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Syntheses of 1-Epikanamycin A and Its 1-*N*-[(*S*)-4-Amino-2-hydroxybutyryl] Derivative

Yoshiaki TAKAHASHI, Tsutomu TSUCHIYA,* Yukiko SUZUKI, Sumio UMEZAWA, Hamao UMEZAWA,† and Shunzo FUKATSU††

Institute of Bioorganic Chemistry, 1614 Ida, Nakahara-ku, Kawasaki 211

†*Institute of Microbial Chemistry, Kamiosaki, Shinagawa-ku, Tokyo 141*

††*Central Research Laboratories, Meiji Seika Kaisha, Ltd, Morooka-cho, Kohoku-ku, Yokohama 222*

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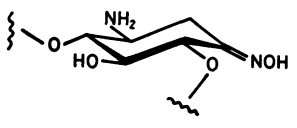
The titled compounds were prepared from 3,6′-bis(*N*-benzyloxycarbonyl)-3″-*N*-(trifluoroacetyl)kanamycin A (**1**). Oxidation of **1** with hydrogen peroxide in the presence of sodium tungstate gave the 1-hydroxyimino derivative, which, after deblocking, gave 1-deamino-1-dehydro-1-hydroxyiminokanamycin A (**6**). Reduction of **6** with Raney nickel–hydrogen in aqueous ammonia gave a mixture of kanamycin A and 1-epikanamycin A (**9**), which were separated through derivation to the corresponding tetrakis-*N*-(*t*-butoxycarbonyl) derivatives, which showed different solubility in chloroform. 1-*N*-[(*S*)-4-Amino-2-hydroxybutyryl]-1-epikanamycin A was prepared from **9** by the zinc acetate–ethyl trifluoroacetate method [to give 3,6′-bis(*N*-benzyloxycarbonyl)-1-epi-3″-trifluoroacetamidokanamycin A] followed by a regiospecific 1-*N*-acylation with (*S*)-4-benzyloxycarbonylamino-2-hydroxybutyryl group and successive deblocking.

Epimerization of a group at special position of amino glycoside antibiotics is of interest in connection with the role of the group in the antibacterial action. Recently we reported the synthesis of 3″-epidihydrostreptomycin,[1] which was active against resistant bacteria. In this paper we report on the epimerization of the 1-amino group of kanamycin A and the preparation of the 1-*N*-[(*S*)-4-amino-2-hydroxybutyryl] derivative of the epimer of kanamycin A. The (*S*)-4-amino-2-hydroxybutyryl side chain is known to enhance the antibacterial activity of kanamycin A as shown by amikacin.[2] Recently Igarashi *et al.*[3] prepared 1-deamino-1-epihydroxykanamycin A through a 1-oxo intermediate obtained by oxidation of a protected free 1-aminokanamycin A with 3,5-di-*t*-butyl-1,2-benzoquinone.[4]

As the starting material, we utilized 3,6′-bis(*N*-benzyloxycarbonyl)-3″-*N*-(trifluoroacetyl)kanamycin A (**1**),[5] which has a free amino group only at C-1, and prepared readily from kanamycin A by the zinc acetate–ethyl trifluoroacetate method.[5] Since oxidation of the 1-$CHNH_2$ group of **1** to ketoxime (–C=NOH) (not to carbonyl) is desirable for the present synthesis, **1** was treated with hydrogen peroxide in the presence of sodium tungstate according to the procedure by Kahr and Berther.[6] The desired 1-hydroxyimino derivative (**2**) was obtained in 86% yield. To confirm the structure, the compound (**2**) was led to the tris(*N*-benzyloxycarbonyl)-per-*O*-acetyl derivative (**5**). Deacylation of **2** with aqueous ammonia in oxolane gave the 3″-amino derivative (**3**), which, after reprotection of the amino group with *N*-(benzyloxycarbonyloxy)succinimide[2] [to give the 3,6′,3″-tris(*N*-benzyloxycarbonyl) derivative (**4**)], was acetylated with acetic anhydride in pyridine to afford the octaacetyl derivative (**5**). Compound **4** was also prepared directly from **6** in high yield by treating with *N*-(benzyloxycarbonyloxy)succinimide.

