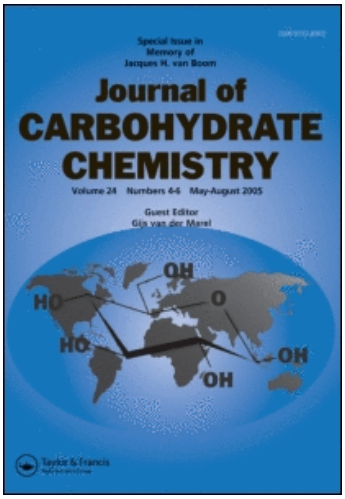

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Partial Protection of Carbohydrate Derivatives. Part 13.[1] Chemical Conversion of Kanamycin A Into 2'-Deoxykanamycin A, 2'-Epi-Kanamycin A, and 2'-Epi-Kanamycin B

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PARTIAL PROTECTION OF CARBOHYDRATE DERIVATIVES. PART 13.[1] CHEMICAL CONVERSION OF KANAMYCIN A INTO 2'-DEOXYKANAMYCIN A, 2'-EPI-KANAMYCIN A, AND 2'-EPI-KANAMYCIN B

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ABSTRACT

Hydrazinolyses of hexa-O-benzoyl-tetra-N-benzyloxycarbonyl- and N-ethoxycarbonylkanamycin A were performed and found to be sufficiently regioselective to give the corresponding 2'-hydroxyl derivatives in good yields under controlled conditions. The products were converted into the corresponding 2'-triflates, which were then subjected to nucleophilic substitution reactions with sodium benzenethioxide, sodium benzoate, and sodium azide to give the corresponding D-mannopyranosyl derivatives in good yields. Deprotection of the phenylthio (10) and azido (12) derivatives, and hydrogenolysis, gave 2'-deoxykankmycin A and 2'-epi-kanamycin B, respectively. Moreover, deprotection of the benzoyl compound 11 gave 2'-epi-kanamycin A.

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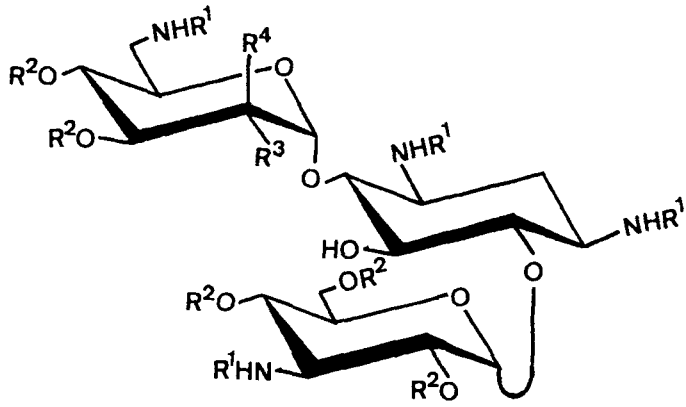
INTRODUCTION

A large number of analogues of aminocyclitol antibiotics have been prepared in an effort to obtain compounds which are safer, more potent, and broader in their spectum of activity. Development of new compounds which are effective against resistant bacteria is particularly important. Chemical modification of dibekacin,[2] amikacin,[3] and tobramycin[4] represent examples of successful attempts to produce semi-synthetic antibiotics. Similar modifications of kanamycin A at the 2'-position have not been achieved because an appropriate procedure for obtaining a free hydroxyl group at this position has been lacking. Recently, we have established a novel procedure for regioselective O-deacylation of fully acylated ribonucleosides by treating them with hydrazine hydrate in acetic acid-pyridine[5] or with hydroxylammonium acetate in pyridine.[6] A similar 2-position deprotection of fully acylated, methyl glycosides has been achieved using hydrazine hydrate in pyridine.[7] These procedures give, in general, the corresponding 2- or 2'-hydroxyl derivatives of glycosyl compounds in good yields. We also have reported recently triflate displacement from several methyl 2-O-trifluoromethylsulfonyl-α-D-glucopyranoside derivatives.[8] Substitution at the 2-position in glucopyranoside derivatives is difficult to perform.[9] Drawing upon a combination of the chemical reactions described above, we have developed a new approach to the chemical modification of kanamycin A at the 2'-position.

