Chemical Transformation of Seldomycin 5 into 3′-Deoxyseldomycin 5 and Related Compounds[1)]

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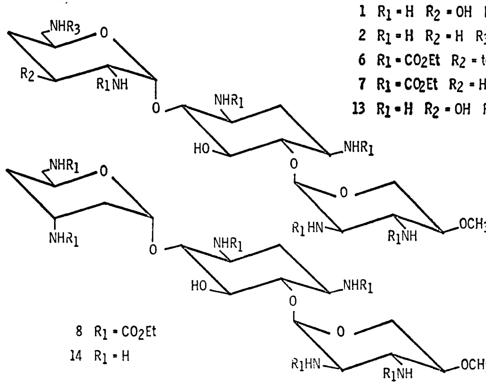
3′-Deoxyseldomycin 5 (**2**), 2′-deamino-3′-epiamino-3′-deoxyseldomycin 5 (**14**), and 2′,3′-epimino-2′-deamino-3′-deoxyseldomycin 5 (**15**) were prepared from seldomycin 5 (**1**). Treatment of hexa-*N*-ethoxycarbonyl (ecb)-3′-*O*-tosylseldomycin 5 with sodium borohydride in DMSO gave the corresponding *N*-ecb-derivatives of **2**, **14**, and **15**. Removal of the ecb groups of these compounds with aq potassium hydroxide gave the free bases **2**, **14**, and **15**, respectively. Structure determination of the products was performed by means of MS, CMR, PMR, and chemical reactions. It was found that **2** exhibits a more potent and broader antibacterial activity than the parent compound **1**, and **14** shows unique antibacterial spectrum against resistant strains, whereas **15** has almost no activity.

Synthesis of 3′-deoxy derivatives of aminoglycoside antibiotics has been studied extensively, since it was disclosed by H. Umezawa and S. Umezawa that resistance of certain clinically isolated bacteria against kanamycin is due to enzymatic 3′-*O*-phosphorylation of the antibiotic, and that 3′-deoxykanamycin, designed rationally upon this mechanism of resistance, exhibits strongly recovered antibacterial activity against the resistant strains.[2)] Seldomycin 5 (SLD-5) (**1**), a new aminoglycoside antibiotic found in our institute, is inactivated, like other aminoglycoside antibiotics, by various resistant strains including those bacteria which produce aminoglycoside 3′-phosphotransferase.[3)] In a previous paper, we reported that the inactivation of SLD-5 by enzymatic 3′-*O*-phosphorylation is inhibited by the change of configuration of the 3′-hydroxyl group of **1**.[4)] In this paper, we describe the synthesis of 3′-deoxy-SLD-5 (**2**) and related compounds as a modification to enhance and broaden the antibacterial activity of **1**. We expected **2** to exhibit more potent activity than the parent compound since **2** has a pseudodisaccharide unit, 3′,4′-dideoxyneamine (**3**), which constructs the common partial structure of gentamicin C_{1a} (GM-C_{1a}) (**4**)[5)] and 3′,4′-dideoxykanamycin B (DKB) (**5**).[6)] The latter two compounds are well-known aminoglycoside antibiotics that have very strong intrinsic activity against various bacteria.

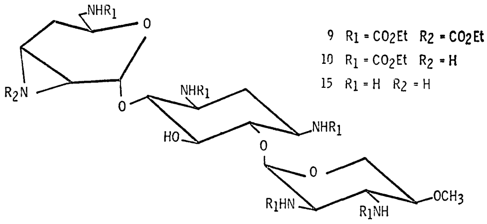
Results and Discussion

Synthesis and Identification of the Products. Attempts were made to convert hexa-*N*-ethoxycarbonyl(ecb)-3′-*O*-tosyl-SLD-5 (**6**)[4)] into 3′-iodo derivative by the usual procedure using sodium iodide, but TLC of the reaction mixture indicated that the reaction is complicated. S_N2 substitution reaction of a tosyloxy group by a nucleophile does not occur smoothly when an approaching nucleophile and a substituent at γ position are in 1,3-diaxial relationship. 1,2,4,6-Tetra-*O*-benzoyl-3-*O*-tosyl-α-D-glucopyranose is recovered on the nucleophilic displacement reaction with sodium benzoate.[7)] 1,2: 5,6-Di-*O*-isopropylidene-3-*O*-tosyl-α-D-glucofuranose is recovered in a 90% yield on reaction with azide ion.[8)] Steric and polar factors affecting reactivity of a sulfonyloxy group of carbohydrate have been summarized[9)] and such steric hindrance has been reported in the field of steroid chemistry.[10)] The results suggest that the α-glycosidic linkage at the 1′ position of **6** blocks the approach of an iodide ion from side α to the 3′ position in our case also. However, penta-*N*-ecb-3′-*O*-tosylkanamycin B was successfully converted into 3′-iodo derivative in an exceptionally good yield.[11)] Successful iodination was attributed to the neighboring group participation of the 2′-ethoxycarbonylamino group.[11)] However, an alterna-