The presence of octaacetyl group in **5**, confirmed by the ^{1}H-NMR spectrum, indicated that all hydroxyl groups including the hydroxyimino group were acetylated. The position of the acetoxyimino group (at C-1) evident from the chemical pathway was further confirmed from the ^{1}H-NMR spectrum. The splitting pattern of each of the C-2-methylene protons (δ 3.00 and 3.56) was pure double doublets, one of them being clearly brought from geminal $^2J_{2a,2b}$ (=16.5 Hz) coupling, indicated that the methylene hydrogens had only one vicinal hydrogen (at C-3). This indicates that another adjacent group to the methylene is the C-acetoxyimino group. A doublet (J 6.5 Hz) was recognized at low field (δ 4.99), which is not assigned to H-1′ or -1″ from the J-value, should be assigned to a proton of the methine (C-6) adjacent to the carbon carring the acetoxyimino group, because only the two anomeric and the methine protons can show a doublet, respectively. Furthermore, the proton (H-6) was found to be coupled to a proton, resonating at low field (δ 5.80) as a triplet (J 6.5 Hz, H-5); this indicates that the hydroxyl group at C-5 is acetylated. The above-described result was rather unexpected because, in tetrakis(*N*-benzyloxycarbonyl)-4″,6″-*O*-cyclohexylidenekanamycin A[7] and other related compounds, 5-*O*-acetylation scarcely occurred under reaction conditions similar to that described here. This easy acetylation may be ascribed to the conformation of the aminocyclitol portion of **4**, which is expected not to be chair as in the case of **5** (see ^{1}H-NMR data in Experimental section).

Hydrogenolysis of the *N*-benzyloxycarbonyl groups of **3** in the presence of palladium black gave the 1-(hydroxyimino)kanamycin A (**6**), in 83% yield (based on **2**), with slight kanamycin A. The presence of the hydroxyimino group was supported by appearance of a resonance at low field (δ 154 ppm, C-1) in the ^{13}C-NMR spectrum (see Table 1). Non-chair conformation of the aminocyclitol portion of **6** was also revealed from the ^{1}H-NMR data. This discrepancy from chair conformation, judged from the J values, will be originated from instability of the chair form I caused by interaction between coplaner



I (unstable)

6-hydroxyl and 1-hydroxyimino groups.[8,9] Possible conformation would be that of slightly flattered skew with 5- and 6-hydroxyl groups pseudoaxial.

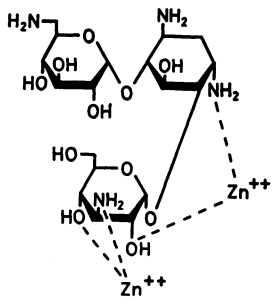
Attempts to produce 1-epikanamycin A (**9**) from **6** by catalytic hydrogenation with palladium catalyst under several conditions failed, only giving kanamycin A. However, when **6** was hydrogenated in the presence of Raney nickel in aqueous ammonia, a mixture of kanamycin A and 1-epikanamycin A (**9**) was produced in a ratio approximately 2 : 1. As the direct separation of the products was proved difficult, several *N*-protected derivatives were prepared to seek suitable derivatives for the separation. Among them, a mixture of tetrakis-(*N*-*t*-butoxycarbonyl) derivatives, prepared by treating the mixture of kanamycin A and 1-epikanamycin A with *O*-*t*-butyl *S*-(4,6-dimethyl-2-pyrimidinyl) thiocarbonate[10] (Boc-*S* reagent), was found successful; the tetra-*N*-Boc-kanamycin A (**7**) and tetra-*N*-Boc-1-epikanamycin A (**8**) were separated by the difference of solubility in chloroform.

Deblocking of **7** gave kanamycin A identical with the authentic sample, and deblocking of **8** gave 1-epikanamycin A (**9**). The 1-epi structure of **9** was established by the small *J*-values, in its ^{1}H-NMR spectrum, relating to H-1 (the aminocyclitol portion took chair form). In the ^{13}C-NMR spectrum, large up-field shifts were observed at C-1 and -6, in comparison to the corresponding shifts of kanamycin A (Table 1).

