SYNTHESIS OF 1-*N*-(D-*THREO*- AND RACEMIC *ERYTHRO*-3-AMINO-2-HYDROXYBUTANOYL)-2′,3′-DIDEOXYKANAMYCIN A

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(Received for publication July 13, 1987)

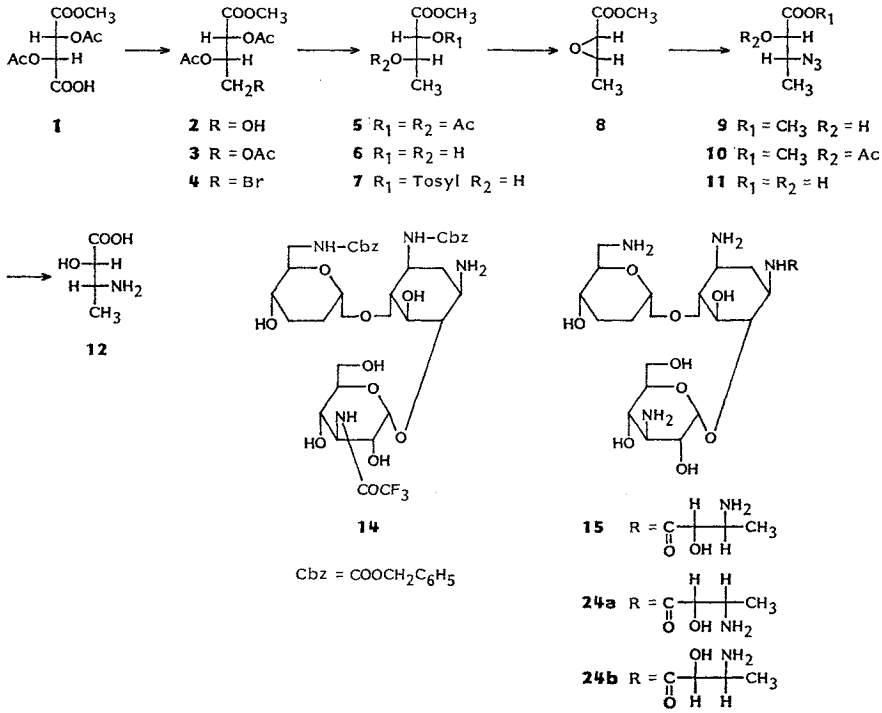
1-*N*-(D-*Threo*-3-amino-2-hydroxybutanoyl)-2′,3′-dideoxykanamycin A has been prepared by coupling of 3,6′-bis(*N*-benzyloxycarbonyl)-2′,3′-dideoxy-3″-*N*-(trifluoroacetyl)kanamycin A with D-*threo*-3-azido-2-hydroxybutanoic acid. A diastereomeric mixture of the *erythro* analog has also been prepared by use of racemic *erythro*-3-azido-2-hydroxybutanoic acid. Synthesis of the D-*threo*- and racemic *erythro*-3-amino-2-hydroxybutanoic acids has been described.

As reported by UMEZAWA in 1970[1], the 1-amino or the 3-amino group of the 2-deoxystreptamine moiety of kanamycin is involved in the binding of the antibiotic with 3′-*O*-phosphotransferases, and the modification of one of these amino groups suggested to produce derivatives active against resistant bacteria. In fact, butirosins[2] and amikacin[3], which contain the (2*S*)-4-amino-2-hydroxybutanoyl (AHB) residue attached to the 1-amino group of the parent antibiotics inhibit the growth of a wide variety of resistant bacteria. 1-*N*-Ethylsisomicin[4] (netilmicin) derived from sisomicin is another example. 3-*N*-AHB-Kanamycin also showed the same character although the antibacterial activity is low on the whole[5]. A number of kanamycin derivatives having AHB moieties[6,7] or other kinds of residues[8] have been prepared, but they are all less, or at most, similar in activity in compared to the AHB derivatives. Among them 1-*N*-[(2*S*)-3-amino-2-hydroxypropanoyl]kanamycin A[6] (AHP-KM) showed almost the same activity as that of 1-*N*-AHB-kanamycin A (amikacin). As part of our studies on similar functional modifications, we have undertaken to prepare the derivatives of 2′,3′-dideoxykanamycin A[9] having an 1-*N*-amino acid residue in which C-3 is asymmetric and bears a primary amino group. Since 2′,3′-dideoxykanamycin A has higher antibacterial activity than kanamycin A, the former was used as substrate.