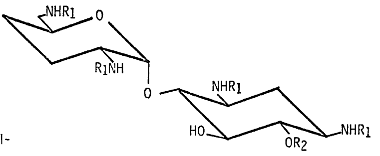


1 $R_1 = H$ $R_2 = OH$ $R_3 = H$
2 $R_1 = H$ $R_2 = H$ $R_3 = H$
6 $R_1 = CO_2Et$ $R_2 =$ tosyloxy $R_3 = CO_2Et$
7 $R_1 = CO_2Et$ $R_2 = H$ $R_3 = CO_2Et$
13 $R_1 = H$ $R_2 = OH$ $R_3 =$ benzyloxycarbonyl

8 $R_1 = CO_2Et$
14 $R_1 = H$



9 $R_1 = CO_2Et$ $R_2 = CO_2Et$
10 $R_1 = CO_2Et$ $R_2 = H$
15 $R_1 = H$ $R_2 = H$



3 $R_1 = H$ $R_2 = H$
4 $R_1 = H$ $R_2 =$ 3-deoxy-4-C-methyl-3-methylamino-β-L-arabinopyranosyl (garosaminyl)
5 $R_1 = H$ $R_2 =$ 3-deoxy-3-amino-α-D-glucopyranosyl
11 $R_1 = CO_2Et$ $R_2 =$ 3-N-ethoxycarbonyl-garosaminyl
12 $R_1 = CO_2Et$ $R_2 = H$

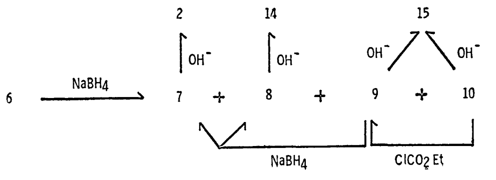


TABLE 1. CMR CHEMICAL SHIFTS OF COMPOUNDS **7**, **9**, **10**, AND **11**[a)]

Compound	**7**		**11**[15),f)]	**9**		**10**	
Solvent	DMSO-d_6	pyr.-d_5[b)]	DMSO-d_6	DMSO-d_6	pyr.-d_5[b)]	DMSO-d_6	pyr.-d_5[b)]
C-1′	98.3	100.0	98.3	93.4	94.5	95.2	97.2[e)]
C-2′	50.7	51.5	50.2	36.5[c)]	37.2[d)]	27.7	28.3
C-3′	23.5	25.0	23.9	36.1[c)]	36.8[d)]	29.4	30.5
C-4′	27.2	28.1	27.5	26.9	27.4	27.7	28.3
C-5′	66.6	68.1	67.1	64.0	65.0	64.0	65.3
C-6′	44.6	45.5	45.0	44.0	44.9	44.2	45.2
C-1	50.7	51.9	50.8	50.7	51.8	50.7	52.0
C-2	35.0	36.0	34.7	35.1	35.8	35.1	36.0
C-3	49.9	51.1	50.2	49.2	50.3	49.6	50.7
C-4	82.1	83.0	82.4	80.1	80.6	79.5	81.1
C-5	74.8	76.3	74.9	74.7	75.7	74.5	75.7
C-6	79.6	81.8	81.8	79.4	80.0	79.0	79.9
C-1″	96.9	98.6	98.8	96.7	97.7	96.5	97.9[e)]
C-2″	54.4	55.5	64.5	54.4	55.2	54.4	55.4
C-3″	53.2	54.5	58.6	53.2	54.3	53.3	54.5
C-4″	76.2	78.5	73.1	76.4	78.0	76.4	78.5
C-5″	59.5	61.4	69.6	59.6	62.4	59.5	61.1
OCH_3	57.5	58.2	—	57.6	58.2	57.6	58.3
$CO_2CH_2\underline{CH_3}$	14.4	14.8	14.5	14.4	14.4	14.5	14.8
					14.8		
$CO_2\underline{CH_2}CH_3$	59.5	60.6	59.7	59.6	60.1	59.5	60.3
	60.0		60.5		60.3		
					61.0		
CO	155.7	156.8	155.7	155.9	156.5	155.6	156.6
	155.8	157.5	156.1	156.6	157.1	155.9	157.4
	156.5	157.7		162.5	157.3	156.5	157.5
		158.0			157.5		157.7
					163.2		

a) Reported in ppm from internal TMS. b) Pyridine-d_5 containing 10% DMSO-d_6 to increase solubility of the sample. c) d) e) Interconvertible. f) Data on N–CH_3 and C–CH_3 omitted.

tive explanation might be given by the present result.***

Bell *et al.* reported that treatment of several sulfonates with sodium borohydride in dimethyl sulfoxide (DMSO) or diglyme gives deoxygenated products.[12)] Application of this method to carbohydrate derivatives has been reported.[13,14)] We have adopted the reaction to reduce **6**.

