure-activity relationship.

This paper describes the synthesis and activity of 6-*O*-(3-amino-3-deoxy-α-D-glucosyl)apramycin (**17**) and 5-*O*-β-D-ribofuranosylapramycin (**24**) along with two ribofuranosyl isomers (**25** and **26**).



1 Apramycin

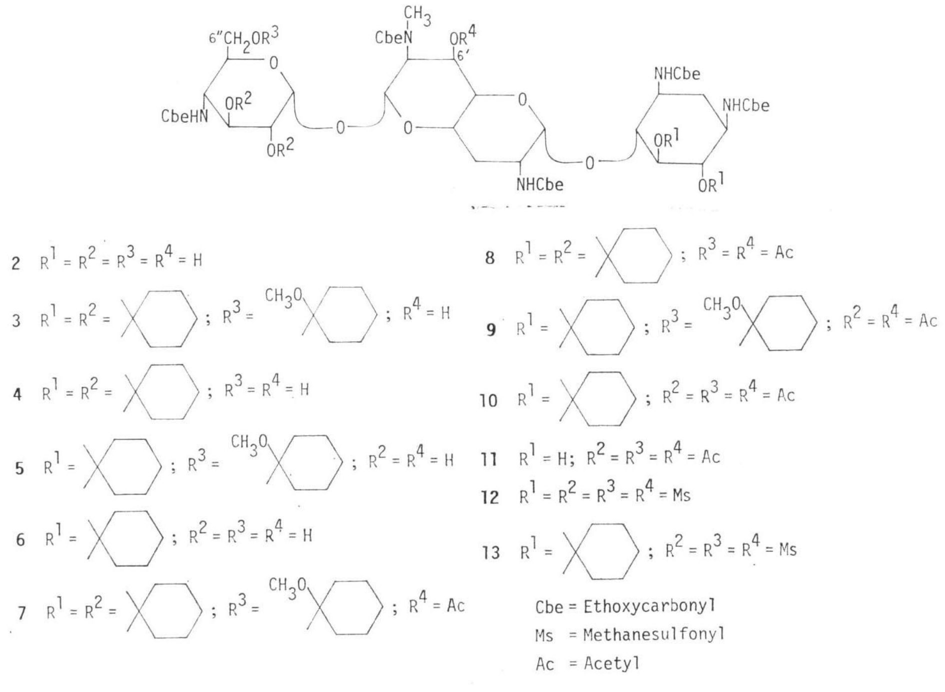
Synthesis

Preparation of suitably blocked intermediates was considered to be prerequisite for glycosidation of the 5- or 6-hydroxyl group of apramycin. In the total synthesis of kanamycin A[4], B[5] and C[6], a protected kanamine, neamine or paromamine having free hydroxyl groups at both C-5 and C-6 of DOS was used as a key intermediate for glycosidation with the protected 3-amino-3-deoxyglucosyl chloride[7,8]. The blocked apramycin (**11**) with free hydroxyls at the 5- and 6-positions was prepared by a sequence of reactions using ethoxycarbonyl group (Cbe) for the protection of amino groups, and cyclohexylidene and acetyl groups for protecting hydroxyl groups.

Apramycin (**1**) was treated with ethyl chloroformate in aqueous methanol to give penta-*N*-Cbe-apramycin (**2**), which was heated with 1,1-dimethoxycyclohexane in the presence of *p*-toluenesulfonic acid to give a mixture of the tri-, di- and monocyclohexylidene derivatives (**3**, **4**, **5** and **6**). Chromatography of the mixture on silica gel gave the desired product **6** in 10% yield, together with compounds **3**, **4** and **5** in 26%, 28% and 9% yields, respectively. The PMR spectra of these compounds showed the presence of three cyclohexylidene (δ 0.85 ~ 1.9 ppm) and one methoxy (δ 3.13 ppm) groups in **3**, two cyclohexylidene in **4**, two cyclohexylidene and one methoxy in **5**, and one cyclohexylidene in **6**. Silica gel TLC indicated that a treatment of **3** with 75% aqueous acetic acid - acetone (3: 5) at room temperature for an hour gave **4**. Prolonged hydrolysis (3.5 days) under the same condition changed **4** to **6**. **5** was also converted to **6** by similar hydrolysis for an hour. Thus **6** was obtained in the overall yield of 61% from **2** by selective deprotection of the mixture without separating each component. The structure of **6** was confirmed by converting it to the tetra-*O*-mesyl derivative (**13**) which afforded, upon methanolysis, di-*N*-Cbe-2-deoxystreptamine (**14**)[9]. The hexa-*O*-mesyl derivative (**12**) prepared from **2** did not give **14**. The above results indicate that the cyclohexylidene group of **6** was on the 5- and 6-hydroxyls of DOS. The structures of **3**, **4** and **5** were assigned as shown in Fig. 1 taking into account the reduced steric hindrance of 6''-OH compared to 6'-OH.

Acetylation of the remaining hydroxyl groups of **3**, **4**, **5** and **6** with acetic anhydride in pyridine yielded the corresponding mono-, di-, tri- and tetra-*O*-acetyl derivatives (**7**, **8**, **9** and **10**), respectively. The tetra-*O*-acetyl derivative, **10**, was treated with 0.5 N hydrochloric acid - acetone (1: 32) at room

Fig. 1.



Cbe = Ethoxycarbonyl
Ms = Methanesulfonyl
Ac = Acetyl

temperature affording the 5,6-dihydroxy derivative (**11**) in 94% yield. Glycosidation of **11** with the blocked 3-amino-3-deoxyglucosyl chloride (**15**)[7,8] in dry *N,N*-dimethylformamide in the presence of mercuric cyanide gave the desired 6-glucosyl derivative **16** in 14% yield, which was also obtained in 11% yield by condensation in methylene chloride - dioxane (6: 1) in the presence of silver carbonate and silver perchlorate. Catalytic hydrogenation of **16** with 10% palladium on charcoal followed by hydrazinolysis and subsequent chromatographic purification afforded the final product **17** in 41% yield.

The PMR spectrum of **17** showed four doublets in the anomeric region at δ 5.14 (J=3.8 Hz), 5.24 (J=8.4 Hz), 5.50 (J=3.8 Hz) and 5.77 (J=3.8 Hz). The second through the fourth signals were in good agreement with those of three anomeric protons of **1** occurring at δ 5.20 (J=8.3 Hz), 5.47 (J=3.4 Hz) and 5.72 (J=3.8 Hz). The first doublet of **17** at δ 5.14 was thus assigned to a new anomeric proton resulting from the 3-AG moiety introduced and its coupling constant (3.8 Hz) indicated an α-glycosidic linkage.

The CMR signals of **17** (Table 1) are in good agreement with those of **1**[10] and the 3-AG portion of kanamycin B[11], except that the C-5 and C-6 signals of **17** are coincident with those of kanamycin B rather than apramycin. Glycosidation at the 6-position of DOS is known to induce a downfield shift of *ca.* 10 ppm for C-6 and an upfield shift of *ca.* 1.5 ppm for C-5[12]. The C-6 signal of **17**, which was assigned on the basis of a β-carbon shift of 4.2 ppm at acidic pD, showed a downfield shift of 10.9 ppm, and the C-5 signal showed an upfield shift of 1.4 ppm from those of **1**. The above data indicate that the glycosidation took place on the 6-hydroxyl group and, therefore, **17** is 6-*O*-(3-amino-3-deoxy-α-D-glucopyranosyl)apramycin.