Table 1. The ^{13}C chemical shifts[a] of kanamycin A[b] (KMA), 1-deamino-1-dehydro-1-hydroxyimino-kanamycin A (**6**), 1-epikanamycin A (**9**), and 1-*N*-[(*S*)-4-amino-2-hydroxybutyryl]-1-epikanamycin A (**14**) measured in 20% ND_3 in D_2O

Carbon	KMA	6	9	14
1	51.3	154.4	45.6[d]	44.8
2	36.3	28.3	34.5	32.8
3	49.8	50.0	47.4[d]	48.0
4	88.4	85.0[e]	88.8[e]	87.8[e]
5	74.8	74.9[e]	72.1[f]	72.9[e]
6	88.6	78.6[e]	79.0[e]	76.2
1′	100.5	98.8[e]	100.8[e]	100.7
2′	72.5	72.5[d]	72.8	72.7[e]
3′	73.6[e]	73.6[d′]	73.7[g]	73.6[h]
4′	71.8	72.0[d]	71.8[d′]	71.8
5′	73.9[e]	73.9[d′]	73.9[g]	73.9[h]
6′	42.6	42.7	42.6	42.6
1″	100.8[e]	97.2	95.4	95.3
2″	72.7[e]	72.5[d]	72.1[e]	72.0
3″	55.1	55.3	54.7	54.8
4″	70.0	70.2	70.4[d′]	70.3
5″	73.0	73.4[d′]	72.8[f]	72.9
6″	61.0	61.4	61.3	61.3
1‴				177.3
2‴				70.5[e]
3‴				37.1
4‴				37.8[e]

a) In ppm downfield from TMS calculated as $\delta^{TMS} = \delta^{dioxane} + 67.4$ ppm. b) Shift assignments were based on the shifts of kanamycin A reported[11]. Selective irradiation at (denoted c) or near (denoted d or d′; carried out when c was inadequate) the resonance of the proton attached to the carbon, respectively. e—h) The values cited may be reversed, respectively.



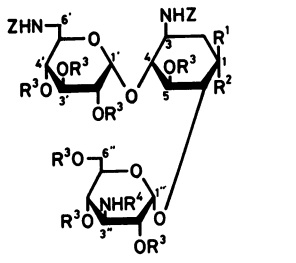
II

The 1-epikanamycin A (**9**) was led to 1-*N*-[(*S*)-4-amino-2-hydroxybutyryl]-1-epikanamycin A (**14**). For that, selective protections of the 3-, 6′-, and 3″-amino groups of **9** are requisite. This was performed by applying the zinc acetate-ethyl trifluoroacetate method.[5] In spite of the 1-epiamino structure of **9**, zinc ions completely protected the 1- and 3″-amino groups as in the case of kanamycin A. If Nagabhushan's proposal[11] for nickel, copper and cobalt is applied to this case, zinc chelation such as II may be considered as a candidate. Treatment of **9** with *N*-(benzyloxycarbonyloxy)succinimide[2] in the presence of zinc acetate in dimethyl sulfoxide gave 3,6′-bis(*N*-benzyloxycarbonyl)-1-epikanamycin A (**10**) in 83% yield after purification. The 3″-amino protection of **10** was also readily carried out by using ethyl trifluoroacetate to give the corresponding 3″-trifluoroacetamide derivative (**11**) in almost quantitative yield.

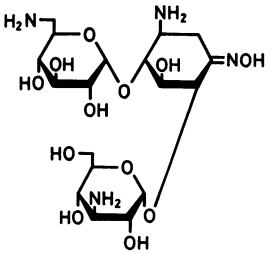
Coupling (*S*)-4-benzyloxycarbonylamino-2-hydroxybutyric acid to the free amino group at C-1 of **11** was readily performed by utilizing the active ester[2] of the acid to give the product (**12**) in 79% yield. De(trifuoroacetyl)ation of **12** (to give **13**) followed by catalytic de(benzyloxycarbonyl)ation gave the final product (**14**) in 92% yield. The structure of **14** was elucidated by the ^{1}H-NMR spectral data. The resonances of H-1 proton, confirmed by the decoupling method, appeared at δ 4.51 as a narrow quartet, the shift-value being fairly high in comparison to that (δ 3.5) of 1-epikanamycin A (**9**); this difference in shift-value indicates that the 1-amino group of **9** was acylated as expected. The ^{13}C-NMR spectrum of **14** was shown in Table 1.