Treatment of **6** with sodium borohydride in DMSO afforded the following four products after chromatographic separation; hexa-*N*-ecb-3′-deoxy-SLD-5 (**7**) (20.1%), hexa-*N*-ecb-2′-deamino-3′-epiamino-3′-deoxy-SLD-5 (**8**) (23.3%), 1,3,6′,2″,3″-penta-*N*-ecb-2′,3′-*N*-ecb-epimino-2′-deamino-3′-deoxy-SLD-5 (**9**) (21.8%), and 1,3,6′,2″,3″-penta-*N*-ecb-2′,3′-epimino-2′-deamino-3′-deoxy-SLD-5 (**10**) (14.8%). The structure of **7** was elucidated by comparison of its CMR spectrum with that of penta-*N*-ecb-GM-C_{1a}[15)] (**11**) (Table 1). The chemical shifts for C-1′—C-6′ and C-1—C-5 of **7** correspond to those for the carbons of **11** which has a common disaccharide unit, 1,3,2′,6′-tetra-*N*-ecb-3′,4′-dideoxyneamine (**12**). The ecb groups of **7** were removed by aq methanolic potassium hydroxide to give 3′-deoxy-SLD-5 free base (**2**). Its structure was confirmed by MS, PMR, and CMR spectra.[1)] Compound **2** consumed 1.00 mol/mol periodate (theoretical value: 1.00); **1** consumed 2.90 (theoretical value: 2.00) under carefully controlled conditions. Although we have tentatively attributed the excess uptake of periodate

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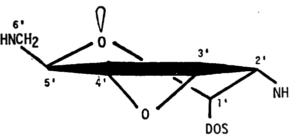
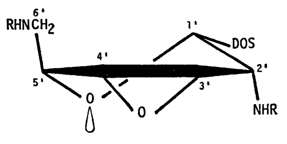
*** In the case of kanamycin B, the smooth displacement of the 3′-tosyloxyl group by iodide ion might occur with the aid of the neighboring 4′-hydroxyl group participation to form a 3′,4′-anhydro derivative as an intermediate, followed by diequatorial opening of the epoxide ring [N. R. Williams, *Adv. Carbohydr. Chem. Biochem.*, **25**, 109 (1970)] due to the regiospecific attack of iodide ion at the 3′ position. The cause of regiospecifity can be explained as follows. The epoxide is considered to adopt one of the conformers (i) or (ii). If the epoxide exists as conformer (i), the attack of iodide ion at 4′ position might be hindered by the 1,3-diaxial electrostatic repulsion between the approaching iodide ion and the lone pair electrons of the pyranose-ring oxygen. If the epoxide exists as conformer (ii), the attack of iodide ion at 4′ position might sterically be hindered by the adjacent *quasi*-axial 5′-ethoxycarbonylaminomethyl group, thus resulting in the regiospecific formation of the 3′-iodo derivative. In the case of compound **6**, no such epoxide intermediate can be formed since **6** has no 4′-hydroxyl group.



(i)



(ii)

Table 2. CMR chemical shifts of compounds **14** and **15**[a)]

	14 (3′-epiamino-)			**15** (2′,3′-epimino-)		
	Free base	PD 1.5	*M*[b)]	Free base	PD 1.7	*M*
C-1′	100.4	98.5	d	96.3	91.4	d
C-2′	36.6[c)]	32.8	t	29.2[d)]	36.1	d
C-3′	42.9	42.9	d	31.2[d)]	36.1	d
C-4′	35.4[c)]	29.2	t	27.3	21.9	t
C-5′	69.1	64.5	d	66.8	63.3	d
C-6′	44.9	43.6	t	45.3	43.3	t
C-1	51.3	50.9	d	51.4	50.9	d
C-2	36.9	28.9	t	36.4	28.8	t
C-3	50.0	48.7	d	49.8	49.1	d
C-4	88.0	80.8	d	83.7	78.0	d
C-5	75.1	73.9	d	75.6	74.7	d
C-6	86.9	81.2	d	87.1	81.4	d
C-1″	100.4	94.7	d	100.4	94.9	d
C-2″	56.2	50.7	d	56.1	50.6	d
C-3″	54.9	49.9	d	54.8	49.8	d
C-4″	80.3	73.9	d	80.2	73.9	d
C-5″	60.7	61.7	t	60.7	61.8	t
OCH_3	58.8	58.5	q	58.8	58.6	q

a) Measured in D_2O solution with dioxane as an internal reference and reported by ppm from TMS. b) Multiplicity by off-resonance. c), d) Interconvertible.