Ribosylation of blocked neamine or paromamine having free hydroxyls at the 5- and 6-positions has been reported to give only 5-ribosyl derivative in one case[13] and only 6-ribosyl derivative by others[14,15]. In the present study, the 6-hydroxyl group of **1** was protected with a tetrahydropyranyl (THP) group as in the case of regiospecific synthesis[15] of ribostamycin and butirosin.

The 5,6-dihydroxy derivative **11** was allowed to react with 3,4-dihydro-2*H*-pyran in the presence of *p*-toluenesulfonic acid to give two mono-*O*-THP derivatives **19** and **20** in 32% and 31% yield, respectively, both of which were a mixture of diastereoisomers[16] shown by TLC. Condensation of **19** with the blocked ribosyl chloride (**18**)[17] in toluene in the presence of mercuric cyanide afforded a mixture of **21** and **22** in 16% yield along with **23** in 32% yield. Deblocking of the mixture of **21** and **22** by heating with hydrazine followed by chromatographic separation afforded the 5β anomer **24** in 13% yield and the 6α anomer **25** in 24% yield. Similarly, deblocking of **23** gave the 6β anomer **26** in 37% yield. These results indicated that the THP group of **19** was cleaved during the reaction probably due to the protons liberated. On the other hand, ribosylation of **20** with **18** under similar conditions gave **22** in 15% yield and **23** in 55% yield, which afforded **25** and **26** but the 5β isomer **24** was not obtained. Direct ribosylation of **11** gave **22** in 12% yield and **23** in 61% yield, indicating that the ribosylation occurs preferentially on the 6-position irrespective of whether the 5-hydroxyl is protected or not.

The PMR spectra of the ribosylated apramycin derivatives showed an additional anomeric proton resonance at δ 5.36 (d, J=1 Hz) in **24**, at δ 5.29 (d, J=3.5 Hz) in **25**, and at δ 5.18 (s) in **26**, indicating that the configuration of ribosyl moiety is β for **24** and **26** and α for **25**. The CMR data on **24** and **25** could not be obtained because of a short supply of these samples prepared, but **26** was subjected to run the CMR spectrum at alkaline (pD > 11) and acid (pD < 1) media (Table 1). The CMR spectrum of **26** showed a deshielding of the C-6 signal by 8.0 ppm compared to that of **1**, while other signals of **26** accorded with those of **1** and the ribose moiety of ribostamycin. In ribostamycin[18] and xylostasin[12],

Table 1. CMR spectra of apramycin derivatives, **17** and **26**, and related compounds (in D_2O, 20 MHz).

	Apramycin (**1**)*			**17**			Kanamycin B[11]			**26**			Ribostamycin[18]			Neamine[18]
Carbon	pD>11	pD<1	$\Delta\delta$	pD>11	pD<1	$\Delta\delta$	pD>11	pD<1	$\Delta\delta$	pD>11	pD<1	$\Delta\delta$	Free base	Sulfate	$\Delta\delta$	Free base
DOS moiety																
1	51.1	50.6	0.5	51.2	50.4	0.8	50.5	50.5	0	50.0	49.3	0.7	51.2	51.4	−0.2	51.4
2	36.0	29.1	6.9	36.1	28.7	7.4	36.5	28.6	7.9	35.8	29.0	6.8	36.7	32.0	4.7	36.5
3	50.2	49.4	0.8	50.2	49.2	1.0	50.3	49.3	1.0	49.8	49.1	0.7	51.2	49.8	1.4	50.3
4	87.4	78.7	8.7	87.3	78.6	8.7	87.4	77.5	9.9	86.6	77.9	8.7	83.0	79.2	3.8	87.7
5	76.7	75.9	0.8	75.3	75.0	0.3	75.2	75.1	0.1	76.5	75.4	1.1	85.0	86.0	−1.0	76.9
6	77.8	73.3	4.5	88.7	84.5	4.2	88.6	84.5	4.1	85.8	81.6	4.2	78.4	74.4	4.0	78.1
Octadiose moiety																
1′	101.5	96.1	5.4	101.1	96.2	4.9				101.2	96.0	5.2				
2′	49.7	48.8	0.9	49.8	48.8	1.0				49.8	48.8	1.0				
3′	32.6	27.7	4.9	32.4	27.7	4.7				32.8	27.6	5.2				
4′	67.9	66.9	1.0	67.9	66.9	1.0				67.9	66.9	1.0				
5′	71.0	70.5	0.5	71.0	70.6	0.4				71.0	70.5	0.5				
6′	66.2	63.6	2.6	66.2	63.6	2.6				66.1	63.6	2.5				
7′	62.3	60.3	2.0	62.3	60.3	2.0				61.7	59.8	1.9				
8′	96.4	93.7	2.7	96.4	93.8	2.6				96.4	93.8	2.6				
$N-CH_3$	32.9	31.1	1.8	32.8	31.1	1.7				32.8	31.1	1.7				
4-AG moiety																
1″	95.4	95.4	0	95.4	95.4	0				95.4	95.4	0				
2″	71.7	71.1	0.6	71.7	71.1	0.6				71.7	71.1	0.6				
3″	74.2	70.1	4.1	74.2	70.2	4.0				74.2	69.4	4.8				
4″	53.1	53.1	0	53.1	53.0	0.1				53.1	53.0	0.1				
5″	73.4	69.0	4.4	73.4	69.0	4.4				73.4	69.0	4.4				
6″	61.7	61.2	0.5	61.7	61.2	0.5				61.3	60.3	1.0				
3-AG or ribose moiety																
1‴				100.8	101.4	−0.6	101.4	101.4	0	109.5	109.5	0	109.1	111.0	−1.9	
2‴				72.5	68.9	3.6	73.0	69.0	4.0	75.7	75.4	0.3	75.7	76.1	−0.4	
3‴				55.1	55.7	−0.6	56.5	55.8	0.7	70.2	70.2	0	70.5	70.0	0.5	
4‴				70.0	66.2	3.8	71.3	66.3	5.0	83.2	83.2	0	83.4	83.3	0.1	
5‴				73.0	73.8	−0.8	74.0	73.7	0.3	62.3	61.2	1.1	62.6	62.1	0.5	
6‴				61.2	60.7	0.5	62.0	60.8	1.2							

* CMR data; the assigment of signals made according to reference 10.

glycosidation is known to cause a downfield shift of *ca.* 8 ppm for the carbon at glycosidation site to give a peak at around 85 ppm, which does not shift on protonation. In **26**, a signal at 85.8 ppm assigned to the ribosylated carbon showed a protonation shift of 4.2 ppm. This indicates that the additional sugar in **26** is located at the C-6 of DOS, but not at the C-5. Thus, **26** is 6-*O*-(β-D-ribofuranosyl) derivative of **1**. Therefore, the other β-anomer **24** is 5-*O*-(β-D-ribofuranosyl)apramycin, which has the same stereochemistry as ribostamycin. The α-anomer **25** was assigned to be 6-*O*-(α-D-ribofuranosyl)-apramycin, because of the predominant reactivity of the 6-hydroxyl over 5-hydroxyl of **1**.

Fig. 3.