Table 2. Minimal inhibitory concentration (μg/ml) of **9**, **14**, and kanamycin A

	Kanamycin A	9	14
Staphylococcus aureus FDA 209P	0.78	3.12	12.5
Staphylococcus aureus AP01	25	>100	100
Micrococcus luteus PCI 1001	25	>100	>100
Bacillus subtilis NRRL B-558	0.39	3.12	3.12
Escherichia coli K-12	0.78	6.25	12.5
Escherichia coli K-12 ML1629	>100	>100	50
Salmonella typhi T-63	0.39	3.12	25
Pseudomonas aeruginosa A3	25	3.12	25



	R^1	R^2	R^3	R^4
1	NH_2	H	H	$COCF_3$
2	=NOH		H	$COCF_3$
3	=NOH		H	H
4	=NOH		H	Z
5	=NOAc		Ac	Z



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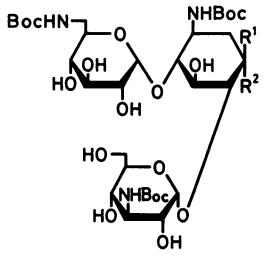
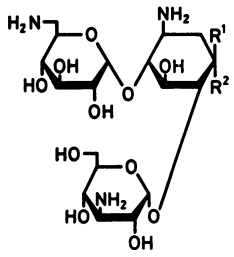
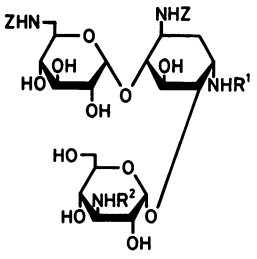


	R^1	R^2
7	NHBoc	H
8	H	NHBoc



	R^1	R^2
Kanamycin A	NH_2	H
9	H	NH_2
14	H	(S) $NHCOCHCH_2CH_2NH_2$, OH



	R^1	R^2
10	H	H
11	H	$COCF_3$
12	(S) $COCHCH_2CH_2NHZ$, OH	$COCF_3$
13	(S) $COCHCH_2CH_2NHZ$, OH	H

Z: $CO_2CH_2C_6H_5$,
Boc: $CO_2C(CH_3)_3$

Antibacterial spectra of **9** and **14** were shown in Table 2 with the spectrum of kanamycin A. This result shows that epimerization of the 1-amino group of kanamycin A greatly reduced the original activity. 1-(Hydroxyimino)kanamycin A (**6**) was almost devoid of antibacterial activity.

Experimental

Specific rotations were measured with a Perkin–Elmer Model 241 polarimeter. ^{1}H- and ^{13}C-NMR spectra were recorded in the FT mode with a Bruker WM 250 spectrometer operating at 250 and 62.9 MHz, respectively. In the ^{1}H-NMR spectra, tetramethylsilane (δ=0) was used as the internal standard in both organic solvents and deuterium oxide. Thin-layer chromatography (TLC) was carried out on E. Merck precoated silica gel 60 plates with the spray of sulfuric acid or 0.5% ninhydrin in pyridine for detection. For silica-gel column chromatography, Wakogel C-200 was used.

*3,6′-Bis(*N*-benzyloxycarbonyl)-1-deamino-1-dehydro-1-hydroxyimino-3″-*N*-(trifluoroacetyl)kanamycin A (**2**).* To a solution of **1** (7.73 g, as the base) and sodium tungstate dihydrate (0.6 g) in aqueous oxolane (1 : 2, 160 ml) was added 30% aqueous hydrogen peroxide (8.8 ml), and the solution was kept at room temperature overnight. On checking by TLC with chloroform–methanol–20% aqueous acetic acid (1 : 1.2 : 1, lower layer), the solution showed a single spot at R_f 0.4 (*cf* **1**: R_f 0.25). Concentration of the pale-yellow solution gave a residue, which was washed with water, dried, and washed with ether to give a solid, 6.76 g (86%), $[\alpha]_D^{24}$ +100° (*c* 2, *N*,*N*-dimethylformamide).

^{1}H-NMR (pyridine-d_5–D_2O=30 : 1): δ=4.00 (1H dd, $J_{1',2'}$ 3.5, $J_{2',3'}$ 9.5 Hz, H-2′), 4.26 (1H dd, $J_{1'',2''}$ 3.5, $J_{2'',3''}$ 10.5 Hz, H-2″), 5.08 (1H t, $J_{3'',4''}$ 10.5 Hz, H-3″), 5.42 (1H d, H-1″), 5.65 (1H d, H-1′).

Found: C, 50.09; H, 5.36; N, 6.48; F, 6.47%. Calcd for $C_{36}H_{45}F_3N_4O_{17}$: C, 50.12; H, 5.26; N, 6.49; F, 6.61%.