by **1** to non-Malapradian oxidation of vicinal amino and methoxyl groups,[16)] the present result together with another result concerning periodate oxidation of 6′-*N*-benzyloxycarbonyl-SLD-5 (**13**)[17)] (consumption: 1.52, theoretical value: 2.00), indicate that our previous speculation was incorrect. The over-oxidation seems to take place only when both the 3′-hydroxyl and 6′-amino groups exist in the molecule.

The structure of **8** was determined retrospectively on the basis of the following spectroscopic data for de-ethoxycarbonylated compound, 2′-deamino-3′-epi-amino-3′-deoxy-SLD-5 free base (**14**). i) High resolution mass spectrum indicates that the molecular weight of **14** is 434.2816, which is isomeric with **2**. The deoxygenated structure was supported by the appearance of a prominent peak at m/e 129.[18)] ii) In PMR spectrum of **14**, the H-1′ signal appeared at τ 4.68 as a broad double doublet with J=*ca.* 5 and *ca.* 2 Hz, which indicates that the 2′ position has no substituent. iii) In CMR (Table 2), the signal of C-2′ appeared at 36.6 ppm as a triplet by off-resonance and that of C-3′ at 42.9 ppm as a doublet. Protonation-β-shift of the spectrum was in line with the structure. No evidence was obtained that 3′-amino group is axially oriented. However, its orientation was suggested from the reaction mechanism.

Compounds **9** and **10** gave the same product, 2′,3′-epimino-2′-deamino-3′-deoxy-SLD-5 free base (**15**), by alkaline hydrolysis to remove ecb groups. The structure of **15** was determined by the following data. i) Mass spectrum of **15** gives a (M+1) and M peak at m/e 433 and 432, respectively. ii) PMR spectrum of **15** shows no signal of an olefinic proton. iii) In CMR spectrum of **15** (Table 2), two signals attributable to C-2′ and C-3′ appear at 29.2 and 31.2 ppm (or *vice versa*) as doublets, respectively. These signals shifted *ca.* 5—7 ppm downfield by acidifying the media, C-1′ and C-4′ signals shifted *ca.* 5 ppm upfield.

Elemental analyses of **9** and **10** indicate that **9** has

Table 3. Minimum inhibitory concentrations of compounds **1**, **2**, **4**, **5**, **14**, and **15** against sensitive strains (mcg/ml)

Strain	**1**[a)]	**2**	**14**	**15**	**4**[b)]	**5**[c)]
Staphylococcus aureus 209-P	0.78	1.56	3.12	100	0.4	0.2
Smith	0.4	0.4	0.78	50	0.1	0.2
Newman	1.56	0.78	1.56	100	0.4	0.4
Staphylococcus epidermidis	0.78	0.4	1.56	50	0.05	0.2
Batillus subtilis ATCC 6633	0.4	0.4	0.78	25	0.05	0.1
Escherichia coli NIHJC-2	3.12	1.56	12.5	100	0.78	1.56
GN2411-5	3.12	1.56	12.5	100	0.78	1.56
Juhl	3.12	3.12	12.5	100	0.78	1.56
3100	6.25	3.12	25	100	0.78	1.56
Klebsiella pneumoniae ♯8045	1.56	0.78	6.25	100	0.2	0.2
Salmonella enteritidis G-14	12.5	12.5	50	100	1.56	6.25
Salmonella typhimurium E-9	3.12	1.56	6.25	100	0.4	0.4
Shigella sonnei ATCC 9290	6.25	3.12	12.5	100	0.78	1.56
Pseudomonas aeruginosa BMH ♯1	6.25	1.56	6.25	100	0.4	0.4
BMH ♯10	3.12	0.78	1.56	100	0.2	0.2
E-2	25	6.25	25	100	0.78	0.78
Serratia marcescens T-55	6.25	6.25	12.5	100	0.78	3.12
T-26	12.5	12.5	25	100	1.56	12.5
176VA	12.5	12.5	25	100	0.78	6.25
Providencia KY-3947	12.5	12.5	12.5	100	0.78	3.12
Proteus vulgaris ATCC 6897	12.5	6.25	25	100	0.78	1.56
Proteus rettgeri KY-4288	6.25	1.56	12.5	100	0.4	1.56
Proteus morganii KY-4298	6.25	3.12	12.5	100	0.78	0.78

a) Seldomycin 5. b) Gentamicin C_{1a}. c) 3′,4′-Dideoxykanamycin B.

