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Deoxygenation of Amino-Glycoside Antibiotics *via* Anhydro Intermediates. II.[1] Improved Syntheses of 3′,4′-Dideoxykanamycin A and 4′-Deoxykanamycin A

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A new deoxygenation of kanamycin A leading to 3′,4′-dideoxykanamycin A and 4′-deoxykanamycin A is described. The key stage in the syntheses involves the formation of the 3′,4′-anhydro-4′-epi derivative (**5**) followed by its conversion to the 3′Δ and 4′-deoxy derivative through the iodohydrin. Compound **5** was prepared by the treatment of 2′,3′,2″-tri-*O*-benzoyl-4″,6″-*O*-cyclohexylidene-4′-*O*-methylsulfonyl-tetra-*N*-*tert*-butyloxycarbonylkanamycin A (**4**) with sodium methoxide.

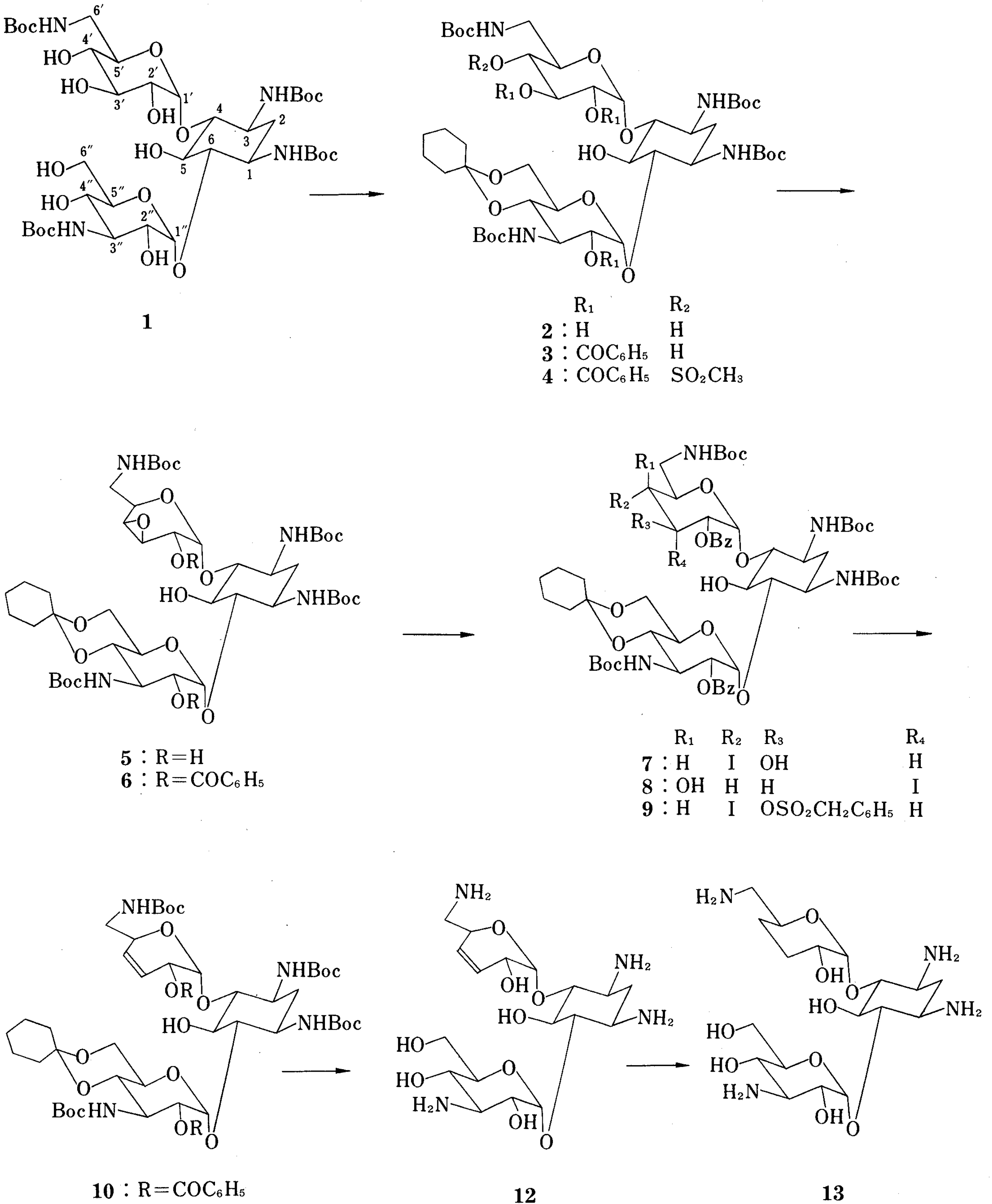
Keywords——synthesis; kanamycin A; deoxygenation; 3′,4′-dideoxykanamycin A; 4′-deoxykanamycin A; 3′,4′-anhydro-4′-epi derivative