*3,6′,3″-Tris(*N*-benzyloxycarbonyl)-1-deamino-1-dehydro-1-hydroxyiminokanamycin A (**4**).* A solution of **2** (6.38 g) in a mixture of 2 M (1 M=1 mol dm^{-3}) aqueous ammonia–oxolane (1 : 1, 250 ml) was kept at room temperature overnight. The solution showed a single spot at R_f 0.2 (*cf* **2**: R_f 0.3), on TLC with chloroform–methanol–28% aqueous ammonia (1 : 1 : 1, lower layer). Concentration gave a crude solid of **3** (6.58 g). To a solution of the solid (100 mg) in aqueous *N*,*N*-dimethylformamide (1 : 2, 3 ml) was added *N*-(benzyloxycarbonyloxy)-succinimide[2] (32 mg) and the solution was kept for 1 h at room temperature. Concentration gave a syrup, which was washed with water, then with ether to give a chromatographically homogeneous solid, 88 mg (85% based on **2**), $[\alpha]_D^{24}$ +95° (*c* 1, *N*,*N*-dimethylformamide).

Found: C, 55.13; H, 5.78; N, 6.11%. Calcd for $C_{42}H_{52}N_4O_{18}\cdot H_2O$: C, 54.90; H, 5.92; N, 6.10%.

*1-Acetoxyimino-5,2′,3′,4′,2″,4″,6″-hepta-*O*-acetyl-3,6′,3″-tris(*N*-benzyloxycarbonyl)-1-deamino-1-dehydrokanamycin A (**5**).* To a solution of **4** (140 mg) in pyridine (2.8 ml) was added acetic anhydride (0.3 ml), and the solution was kept overnight at room temperature. Addition of water (0.3 ml) followed by standing for 0.5 h at room temperature and concentration of the solution gave a syrup, which was dissolved in chloroform. The solution was then processed in a usual manner to give a crude product, which was passed through a short column of silica gel with chloroform–ethyl methyl ketone (3 : 1) to give a pure solid of **5**, 147 mg (78%), $[\alpha]_D^{23}$ +90° (*c* 1, chloroform).

^{1}H-NMR (pyridine-d_5): δ=2.00, 2.01, 2.03, 2.05 (6H), 2.16, 2.17, 2.24 (each s, Ac); 3.00 (1H dd, $J_{2a,2e}$ 16.5, $J_{2a,3}$ 11.5 Hz, H-2a), 3.56 (1H dd, $J_{2e,3}$ 5 Hz, H-2e), 4.99 (1H d, $J_{5,6}$ 6.5 Hz, H-6), 5.80 (1H t, $J\approx$6.5 Hz, H-5); Irradiation of H-6 collapsed the triplet of H-5 to a doublet, and irradiation of H-5 caused the signals at $\delta\approx$4.70 (H-4) to simplify.

Found: C, 56.31; H, 5.54; N, 4.53%. Calcd for $C_{58}H_{68}N_4O_{26}$: C, 56.06; H, 5.75; N, 4.42%.

*1-Deamino-1-dehydro-1-hydroxyiminokanamycin A (**6**).* A solution of crude **3** (6.20 g; described in the preparation of **4**) in aqueous oxolane (1 : 2, 300 ml) was hydrogenated in the presence of palladium black and acetic acid (5 ml) under atmospheric pressure of hydrogen for 2 h at room temperature. After filtration, the solution was neutralized with 28% aqueous ammonia (5 ml), and concentrated to give a syrup. Separation of the products by column chromatography of the syrup

with CM-Sephadex C-25 with aqueous ammonia (0→0.15 M, the concentration was gradually changed) gave a solid of **6** (from the earlier frations), 3.06 g (83% as the hemicarbonate, based on **2**) and a solid of kanamycin A (from the later fractions), 0.17 g (4.7% as the hemicarbonate, based on **2**). **6**: $[\alpha]_D^{22}$ +180° (*c* 1, water); ^{1}H-NMR (20% ND_3 in D_2O): δ= 2.06 (1H dd, H-2a), 2.74 (1H dd, H-6'a), 3.00 (1H dd, H-6'b), 3.04 (1H t, H-3″), 3.09 (1H dd, H-2e), ≈3.17 (1H m, H-3), 3.25 (1H t, H-4″), 3.27 (1H t, H-4'), 3.41 (1H dd, H-2″), 3.53 (1H dd, H-4), 3.56 (1H dd, H-2'), 3.69 (1H t, H-3'), 3.92 (1H t, H-5), 4.24 (1H d, H-6), 4.92 (1H d, H-1″), 5.35 (1H d, H-1'); $J_{2a,2e}$ 15.0, $J_{2a,3}$ 11.0, $J_{2e,3}$ ≈4.5, $J_{3,4}$ ≈9, $J_{4,5}=J_{5,6}$ 6.0, $J_{1',2'}$ 3.8, $J_{2',3'}=J_{3',4'}=J_{4',5'}$ 9.5, $J_{5',6'a}$ 7.5, $J_{5',6'b}$ 2.5, $J_{6'a,6'b}$ 13.5, $J_{1'',2''}$ 3.8, $J_{2'',3''}=J_{3'',4''}=J_{4'',5''}$ 10.0 Hz.