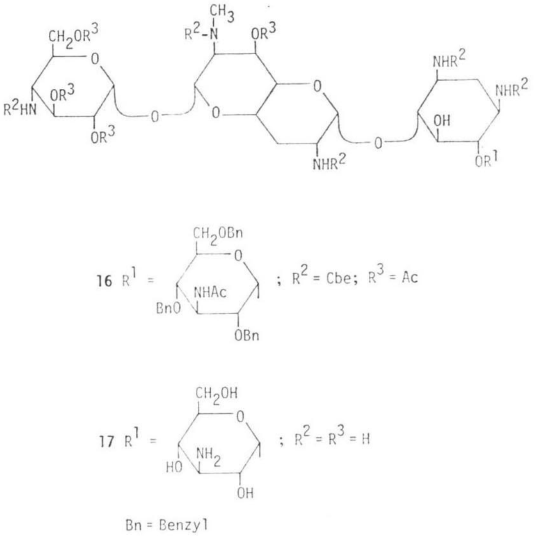
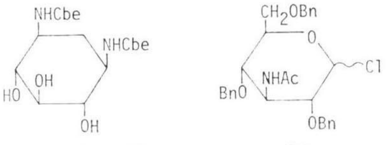


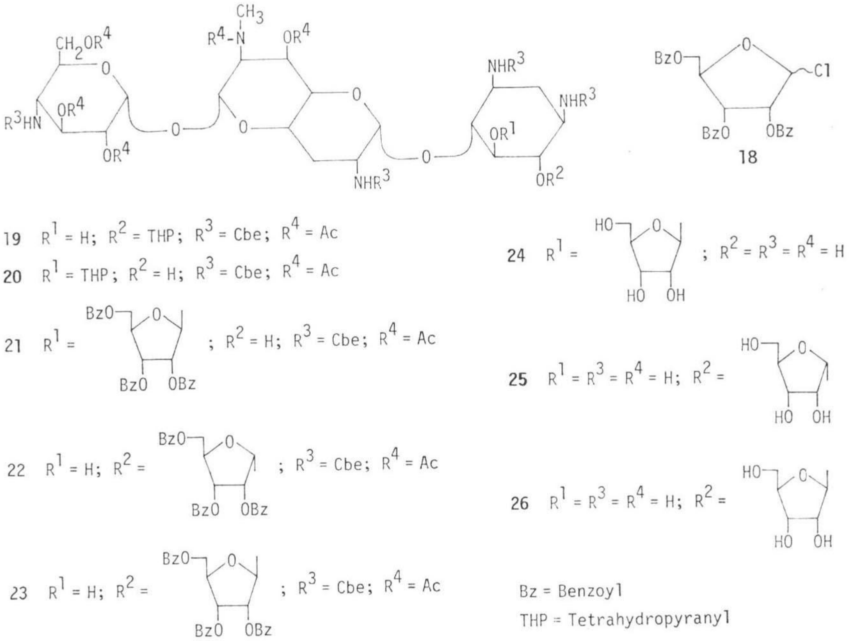
Fig. 2.



14 Di-N-Cbe-DOS

15

Fig. 4.



Antimicrobial Activity

The minimum inhibitory concentrations (MIC) of **17**, **24**, **25** and **26** were determined by a two-fold agar dilution method against both aminoglycoside-sensitive and -resistant organisms in comparison with **1**. As shown in Table 2, the 6-*O*-(3-amino-3-deoxy-D-glucosyl) derivative, **17**, was at least as active as **1**

Table 2. Antibacterial activity of apramycin derivatives (**17**, **24**, **25** and **26**) and apramycin (**1**).

Organism	Aminoglycoside-modifying enzyme*	MIC (mcg/ml)				
		17	**24**	**25**	**26**	Apra-mycin
E. coli NIHJ		1.6	1.6	6.3	50	3.1
〃 ML1630	APH (3′)-I	1.6	6.3	25	>100	3.1
〃 NR-79/W677	AAC (6′)	0.8	0.8	3.1	50	3.1
〃 JR35/C600	APH (3′)-I	0.4	1.6	6.3	100	1.6
〃 A20107	APH (3′)-II	3.1	3.1	12.5	100	6.3
〃 JR66/W677	APH (3′)-II, ANT (2″)	3.1	6.3	25	100	3.1
〃 R5	AAC (6′)-I	3.1	1.6	6.3	50	3.1
〃 JR88	AAC (3)-I	0.8	1.6	6.3	50	3.1
〃 A20732	ANT (2″)	1.6	1.6	6.3	50	3.1
〃 A9624	**	50	100	100	>100	>100
K. pneumoniae D11		0.4	0.8	6.3	50	0.8
〃 22–3038	APH (3′)-II, ANT (2″)	3.1	3.1	25	>100	3.1
E. cloacae A20364	APH (3′)-I	3.1	3.1	12.5	100	3.1
〃 A21006	APH (3′)-II	3.1	3.1	12.5	100	3.1
S. marcescens A20019		3.1	6.3	12.5	>100	3.1
〃 A21247	APH (3′)-I, ANT (2″)	3.1	12.5	50	>100	3.1
Pr. vulgaris A9436		0.4	0.8	1.6	12.5	0.4
Prov. stuartii A20894	AAC (2′)	1.6	0.8	3.1	50	3.1
Alcalig. sp. A21383	**	>100	>100	>100	>100	>100
Ps. aeruginosa A9930		1.6	0.8	1.6	25	3.1
〃 A20653	APH (3′)-I, II	25	50	50	>100	12.5
〃 #130	AAC (3)-I, APH (3′)	12.5	6.3	50	50	6.3
〃 A20601	AAC (3)-I	12.5	25	50	>100	6.3
〃 A20896	AAC (3)-II	50	50	100	>100	12.5
〃 GN-315	AAC (6′)-IV	6.3	12.5	25	>100	3.1
〃 GN-4925	AAC (6′)-III	6.3	12.5	25	>100	1.6
〃 A21509	**	50	100	100	>100	25
S. aureus Smith		0.8	3.1	6.3	100	0.8
A20239	APH (3′)-I, II	6.3	25	12.5	>100	3.1
B. brevis IFO 12334	ANT (4′)	0.8	3.1	12.5	100	1.6
B. subtilis PCI 219		0.2	1.6	1.6	12.5	0.4

* Abbreviation for aminoglycoside-modifying enzymes, see reference 21.

** Permeability mutant.

against most organisms tested except *Pseudomonas* strains. Especially, **17** was 2~4 times more active than **1** against *E. coli* strains producing aminoglycoside-modifying enzymes. Against *Pseudomonas aeruginosa*, however, **17** was less active than **1**. The 5-*O*-β-ribosyl derivative, **24**, was the most active of the three ribosyl congeners, although it was slightly less active than **17**. The activity of **24** compared favorably to that of **1** against most sensitive and resistant strains of *E. coli*, *K. pneumoniae* and *E. cloacae*. **24** was generally less active than **1** against *S. marcescens*, *P. aeruginosa* and Gram-positive bacteria. **25** was 4~8 fold less active than **1** against most of the organisms tested and **26** was even less active.