RESULTS AND DISCUSSION

Treatment of kanamycin A derivatives 1 and 2 with 2 molar equivalents of hydrazine hydrate in pyridine at room temperature for 24 h gave the corresponding 2'-hydroxyl derivatives 3 (65% yield) and 4 (67% yield), respectively. In order to confirm the position of the free hydroxyl group, 3 was subjected to the treatment as follows: methylsulfonylation with methanesulfonyl chloride in



	R^1	R^2	R^3	R^4
1:	-CO-OBn	Bz	OBz	H
2:	-CO-OEt	Bz	OBz	H
3:	-CO-OBn	Bz	OH	H
4:	-CO-OEt	Bz	OH	H
5:	-CO-OBn	Bz	OTf	H
6:	-CO-OEt	Bz	OTf	H
7:	-CO-OBn	H	H	SPh
8:	-CO-OBn	H	H	OBz
9:	-CO-OBn	H	H	N_3
10:	-CO-OEt	Bz	H	SPh
11:	-CO-OEt	Bz	H	OBz
12:	-CO-OEt	Bz	H	N_3
13:	H	H	H	H
14:	H	H	H	OH
15:	H	H	H	NH_2

pyridine at 0°C, debenzoylation with saturated methanolic ammonia at room temperature, hydrogenolysis with hydrogen on palladium black, chromatography on a column of CM sephadex C-25 with 0.3 M aqueous ammonia. This procedure gave kanamycin A monomesylate, whose hydrolyzate obtained by refluxing with 6 M hydrochloric acid was chromatographically compared with that of kanamycin A similarly obtained. A paper chromatogram (6:4:3:1 - 1-butanol-pyridine-water acetic acid[10]) of the former gave spots for 2-deoxy-streptamine (DST) and 3-amino-3-deoxy-D-glucose ($R_{f\ DST}$ = 2.66), but unlike kanamycin A itself, gave no spot for 6-amino-6-deoxy-D-glucose ($R_{f\ DST}$ = 1.53). ^{1}H NMR spectrum (90 MHz in D_2O) of the mesylate gave a singlet at δ3.10 ($-SO_2CH_3$) and doublets at δ5.12 ($J_{1",2"}$ 3.5 Hz, H-1") and δ5.91 ($J_{1',2'}$ 4.0 Hz, H-1'). Irradiation at δ4.51 (H-2') made the δ5.91 doublet a singlet. While the spectrum of kanamycin A gave two doublets at δ5.10 ($J_{1",2"}$ 3.0 Hz, H-1") and at δ5.56 ($J_{1',2'}$ 3.0 Hz, H-1'), irradiation of δ3.66 (H-2' and H-2") transformed both of the doublets to singlets. Therefore, we concluded that the O-debenzoylation was induced at 2'-position with high regioselectivity. Compound 4 was, however, not suitable for sequential treatments to obtain the corresponding 2'-mesylate, since N-deethoxycarbonylation with aqueous barium hydroxide solution at reflux brought about substitution and elimination reactions. The results obtained from degradation of 3 indicated that 2'-O-debenzoylation also should have occurred in formation of 4. It was of interest to note that these reactions gave no products bearing a free hydroxyl group at the 2"-position. The reason for this selectivity could be either that the 3"-amido group is sufficiently large to inhibit reaction at the 2"-position or that hydrogen bonding between the 2'-benzoyloxy group and 5-hydroxyl groups activates the 2'-benzoyloxy group. Treatment of 3 and 4 with trifluoromethanesulfonic anhydride (2 molar equivalents) at -10 °C in pyridine gave the triflates 5 (82 % yield) and 6 (96 % yield), respectively.