The aminoglycoside antibiotics are valuable and widely used as chemotherapeutic agents because of their remarkable activity against gram-negative bacteria which are not readily susceptible to other antibiotics. Their widespread clinical use has led to the emergence of a growing number of resistant strains whose resistance results from enzymatic modification of the antibiotics.[2] Chemical modification studies to overcome this problem have been done with many natural aminoglycosides. In the case of kanamycin B, removal of both the 3′- and 4′-hydroxyl groups gave a clinically useful compound, 3′,4′-dideoxykanamycin B,[3] and removal of the 3′-hydroxyl group gave tobramycin.[4]

The deoxygenation has usually been done by reductive removal of the sulfonate derivatives of the target hydroxyl groups. However, this method often requires vigorous conditions and furthermore, in compound possessing hydroxyl groups at C-2′, C-3′, and C-4′, such as kanamycin A, selective sulfonations of C-3′ and C-4′ or of only C-4′ were reported to be quite difficult and many steps were required.[5,6]

In the previous paper,[1] we reported that the 3′,4′-anhydro-4′-epi derivative of kanamycin B, which was obtained in high yield by making use of the low reactivity of the 4′-hydroxyl group toward benzoylation with benzoyl chloride in pyridine, was a useful intermediate for 3′,4′-dideoxykanamycin B and 4′-deoxykanamycin B. We now show that the selective benzoylation method is easily applicable to the transformation of kanamycin A, and that the 3′,4′-anhydro-4′-epi derivative is a good intermediate for 3′4′-dideoxykanamycin A and 4′-deoxykanamycin A.

Kanamycin A was treated with Boc-S reagent[7] to provide the tetra-*N*-Boc derivative (**1**) in 75% yield. The 4″,- and 6″-hydroxyl groups of **1** were selectively protected by the formation of the cyclohexylidene derivative in the usual manner.[1] As reported in the previous paper,[1] the 4′-hydroxyl group was least reactive toward benzoylation with benzoyl chloride in pyridine. Therefore, the 2′-, 3′-, and 2″-hydroxyl groups of **2** were preferentially benzoylated with benzoyl chloride (3.5 mol to **2**) in pyridine at 5°C to give the 2′,3′,2″-tri-*O*-benzoate product (**3**) in 75% yield. Mesylation of **3** with mesyl chloride in pyridine gave the 4′-*O*-mesyl derivative (**4**) in 89% yield. In the ^1H nuclear magnetic resonance (NMR) spectrum, a three-proton singlet due to SO_2CH_3 appeared at δ 2.9 ppm. The treatment of **4** with methanolic sodium methoxide gave the 3′,4′-anhydro-4′-epi derivative (**5**) together with a minor compound which was slower-moving on thin layer chromatography (TLC). The best conditions were to use sodium methoxide (2.4 equivalent to **4**) in chloroform–methanol at 0°C,[8] affording **5** as a major product together with a trace amount of the slower moving material on TLC. **5** was re-*O*-benzoylated to the 2′,2″-di-*O*-benzoate (**6**) with benzoyl chloride. Successive treatment of **6** sodium iodide, sodium acetate, and acetic acid in acetone afforded the iodohydrin (**7**). Formation of the anhydro-ring opened isomer (**8**) was not observed in this reaction. The iodohydrin **7** should be obtained by abnormal anhydro-ring opening, the mechanism of which was discussed in connection with the anhydro-ring opening of kanamycin B.1)