Discussion

The naturally occurring 4,5- or 4,6-disubstituted DOS-containing aminoglycoside antibiotics have either a 5-*O*-furanosyl substitution in the β-configuration or a 6-*O*-pyranosyl group in the α-configuration. In an attempt to increase the intrinsic activity or to broaden the antimicrobial spectrum of apramycin, 6-*O*-(3-amino-3-deoxy-α-D-glucopyranosyl)apramycin (**17**) and 5-*O*-(β-D-ribofuranosyl)apramycin (**24**) were synthesized. Both **17** and **24** have the same stereochemistry as that of the naturally occurring aminoglycoside antibiotics, and they showed improved antibacterial activity over apramycin against most test organisms except *Pseudomonas* strains. The 6α and 6β positional isomers (**25** and **26**), co-produced in the synthesis of the 5-*O*-β-ribosyl derivative (**24**), were less active than **24**. This is consistent with the published data on synthetic 6-*O*-furanosyl derivatives, 6-*O*-(β-D-ribofuranosyl)paromamine[14)], 6-*O*-(β-D-ribofuranosyl)neamine[14,19)], 6-*O*-(α-D-arabinofuranosyl)paromamine[14)] and 6-*O*-(α- and β-D-3-amino-3-deoxyglucofuranosyl)neamine[20)], all of which were reported to be weakly active or nearly inactive. As shown in Table 1, the 6-*O*-α-ribosyl derivative (**25**) was more active than the 6β isomer (**26**), although both were less active than the natural-type 5β isomer **24**. This was also the case reported by SITRIN *et al*[20)] for a pair of 6α and 6β isomers of 6-*O*-(3-amino-3-deoxyglucofuranosyl)-neamine. SUAMI *et al*[19)] have reported that 6α derivatives were also more active than the corresponding β isomers in 6-*O*-pyranosides, 6-*O*-D-glucopyranosylneamine and 6-*O*-D-galactopyranosylneamine.

Experimental

Silica gel column chromatography was carried out on Wakogel C-100. Thin-layer chromatography was run on a silica gel plate 60 F_{254} (Merck), spraying reagent: anthrone and/or ninhydrin. Proton nuclear magnetic resonance spectra (PMR) were determined on a JNM C-60HL instrument using TMS as either an internal or an external standard. Carbon-13 nuclear magnetic resonance (CMR) spectra were recorded on a Varian FT-80A spectrometer and shifts were expressed in ppm downfield from TMS with dioxane as an internal standard (67.4 ppm). Infrared spectra were run on a JASCO IRA-1 spectrophotometer. Melting points were taken on a Yanagimoto melting point apparatus and were uncorrected. Optical rotation were determined on a JASCO model DIP automatic polarimeter.

1,2′,3,4′′,7′-Penta-*N*-ethoxycarbonylapramycin (2)

To a stirred solution of 3.78 g (7.0 mmoles) of apramycin and 3.27 g (31 mmoles) of sodium carbonate in 65 ml of water - methanol (10: 3) was added dropwise 4.18 g (39 mmoles) of ethyl chloroformate at room temperature. The reaction mixture was stirred overnight and concentrated to remove most of the organic solvent *in vacuo*. The concentrate was passed through a column of HP-10 (100 ml), which was washed with 400 ml of water, and eluted with EtOH - water (2: 1). The appropriate fraction was evaporated to dryness *in vacuo* to afford 5.16 g (82%) of **2**, mp 177~181°C, $[\alpha]_D^{25}+101°$ (*c* 0.5, acetone). IR (KBr): 3400, 1700, 1540, 1030 cm^{-1}. PMR (DMSO-d_6): δ 0.95~1.4 (15H, m, five OCH_2CH_3), 4.85~5.2 (3H, anomeric protons). TLC: Rf 0.80 (S-114)*, Rf 0.35 (AcOEt - EtOH - conc. NH_4OH, 30:60:1).

Anal. Calcd. for $C_{36}H_{61}N_5O_{21}\cdot H_2O$: C, 47.11; H, 6.92; N, 7.63.
Found: C, 47.22; H, 7.00; N, 7.38.

Cyclohexylidenation of 2

A solution of 535 mg (0.6 mmole) of **2**, 25 mg of *p*-toluenesulfonic acid and 2.6 ml of 1,1-dimethoxycyclohexane in 6 ml of dry DMF was heated at 55°C for 45 minutes, which showed Rf 0.10 (**6**), 0.16 (**5**), 0.21 (**4**) and 0.36 (**3**) by TLC (EtOH - $CHCl_3$ = 1: 15). The reaction mixture was cooled to room temperature, neutralized with $NaHCO_3$ and evaporated to dryness *in vacuo*. The residue was triturated with 3 ml of water to give 638 mg of a solid, which was chromatographed on a silica gel column (32 g) using MeOH - $CHCl_3$ (1: 100~1: 20) as eluant, affording 183 mg (26%) of **3**, 174 mg (28%) of **4**, 57 mg (9%) of **5**, and 59 mg (10%) of **6**.

Compound **3**; mp 155~158°C, $[\alpha]_D^{22.5}+73°$ (*c* 0.275, acetone). IR (KBr): 2940, 1720 (sh), 1705, 1535, 1260, 1110, 1030 cm^{-1}. PMR (acetone-d_6): δ 0.85~1.9 (47H, m, three cyclohexylidenes, five OCH_2CH_3, 2-H_{ax} & 3′-H_{ax}), 3.13 (3H, s, OCH_3), 5.05~5.65 (3H, anomeric protons).

Anal. Calcd. for $C_{55}H_{89}N_5O_{27}$: C, 56.35; H, 7.65; N, 5.97.
Found: C, 56.40; H, 7.92; N, 5.94.

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* S-114: AcOMe - *n*-PrOH - conc. NH_4OH = 45: 105: 60

Compound **4**; mp 171~175°C, $[\alpha]_D^{22.5}+65°$ (*c* 0.208, acetone). IR (KBr): 2940, 1720 (sh), 1705, 1535, 1260, 1110, 1030 cm^{-1}. PMR (acetone-d_6): δ 0.8~1.9 (37H, m, two cyclohexylidenes, five OCH_2-CH_3, 2-H_{ax} & 3′-H_{ax}), 5.05~5.65 (3H, anomeric protons).

Anal. Calcd. for $C_{48}H_{77}N_5O_{21}\cdot\frac{1}{2}H_2O$: C, 53.92; H, 7.35; N, 6.55.
Found: C, 53.90; H, 7.56; N, 6.16.

Compound **5**; mp 161~165°C, $[\alpha]_D^{22.5}+95°$ (*c* 0.243, acetone). IR (KBr): 2940, 1720 (sh), 1705, 1535, 1260, 1110, 1030 cm^{-1}. PMR (acetone-d_6): δ 0.8~1.9 (37H, m, two cyclohexylidenes, five OCH_2-CH_3, 2-H_{ax} & 3′-H_{ax}), 3.03 (3H, s, OCH_3), 5~5.5 (3H, anomeric protons).

Anal. Calcd. for $C_{49}H_{81}N_5O_{22}$: C, 53.89; H, 7.48; N, 6.41.
Found: C, 53.84; H, 7.71; N, 6.10.

Compound **6**; mp 167~171°C, $[\alpha]_D^{22.5}+75°$ (*c* 0.255, acetone). IR (KBr): 2940, 1720 (sh), 1700, 1535, 1260, 1030 cm^{-1}. PMR (acetone-d_6): δ 1.05~1.45 (15H, m, five OCH_2CH_3), 1.45~1.9 (10H, m, cyclohexylidene), 5.1~5.4 (3H, anomeric protons).

Anal. Calcd. for $C_{42}H_{69}N_5O_{21}\cdot\frac{1}{2}H_2O$: C, 51.01; H, 7.13; N, 7.08.
Found: C, 51.14; H, 7.39; N, 6.59.