We next conducted nucleophilic substitution reactions on 5 and 6 according to a previously reported procedure. In separate

reactions, compound 5 was treated with sodium benzenthioxide, sodium benzoate, and sodium azide. Because of partial O-debenzoylation during these reactions, all of the resulting mixtures were O-debenzoylated with methanolic ammonia to give 1,3,6',3"-tetra-N-benzyloxycarbonyl-2'-deoxy-2'phenylthio-2'-epi-kanamycin A (7), 2'-epi-kanamycin A (8),and 2'-azido-2'deoxy-2'-epi-kanamycin A (9), respectively[11] (Entries 1-3, TABLE 1). In a similar manner, compound 6 was converted into 3',4',2",4",6"-penta-O-benzoyl-1,3,6',3"-tetra-N-ethoxycarbonyl-2'-deoxy-2'-phenylthio-2'-epi-kanamycin A (10), 2',3',4',2",4",6"-hexa-O-benzoyl-1,3,6',3"-tetra-N-ethoxycarbonyl-2'-epi-kanamycin A (11), and 3',4',2",4",6"-penta-O-benzoyl-1,3,6',3"-tetra-N-ethoxycarbonyl-2'-azido-2'-deoxy-2'-epi-kanamycin A (12), respectively (Entries 4-6, TABLE 1). Successive treatment of 10 with sodium methoxide in methanol, Raney nickel, and barium hydroxide in water gave 2'-deoxykanamycin A (13) in 47 %

TABLE 1

Nucleophilic Substitution of 2'-Trifluormethylsulfonylkanamycin A[a]

Entry	2'-Triflate	Nucleophile (equiv.)	Temp. (°C)	Time (h)	Yield (%)
1	5	NaSPh (2)	70-75	12	73[b]
2	5	NaOBz (4)	70-75	18	65[b]
3	5	NaN_3 (2)	70-75	18	75[b]
4	6	NaSPh (1.2)	80-90	3	80[c]
5	6	NaOBz (3)	80-90	7	69[c]
6	6	NaN_3 (3)	80-90	4	82[c]

[a]All of the reactions were performed in N,N-dimethylformamide.

[b]These products were isolated by chromatography on a column of silica gel after O-debenzoylation with methanolic ammonia.

[c]These products were isolated as the corresponding benzoates by chromatography on a column of silica gel.

yield. Similar treatment of 11 afforded 14 (45 % yield). Successive treatment of 12 with sodium methoxide in methanol, palladium black and hydrogen, and aqueous barium hydroxide gave 15 in 41 % yield.

The results from this study demonstrate that regioselective hydrazinolysis, when followed by trifluoromethylsulfonylation and nucleophilic substitution, represents an effective method for chemical modification of kanamycin A at the 2'-position.

EXPERIMENTAL

General Procedures. ^{1}H NMR spectra were recorded with Varian T-60, Varian EM-390, and Bruker (250 MHz) instruments, using tetramethylsilane as an internal standard. IR spectra were recorded with a Hitachi 285 instrument. Specific rotations were determined with Hitachi PO-B, Carl-Zeiss LEP A-1, and JASCO DIP-SL instruments. TLC was performed on Merck silica gel 60 F_{254} pre-coated plates (thickness 0.25 mm), and paper chromatography was carried out on Toyo-Roshi paper No. 50. Column chromatography was performed on Wakogel C-300 using 30 times as much as the weight of the sample. Elemental analyses were performed by Miss Mikiko Aoki, Laboratory of Organic Analysis, Department of Chemistry, Tokyo Institute of Technology, and by the members of Organic Analysis, Institute of Physical and Chemical Research.

2',3',4',2",4",6"-Hexa-O-benzoyl-1,3,6',3"-tetra-N-benzyloxycarbonylkanamycin A (1). To an ice-cold solution of 1,3,6',3"-tetra-N-benzyloxycarbonylkanamycin A[12] (10.2 g, 10 mmol) in pyridine (150 mL), benzoyl chloride (30 mL) was added dropwise and the reaction mixture was stirred at room temperature overnight. The resulting mixture was poured into ice-water, and extracted with chloroform (800 mL). The organic layer was washed successively with 5 % aqueous potassium hydrogen sulfate, aqueous saturated sodium bicarbonate, and water. After drying the organic layer over anhydrous magnesium sulfate, it was evaporated to a syrup,

which was then purified by column-chromatography (chloroform-methanol, 200:1 v/v; 2000 mL) to give 1 (14.1 g, 86 % yield) as an amorphous powder, $[\alpha]_D^{23}$ +78° (c 1.5 in chloroform).