(2*S*,3*R*)-3-Amino-2-hydroxybutanoic acid (D-*threo*-3-amino-2-hydroxybutanoic acid, **12**) was prepared first. 2,3-Di-*O*-acetyl-L-tartaric acid monomethyl ester[10] (**1**) having the 2*R*,3*R* configurations was reduced with borane in oxolane to give methyl L-*threo*-2,3-di-*O*-acetyl-2,3,4-trihydroxybutanoate[†,11] (**2**). The structure was confirmed by conversion to its tri-*O*-acetyl derivative[11] (**3**). Treatment of **2** with triphenylphosphine and carbon tetrabromide in pyridine gave the slightly unstable 4-bromo derivative (**4**), which was reduced with tributylstannane in the presence of 2,2′-azobis(isobutyronitrile) (AIBN) to give the 4-deoxy derivative (**5**). The methyl group of **5** was confirmed by its ^{1}H NMR spectrum. ZEMPLÉN deacetylation of **5** gave syrupy methyl L-*threo*-2,3-dihydroxybutanoate (**6**). *p*-Toluenesulfonylation using limited amounts of tosyl chloride gave mainly the 2-*O*-tosyl derivative (**7**), which, by treatment with sodium methoxide in methanol, gave the L-*erythro*-2,3-epoxide (**8**). Although the compound was isolated only in methanol solution because of its volatility, the presence of the epoxy group was supported by its ^{1}H NMR spectrum (see Table 1). Treatment

† BESTMANN and SCHMIECHEN[11] have prepared **2** by a different route.

Chart 1.


1
2 R = OH
3 R = OAc
4 R = Br
5 R1 = R2 = Ac
6 R1 = R2 = H
7 R1 = Tosyl R2 = H
8
9 R1 = CH3 R2 = H
10 R1 = CH3 R2 = Ac
11 R1 = R2 = H
12
14
Cbz = COOCH2C6H5
15
24a
24b

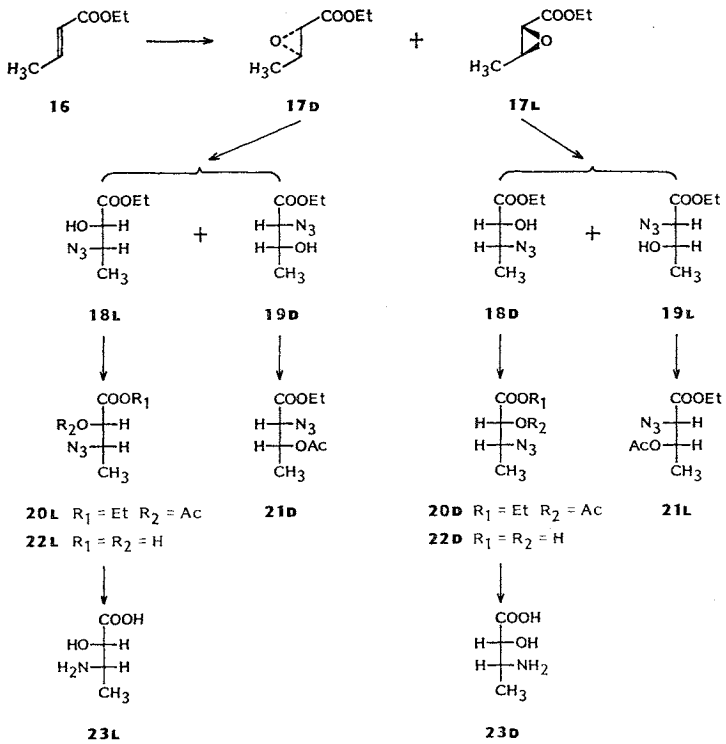

16
17D
17L
18L
19D
18D
19L
20L R1 = Et R2 = Ac
22L R1 = R2 = H
21D
20D R1 = Et R2 = Ac
22D R1 = R2 = H
21L
23L
23D

Table 1. ^{1}H NMR spectra of compounds **1**, **3**~**12** and **17**~**23** in $CDCl_3$ (unless otherwise stated).