1

	R_1	R_2
2 :	H	H
3 :	COC_6H_5	H
4 :	COC_6H_5	SO_2CH_3

5 : R=H
6 : $R=COC_6H_5$

	R_1	R_2	R_3	R_4
7 :	H	I	OH	H
8 :	OH	H	H	I
9 :	H	I	$OSO_2CH_2C_6H_5$	H

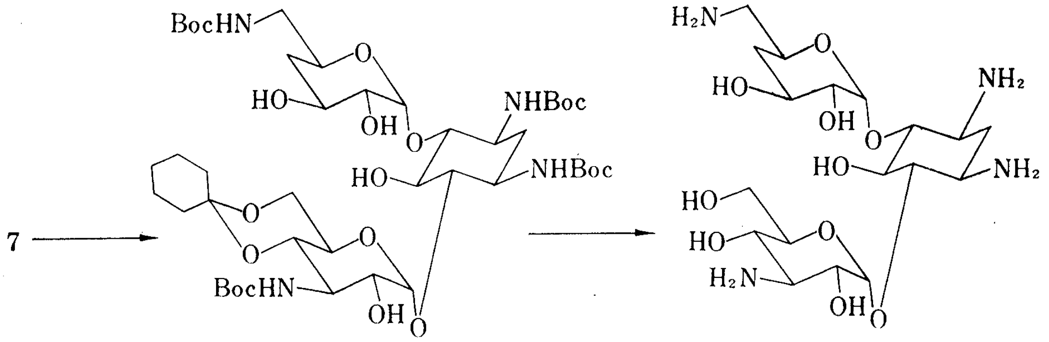
10 : $R=COC_6H_5$
11 : R=H

12

13

Chart 1

The iodohydrin (**7**) was treated with α-toluenesulfonyl chloride in pyridine and the resulting 3′-α-toluenesulfonate (**9**) was heated at 90°C for 30 min to give the 3′-ene derivative (**10**) in 72% yield. Removal of the benzoyl groups with methanolic sodium methoxide gave **11** and the successive treatment of **11** with 95% trifluoroacetic acid afforded the 3′Δ kanamycin A (**12**). Catalytic hydrogenation of **12** gave 3′,4′-dideoxykanamycin A (**13**).[5] Catalytic hydrogenation of the crude iodohydrin (**7**) followed by treatment with methanolic sodium methoxide and 95% trifluoroacetic acid gave 4′-deoxykanamycin A (**15**)[6] *via* **14**. The ^{1}H NMR, TLC and biological activity of **13** and **15** were identical with those of authentic samples.



14 **15**

Chart 2

Experimental

All melting points are uncorrected. Optical rotations were measured with a Perkin-Elmer Model 241 polarimeter. ^{1}H NMR spectra were recorded at 60 MHz with a Varian S-60T spectrometer. TLC was performed on Merck silica gel plates, No. 5714.

Tetra-*N*-*tert*-butoxycarbonylkanamycin A (1)——The Boc-S reagent (43.2 g) in 1,4-dioxane (200 ml) was added to a solution of kanamycin A (14.5 g) in aqueous triethylamine (23%, 156 ml), and the mixture was stirred for 24 h at room temperature. The syrupy mixture was poured into water (600 ml) and the resulting precipitate was filtered, washed successively with 0.1 M hydrochloric acid and water, and dried to give **1** as a white powder (20 g, 75%); mp 238—255°C (dec.), $[\alpha]^{25}_D$ +74° (c=1.0, DMF). *Anal.* Calcd for $C_{38}H_{68}N_4O_{19}$: C, 51.56; H, 7.76; N, 6.33. Found: C, 51.40; H, 7.60; N, 6.41.

4″,6″-*O*-Cyclohexylidene-tetra-*N*-*tert*-butoxycarbonylkanamycin A (2)——*p*-Toluenesulfonic acid hydrate (400 mg) and 1,1-dimethoxycyclohexane (20 ml) were added to a solution of **1** (10.5 g) in *N*,*N*-dimethylformamide (200 ml), and the mixture was allowed to stand at room temperature overnight. The resulting solution contained five compounds (TLC, chloroform–methanol (7: 1), *Rf* 0.1 (starting material), 0.28 (**2**), 0.47 (trace), 0.49 (trace), 0.50 (trace). After neutralization with triethylamine (3 ml), the solution was concentrated to a syrup and water was added. The resulting precipitate was filtered off, washed with water and dried. The crude product was dissolved in hot methanol and, on cooling, the solution gave pure **2** (9.5 g, 83%); mp 250—265°C (dec.), $[\alpha]^{25}_D$ +67° (c=1.0, pyridine). *Anal.* Calcd for $C_{44}H_{76}N_4O_{19}$: C, 54.75; H, 7.95; N, 5.81. Found: C, 54.38; H, 7.46; N, 5.42.