Anal. Calcd for $C_{92}H_{84}N_4O_{25}$: C, 67.14; H, 5.15; N, 3.40. Found: C, 67.41; H, 5.09; N, 3.63.

2',3',4',2",4",6"-Hexa-O-benzoyl-1,3,6',3"-tetra-N-ethoxycarbonylkanamycin A (2). Treatment of 1,3,6',3"-tetra-N-ethoxycarbonylkanamycin A[4] (7.73 g, 10 mmol) with benzoyl chloride (12.2 mL) in the same manner as above gave 2 (13.6 g, 97 % yield) as an amorphous powder, $[\alpha]_D^{25}$ +100.7° (c 0.9 in chloroform).

Anal. Calcd for $C_{72}H_{76}N_4O_{25}$: C, 61.87; H, 5.49; N, 4.01. Found: C, 61.78; H, 5.42; N, 3.87.

3',4',2",4",6"-Penta-O-benzoyl-1,3,6',3"-tetra-N-benzyloxycarbonylkanamycin A (3). To a solution of 1 (16.43 g, 10 mmol) in pyridine (100 mL), hydrazine hydrate (1.0 mL, 20 mmol) was added and the reaction mixture was stirred at room temperature for 24 h. The resulting solution was mixed with chloroform (300 mL), and washed successively with 1 M hydrochloric acid, an aqueous saturated solution of sodium hydrogen carbonate, and water. The organic layer was dried over anhydrous magnesium sulfate, the drying agent was removed, and the solvent was evaporated to leave a white solid. The solid was subjected to column chromatographic separation (chloroform-methanol, 100:1 v/v; 3000 mL) to give 3 (11.2 g, 65 % yield), mp 134-137 °C (from ethanol), $[\alpha]_D^{23}$ +81° (c 1.0 in chloroform), R_f 0.47 (benzene-ethyl acetate, 2:3 v/v).

Anal. Calcd for $C_{85}H_{80}N_4O_{24}$: C, 66.23; H, 5.23; N, 3.63. Found: C, 65.95; H, 5.17; N, 3.50.

Methylsulfonylation study of 3. To an ice cold solution of 3 (0.46 g, 0.3 mmol) in pyridine (10 mL), methylsulfonyl chloride (0.08 mL, 1 mmol) was added dropwise, and the reaction mixture was

stirred at room temperature for 1 d. The resulting solution was poured into ice water and extracted with chloroform. The organic layer was successively washed with 1 M hydrochloric acid, saturated aqueous sodium hydrogencarbonate, and water, and dried over anhydrous magnesium sulfate. After filtering the desiccant, the resulting solution was evaporated to a syrup. The syrup was subjected to chromatographic purification on a column of silica gel (chloroform-methanol, 100:1 v/v) to give the corresponding monomesylate (0.45 g, 93 % yield), which was homogeneous in TLC [R_f 0.45 (benzene-ethyl acetate, 6:4 v/v)]. The mesylate was treated with methanolic ammonia (10 mL) at room temperature for 2 d, and the solution was evaporated to a syrup. It was dissolved in dioxane-acetic acid-water (2:2:1 v/v, 20 mL), and the solution was stirred under hydrogen atmosphere in the presence of palladium black (0.05 g) at room temperature for 6 h. After filtering the catalyst, the resulting solution was evaporated to a syrup, which was subjected to chromatographic separation on a column of CM sephadex C-25 (NH_4^+ form) (20 mL) by the use of ∿ 0.3 M aqueous ammonia to give kanamycin A monomesylate (0.09 g, 54 % yield based on 3), R_f 0.51 (29 % aqueous ammonia – methanol – chloroform, 1:2:1 v/v), ^{1}H NMR (90 MHz in deuterium oxide) δ (DSS) 3.10 (s, 3, $-SO_2CH_3$), 5.12 (d, 1, $J_{1",2"}$ 3.5 Hz, H-1"), 5.91 (d, 1, $J_{1',2'}$ 4.0 Hz, H-1') [cf. kanamycin A gave 5.10 (d, 1, $J_{1",2"}$ 3.0 Hz, H-1") and 5.56 (d, 1, $J_{1',2'}$ 3.0 Hz, H-1')]. The mesylate (20 mg) was dissolved in 6 M hydrochloric acid (5 mL) and the solution was stirred at 90-95 °C for 6 h. After cooling down to room temperature, the resulting solution was neutralized with aqueous sodium hydrogen carbonate and subjected to paper chromatographic analysis (1-butanol – pyridine – water – acetic acid, 6:4:3:1 v/v; descending for 3 d[10]), giving three spots of $R_{f\ DST}$ 1.00, 2.27, and 2.66 [cf. hydrolyzate of kanamycin A gave three spots of $R_{f\ DST}$ 1.00 (2-deoxystreptamine), 1.53 (6-amino-6-deoxy-D-glucose), and 2.66 (3-amino-3-deoxy-D-glucose)].