Selective Preparation of 5,6-*O*-Cyclohexylidene-1,2′,3,4′′,7′-penta-*N*-ethoxycarbonylapramycin (**6**)

A solution of 7.82 g (8.7 mmoles) of **2**, 0.357 g of *p*-toluenesulfonic acid and 35 ml of 1,1-dimethoxycyclohexane was heated at 50°C for 50 minutes, which showed Rf 0.10 (**6**), 0.16 (**5**), 0.21 (**4**), and 0.36 (**3**) by TLC (EtOH - $CHCl_3$, 1: 15). The reaction mixture was treated with 274 mg of $NaHCO_3$ and evaporated to dryness *in vacuo* to give a solid, which was dissolved in 75% aqueous AcOH - acetone (47 ml: 78 ml). The solution was allowed to stand at room temperature. After 1 hour two spots at Rf 0.16 (**5**) and Rf 0.36 (**3**) disappeared with increased intensity of the spots at Rf 0.10 (**6**) and Rf 0.21 (**4**). After 3.5 days the spot of Rf 0.21 weakened and **6** became a major product. The reaction mixture was evaporated to dryness *in vacuo* below 35°C. The residue was dissolved in 30 ml of acetone and neutralized with aqueous $NaHCO_3$ (2.1 g in 30 ml of water) and evaporated to dryness *in vacuo*. The residue was again dissolved in 30 ml of acetone. Insoluble material was removed by filtration and the filtrate was chromatographed on a silica gel column (293 g) which was pretreated with 30 ml of triethylamine. After the filtrate was passed through the column, it was eluted with MeOH - $CHCl_3$ (1: 50~1: 15), affording 5.176 g (61%) of **6**, mp 168~171°C, and 0.989 g (11%) of **4**, mp 171~175°C, which were identical with the products directly derived from **2** as described above, respectively, in all respects of IR, PMR, TLC and microanalyses.

Acetylation of **3**, **4**, **5** and **6**

A solution of 1.5 g (1.5 mmoles) of **6** in a mixture of 5 ml of acetic anhydride and 10 ml of dry pyridine was allowed to stand at room temperature overnight and evaporated to dryness *in vacuo* to give a residue, which was triturated with 10 ml of water, affording 1.71 g (97%) of **10**, mp 140~150°C, $[\alpha]_D^{25}+82°$ (*c* 0.5, acetone). IR (KBr): 1750 (sh), 1725, 1710, 1530, 1230, 1030 cm^{-1}. PMR ($CDCl_3$): δ 0.9~1.7 (22H, five OCH_2CH_3, 5H from a cyclohexylidene, 2-H_{ax} & 3′-H_{ax}), 1.7~2.5 (19H, four $COCH_3$, 5H from a cyclohexylidene, 2-H_{eq} & 3′-H_{eq}), 2.88 (3H, s, NCH_3), 5.25~5.75 (3H, anomeric protons).

Anal. Calcd. for $C_{50}H_{77}N_5O_{25}\cdot\frac{1}{2}H_2O$: C, 51.90; H, 6.79; N, 6.05.
Found: C, 52,12; H, 6.92; N, 5.77.

Acetylation of 36 mg (0.033 mmole) of **5** in a manner similar to that described above gave 38 mg (95%) of **9**. IR (KBr): 1750 (sh), 1725, 1710, 1230, 1030 cm^{-1}. PMR ($CDCl_3$): δ 0.95~1.6 (17H, 2-H_{ax}, 3′-H_{ax} & five OCH_2CH_3), 1.24 (15H, t, J=7Hz, five OCH_2CH_3), 1.6~2.6 (31H, two cyclohexylidenes, 2-H_{eq}, 3′-H_{eq} & three $COCH_3$), 2.85 (3H, s, NCH_3), 5.3~5.7 (3H, anomeric protons).

Anal. Calcd. for $C_{55}H_{87}N_5O_{25}\cdot\frac{1}{2}H_2O$: C, 53.82; H, 7.23; N, 5.71.
Found: C, 53.71; H, 7.20; N, 5.47.

Similarly, acetylation of 153 mg (0.14 mmole) of **4** afforded 159 mg (96%) of **8**, mp 159~164°C, $[\alpha]_D^{22.5}+59°$ (*c* 0.16, acetone). IR (KBr): 1720 (sh), 1710, 1240, 1030 cm^{-1}. PMR ($CDCl_3$): δ 0.85~1.6 (17H, five OCH_2CH_3, 2-H_{ax} & 3′-H_{ax}), 1.6~2.55 (28H, two cyclohexylidenes, two $COCH_3$, 2-H_{eq} & 3′-H_{eq}), 2.85 (3H, broad singlet, NCH_3), 5.05~5.7 (3H, anomeric protons).

Anal. Calcd. for $C_{52}H_{81}N_5O_{23}\cdot\frac{1}{2}H_2O$: C, 54.16; H, 7.17; N, 6.07.
Found: C, 54.21; H, 7.30; N, 5.72.

Similarly, 158 mg (95%) of **7**, mp 153~158°C, $[\alpha]_D^{22.5}+65°$ (*c* 0.193, acetone), was obtained from 160 mg (0.14 mmole) of **3**. IR (KBr): 1720 (sh), 1705, 1230, 1030 cm^{-1}. PMR ($CDCl_3$): δ 0.85~1.55 (17H, five OCH_2CH_3, 2-H_{ax} & 3′-H_{ax}), 1.55~2.6 (35H, three cyclohexylidenes, $COCH_3$, 2-H_{eq} & 3′-H_{eq}), 2.83 (3H, broad singlet, NCH_3), 5~5.65 (3H, anomeric protons).

Anal. Calcd. for $C_{57}H_{91}N_5O_{23}\cdot\frac{1}{2}H_2O$: C, 55.96; H, 7.58; N, 5.72.
Found: C, 55.95; H, 7.67; N, 5.44.

2′′,3′′,6′,6′′-Tetra-*O*-acetyl-1,2′,3,4′′,7′-penta-*N*-ethoxycarbonylapramycin (**11**)

To a solution of 2.56 g (2.2 mmoles) of **10** in 24 ml of acetone was added 0.75 ml of 0.5 N HCl. The mixture was allowed to stand at room temperature for 20 hours, during which a spot at Rf 0.47 due to **10** disappeared and a new spot at Rf 0.17 due to **11** became predominant by TLC (EtOH - $CHCl_3$, 1:15). The resulting mixture was treated with aqueous AcOK (37 mg/2 ml) and evaporated to dryness *in vacuo*. A trace of the solvent remained was removed by azeotropic distillation with toluene (5 ml ×3). The residue was dissolved in 15 ml of chloroform and the insoluble material was removed by filtration. The filtrate was chromatographed on a silica gel column (89 g) using MeOH - $CHCl_3$ (1:30~1:15) to give 2.23 g (94%) of **11**, mp 158~163°C, $[\alpha]_D^{25}+93°$ (*c* 0.5, acetone). IR (KBr): 1740 (sh), 1720 (sh), 1700, 1535, 1235, 1030 cm^{-1}. PMR ($CDCl_3$): δ 1.05~1.5 (15H, five OCH_2CH_3), 1.95~2.2 (12H, four $COCH_3$), 2.87 (3H, br.s, NCH_3), 4.9~5.75 (3H, br., anomeric protons). TLC: Rf 0.17 (EtOH - $CHCl_3$, 1:15).

Anal. Calcd. for $C_{44}H_{69}N_5O_{25}\cdot H_2O$: C, 48.66; H, 6.59; N, 6.45.
Found: C, 48.64; H, 6.60; N, 6.13.