TABLE 4. MINIMUM INHIBITORY CONCENTRATIONS OF COMPOUNDS **1**, **2**, **4**, **5**, **14**, AND **15** AGAINST RESISTANT STRAINS (mcg/ml)

Strain	Inactivating enzyme[a]	**1**[b]	**2**	**14**	**15**	**4**[c]	**5**[d]
Escherichia coli R-5	APH(3′)-I	100	3.12	6.25	100	0.4	0.78
R-12	ANT(2″)	3.12	3.12	6.25	100	6.25	25
R-16	APH(3′)-I	100	0.78	1.56	50	0.2	0.4
R-18*	ANT(2″)	25	3.12	12.5	100	0.78	0.78
R-19	AAC(3)-I	50	100	6.25	100	12.5	0.4
R-20	APH(3′)-I	100	1.56	12.5	100	0.4	0.78
57R/W677	ANT(2″)	6.25	6.25	12.5	100	25	100
76-2*	ANT(2″)	50	3.12	25	100	12.5	25
Pseudomonas aeruginosa R-3	AAC(6′)	100	100	100	100	6.25	50
R-4	AAC(3)-I	100	100	25	100	12.5	0.78
R-5	APH(3′)-I,II	25	6.25	12.5	100	0.4	0.4
R-9	AAC(6′)	100	100	100	100	25	100
R-10	APH(3′)-I	100	3.12	12.5	100	0.4	0.4
R-12	AAC(3)-II	100	100	100	100	100	100
Serratia marcescens 1065	AAC(6′)	100	100	100	100	12.5	100
Providencia 164	AAC(2′)	100	100	25	100	25	50
Klebsiella pneumoniae Y-58*	ANT(2″)	25	1.56	6.25	100	6.25	12.5
Y-60*	ANT(2″)	25	3.12	12.5	100	12.5	50

a) Figure in parentheses indicates the position where the antibiotic is enzymatically modified. b) Seldomycin 5. c) Gentamicin C$_{1a}$. d) 3′,4′-Dideoxykanamycin B. * Strains which inactivate streptomycin.

six ecb groups whereas **10** has five. This is supported by the fact that **10** was converted into **9** by treatment with ethyl chloroformate. A comparison of the CMR spectra of both compounds (Table 1) reveals that the 2′,3′-aziridine ring of **10** bears no ecb group. The C-2′ and C-3′ signals of **9** appear at 36.5 and 36.1 ppm, those of **10** at 27.7 and 29.4 ppm accompanied by slight downfield shifts of C-1′ and C-4′. The peak appearing at 162.5 ppm in the spectrum of **9** is attributable to the carbonyl carbon of ecb group attached at the aziridine nitrogen. The fact that treatment of **9** with sodium borohydride in DMSO afforded **7** and **8** leads to the conclusion that, in the reaction of **6** with sodium borohydride, **9** was initially formed and then transformed into **7** and **8** by ring-opening of the aziridine due to an attack of hydride ion. It is not obvious whether **7** was formed, besides this mechanism, directly by the replacement of the 3′-tosyloxyl group with hydride. The course of formation of **10** remains unclarified. The formation might take place by hydrolysis of the ecb group attached to the aziridine nitrogen due to basic property of sodium borohydride. Recently, Okutani *et al.* reported that 2′,3′-epimino derivatives play the role of intermediate during the course of synthesis of 3′-deoxy and related compounds from butirosin 3′-phosphate *etc.*[19]

*Antibacterial Activities of **2**, **14**, and **15**.* The *in vitro* antibacterial activities of **2**, **14**, and **15** were measured and compared with those of SLD-5 (**1**), GM-C$_{1a}$ (**4**), and DKB (**5**) (Tables 3 and 4). Compound **2** exhibits, as compared with **1**, both enhanced activity against gram-negative sensitive strains and strongly recovered potency against resistant strains which produce aminoglycoside 3′-phosphotransferase [APH(3′)]. Activity of **2**, however, is less potent than that of the two reference compounds, **4** and **5**, against both sensitive and resistant strains except aminoglycoside 2″-nucleotidyltransferase [ANT(2″)]-producing strains. The result of the test *in vivo* (Table 5) showed a similar tendency to that in the *in vitro* experiment. The difference in activity seems to arise from the structure of the monosaccharide attached to the 6 position of the deoxystreptamine core, since other portions of the molecules of these compounds are quite the same. We infer that, as compared with 2″-equatorial hydroxyl group, the 2″-equatorial amino group characteristically involved in the structures of **1** and **2** does not function to strengthen antibacterial activity.