3',4',2",4",6"-Penta-O-benzoyl-1,3,6',3"-tetra-N-ethoxycarbonylkanamycin A (4). Treatment of 2 (4.2 g, 3 mmol) with

hydrazine hydrate (0.48 mL, 10 mmol) in the same manner as above gave 4 (2.60 g, 67 % yield) as an amorphous powder, $[\alpha]_D^{25}$ +85.5° (c 1.0 in chloroform), R_f 0.49 (chloroform-methanol, 9:1 v/v), R_f 0.44 (benzene-ethyl acetate, 2:3 v/v).

Anal. Calcd for $C_{65}H_{72}N_4O_{24}$: C, 60.37; H,5.61; N, 4.33. Found: C, 60.23; H, 5.70; N, 4.81.

3',4',2",4",6"-Penta-O-benzoyl-1,3,6',3"-tetra-N-benzyloxycarbonyl-2'-O-trifluoromethylsulfonylkanamycin A (5). To a solution of 3 (1.15 g, 1 mmol) in a mixture of pyridine (10 mL) and dichloromethane (40 mL), trifluoromethanesulfonic anhydride[13] (0.3 mL) was added at -10 °C and the reaction mixture was stirred for 30 h at room temperature. The resulting solution was poured into ice-water and the resulting mixture was stirred for 1 h at room temperature. The mixture was extracted with chloroform (50 mL) and washed successively with 1 M hydrochloric acid (100 mL x 3), saturated aqueous sodium hydrogen carbonate (100 mL x 2), and water. The organic layer was dried over anhydrous magnesium sulfate, and evaporated to a syrup after filtering off the desiccant. The syrup was subjected to column chromatographic separation (chloroform-methanol, 99:1 v/v; 1000 mL) to give 5 (1.39 g, 84 % yield), mp 110-113 °C (from ethanol), $[\alpha]_D^{23}$ +75° (c 1.3 in chloroform), R_f 0.44 (benzene-ethyl acetate, 6:4 v/v).

Anal. Calcd for $C_{86}H_{79}N_4O_{26}SF_3$: C, 61.72; H, 4.76; N, 3.53; S, 1.92. Found: C, 61.68; H, 4.67; N, 3.51; S, 2.25.

3',4',2",4",6"-Penta-O-benzoyl-1,3,6',3"-tetra-N-ethoxycarbonyl-2'-O-trifluoromethylsulfonylkanamycin A (6). Treatment of 4 (3.9 g, 3 mmol) with trifluoromethanesulfonic anhydride (1 mL) in pyridine (10 mL) – dichloromethane (80 mL) in the same manner as above gave 6 (4.1 g, 96 % yield) as an amorphous powder, $[\alpha]_D^{25}$ +110.5° (c 0.7 in chloroform) and ν_{max} (KBr) 1140 and 1415 cm^{-1} ($-OSO_2-$), R_f 0.41 (benzene-ethyl acetate, 6:4 v/v).