Found: C, 42.28; H, 6.70; N, 10.43%. Calcd for $C_{18}H_{34}N_4O_{12}\cdot 1/2\,H_2CO_3$: C, 42.04; H, 6.48; N, 10.60%.

*1,3,6',3″-Tetrakis(*N-t-*butoxycarbonyl)kanamycin A (**7**) and 1,3,6',3″-Tetrakis(*N-t-*butoxycarbonyl)-1-epikanamycin A (**8**).* A solution of **6** hemicarbonate (2.00 g) in 1 M aqueous ammonia (40 ml) was shaken in the presence of Raney nickel under 3 atmospheric pressure of hydrogen for 5 h at room temperature. On checking by TLC with chloroform–ethanol–1-propanol–17% aqueous ammonia (2 : 7 : 4 : 7), the solution showed a single spot at R_f 0.16 (*cf* **6**: R_f 0.23). Filtration followed by concentration gave a residue, which was passed through a column of CM-Sephadex C-25 with aqueous ammonia (concentration gradually changed from 0 to 0.15 M) and the ninhydrin-positive fractions were concentrated to give a mixture (1.21 g) of kanamycin A and 1-epikanamycin A (**9**).

To an aqueous solution (4 ml) of the mixture (1.00 g) were added triethylamine (3.5 ml) and Boc-*S* reagent (3.0 g, dissolved in 8 ml 1,4-dioxane), and the mixture was stirred for 24 h at 40°C. Concentration gave a residue, which was extracted with chloroform. The nonextractable residue (1.05 g), which gave R_f 0.47 on TLC with chloroform–methanol–20% aqueous acetic acid (1 : 1 : 1, lower layer) was washed successively with acetone to give a solid of **7**, 0.97 g (34%), $[\alpha]_D^{23}$ +79° (*c* 1, pyridine); Found: C, 50.44; H, 7.54; N, 6.46%. Calcd for $C_{38}H_{68}N_4O_{19}\cdot H_2O$: C, 50.54; H, 7.81; N, 6.20%.

The chloroform layer was washed with 0.5 M aqueous sodium hydroxide to remove the concomitant 4,6-dimethyl-2-pyrimidinethiol, and then with 3% aqueous sodium sulfate, dried over sodium sulfate, and concentrated to give a solid of **8**, 0.51 g (18%), which gave R_f 0.55 on TLC with the above-described developing mixture, $[\alpha]_D^{23}$ +81° (*c* 1, pyridine); Found: C, 50.79; H, 7.55; N, 6.16%. Calcd for $C_{38}H_{68}N_4O_{19}\cdot H_2O$: C, 50.54; H, 7.81; N, 6.20%.

*1-Epikanamycin A (**9**).* A solution of **8** (1.74 g, as the monohydrate) in aqueous trifluoroacetic acid (1 : 19, 17 ml) was kept for 30 min at room temperature. Evaporation *in vacuo* at room temperature with several additions of toluene gave a residue, which was charged on a column of CM-Sephadex C-25 and developed with aqueous ammonia (0→0.15 M) to give **9** as the carbonate, 960 mg (91%), $[\alpha]_D^{25}$ +166° (*c* 2, water); ^{1}H-NMR (20% ND_3 in D_2O): δ=1.45 (1H slightly deformed dt, H-2a), 1.95 (1H dt, H-2e), 2.74 (1H dd, H-6'a), 2.95 (1H dd, H-6'b), 3.10 (1H t, H-3″), ≈3.11 (1H m, H-3), 3.26 (1H t, H-4'), 3.28 (1H t, H-4), 3.28 (1H t, H-4″), 3.45 (1H dd, H-2″), ≈3.48 (1H narrow m, H-1), 3.55 (1H dd, H-2'), 3.63 (1H dd, H-6), 3.67 (1H t, H-3'), ≈3.75 (1H m, H-5'), 3.92 (1H t, H-5), 4.97 (1H d, H-1″), 5.32 (1H d, H-1'); $J_{1,2a}=J_{1,2e}$≈3.5, $J_{1,6}$ 4.0, $J_{2a,2e}$ 14.0, $J_{2a,3}$ ≈13, $J_{2e,3}$ ≈3.5, $J_{3,4}=J_{4,5}=J_{5,6}$ 10, $J_{1',2'}$ 3.8, $J_{2',3'}=J_{3',4'}=J_{4',5'}$ 9.5, $J_{5',6'a}$ 7.5, $J_{5',6'b}$ 2.5, $J_{6'a,6'b}$ 13.5, $J_{1'',2''}$ 3.8, $J_{2'',3''}=J_{3'',4''}=J_{4'',5''}$ 10 Hz.