TABLE 5. ED$_{50}$ VALUES OF COMPOUND **2** AGAINST EXPERIMENTAL INFECTIONS IN MICE (mg/kg)[a]

Organism	**1**	**2**	**5**
Escherichia coli Juhl	6.75	3.9	0.62
R-20	200	4.5	2.8
Pseudomonas aeruginosa BMH♯1	265	150	18.8
R-5	180	52.5	—

a) Acute systemic infections in mice were produced by intraperitoneal inoculation. The dosage regimen for all infections was 1 or 2 h post-infection, all the compounds being administered subcutaneously.

It seems that **1** is inactive against four of the six ANT-producing resistant strains tested in spite of its lack of a 2″-hydroxyl group in the structure. The four strains which are moderately resistant to **1** inactivate streptomycin[20] (MIC: >100 mcg/ml, data not given in Table 4). The other two strains which are sensitive to **1** do not inactivate streptomycin (MIC: 3.12—12.5 mcg/ml). It should be noted that this inactivation of **1** was inhibited by deoxygenation or epimerization[4] at the 3′ position of **1**. The results suggest that in **1** the 3′-hydroxyl group might function as a substrate for the ANTs, or these four strains might produce, besides ANT, other enzymes which modify the 3′-hydroxyl group of **1**.

Compound **15** lacks antibacterial activity. It is considered that construction of the aziridine ring deforms chair conformation of the 2,6-diaminohexose unit, causing the loss of activity.

The potency of **14** against sensitive strains is less strong than that of **1** except against *Pseudomonas*. However, the antibacterial spectrum of **14** against resistant strains was found to be interesting. Although less potent than **2**, it exhibits recovered activity against resistant strains which produce APH(3′)-I or aminoglycoside 2′-acetyltransferase [AAC(2′)]. This is understandable since the targets of these enzymes have been removed from the substrate. Compound **14** is active against AAC(3)-I-producing strains, indicating that 2′-deamino-3′-epiamino-3′-deoxygenation offers a new mode of modification to the established methods, such as 1-*N*-ω-amino-α-hydroxyalkanoylation,[21] 1-*N*-ethylation,[22] and 1-*N*-ω-amino-α-hydroxyalkylation,[23] for recovering activity of aminoglycoside antibiotics against AAC(3)-I-producing strains. Against the four ANT-producing strains, **14** shows improved activity than **1**. Compound **14** is an unique aminoglycoside antibiotic both in its structure and its width of antibacterial spectrum against resistant strains.

The LD$_{50}$ value of **2** was estimated to be 300 mg/kg (mice, intraveneously).

Experimental

General. Melting points were determined with a Yanaco micro melting point apparatus and are uncorrected. Varian T-60, JEOL JNM-FX 100, or JEOL PS-100 was employed for NMR measurements with TMS or DSS as an internal reference. Samples of the free bases were decarbonated in the usual way[24] just before the NMR measurements. Thin layer chromatography (TLC) was carried out with Merck TLC plate and spots were located by iodine vapor and/or ninhydrin. Mass spectra were obtained with JEOL JMS-01SG-2 at 75 eV using direct insertion probe. Optical rotations were measured with a Hitachi-Perkin-Elmer 141 polarimeter at ambient temperature. Commercial DMSO and sodium borohydride were used without further purification. Quantitative determination of periodate uptake was made as reported.[16] No effort was made to crystallize the products. Antibacterial activity was determined by the agar dilution method at pH 7.2 specified by the Japan Society of Chemotherapy.

*Reaction of **6** with Sodium Borohydride.* To a solution of **6** (25.0 g) in DMSO (500 ml) was added sodium borohydride (12.5 g) in one portion and the solution was stirred at 65 °C for 17 h. DMSO was evaporated *in vacuo* and water (600 ml) was added to the residue. The mixture was extracted with chloroform (300 ml × 10) and the organic layer was washed with water (50 ml × 3), dried with anhydrous sodium sulfate and evaporated to give white powder. The residue was charged to a silica gel column (Merck, 2.2 kg, 8 cm × 128 cm) and the column was washed with chloroform (7 liter), followed by elution with a mixture of chloroform–methanol (fraction *ca.* 200 ml each); eluent: Frs. 1—101, chloroform–methanol 30: 1; Frs. 102—199, 20: 1; Frs. 200—269, 10: 1. Fractions were monitored by TLC.

Frs. 56—72: hexa-*N*-ecb-3′-deoxy-SLD-5 (**7**), 4.20 g as white powder. Mp 278—279 °C, $[\alpha]^{25}_{\mathrm{D}}$ +70.7 (*c* 0.382, DMF).