Anal. Calcd for $C_{86}H_{79}N_4O_{26}SF_3$: C, 56.62; H, 5.02; N, 3.93; S, 2.25. Found: C, 56.56, H, 5.05; N, 3.88; S, 2.03.

1,3,6',3"-Tetra-N-benzyloxycarbonyl-2'-deoxy-2'-phenylthio-2'-epi-kanamycin A (7). To a solution of benzenethiol (0.25 mL) in DMF (6 mL), 2 M methanolic sodium methoxide (1.0 mL) was added and the solution was stirred at room temperature for 1 h. Compound 5 (1.66 g, 1 mmol) was added and the temperature raised to 70-75 °C for 12 h. After cooling down to room temperature, the resulting solution was treated with methanolic ammonia (20 mL) at room temperature for 2 d. The solvent was evaporated to a syrup, which was then subjected to chromatographic separation on a column of silica gel (chloroform-methanol, 19:1 v/v; 1000 mL) to give 7 (0.81 g, 73 % yield) as an amorphous powder, $[\alpha]_D^{23}$ +53 (c 0.6 in DMF), R_f 0.36 (chloroform-methanol, 8:2 v/v).

Anal. Calcd for $C_{56}H_{64}N_4O_{18}S \cdot H_2O$: C, 59.45; H, 5.83: N, 4.95; S, 2.83. Found: C, 59.74; H, 5.92; N, 5.42; S, 2.55.

1,3,6',3"-Tetra-N-benzyloxycarbonyl-2'-epi-kanamycin A (8). Treatment of 5 (0.84 g, 0.5 mmol) with sodium benzoate (0.29 g, 2 mmol) at 70-75 °C for 18 h in the same manner as above gave 8 (0.33 g, 65 % yield) as an amorphous powder, $[\alpha]_D^{23}$ +43° (c 0.4 in DMF), R_f 0.30 (chloroform-methanol, 8:2 v/v).

Anal. Calcd for $C_{50}H_{60}N_4O_{19} \cdot H_2O$: C, 57.80; H, 6.01; N, 5.39. Found: C, 57.73; H, 5.70; N, 5.20.

2'-Azido-1,3,6',3"-tetra-N-benzyloxycarbonyl-2'-deoxy-2'-epi-kanamycin A (9). Treatment of 5 (0.84 g, 0.5 mmol) with sodium azide (0.065 g, 1 mmol)at 70-75 °C for 18 h in the same manner as above gave 9 (0.42 g, 79 % yield) as an amorphous powder, $[\alpha]_D^{23}$ +75° (c 0.6 in DMF), ν_{max} (KBr) 2100 cm^{-1}($-N_3$), R_f 0.38 (chloroform-methanol, 8:2 v/v).

Anal. Calcd for $C_{50}H_{59}N_7O_{18} \cdot H_2O$: C, 56.43; H, 5.68; N, 9.21. Found: C, 56.10; H, 5.52; N, 8.95.

3',4',2",4",6"-Penta-O-benzoyl-2'-deoxy-1,3,6',3"-tetra-N-ethoxycarbonyl-2'-phenylthio-2'-epi-kanamycin A (10). To a solution of 6 (0.39 g, 0.3 mmol) in DMF (10 mL), sodium benzene-

thioxide (0.05 g, 0.4 mmol) was added and the reaction mixture was stirred at 70-80 °C for 3 h. The resulting mixture was diluted with ethyl acetate (100 mL), and washed with saturated aqueous sodium chloride. The organic layer was dried over anhydrous magnesium sulfate. The solution was, after filtering off the desiccant, evaporated to leave a syrup, which was subjected to chromatographic separation on a column of silica gel (benzene-ethyl acetate, 9:1 v/v; 700 mL) to give 10 (0.32 g, 80 % yield) as an amorphous powder, $[\alpha]_D^{25}$ +88° (c 1.0 in chloroform), R_f 0.49 (benzene-ethyl acetate, 6:4 v/v).

Anal. Calcd for $C_{71}H_{76}N_4O_{23}S$: C, 61.55; H, 5.53; N, 4.04; S, 2.31. Found: C, 61.01; H, 5.58; N, 3.93; S, 2.31.