	$COOCH_3$ (s)	2-H	3-H	4-H	Ac (each s)	$J_{2,3}$(Hz)	$J_{3,4}$(Hz)
1[a]	3.78	5.65, 5.68 ABq	5.65, 5.68 ABq		2.11, 2.13	2.5	
3	3.76	5.27 d	5.57 dt	4.16 dd, 4.28 dd	2.07, 2.10, 2.21	3	6, 6 ($J_{4,4'}$=11)
4	3.80	5.1~6.0	5.1~6.0	5.1~6.0	2.07, 2.25		
5	3.75	5.10 d	5.41 dq	1.31 d	2.05, 2.20	3	7
6	3.83	4.03 d	4.09 dq	1.31 d		2.5	6.5
7[b]	3.68	4.75 d	4.18 dq	1.22 d		4	6.5
8[c]	3.81	3.59 d	3.27 dq	1.39 d		4	5
9	3.83	4.12 dd[e]	3.77 dq	1.46 d		2.5	6.5
10	3.81	5.05 d	3.96 dq	1.36 d	2.22	4	6.5
11[a]		4.10 d	3.77 dq	1.38 d		3	7
12[d]		4.05 d	3.59 dq	1.37 d		5	6.5
17		3.17 d	3.22 dq	1.40 d		2	5
18		4.31 d	3.77 dq	1.32 d		3.5	7
19		3.96 d	4.15 dq	1.28 d		5.5	6.5
20		5.12 d	3.87 dq	1.37 d	2.20	3.5	7
21		4.19 d	5.30 dq	1.30 d	2.10	4.5	6.5
22[a]		4.25 d	3.72 dq	1.28 d		3.5	7
23[c]		4.25 d	3.75 dq	1.24 d		3	6.5

[a] In CD_3OD. [b] δ 2.46 (3H, s, Ts(CH_3)). [c] The sample contains MeOH. [d] In D_2O. [e] $J_{2,OH}$=6 Hz.

of **8** with sodium azide in the presence of ammonium chloride in methanol (110°C in a sealed tube) gave methyl D-*threo*-3-azido-2-hydroxybutanoate (**9**) of 2*S*, 3*R* stereochemistry in a good yield. No 2-azido derivative was isolated. The presence of a hydroxyl group at C-2 was demonstrated by the ^{1}H NMR spectrum of the 2-*O*-acetyl derivative (**10**). Hydrolysis of **9** with acidic resin (to give **11**) followed by catalytic reduction gave the desired amino acid, D-*threo*-3-amino-2-hydroxybutanoic acid (**12**). Comparison of the physical data of **12** with those of the corresponding L-isomer[12] indicates that **12** and its L-isomer are the mirror of images each other as expected. (2*RS*,3*R*)-3-Amino-2-hydroxybutanoic acid[13] was reported as its L-leucyl peptide in the syntheses of bestatin-related compounds.

In order to couple the new amino acid (**12**) with kanamycin, the *N*-hydroxysuccinimide ester (**13**) of 3-azido-2-hydroxybutanoic acid (**11**) was prepared and coupled to 3,6′-bis(*N*-benzyloxycarbonyl)-2′,3′-dideoxy-3″-*N*-(trifluoroacetyl)kanamycin A[9] (**14**). The latter compound was prepared by the $Zn(OAc)_2$-CF_3COOEt method[14]. Coupling in the usual manner[9] followed by deblocking and hydrogenation as described[9,14] gave 1-*N*-(D-*threo*-3-amino-2-hydroxybutanoyl)-2′,3′-dideoxykanamycin A (**15**).

Next, preparation of (2*S*,3*S*)-3-amino-2-hydroxybutanoic acid (L-*erythro*-3-amino-2-hydroxybutanoic acid, **23**L) was tried for another coupling. Oxidation of ethyl (*E*)-crotonate (**16**) with *m*-chloroperbenzoic acid according to BALDWIN and FLEMING[15] gave a racemic mixture of the oxirane (**17**). Opening of the epoxide ring with sodium azide in the presence of ammonium chloride in boiling aqueous ethanol gave a mixture of racemic ethyl *erythro*-3-azido-2-hydroxybutanoate (**18**) and ethyl *erythro*-2-azido-3-hydroxybutanoate (**19**), which were chromatographically separated. The structures of **18** and **19** were confirmed by their *O*-acetyl derivatives (**20**, **21**): **20** showed a doublet (2-H) in low field (δ 5.12), whereas **21** gave a doublet of quartets (3-H) at δ 5.30 (see Table 1). This indicates that the acetoxyl groups of **20** and **21** are attached at C-2 and C-3, respectively. Acidic hydrolysis of **18**

Table 2. Antibacterial activity (MIC, μg/ml) of **15**, an equal mixture of **24a** and **24b**, and amikacin.