5,6-*O*-Cyclohexylidene-1,2′,3,4′′,7′-penta-*N*-ethoxycarbonyl-2′′,3′′,6′,6′′-tetra-*O*-mesylapramycin (**13**)

To a solution of 200 mg (0.2 mmole) of **6** in 2 ml of dry pyridine was added 300 mg (2.6 mmoles) of methanesulfonyl chloride with cooling. The mixture was allowed to stand for 2 days and evaporated to dryness *in vacuo*. The residue was washed with aqueous $NaHCO_3$ (630 mg/10 ml) and then 5 ml of water to give 255 mg (97%) of **13**, mp 173~177°C, $[\alpha]_D^{22.5}+50°$ (*c* 0.2, acetone). IR (KBr): 1710, 1530, 1370, 1175, 1035 cm^{-1}. PMR (acetone-d_6): δ 1.03~1.75 (25H, m, cyclohexylidene & five OCH_2CH_3), 2.95~3.35 (15H, four SO_2CH_3 & NCH_3), 5.06~5.3 (3H, br., anomeric protons).

Anal. Calcd. for $C_{46}H_{77}N_5O_{29}S_4$: C, 42.75; H, 6.01; N, 5.42; S, 9.92.
Found: C, 42.97; H, 6.20; N, 5.31; S, 9.78.

Decomposition of **13** with HCl - MeOH

To a solution of 0.259 g (0.20 mmole) of **13** in 26 ml of dry MeOH was added 3.37 g (31 mmoles) of ethyl chloroformate. The mixture was heated under reflux for 10 hours. Another 5.06 g (46.6 mmoles) of ethyl chloroformate was added to this mixture, which was again heated under reflux for 26 hours and evaporated to dryness *in vacuo*. The residue was chromatographed on a silica gel column (13 g) using MeOH - $CHCl_3$ (1:100~1:5) to afford 34 mg (55%) of di-*N*-ethoxycarbonyl-2-deoxystreptamine (**14**), mp 235~236°C (dec.) (lit. 231~232°C)[9]. IR (KBr): 3330, 1690, 1550, 1540, 1310, 1045 cm^{-1}. PMR (D_2O): δ 1.24 (6H, t, J=7Hz, two CH_2CH_3), 2.07 (1H, dt, J=13 & 4Hz, 2-H_{eq}), 3.15~3.85 (5H, m, 1, 3, 4, 5 & 6-H), 4.10 (4H, q, J=7Hz, two OCH_2CH_3). TLC: Rf 0.55 (EtOH - AcOEt - conc. NH_4OH, 15:30:2, conc. sulfuric acid).

Anal. Calcd. for $C_{12}H_{22}N_2O_7\cdot H_2O$: C, 44.44; H, 7.46; N, 8.64.
Found: C, 44.77; H, 7.01; N, 8.24.

Compound **14** was identical with an authentic specimen[9] prepared from 2-deoxystreptamine in all respects (TLC, IR, PMR and mixed melting point).

1,2′,3,4′′,7′-Penta-*N*-ethoxycarbonyl-2′′,3′′,5,6,6′,6′′-hexa-*O*-mesylapramycin (**12**)

Mesylation of 300 mg (0.33 mmole) of **2** in a similar manner to that in **13** gave 435 mg (95%) of **12**, mp 174~178°C, $[\alpha]_D^{25}+59°$ (*c* 0.5, acetone). IR (KBr): 1720 (sh), 1710, 1530, 1350, 1175, 1035 cm^{-1}. PMR (acetone-d_6): δ 1.05~1.47 (15H, m, five CH_2CH_3), 2.95~3.4 (21H, six SO_2CH_3 & NCH_3).

Anal. Calcd. for $C_{42}H_{73}N_5O_{33}S_6$: C, 36.86; H, 5.38; N, 5.12; S, 14.06.
Found: C, 36.91; H, 5.29; N, 4.69; S, 13.46.

Decomposition of **12** with HCl - MeOH

To a solution of 300 mg (0.22 mmole) of **12** in 30 ml of dry MeOH was added 4.88 g (45 mmoles) of ethyl chloroformate. The mixture was refluxed for 5 hours. After the addition of another 4.88 g (45 mmoles) of ethyl chloroformate, the mixture was again heated under reflux for 18 hours and evaporated to dryness *in vacuo* to give a dark-brown residue. A solution of the residue in 3 ml of acetone was placed on a silica gel column (15 g), which was washed with $CHCl_3$ (50 ml) and eluted stepwise with MeOH - $CHCl_3$ (1: 100, 1: 50, 1: 20, 1: 10, 1: 5 and 1: 3). The eluate was collected in glass tubes in 10-ml fractions, which were monitored by TLC and grouped into the following four fractions. Each fraction was evaporated to dryness *in vacuo* to give amorphous powder.

Fraction I: 39 mg from tube Nos. 16 and 17; TLC (a)* Rf 0.38, 0.48 (major), 0.57 (major), 0.63; (b)** Rf *ca.* 1.0.

Fraction II: 174 mg from tube Nos. 19 and 20; TLC (a) Rf 0.24, 0.30 (major), 0.38; (b) Rf *ca.* 1.0.

Fraction III: 48 mg from tube Nos. 21 ~ 24; TLC (a) Rf 0.10, 0.17 (major), 0.28, 0.32; (b) Rf *ca.* 1.0.

Fraction IV: 9 mg from tube Nos. 27 ~ 35; TLC (a) Rf 0.00, 0.03; (b) 0.10, 0.20, 0.35, 0.52, 0.58, 0.64, 0.75, 0.83.

Fraction IV, in which **14** should be contained if it were produced from **12**, did not show any spot at around Rf 0.40 due to **14** in TLC using EtOH - AcOEt - H_2O (15: 30: 2) as shown above. The main component of Fraction II was the starting material **12**. The expected decomposition product of **12** was supposed to be contained in Fraction I or Fraction III. Both fractions were subjected again to silica gel column chromatography, but did not give any pure product enough to be confirmed its structure.

6-*O*-(3-Acetamido-2,4,6-tri-*O*-benzyl-3-deoxy-α-D-glucopyranosyl)-2′′,3′′,6′,6′′-tetra-*O*-acetyl-1,2′,3,4′′,7′-penta-*N*-ethoxycarbonylapramycin (16)

To a solution of 0.873 g (0.82 mmole) of **11** in 10 ml of dry DMF was added 3 g of anhydrous calcium sulfate and the suspension was stirred under nitrogen overnight. Another 3 g of anhydrous calcium sulfate was added and the mixture was stirred for 2.5 hours. To the mixture was added a solution of 0.974 g (1.9 mmoles) of blocked 3-aminoglucosyl chloride **15** in 5 ml of dry DMF followed by 1.93 g of mercuric cyanide. The reaction mixture was heated at 80 ~ 85°C for 65 hours with stirring under nitrogen, cooled to room temperature and filtered. The filtrate was evaporated to dryness *in vacuo*. The residue was dissolved in 10 ml of chloroform. Insoluble material was removed by filtration and the filtrate was chromatographed on a silica gel column (61 g) employing MeOH - $CHCl_3$ (1: 100 ~ 1: 20) as eluant. The fractions which showed Rf 0.17 by TLC (MeOH - $CHCl_3$, 1: 30) were combined and evaporated to afford 0.174 g (14%) of **16**, mp 149 ~ 154°C, $[\alpha]_D^{26}$ +83° (*c* 0.635, $CHCl_3$). IR (KBr): 3440 ~ 3340, 1740, 1725 ~ 1705, 1530, 1230, 1065, 1030 cm^{-1}. PMR ($CDCl_3$): δ 1.0 ~ 1.5 (15H, five OCH_2CH_3), 1.79 (3H, $NCOCH_3$), 1.9 ~ 2.2 (12H, four $OCOCH_3$), 2.86 (3H, s, NCH_3), 7.1 ~ 7.4 (15H, m, benzene ring protons).