Found: C, 41.91; H, 7.27; N, 9.98%. Calcd for $C_{18}H_{36}N_4O_{11}\cdot H_2CO_3$: C, 41.76: H, 7.01; N, 10.25%.

*3,6'-Bis(*N-*benzyloxycarbonyl)-1-epikanamycin A (**10**).* To a suspended mixture of **9** (100 mg, as the carbonate) in dimethyl sulfoxide (1.5 ml, dried over molecular sieves 4A) was added zinc acetate dihydrate (180 mg), and the mixture was stirred overnight at room temperature. To the resulting clear solution was gradually added *N*-(benzyloxycarbonyloxy)-succinimide (105 mg) and the mixture was kept for 1 h at room temperature. Addition of ether gave a syrup, which was thoroughly washed with ether. The resulting syrup was charged on a column of Amberlite CG 50 (20 ml, an equal-volume mixture of H^+ and NH_4^+ forms was used) and, after washing the column with aqueous oxolane (1 : 1), the product was developed with aqueous oxolane (1 : 1) containing ammonia (the concentration gradually changed from 0.5 to 1 M). The fractions containing **10** [checked by TLC with chloroform–methanol–aqueous 20% acetic acid=5 : 7 : 5 (lower layer) giving R_f 0.32] were concentrated to a volume of ≈5 ml and further concentrated with occasional additions of water and occasional introductions of carbon dioxide (without this procedure, intermolecular *N*-acyl migration or 1,3-ureylene compound[12] may occur to give a contaminated mixture) to give a zinc-ion free (tested by diphenylcarbazide-ammonia[13]) solid of **10** as the carbonate, 124 mg (83%), $[\alpha]_D^{24}$ +95° (*c* 1, aqueous oxolane=1 : 2).

Found: C, 51.56; H, 6.16; N, 6.84%. Calcd for $C_{34}H_{48}N_4O_{15}\cdot H_2CO_3$: C, 51.59; H, 6.19; N, 6.88%.

*3,6'-Bis(*N-*benzyloxycarbonyl)-1-epi-3″-*N-*(trifluoroacetyl)kanamycin A (**11**).* To a solution of **10** (130 mg, as the carbonate) in dry dimethyl sulfoxide (0.8 ml) was added ethyl trifluoroacetate (0.025 ml) and the solution was kept for 20 min at room temperature. On checking by TLC with chloroform–methanol–aqueous 20% acetic acid=5 : 7 : 5 (lower layer), the solution showed a single spot at R_f 0.5. Addition of ether gave a syrup, which was thoroughly washed with ether. The syrup was dissolved in small volume of methanol (0.5 ml) and precipitated by addition of ether. The procedure was repeated twice more to give a dimethyl sulfoxide-free solid of **11**, 137 mg (99%), $[\alpha]_D^{23}$ +93° (*c* 1, methanol).

Found: C, 50.27; H, 5.90; N, 6.19; F, 6.84%. Calcd for $C_{36}H_{47}F_3N_4O_{16}\cdot 1/4\,H_2CO_3$: C, 50.38; H, 5.54; N, 6.48; F, 6.59%.