Test organisms[a]	**15**	**24a** and **24b**	Amikacin
Staphylococcus aureus FDA 209P	1.56	3.12	0.78
S. aureus Smith	0.78	3.12	0.78
S. aureus Ap01	3.12	6.25	3.12
Bacillus subtilis PCI 219	0.78	1.56	0.39
Escherichia coli K-12	3.12	6.25	0.78
E. coli K-12 ML 1629	6.25	25	3.12
E. coli K-12 LA290 R55	12.5	25	3.12
E. coli W677	3.12	6.25	0.78
E. coli JR66/W677	12.5	25	3.12
E. coli JR225	6.25	6.25	0.78
Mycobacterium smegmatis ATCC 607[b]	3.12	6.25	0.78
Klebsiella pneumoniae PCI 602	12.5	12.5	3.12
K. pneumoniae 22 No. 3038	25	25	6.25
Proteus vulgaris OX 19	6.25	12.5	1.56
P. rettgeri GN 311	6.25	3.12	0.78
Serratia marcescens	25	25	3.12
Providencia sp. Pv16	6.25	6.25	1.56
Pseudomonas aeruginosa A3	3.12	3.12	0.39
P. aeruginosa TI 13	25	50	3.12
P. aeruginosa GN 315	>100	>100	50

[a] Agar dilution streak method (Mueller-Hinton agar, 17 hours, 37°C).
[b] 48 hours.

gave the free acid (**22**), which by catalytic reduction, gave racemic (*SS* and *RR*) *erythro*-3-amino-2-hydroxybutanoic acid (**23**).

Since the separation of the racemete **18** or **22** to the optically pure isomers seemed difficult, the active ester of the racemic mixture (**22**) was prepared and coupled with **14** in the hope of obtaining two kanamycin derivatives of different mobility. However, both the direct coupling products and their deblocked (and hydrogenated) final products (**24a** and **24b**) had the same mobility. Thus a mixture of the L-*erythro*(2*S*,3*S*) (**24a**) and D-*erythro*(2*R*,3*R*) isomers (**24b**) in approximately equal amounts was obtained.

The antibacterial activities of **15**, the mixture of **24a** and **24b**, and amikacin are shown in Table 2. Although **24** is a mixture of the two diastereomers, it is estimated that **15** and **24** (possibly **24a**?) have approximately equal activities. Both compounds are less active than amikacin. This decreased activity in comparison to amikacin could be due to the difficulty of the C-3 amino group of the amino acid residue to face (by the terminal methyl group) to a suitable direction for optimum antibacterial activity irrespective of the absolute configurations at C-3.

Experimental

Optical rotations were measured with a Perkin-Elmer 241 polarimeter. TLC was carried out on Wakogel B-5 silica gel with detection by spraying with concentrated sulfuric acid, followed by slight heating. Column chromatography was performed on Wakogel C-200. IR spectra were measured with a Jasco A-202 grating spectrophotometer. ^{1}H NMR spectra were recorded at 250 and 90 MHz with a Bruker 250 and Varian EM-390 spectrometers, respectively.

Methyl L-*Threo*-2,3-di-*O*-acetyl-2,3,4-trihydroxybutanoate (**2**)

To an ice-cold solution of **1** (3.31 g) in oxolane (10 ml) was added, dropwise, under an atmosphere of nitrogen 1 M borane in oxolane (27 ml) and the mixture was stirred for 5 hours at 40°C. TLC of

the solution with $CHCl_3$ - MeOH - 28% aq NH_3 (3 : 1 : 0.1) gave two spots of Rf 0.75 (**2**) and 0.3 (minor, **1**). After the addition of water (1 ml) the solution was concentrated and the residue was extracted with $CHCl_3$. The solution was washed with 5% aq $NaHCO_3$ and water, dried ($MgSO_4$), and concentrated to give a syrup of **2**, 1.79 g (58%).

Methyl L-*Threo*-2,3,4-tri-*O*-acetyl-2,3,4-trihydroxybutanoate (**3**)

A mixture of **2** (28.4 mg) and acetic anhydride (0.3 ml) in pyridine (1 ml) was kept at room temp for 30 minutes. TLC of the solution with C_6H_6 - EtOAc (2 : 1) gave a single spot (**3**, Rf 0.8; *cf.* **2**: Rf 0.4). Addition of water (0.2 ml) followed by evaporation gave a syrup of **3**, 33.2 mg (99%): $[\alpha]_D^{22}$ −28° (*c* 1, MeOH) (literature[10] $[\alpha]_D^{20}$ −30° (MeOH)).