Anal. Calcd. for $C_{73}H_{100}N_6O_{30} \cdot 2H_2O$: C, 55.58; H, 6.64; N, 5.33.
Found: C, 55.83; H, 6.62; N, 5.10.

Evaporation of the eluate showing Rf 0.04 by TLC recovered 0.635 g (73%) of **11**.

6-*O*-(3-Amino-3-deoxy-α-D-glucopyranosyl)apramycin (17)

A solution of 296 mg (0.19 mmole) of **16** in 4 ml of glacial acetic acid was hydrogenated in the presence of 100 mg of 10% palladium on charcoal at 50°C overnight. The mixture was filtered and the filtrate evaporated to dryness *in vacuo* to afford a residue, which was then heated with 10 ml of 100% hydrazine hydrate in a sealed tube at 123 ~ 128°C for 25 days. The resultant solution was evaporated to dryness *in vacuo* giving a syrup, which was chromatographed on a column of Amberite CG-50 (NH_4^+, 30 ml) employing 0.1 ~ 0.5 N aqueous ammonia as eluant, affording 91 mg of the crude product showing a major spot at Rf 0.15 and a minor one at Rf 0.20 by TLC (S-118***). Further purification by CM-Sephadex C-25 (NH_4^+, 80 ml) column chromatography using 0.05 N aqueous ammonia gave 57 mg (41%) of **17** Rf 0.15, mp 205 ~ 208°C, $[\alpha]_D^{24.5}$ +169° (*c* 0.508, water). IR (KBr): 3040, 1600, 1390, 1080, 1030, 990 cm^{-1}. PMR (D_2O+DCl, pD 1): δ 1.5 ~ 2.75 (4H, m, 2-H_{ax}, 2-H_{eq}, 3′-H_{ax} & 3′-H_{eq}), 2.76 (3H, s, NCH_3), 3.05 ~ 4.35 (22H, m, O–CH, N–CH & CH_2OH), 5.14 (1H, d, *J*=3.75 Hz, 1′′′-H), 5.24

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* (a) EtOH - $CHCl_3$=1: 15; Compound **14** Rf 0.00 ~ 0.01; Compound **12** Rf 0.30.

** (b) EtOH - AcOEt - H_2O=15: 30: 2; Compound **14** Rf 0.40; Compound **12** Rf *ca.* 1.0.

*** S-118: $CHCl_3$ - MeOH - conc. NH_4OH (1: 2: 1)

(1H, d, J=8.4 Hz, 8′-H), 5.5 (1H, d, J=3.75 Hz, 1″-H), 5.77 (1H, d, J=3.75 Hz, 1′-H).

Anal. Calcd. for $C_{27}H_{54}N_6O_{16}\cdot\frac{5}{2}H_2CO_3$: C, 40.55; H, 6.81; N, 9.62.
Found: C, 40.52; H, 6.99; N, 9.57.

Protection of 5- or 6-Hydroxyl Group of 2″,3″,6′,6″-Tetra-*O*-acetyl-1,2′,3,4″,7′-penta-*N*-ethoxycarbonylapramycin (**11**) with a Tetrahydropyranyl Group

To a solution of 2.0 g (1.9 mmoles) of **11** in 10 ml of dry DMF was added 5 ml of 3,4-dihydro-2*H*-pyran followed by 0.030 g of *p*-toluenesulfonic acid. The reaction mixture was stirred at room temperature for 1 hour, treated with 0.1 ml triethylamine and then evaporated to dryness *in vacuo*. A solution of the residue in 30 ml of chloroform was washed with aqueous $NaHCO_3$ (20 mg/7 ml) and 5 ml of water, dried (Na_2SO_4) and evaporated *in vacuo* to afford a solid, which was chromatographed on a silica gel column (100 g). A minor impurity was eluted with EtOH - $CHCl_3$, 1: 100 and then the desired products were eluted with EtOH - $CHCl_3$, 1: 30, giving 0.695 g (32%) of **19**, Rf 0.22 and 0.26, and 0.659 g (31%) of **20**, Rf 0.32 and 0.36 by TLC (EtOH - $CHCl_3$, 1: 20), both of which were a mixture of diastereoisomers.

Compound **19**; mp 146 ~ 149°C, $[\alpha]_D^{22.5}$ +83° (c 0.473, acetone). IR (KBr): 3360, 1740, 1715, 1535, 1230, 1025 cm^{-1}. PMR (benzene-d_6): δ 0.8 ~ 1.3 (15H, m, five OCH_2CH_3), 1.3 ~ 2.1 (18H, m, four $COCH_3$ & $CH_2CH_2CH_2$).

Anal. Calcd. for $C_{49}H_{77}N_5O_{26}\cdot H_2O$: C, 50.29; H, 6.80; N, 5.98.
Found: C, 50.45; H, 7.12; N, 5.60.

Compound **20**, mp 147 ~ 150°C, $[\alpha]_D^{22.5}$ +70° (c 0.453, acetone). IR (KBr): 3320, 1745, 1715, 1530, 1235, 1025 cm^{-1}. PMR (benzene-d_6): δ 0.8 ~ 1.3 (15H, m, five OCH_2CH_3), 1.3 ~ 2.1 (18H, m, four $COCH_3$ & $CH_2CH_2CH_2$).

Anal. Calcd. for $C_{49}H_{77}N_5O_{26}\cdot H_2O$: C, 50.27; H, 6.80; N, 5.98.
Found: C, 50.32; H, 6.98; N, 5.52.

Glycosidation of **19** with 2,3,5-Tri-*O*-benzoyl-D-ribofuranosyl Chloride (**18**)

A solution of 662 mg (0.58 mmole) of **19** in 60 ml of dry toluene was concentrated to 10 ml under vacuum, to which was added another 60 ml of dry toluene and the solution was concentrated again to 30 ml to remove the moisture. The reaction vessel was flushed with dry nitrogen and to this were placed 7 g of anhydrous calcium sulfate and a solution of 2 g (2.9 mmoles) of 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride (**18**) in 7 ml of dry toluene. After stirring for 2.5 hours, 0.060 g of mercuric cyanide was added to the mixture, which was heated at 53 ~ 54°C for 30 hours while stirring under nitrogen. The resultant mixture was filtered and the filtrate was washed with aqueous $NaHCO_3$ (1 g/10 ml) and water (7 ml × 2), dried (Na_2SO_4) and concentrated to 20 ml. The solution was chromatographed on a silica gel column (33 g) using EtOH - $CHCl_3$ (1: 100 ~ 1: 50) as eluant to afford 0.274 g (32%) of **23**, mp 141 ~ 143°C, Rf 0.20 and 0.135 g (16%) of a mixture of **21** and **22**, mp 144 ~ 147°C, Rf 0.18 by TLC (EtOH - $CHCl_3$, 1: 20).