2',3',4',2",4",6"-Hexa-O-benzoyl-1,3,6',3"-tetra-N-ethoxycarbonyl-2'-epi-kanamycin A (11). Treatment of 6 (0.39 g, 0.3 mmol) with sodium benzoate (0.14 g, 1 mmol) at 80-90 °C for 7 h in the same way as described above gave 11 (0.27 g, 69 % yield) as an amorphous powder, $[\alpha]_D^{23}$ +41.7° (c 0.6 in chloroform), R_f 0.30 (benzene-ethyl acetate, 6:4 v/v).

Anal. Calcd for $C_{72}H_{76}N_4O_{25}$: C, 61.87; H, 5.49; N, 4.01. Found: C, 61.65; H, 5.50; N, 3.88.

2'-Azido-3',4',2",4",6"-penta-O-benzoyl-2'-deoxy-1,3,6',3"-tetra-N-ethoxycarbonyl-2'-epi-kanamycin A (12). Treatment of 6 (0.39 g, 0.3 mmol) with sodium azide (0.065 g, 1 mmol) at 80-90 °C for 4 h in the same manner as above gave 12 (0.33 g, 82 % yield) as an amorphous powder, $[\alpha]_D^{23}$ +47.1° (c 0.6 in chloroform), R_f 0.44 (benzene-ethyl acetate, 6:4 v/v).

Anal. Calcd for $C_{65}H_{73}N_7O_{24}\cdot H_2O$: C, 58.42; H, 5.51; N, 7.34. Found: C, 58.49; H, 5.42; N, 6.99.

2'-Deoxykanamycin A (13). To a solution of 10 (1.5 g, 1.08 mmol) in ethanol (35 mL), was added Raney nickel W-2 (10 mL, ethanol wet) and stirred under reflux for 30 min. The resulting solution was, after filtering off the catalyst, evaporated to a

syrup, which was then subjected to chromatographic purification on a column of silica gel (chloroform-methanol, 99:1 v/v), and the fractions [R_f 0.47 (benzene-ethyl acetate, 6:4 v/v)] were combined and the solvent was evaporated to give a syrup. The resulting syrup was dissolved in anhydrous methanol (30 mL) and 28 % methanolic sodium methoxide solution was added until pH 11 was reached. After leaving the solution for 30 min at room temperature, it was neutralized with 1 M hydrochloric acid and evaporated to a syrup. The syrup was dissolved in 0.2 M aqueous barium hydroxide and refluxed for 3 h. After cooling to room temperature, the solution was neutralized with dry ice and the insoluble precipitates were filtered. Treatment of the filtrate on a column of Amberlite CG-50 (NH_4^+ form) (20mL) by the use of ~ 0.3 M aqueous ammonia gave 13 (0.19 g 47 % yield) as an amorphous powder, $[\alpha]_D^{25}$ +124.8° (c 0.9 in water), ^{1}H NMR (20 %-deuterium ammonia solution of deuterium oxide) δ 1.36 (q, 1, $J_{1,2_{ax}} = J_{2_{ax},2_{eq}} = J_{2_{ax},3}$ 12.0 Hz, H-2_{ax}), 1.73 (dt, 1, $J_{2'_{ax},2'_{eq}} = J_{2'_{ax},3'}$ 12.5 Hz, H-$2'_{ax}$), 2.06 (dt, 1, $J_{1,2_{eq}} = J_{2_{eq},3}$ 4.0 Hz, H-2_{eq}), 2.26 (dd, 1, $J_{2'_{eq},3'}$ 4.5 Hz, H-$2'_{eq}$), 3.49 (dd, 1, $J_{2'',3''}$ 12.0 Hz, H-2"), 2.8-4.2 (m, 16, overlapping ring protons), 5.02 (d, 1, $J_{1'',2''}$ 4.0 Hz, H-1"), 5.45 (broad d, 1, $J_{1',2'_{ax}}$ 3.8 Hz, $J_{1',2'_{eq}}$ < 1.0 Hz, H-1').