Methyl L-*Threo*-2,3-di-*O*-acetyl-4-bromo-2,3-dihydroxybutanoate (**4**)

A mixture of **2** (920 mg), triphenylphosphine (1.86 g), carbon tetrabromide (1.20 g) and pyridine (20 ml) was kept at room temp for 30 minutes. TLC of the solution with C_6H_6 - EtOAc (10 : 1) gave a spot at Rf 0.85 (**4**). After addition of MeOH (5 ml), the solution was concentrated and the product purified by column chromatography with C_6H_6 - EtOAc (30 : 1) to give a syrup of **4**, 745 mg (64%): $[\alpha]_D^{22}$ +20° (*c* 1.3, MeOH).

Anal Calcd for $C_9H_{13}BrO_6 \cdot \frac{1}{2}H_2O$: C 35.32, H 4.58, Br 26.11.
Found: C 35.41, H 4.45, Br 25.68.

Methyl L-*Threo*-2,3-di-*O*-acetyl-2,3-dihydroxybutanoate (**5**)

To a solution of **4** (400 mg) in toluene (4 ml) were added tributylstannane (560 mg) and AIBN (3 mg) and the mixture was stirred at 70°C under an atmosphere of nitrogen for 2.5 hours. Concentration gave a syrup that was extracted with acetonitrile (10 ml). The solution was washed with pentane (8 ml×2) and concentrated. Column chromatography of the residue with C_6H_6 - EtOAc (30 : 1) gave a syrup of **5**, 247 mg (84%): $[\alpha]_D^{22}$ +17° (*c* 1.1, MeOH).

Anal Calcd for $C_8H_{14}O_6$: C 49.54, H 6.47.
Found: C 49.33, H 6.41.

Methyl L-*Threo*-2,3-dihydroxybutanoate (**6**)

To a solution of **5** (79 mg) in MeOH (2 ml) was added 0.1 M sodium methoxide in MeOH (1 ml) and the solution was kept at room temp for 30 minutes. After introduction of CO_2, the reaction mixture was concentrated and the residue extracted with $CHCl_3$. Filtration followed by evaporation gave a syrup of **6**, 47.6 mg (98%): $[\alpha]_D^{22}$ +21° (*c* 1, MeOH).

Anal Calcd for $C_5H_{10}O_4 \cdot H_2O$: C 39.47, H 7.95.
Found: C 39.71, H 7.92.

Methyl L-*Threo*-2,3-dihydroxy-2-*O*-(*p*-toluenesulfonyl)butanoate (**7**)

To a solution of **6** (324 mg, 2.42 mM) in dry pyridine (15 ml) was added *p*-toluenesulfonyl chloride (460 mg, 2.40 mM) and the solution was kept at room temp for 1 hour. The resulting solution showed, on TLC with C_6H_6 - EtOAc (2 : 1), two spots of Rf 0.2 (**6**) and Rf 0.5 (**7**). Additional *p*-toluenesulfonyl chloride (230 mg, then 180 mg after further 1 hour) was added. After 3 hours, the solution showed three spots of **6**, **7** (major) and the di-*O*-tosyl derivative (Rf 0.85). Addition of water (1 ml) followed by concentration gave a residue that was extracted with $CHCl_3$. The solution was washed with 5% aq $NaHCO_3$, dried ($MgSO_4$), and concentrated. The products were separated by column chromatography with C_6H_6 - EtOAc (30 : 1) to give **7** as a solid, 281 mg (40%), **6**, 77 mg (24%), and the ditosylate, 115 mg (16%). **7**: $[\alpha]_D^{22}$ +33° (*c* 1.5, MeOH).

Anal Calcd for $C_{12}H_{16}O_6S$: C 49.98, H 5.59.
Found: C 50.25, H 5.52.

Methyl L-*Erythro*-2,3-anhydro-2,3-dihydroxybutanoate (**8**)

To a cold solution of **7** (53.7 mg) in MeOH (0.5 ml) was added 1 M sodium methoxide in MeOH (0.5 ml). After 5 minutes in the cold (0~5°C), the solution showed, on TLC with C_6H_6 - EtOAc (10 : 1), a single spot of Rf 0.5 (**8**; *cf.* **7**: Rf 0.1). Neutralization with 1 M HCl - MeOH followed by distillation gave a MeOH solution of **8**, 110 mg.