The mixture of **21** & **22**; PMR ($CDCl_3$): δ 0.95 ~ 1.5 (15H, five OCH_2CH_3), 1.95 ~ 2.15 (12H, four $COCH_3$), 2.85 (3H, br. s, NCH_3), 3.2 ~ 5.8 (35H, N–CH, O–CH, CH_2O & anomeric protons), 7.2 ~ 8.2 15H, m, three benzene rings).

Anal. Calcd. for $C_{70}H_{89}N_5O_{32}\cdot\frac{5}{2}H_2O$: C, 53.98; H, 6.08; N, 4.50.
Found: C, 53.87; H, 6.09; N, 4.45.

Compound **23**; $[\alpha]_D^{25}$ +82° (c 0.628, $CHCl_3$). IR (KBr): 1725, 1530, 1275, 1230, 1110, 1030, 715 cm^{-1}. PMR ($CDCl_3$): δ 0.95 ~ 1.5 (15H, five OCH_2CH_3), 1.4 ~ 2.15 (12H, four $COCH_3$), 2.86 (3H, br. s, NCH_3), 3.2 ~ 5.75 (35H, N–CH, O–CH, CH_2O & anomeric protons), 7.2 ~ 8.15 (15H, m, three benzene rings).

Anal. Calcd. for $C_{70}H_{89}N_5O_{32}\cdot H_2O$: C, 54.93; H, 5.99; N, 4.58.
Found: C, 55.05; H, 5.90; N, 4.22.

Glycosidation of **20** with **18**

Condensation of 625 mg (0.54 mmole) of **20** with 2 g of the blocked D-ribofuranosyl chloride (**18**) under similar conditions to those described above gave 449 mg (55%) of **23**, mp 140 ~ 142°C, $[\alpha]_D^{25}$ +80° (c 0.625, $CHCl_3$), Rf 0.20 and 121 mg (15%) of **22**, mp 145 ~ 148°C, $[\alpha]_D^{25}$ +83° (c 0.620, $CHCl_3$), Rf 0.18 by TLC (EtOH - $CHCl_3$, 1: 20).

Compound **22**; IR (KBr): 1725, 1530, 1275, 1230, 1110, 1030, 715 cm^{-1}. PMR ($CDCl_3$): δ 0.9~1.5 (15H, five OCH_2CH_3), 1.9~2.2 (12H, four $COCH_3$), 2.85 (3H, br. s, NCH_3), 3.2~5.85 (35H, N–CH, O–CH, CH_2O & anomeric protons), 7.2~8.3 (15H, m, three benzene rings).

Anal. Calcd. for $C_{70}H_{89}N_5O_{32}\cdot\frac{5}{2}H_2O$: C, 53.98; H, 6.08; N, 4.50.
Found: C, 53.93; H, 5.85; N, 4.48.

Compound **23** prepared from **20** was identical with that from **19** in all respects of mp, IR, PMR, TLC and elemental analyses.

Glycosidation of 11 with 18

Condensation of 100 mg (0.094 mmole) of **11** with **18** in dry dichloromethane - toluene (1: 2) in a manner similar to that described above yielded 86 mg (61%) of **23**, mp 139~142°C, Rf 0.20 and 18 mg (12%) of **22**, mp 147~149°C, Rf 0.18 by TLC (EtOH - $CHCl_3$, 1: 20).

Compounds **22** and **23** obtained from **11** were identical with those from **20** in all respects (mp, IR, PMR, TLC and elemental analyses).

5-*O*-β-D-Ribofuranosylapramycin (24) and 6-*O*-α-D-Ribofuranosylapramycin (25)

Heating of 132 mg (0.087 mmole) of the mixture of **21** and **22** obtained from **19** with 10 ml of 80% hydrazine hydrate in a sealed tube at 123~127°C for 2.5 days followed by evaporation of the hydrazine *in vacuo* afforded a syrup, which was chromatographed on an Amberite CG-50 (NH_4^+, 15 ml) column employing 0.1 N aqueous ammonia gave 18 mg of crude **24** together with 30 mg of crude **25**. The crude products were purified individually by CM-Sephadex C-25 (NH_4^+, 10~20 ml) chromatography using 0.05 N aqueous ammonia as eluant to afford 7.5 mg (13%) of **24**, Rf 0.11, and 14 mg (24%) of **25**, Rf 0.18 by TLC (S-118, *cf*, apramycin, Rf 0.20).

Compound **24**; darkened over 210°C with no definite melting point. PMR (D_2O+DCl, pD 1): δ 1.5~2.8 (4H, m, 2-H_{ax}, 2-H_{eq}, 3′-H_{ax} & 3′-H_{eq}), 2.85 (3H, s, NCH_3), 3.2~3.8 (21H, N–CH, O–CH & CH_2OH), 5.23 (1H, d, J=8.25 Hz, 8′-H), 5.36 (1H, d, J=1Hz, 1‴-H), 5.5 (1H, d, J=3.75 Hz, 1″-H), 5.9 (1H, d, J=3.75 Hz, 1′-H).

Anal. Calcd. for $C_{26}H_{49}N_5O_{15}\cdot\frac{7}{2}H_2CO_3$: C, 39.87; H, 6.35; N, 7.88.
Found: C, 39.87; H, 5.95; N, 8.13.

Compound **25**; darkened over 205°C. PMR (D_2O+DCl, pD 1): δ 1.1~2.8 (4H, 2-H_{ax}, 2-H_{eq}, 3′-H_{ax} & 3′-H_{eq}), 2.84 (3H, s, NCH_3), 3.2~4.5 (21H, N–CH, O–CH & CH_2OH), 5.25 (1H, d, J=8.25Hz, 8′-H), 5.29 (1H, d, J=3.45 Hz, 1‴-H), 5.53 (1H, d, J=3.75 Hz, 1″-H), 5.87 (1H, d, J=3.75 Hz, 1′-H).

Anal. Calcd. for $C_{26}H_{49}N_5O_{15}\cdot 3H_2CO_3$: C, 40.61; H, 6.46; N, 8.16.
Found: C, 40.68; H, 6.18; N, 8.41.

25 was also prepared from **22**. Deprotection of 96 mg (0.064 mmole) of **22** with 80% hydrazine hydrate followed by purification employing Amberite CG-50 (NH_4^+) and CM-Sephadex C-25 (NH_4^+) columns in a manner similar to that described above gave 8.8 mg (21%) of **25**, which was identical with that described above.

6-*O*-β-D-Ribofuranosylapramycin (26)

Deprotection of 429 mg (0.28 mmole) of **23** followed by purification in a manner similar to that described in **24** gave 116 mg (61%) of pure **26**, mp 189~191°C, $[\alpha]_D^{27.5}$ +76° (c 5, water), Rf 0.18 by TLC (S-118); *cf.* apramycin, Rf 0.20. PMR (D_2O+DCl, pD 1): δ 1.5~2.75 (4H, m, 2-H_{ax}, 2-H_{eq}, 3′-H_{ax} & 3′-H_{eq}), 2.86 (3H, s, NCH_3), 3.1~3.8 (21H, N–CH, O–CH & CH_2OH), 5.18 (1H, s, 1‴-H), 5.24 (1H, d, J=7.8 Hz, 8′-H), 5.5 (1H, d, J=3.3 Hz, 1″-H), 5.83 (1H, d, J=3.75 Hz, 1′-H).

Anal. Calcd. for $C_{26}H_{49}N_5O_{15}\cdot 2H_2CO_3$: C, 42.26; H, 6.71; N, 8.80.
Found: C, 41.92; H, 6.78; N, 9.18.

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