Found: C, 50.28; H, 7.41; N, 9.63; S, 0.00%. Calcd for $C_{36}H_{62}N_6O_{18}$: C, 49.87; H, 7.22; N, 9.70; S, 0.00%. Frs. 77—108: hexa-*N*-ecb-2′-deamino-3′-epiamino-3′-deoxy-SLD-5 (**8**), 4.85 g as white powder. Mp 237—241 °C, $[\alpha]^{22}_{\mathrm{D}}$ +63.0 (*c* 0.437, DMF).

Found: C, 49.97; H, 7.44; N, 9.61; S, 0.00%. Calcd for $C_{36}H_{62}N_6O_{18}$: C, 49.87; H, 7.22; N, 9.70; S, 0.00%. Frs. 109—138: 1,3,6′,2″,3″-penta-*N*-ecb-2′,3′-*N*-ecb-epimino-2′-deamino-3′-deoxy-SLD-5 (**9**), 4.55 g as slightly yellow powder. Mp 235—238 °C, $[\alpha]^{20}_{\mathrm{D}}$ +68.9 (*c* 0.409, DMF).

Found: C, 50.78; H, 7.40; N, 9.32; S, 0.00%. Calcd for $C_{36}H_{60}N_6O_{18}$: C, 49.98; H, 7.01; N, 9.72; S, 0.00%. Frs. 170—178: an unidentified product, 0.590 g as white powder. Frs. 195—231: 1,3,6′,2″,3″-penta-*N*-ecb-2′,3′-epimino-2′-deamino-3′-deoxy-SLD-5 (**10**), 3.10 g as white powder. Mp 272—273 °C, $[\alpha]^{23}_{\mathrm{D}}$ +54.1 (*c* 0.455, DMF).

Found: C, 49.46; H, 7.29; N, 10.29; S, 0.00%. Calcd for $C_{33}H_{56}N_6O_{16}$: C, 49.98; H, 7.13; N, 10.60; S, 0.00%. Frs. 234—244: an unidentified product, 0.21 g as white powder.

*3′-Deoxy-SLD-5 Free Base (**2**).* Compound **7** (3.80 g) was dissolved in methanol (130 ml) and 10.2 M aq potassium hydroxide (130 ml) was added to the solution. The homogeneous solution was heated in a sealed vessel at 140 °C for *ca.* 6 h. After being cooled to room temperature, the solution was acidified to pH 4 by addition of concd hydrochloric acid under ice-cooling. The ppt formed was filtered off and the filtrate was charged to a column of Amberlite CG-50 (ammonium cycle, 500 ml). The column was washed with water (500 ml) and eluted with aq ammonia; eluent: Frs. 1—86, 0.1 M-ammonia; Frs. 87—160, 0.2 M-ammonia (fraction: 20 ml each). Frs. 102—110: compound **2** containing a small amount of impurities, 0.95 g. Frs. 111—126: pure **2**, 0.80 g as white powder. Mp 180—210 °C, $[\alpha]^{25}_{\mathrm{D}}$ +105 (*c* 0.564, water); R_f value (relative to SLD-5 on silica gel TLC) 1.21 (1-butanol–ethanol–chloroform–conc ammonia 4: 5: 2: 5). PMR (D_2O): τ 4.93 (d, *J*=4 Hz, H-1′), 5.04 (d, *J*=3 Hz, H-1″), 7.9—9.0 (m, H-2$_{eq}$, H-2$_{ax}$, H-3′$_{eq}$, H-3′$_{ax}$, H-4′$_{eq}$, H-4′$_{ax}$); MS (*m/e*, relative intensity, possible assignment of fragment ion[25]): 435 (1, MH), 335 (8, A5), 307 (40, A7), 289 (37, A8), 274 (18, F2), 173 (12, A10), 163 (25, A11), 145 (100, Cl and A12), 129 (75, B1).

Found: C, 47.51; H, 8.43; N, 18.16%. Calcd for $C_{18}H_{38}N_6O_6 \cdot 1/2H_2CO_3$: C, 47.72; H, 8.46; N, 18.05%. Fractions 102—110 were rechromatographed on CG-50 to give 0.57 g of pure **2** and a trace amount of a compound supposed to be 6-*O*-(2,3-diamino-2,3-dideoxy-4-*O*-methyl-α-D-xylopyranosyl)-2-deoxystreptamine (DOS-seldamine). Mp 142—149 °C, $[\alpha]^{17}_{\mathrm{D}}$ +59.9 (*c* 0.302, water). PMR (D_2O): τ 5.00 (d, *J*=3 Hz, H-1″), 6.53 (s, OCH_3); CMR (D_2O): C-1, 51.1; C-2, 36.8; C-3, 51.1; C-4, 78.8; C-5, 74.9; C-6, 87.4; C-1″, 100.1; C-2″, 56.2; C-3″, 54.7; C-4″, 80.2; C-5″, 60.8; OCH_3, 58.7. MS: 307 (1, MH), 306 (0.5, M), 289 (3, A8), 203 (37, unknown), 173 (7, A10), 163 (10, A11), 145 (100, Cl and A12). This compound is considered to be formed by hydrolysis of the glycosidic linkage under drastic conditions used for the removal of ecb groups. This compound exhibits no antibacterial activity.