Methyl D-*Threo*-3-azido-2-hydroxybutanoate (**9**)

A mixture of a MeOH solution of **8** (110 mg, described in the preparation of **8**), sodium azide (52 mg), ammonium chloride (10 mg) and MeOH (1 ml) was heated in a sealed tube at 110°C for 1 hour. The resulting solution showed, on TLC with C_6H_6 - EtOAc (10 : 1), a single spot at Rf 0.35 (**9**). Concentration gave a residue, that was extracted with $CHCl_3$. Evaporation of the solvent (finally under 1 mmHg, 40°C bath temp) gave a syrup of **9**, 35.5 mg (56% based on **7**): $[\alpha]_D^{25}$ −97° (*c* 1, MeOH); IR (KBr) 2120 cm^{-1} (N_3).

Anal Calcd for $C_5H_9N_3O_3$: C 37.73, H 5.70, N 2.64.
Found: C 37.48, H 5.95, N 2.25.

Methyl D-*Threo*-2-*O*-acetyl-3-azido-2-hydroxybutanoate (**10**)

A mixture of **9** (15.2 mg) and acetic anhydride (0.2 ml) in pyridine (0.5 ml) was kept ar room temp for 30 minutes. TLC of the solution with C_6H_6 - EtOAc (10 : 1) gave a single spot of Rf 0.55. The usual work up gave a syrup of **10**, 18.0 mg (90%): $[\alpha]_D^{25}$ −97° (*c* 1.1, MeOH); IR (KBr) 2120 cm^{-1} (N_3); MS *m/z* 202 $(M+1)^+$.

D-*Threo*-3-azido-2-hydroxybutanoic Acid (**11**)

A mixture of **9** (26.6 mg) and Dowex 50W resin (H^+ form, 0.3 ml, swollen by water; used after the usual pretreatment) in oxolane - water (3 : 2, 0.5 ml) was refluxed for 1 hour. The supernatant layer showed, on TLC with $CHCl_3$ - MeOH - 28% aq NH_3 (3 : 1 : 0.1), a single spot of Rf 0.4 (**11**). Filtration followed by concentration gave a syrup of **11**, 21.6 mg (90%): $[\alpha]_D^{25}$ −88° (*c* 1, MeOH).

D-*Threo*-3-amino-2-hydroxybutanoic Acid (**12**)

A solution of **11** (100 mg) in 1,4-dioxane - water (1 : 1, 2 ml) and acetic acid (0.1 ml) was hydrogenated for 3 hours with palladium black at atmospheric pressure. TLC of the mixture with BuOH - EtOH - $CHCl_3$ - 17% aq NH_3 (4 : 4 : 2 : 3) showed a single spot (Rf 0.2). After filtration, the solution was concentrated and the residue was recrystallized from EtOH to give crystals of **12**, 65 mg (79%): MP 215~216°C (L-isomer of **12**: 215~216°C[12]); $[\alpha]_D^{26}$ −22° (*c* 1, H_2O) (L-isomer, +23.5°[12]), $[\alpha]_D^{26}$ −5° (*c* 1, 5 M HCl) (L-isomer, +5.5°[12]); CD $\Delta\varepsilon_{min}$ −0.19 (at 210 nm in H_2O), $\Delta\varepsilon_{max}$ +0.29 (at 210 nm in 0.5 M HCl).

Anal Calcd for $C_4H_9NO_3$: C 40.33, H 7.62, N 11.76.
Found: C 39.90, H 7.40, N 11.68.

1-*N*-(D-*Threo*-3-amino-2-hydroxybutanoyl)-2′,3′-dideoxykanamycin A (**15**)