Anal. Calcd for $C_{18}H_{36}N_4O_{10}\cdot\frac{1}{2}H_2CO_3\cdot H_2O$: C, 43.70; H, 7.53; N, 11.02. Found: C, 43.65; H, 7.24; N, 10.82.

2'-epi-Kanamycin A (14). A solution of 11 (0.44 g, 0.31 mmol) in anhydrous methanol (30 mL) was treated with 28 % methanolic sodium methoxide solution until pH 11 was reached, and the resulting solution was allowed to stand for 30 min at room temperature. After neutralization with 1 M hydrochloric acid, the resulting solution was evaporated to a syrup, which was then dissolved in 0.2 M aqueous barium hydroxide (30 mL) and stirred under reflux for 8 h. After cooling to room temperature, the solution was neutralized with dry ice and insoluble precipitates

were filtered. Treatment of the filtrate on a column of Amberlite CG-50 (NH_4^+ form) (20 mL) by the use of ~0.3 M aqueous ammonia gave 14 (0.07 g, 47 % yield) as an amorphous powder, $[\alpha]_D^{23}$ +97.5° (c 0.5 in water), ^1H NMR (20 %-deuterium ammonia solution in deuterium oxide), δ 1.24 (q, 1, $J_{1,2_{ax}} = J_{2_{ax},2eq} = J_{2_{ax},3}$ 12.5, H-2_{ax}), 1.98 (dt, 1, $J_{1,2_{eq}} = J_{2_{eq},3}$ 3.8 Hz, H-2_{eq}), 2.8-4.2 (m, 17, overlapping ring protons), 4.08 (dd, 1, $J_{2',3'}$ 3.5 Hz, H-2'), 5.06 (d, 1, $J_{1'',2''}$ 4.5 Hz, H-1"), 5.23 (d, 1, $J_{1',2'}$ 2.0 Hz, H-1').

Anal. Calcd for $C_{18}H_{38}N_4O_{12}\cdot H_2O$: C, 43.02; H, 7.26; N, 11.15. Found: C, 43.40; H, 7.45; N, 11.24.

2'-epi-Kanamycin B (15). To a solution of 12 (0.52 g, 0.39 mmol) in anhydrous methanol (20 mL), was added 2 M methanolic sodium methoxide solution (10 drops), and the resulting solution was allowed to stand at room temperature for 8 h. After neutralizing the solution with 1 M hydrochloric acid, it was evaporated to a syrup, which was then dissolved in ethanol (10 mL) and stirred under hydrogen atmosphere in the presence of palladium black (0.02 g) at room temperature for 5 h. The resulting solution was, after filtering the catalyst, evaporated to a syrup, which was dissolved in 0.2 M aqueous barium hydroxide (30 mL) and the solution was stirred under reflux for 8 h. After cooling the solution to room temperature, it was neutralized with dry ice, and insoluble precipitates were filtered. Treatment of the filtrate on a column of Amberlite CG-50 (NH_4^+ form) (20 mL) by the use of ~ 0.3 M aqueous ammonia, gave 15 (0.087 g, 41 % yield), as an amorphous powder, $[\alpha]_D^{23}$ +88.5° (c 0.5 in water), ^1H (20 %-deuterium ammonia solution in deuterium oxide), δ 1.26 (q, 1, $J_{1,2_{ax}} = J_{2_{ax},2_{eq}} = J_{2_{ax},3}$ 12.5 Hz, H-2_{ax}), 2.05 (dt, 1, $J_{1,2_{eq}} = J_{2_{eq},3}$ 3.5 Hz, H-2_{eq}), 2.8 – 4.2 (m, 17, overlapping ring protons), 5.06 (d, 1, $J_{1'',2''}$ 3.5 Hz, H-1"), 5.20 (d, 1, $J_{1',2'}$ 2.0 Hz, H-1').

Anal. Calcd for $C_{18}H_{37}N_5O_{10}\cdot H_2CO_3$: C, 41.83; H, 7.21; N, 12.84. Found: C, 41.46; H, 6.93; N, 12.66.

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