2′,3′,2″-Tri-*O*-benzoyl-4″,6″-*O*-cyclohexylidene-tetra-*N*-*tert*-butoxycarbonylkanamycin A (3)——Benzoyl chloride (3.0 ml) was added to a solution of **2** (11.4 g) in dry pyridine (200 ml), and the mixture was kept at 5°C overnight. Benzoyl chloride (2.6 ml) was added dropwise at 5°C until the starting material disappeared. After the addition of water (0.5 ml), the solution was concentrated to a syrup and water was added. The resulting precipitate was filtered off, washed with water and dried. Column chromatography over silica gel (300 g) with chloroform–methanol (60: 1) afforded **3** as a white powder (10.2 g, 75%); mp 182—188°C, $[\alpha]^{25}_D$ +100.5° (c=1.1, $CHCl_3$). *Anal.* Calcd for $C_{65}H_{88}N_4O_{22}$: C, 61.10; H, 6.96; N, 4.39. Found: C, 60.93; H, 6.79; N, 4.15.

2′,3′,2″-Tri-*O*-benzoyl-4″,6″-*O*-cyclohexylidene-4′-*O*-methylsulfonyl-tetra-*N*-*tert*-butoxycarbonylkanamycin A (4)——A solution of **3** (1.9 g) and methylsulfonyl chloride (0.4 ml) in pyridine (30 ml) was kept at room temperature for 3 h. After the addition of water (0.2 ml), the solution was concentrated to a syrup and water was added. The resulting precipitate was filtered off, washed with water and dried (2.0 g). Column chromatography over silica gel (45 g) with chloroform–methanol (70: 1) afforded **4** as a white powder (1.78 g, 89%); mp 194—204°C (dec.), $[\alpha]_D^{25}$ +103.3° (c=1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): 2.90 (3H, s, SO_2CH_3), 7.3—8.2 (15H, m, C_6H_5). *Anal.* Calcd for $C_{66}H_{90}N_4O_{24}S$: C, 58.47; H, 6.71; N, 4.13; S, 2.36. Found: C, 58.45; H, 6.52; N, 4.23; S, 2.20.

3′,4′-Anhydro-4″,6″-*O*-cyclohexylidene-4′-epi-tetra-*N-tert*-butoxycarbonylkanamycin A (5)——A solution of **4** (5.1 g) in methanol (50 ml) and chloroform (50 ml) was treated with sodium methoxide (0.7 g), and the solution was stirred at 0°C for 6 h. After neutralization with concd. hydrochloric acid at 5°C, the resulting solution was concentrated and water was added. The resulting precipitate was filtered off, washed with water and dried (3.1 g, 88%). Column chromatography over silica gel (60 g) with chloroform–acetone (7: 2) afforded analytically pure **5** as a white powder (650 mg); mp 190—207°C (dec.), $[\alpha]_D^{25}$ +54.1° (c=1.0, DMF). *Anal.* Calcd for $C_{44}H_{74}N_4O_{18}$: C, 55.79; H, 7.89; N, 5.92. Found: C, 55.20; H, 7.58; N, 6.00.

3′,4′-Anhydro-2′,2″-di-*O*-benzoyl-4″,6″-*O*-cyclohexylidene-4′-epi-tetra-*N-tert*-butoxycarbonylkanamycin A (6) and the Iodohydrin (7)——Benzoyl chloride (0.78 ml) was added to a solution of **5** (1.3 g) in dry pyridine (25 ml), and the solution was kept at room temperature for 2 h. After the addition of water (0.2 ml), the solution was concentrated to a syrup and water was added. The resulting precipitate was filtered off, washed with water and dried to give **6** as a white powder (1.56 g, one spot on TLC). A mixture of **6** (1.56 g), sodium iodide (1.3 g), sodium acetate (60 mg), acetic acid (1.0 ml) and acetone (30 ml) was refluxed for 4 h. The solvent was removed by evaporation under reduced pressure and the residue was triturated with water. The resulting precipitate was filtered off, washed with water and dried (1.6 g). Column chromatography on silica gel (35 g) with chloroform–acetone (9: 1) gave a white powder (1.07 g, 61%) of the crude iodohydrin containing **7**. *Anal.* Calcd for $C_{52}H_{75}IN_4O_{20}$: I, 9.84. Found: I, 9.31.