A mixture of **11** (35.3 mg), *N*-hydroxysuccinimide (35 mg) and dicyclohexylcarbodiimide (56 mg) in cold (0~5°C) oxolane (1.8 ml) was kept for 30 minutes. TLC of the mixture with $CHCl_3$ - MeOH - 28% aq NH_3 (3 : 1 : 0.1) showed a spot of Rf 0.85 (**13**) with disappearance of that at Rf 0.4 (**11**). The mixture was filtered with aid of oxolane and the solution was directly used for the coupling with **14**. To a mixture of **14** (139 mg) and anhydrous sodium carbonate (15 mg) in oxolane - water (5 : 1, 1.2 ml) was added the above oxolane solution (~2 mol) containing **13** and the solution was stirred at room temp for 30 minutes. TLC of the solution with $CHCl_3$ - MeOH - 17% aq NH_3 (9 : 4 : 1) gave a spot of Rf 0.27 (coupled derivative) with disappearance of that (Rf 0.24) of **14**. Aq NH_3 28% (1 ml) was added and the solution was kept at room temp for 1 hour (de-trifluoroacetylation; Rf changed to 0.2). Concentration gave a residue that was washed with water. A solution of the residue and acetic acid (0.5 ml) in oxolane - water (10 : 3, 13 ml) was hydrogenated in the presence of palladium black at atmospheric pressure at room temp for 2 hours. The solution showed, on TLC with BuOH - EtOH - $CHCl_3$ - 17% aq NH_3 (4 : 5 : 2 : 5), a spot of Rf 0.42 (**15**). Filtration and concentration gave a residue that was chromatographed on a column of CM-Sephadex C-25 resin (NH_4^+ form, 30 ml). After washing with water (200 ml), aq NH_3 (0→0.5 M, gradually changed) eluted **15**, 45.2 mg (43% based on **14**, hemihydrate·hemicarbonate) as a solid: $[\alpha]_D^{22}$ +92° (*c* 1, H_2O); IR (KBr) 1640 cm^{-1} (amide), no peak was observed near 2100 cm^{-1}; ^1H NMR (D_2O - DCl, pD 1) δ 1.22 (3H, d, J=5.5 Hz, CH_3), 5.02 (1H, d, J=3 Hz, 1″-H), 5.24 (1H, br s, 1′-H).

Anal Calcd for $C_{22}H_{43}N_5O_{11}\cdot\frac{1}{2}H_2O\cdot\frac{1}{2}H_2CO_3$: C 45.51, H 7.63, N 11.79.
Found: C 45.71, H 7.47, N 11.95.

Racemic Ethyl *Threo*-2,3-anhydro-2,3-dihydroxybutanoate (**17D, 17L**)

Ethyl (*E*)-crotonate (**16**, 3.70 g) and *m*-chloroperbenzoic acid (7.0 g) were refluxed in dichloromethane (40 ml) for 3 hours. On checking by TLC with C_6H_6 - EtOAc (2:1), **16** (Rf ~0) disappeared and **17** was the only product (Rf 0.55). The solution, after washing with aq $NaHCO_3$ (saturated), was stirred with Na_2SO_3 (0.5 g) for 10 minutes, then washed with aq NaCl (saturated), dried ($MgSO_4$), and distilled to give a dichloromethane solution (6.50 g) containing **17** (estimated to be ~4 g): ^{1}H NMR ($CDCl_3$) δ 1.30 (3H, t, CH_3CH_2O), 4.20 (1H, dq, J_{vic}=7 and J_{gem}=10.5 Hz, CH_3CHHO), 4.24 (1H, dq, J_{vic}=7 Hz, CH_3CHHO).

Racemic Ethyl *Erythro*-3-azido-2-hydroxybutanoate (**18D, 18L**) and Racemic Ethyl *Erythro*-2-azido-3-hydroxybutanoate (**19D, 19L**)

A mixture of a dichloromethane solution of racemic **17** (6.50 g, described for **17**), sodium azide (6.81 g) and ammonium chloride (2.52 g) in EtOH (100 ml) and water (5 ml) was refluxed for 3 hours. The solution showed, on TLC with C_6H_6 - MeCOOEt (2:1), two spots of Rf 0.38 (**18D, 18L**) and Rf 0.26 (**19D, 19L**). Concentration gave a residue that was extracted with $CHCl_3$. The organic solution was concentrated and the residue chromatographed with C_6H_6 - EtOAc (100:1) to give a syrup of racemic **18** (**18D, 18L**), 2.15 g (38% based on **16**) and a syrup of racemic **19** (**19D, 19L**), 2.72 (48% based on **16**).

Racemate **18**: IR (KBr) 2120 cm^{-1} (N_3); MS *m/z* 174 $(M+1)^+$; ^{1}H NMR ($CDCl_3$) δ 1.36 (3H, t, CH_3CH_2O), 4.33 (2H, dq, CH_3CH_2O).