*3,6'-Bis(*N-*benzyloxycarbonyl)-1-*N-*[(*S*)-4-benzyloxycarbonylamino-2-hydroxybutyryl]-1-epi-3″-*N-*(trifluoroacetyl)kanamycin A (**12**).* To a solution of **11** (100 mg) in aqueous oxolane (1 : 1, 3 ml) was gradually added *N*-hydroxysuccinimide ester[2] (83 mg) of (*S*)-4-benzyloxycarbonylamino-2-hydroxybutyric acid dissolved in oxolane (1.5 ml), and the mixture was kept at room temperature for 30 min. The resulting acidic solution was neutralized with sodium carbonate (13 mg) and then concentrated. The resulting residue, after being washed with ether, was dissolved in water-saturated 1-butanol (the solid was scarcely soluble in usual organic solvents immiscible with water) and the solution was washed with 1-butanol-saturated aqueous sodium hydrogencarbonate solution (saturated), and 1-butanol-saturated water to remove inorganic impurities. Concentration of the butanol solution gave a residue, which was chromatographed on a silica-gel column with chloroform–methanol=6 : 1 to give a solid of **11**, 98.5 mg (79%), $[\alpha]_D^{25}$ +67° (*c* 1, methanol).

Found: C, 53.11; H, 5.70; N, 6.33; F, 5.16%. Calcd for $C_{48}H_{60}F_3N_5O_{20}$: C, 53.18; H, 5.58; N, 6.46; F, 5.26%.

*1-*N-*[(*S*)-4-Amino-2-hydroxybutyryl]-1-epikanamycin A (**14**).* A solution of **12** (170 mg) in mixture of 2 M aqueous ammonia–oxolane (1 : 1, 8.5 ml) was kept overnight at room temperature. On checking by TLC with chloroform–methanol–20% aqueous acetic acid (1 : 1 : 1, lower layer), the solution showed a single spot of **13** at R_f 0.15 (*cf* **12**: R_f 0.32). Concentration

gave a residue, which was dissolved in aqueous oxolane (1 : 1, 8 ml) containing acetic acid (1.0 ml), and the solution was hydrogenated in the presence of palladium black under atmospheric pressure of hydrogen for 2 h at room temperature. The resulting product was isolated by column chromatography on CM-Saphadex C-25 with aqueous ammonia (0→0.5 M) to give a solid of **14** as the carbonate, 93 mg (92%), $[\alpha]_D^{23}$ +128° (*c* 1, water); ^{1}H-NMR (20% ND_3 in D_2O): δ=1.52 (1H slightly deformed dt, H-2a), 1.75 (1H apparent sextet, $J\approx$7.5, $\approx$7.5, $\approx$7.5, and 14.5 Hz, H-3″a), 1.90 [1H two dt, J=4, $\approx$7.5, $\approx$7.5, and 14.5 (=$J_{3'''a,3'''b}$) Hz, H-3‴b], 2.09 (1H br dt, H-2e), 2.74 (2H t, J7.5 Hz, H-4″ a,b), $\approx$2.97 (H-3), 2.75 (1H dd, H-6′a), 2.98 (1H dd, H-6′b), 3.04 (1H t, H-3″), 3.27 (1H t, H-4″), 3.28 (1H t, H-4′), 3.36 (1H t, H-4), 3.39 (1H dd, H-2″), 3.57 (1H dd, H-2′), 3.69 (1H t, H-3′), $\approx$3.75 (H-5′), 3.82 (dd, H-6), 3.91 (1H t, H-5), 4.22 (1H dd, $J_{2''',3'''a}$ 8.5, $J_{2''',3'''b}$ 4.0 Hz,H-2‴), 4.51 (1H br q, $J\approx$4 Hz, H-1), 4.91 (1H d, H-1″), 5.35 (1H d, H-1′); $J_{1,2a}$=$J_{1,2e}$ $\approx$3.5, $J_{1,6}\approx$4, $J_{2a,2e}$ 14.0, $J_{2a,3}$ $\approx$12, $J_{2e,3}$ $\approx$3.5, $J_{3,4}$ $\approx$9, $J_{4,5}$=$J_{5,6}$ 9.5, $J_{1',2'}$ 3.8, $J_{2',3'}$=$J_{3',4'}$=$J_{4',5'}$ 9.5, $J_{5',6'a}$ 7.5, $J_{5',6'b}$ 2.5, $J_{6'a,6'b}$ 13.5, $J_{1'',2''}$ 3.5, $J_{2'',3''}$=$J_{3'',4''}$=$J_{4'',5''}$ 10 Hz.

Found: C, 42.62; H, 7.10; N, 10.65%. Calcd for $C_{22}H_{43}N_5O_{13}\cdot H_2CO_3$: C, 42.65; H, 7.00; N, 10.81%.

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