*2′-Deamino-3′-epiamino-3′-deoxy-SLD-5 Free Base (**14**).* The ecb groups of **8** (2.50 g) was removed in a similar manner as described above and the product was purified by CG-50 to afford pure **14** (0.70 g), as slightly yellow powder. Mp 134—137 °C, $[\alpha]^{22}_{\mathrm{D}}$ +106 (*c* 0.356, water).

Found: C, 44.75; H, 8.08; N, 16.55%. Calcd for $C_{18}H_{38}N_6O_6 \cdot H_2O \cdot H_2CO_3$: C, 44.34; H, 8.24; N, 16.33%.

PMR (D_2O): τ 4.68 (br dd, *J*=*ca.* 5, *ca.* 2 Hz, H-1′), 5.04 (d, *J*=3 Hz, H-1″); MS: 435 (1, MH), 307 (7, A7), 289 (5, A8), 274 (18, F2), 273 (29, A4), 258 (8, F1), 173 (13, A10), 163 (31, A11), 145 (100, Cl and A12), 129 (64, B1), 112 (25, F4).

*2′,3′-Epimino-2′-deamino-3′-deoxy-SLD-5 Free Base (**15**).*
*From **9***: Alkaline hydrolysis of **9** (2.00 g) and purification of

the crude product were carried out in a similar way to that mentioned above. Pure **15** was obtained as white powder, 0.54 g. Mp 120—149 °C, $[\alpha]^{23}_{D}$ +100 (c 0.667, water).

Found: C, 48.43; H, 8.80; N, 18.57%. Calcd for $C_{18}H_{36}N_6O_6\cdot H_2O$: C, 47.97; H, 8.52; N, 18.65%.

PMR (D_2O): τ 4.42 (d, J=3 Hz, H-1′), 5.06 (d, J=3 Hz, H-1″), at pD 2: τ 3.83 (d, J=4 Hz, H-1′), 4.46 (d, J=*ca.* 0 Hz,[16] H-1″); MS: 433 (1, MH), 432 (0.5, M), 307 (20, A7), 289 (72, A8), 274 (4, F2), 163 (20, A11), 145 (100, C1 and A12), 127 (32, B1). Characteristics of the MS: no fragment peaks attributable to the species arising from the 4-*O*-glycosyl-2-deoxystreptamine were observed, peaks at m/e 347 (25) and 203 (10) appearing. These two fragments probably correspond to D9 and D10 fragments,[18] though hydrogen shift is needed in this case.

From ***10***: De-ethoxycarbonylation of **10** (0.45 g) and chromatography were carried out as described above. Compound **15** was obtained as white powder, 0.12 g. MS, PMR, and antibacterial activity of this material were identical with those obtained with a sample prepared from **9**. A small amount of DOS-seldamine (0.03 g) was also isolated.

Reaction of ***9*** *with Sodium Borohydride.* Sodium borohydride (13 mg) was added to a solution of **9** (25 mg) in DMSO (0.5 ml) and the solution was kept at 65 °C for 13 h. DMSO was evaporated *in vacuo* and the residue was extracted with chloroform (10 ml), washed with water (3 ml×2), and dried. The chloroform solution was then concentrated to *ca.* 1 ml. TLC (chloroform–methanol 10: 1) of the concentrate indicated that the starting material (R_f 0.61) had disappeared and the following products were formed; **7** (R_f 0.74), **8** (R_f 0.64), a trace of **10** (R_f 0.36), and an unidentified product (R_f 0.31).

Reaction of ***10*** *with Ethyl Chloroformate.* To a solution of **10** (50 mg) in DMF (2 ml) were added anhyd. sodium carbonate (16 mg) dissolved in water (0.5 ml) and ethyl chloroformate (17 mg). The solution was stirred overnight. Solvents were removed *in vacuo* and the residue was extracted with chloroform (10 ml), washed with water (2 ml), and dried with anhyd. sodium sulfate. TLC of the chloroform solution showed a single spot of **9**. Chloroform was evaporated to give white powder of **9** (55 mg); mixed mp with the material obtained above: 234—235 °C.

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