Racemate **19**: IR (KBr) 2120 cm^{-1} (N_3); MS *m/z* 174 $(M+1)^+$; ^{1}H NMR ($CDCl_3$) δ 1.34 (3H, t, CH_3CH_2O), 4.30 (2H, q, CH_3CH_2O).

Racemic Ethyl *Erythro*-2-*O*-acetyl-3-azido-2-hydroxybutanoate (**20D, 20L**)

Racemic **18** (104 mg) was treated with acetic anhydride (0.4 ml) in pyridine (2 ml) as described for **10** to give a syrup of racemic **20** (**20D, 20L**), 117 mg (91%): ^{1}H NMR ($CDCl_3$) δ 1.30 (3H, t, CH_3CH_2O), 4.24 (2H, q, CH_3CH_2O).

Anal Calcd for $C_6H_{13}N_3O_4$: C 44.65, H 6.09, N 19.53.
Found: C 44.51, H 5.95, N 19.50.

Racemic Ethyl *Erythro*-3-*O*-acetyl-2-azido-3-hydroxybutanoate (**21D, 21L**)

Racemic **19** (135 mg) was acetylated as described for **20** to give a syrup of racemic **21** (**21D, 21L**), 164 mg (98%): ^{1}H NMR ($CDCl_3$) δ 1.33 (3H, t, CH_3CH_2O), 4.28 (2H, q, CH_3CH_2O).

Anal Calcd for $C_6H_{13}N_3O_4$: C 44.65, H 6.09, N 19.53.
Found: C 44.38, H 6.22, N 19.41.

Racemic *Erythro*-3-azido-2-hydroxybutanoic Acid (**22D, 22L**)

A mixture of racemic **18** (374 mg) and Dowex 50W resin (H^+ form, 4 ml) in oxolane - water (5:3, 8 ml) was refluxed for 1 hour. Work-up as described for **11** gave a syrup of racemic **22**, 291 mg (93%).

Racemic *Erythro*-3-amino-2-hydroxybutanoic Acid (**23D, 23L**)

Racemic **22** (120 mg) was hydrogenated as described for **12** to give crystals of **23**, 75 mg (76%): MP 238~239°C.

Anal Calcd for $C_4H_9NO_3$: C 40.33, H 7.62, N 11.76.
Found: C 39.89, H 7.44, N 11.66.

1-*N*-(D- and L-*Erythro*-3-amino-2-hydroxybutanoyl)-2′,3′-dideoxykanamycin A (**24a, 24b**)

A mixture of **22** (80 mg), *N*-hydroxysuccinimide (72 mg) and dicyclohexylcarbodiimide (111 mg) in cold (0~5°C) oxolane (2.7 ml) was kept for 1 hour. The mixture was filtered to give a solution (2.5 ml) used in coupling with **14**. A mixture of **14** (241 mg), an oxolane solution containing **22** (2.5 ml, described above) and anhydrous sodium carbonate (28 mg) in oxolane - water (5:1, 2 ml) was kept at room temp for 30 minutes. TLC of the solution with $CHCl_3$ - MeOH - 17% aq NH_3 (9:4:1) gave a spot of Rf 0.28 (coupled derivatives) with disappearance of that (Rf 0.24) of **14**. De-trifluoroacetylation with 28% aq NH_3 (1.5 ml) followed by hydrogenation and successive purification of the

products by column chromatography as described for **15** gave a mixture of chromatographically homogeneous solid products of **24a** and **24b**, 60.2 mg (45% based on **14**, hemihydrate·hemicarbonate): $[\alpha]_D^{22}$ +95° (*c* 1, H_2O); IR (KBr) 1640 cm^{-1} (amide); ^1H NMR (D_2O - DCl, pD 1) δ 1.08 (~1.5H, d, J=7.5 Hz, CH_3), 1.13 (~1.5H, d, J=7.5 Hz, CH_3), 5.01 (~0.5H, d, J=3 Hz, 1″-H), 5.04 (~0.5H, d, J=3 Hz, 1″-H), 5.27 (1H, br s, 1′-H).

Anal Calcd for $C_{22}H_{43}N_5O_{11}\cdot\frac{1}{2}H_2O\cdot\frac{1}{2}H_2CO_3$: C 45.51, H 7.63, N 11.79.
Found: C 45.63, H 7.55, N 11.88.

Acknowledgment

We are grateful to Dr. MASA HAMADA and her group of the Institute of Microbial Chemistry for bioassays and to Mr. SABURO NAKADA of the Keio University for elemental analysis.

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