2′,2″-Di-*O*-benzoyl-4″,6″-*O*-cyclohexylidene-3′,4′-dideoxy-3′Δ-tetra-*N-tert*-butoxycarbonylkanamycin A (10)——A solution of the crude iodohydrin **7** (2.6 g) in pyridine (50 ml) was treated with α-toluenesulfonyl chloride (1.5 g), and the solution was kept at 5°C for 1 h. After the addition of methanol (0.5 ml), the resulting solution was heated at 90°C for 1 h. The solution was concentrated and mixed with water, and the resulting precipitate was filtered off, washed with water and dried (2.1 g). Column chromatography on silica gel (50 g) with chloroform–methanol (20: 1) afforded the pure compound **10** (1.3 g, 56%) as a white powder; mp 221—226°C (dec.), $[\alpha]_D^{25}$ +19.1° (c=1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): 5.85 (2H, s, olefinic protons). *Anal.* Calcd for $C_{58}H_{82}N_4O_{19}$: C, 61.13; H, 7.28; N, 4.92. Found: C, 61.18; H, 7.31; N, 4.45.

4″,6″-*O*-Cyclohexylidene-3′,4′-dideoxy-3′Δ-tetra-*N-tert*-butoxycarbonylkanamycin A (11)——Sodium methoxide (100 mg) was added to a solution of **10** (1.2 g) in methanol (20 ml), and the solution was kept at room temperature for 30 min. After neutralization with 1 M hydrochloric acid, the solvent was removed by evaporation and the residue was triturated with water. The resulting precipitate was filtered off, washed with water and dried (1.0 g). Column chromatography on silica gel (20 g) with chloroform–acetone (3: 1) afforded **11** as a white powder (0.76 g, 78%); mp 214—217°C, $[\alpha]_D^{25}$ +20.3° (c=1.0, CH_3OH). *Anal.* Calcd for $C_{44}H_{74}N_4O_{17}$: C, 56.75; H, 8.03; N, 6.02. Found: C, 57.30; H, 8.01; N, 5.65.

3′,4′-Dideoxy-3′-enekanamycin A (12)——A solution of **11** (950 mg) in 95% trifluoroacetic acid (10 ml) was kept at room temperature for 10 min. The solvent was removed by evaporation and the residue was dissolved in water (10 ml). The aqueous solution was neutralized with 1 M sodium hydroxide and charged onto a column of Amberlite CG-50 (NH_4^+ form, 15 ml). The column was washed with water and developed with 0.3 M ammonium hydroxide. The eluate containing **12** was evaporated to dryness to give a colorless solid (340 mg, 75%); mp 210—224°C (dec.), $[\alpha]_D^{25}$ +52° (c=1.0, H_2O); ^1H NMR (D_2O): 5.47 (1H, d, J=4 Hz, H-1″), 5.80 (1H, d, J=4 Hz, H-1′), 6.11 (2H, s, olefinic protons). *Anal.* Calcd for $C_{18}H_{34}N_4O_9 \cdot H_2CO_3$: C, 44.51; H, 7.09; N, 10.93. Found: C, 44.36; H, 7.21; N, 10.54.

3′,4′-Dideoxykanamycin A (13)——A solution of **12** (200 mg) in water (10 ml) was hydrogenated with platinum oxide (15 mg) under atmospheric pressure for 2 h. After removal of the catalyst, the solution was charged onto a column of Amberlite CG-50 (NH_4^+ form, 6 ml). After being washed with water, the column was eluted with 0.3 M ammonium hydroxide to give **13** as a colorless solid (185 mg, 90%).

This compound was confirmed to be identical with an authentic sample of 3′,4′-dideoxykanamycin A in all respects including biological activity.

4″,6″-*O*-Cyclohexylidene-4′-deoxy-tetra-*N-tert*-butoxycarbonylkanamycin A (14)——The crude iodohydrin **7** (1.0 g) in a mixture of methanol (20 ml) and 1,4-dioxane (5 ml) was hydrogenated for 4 h under atmospheric pressure with Raney-nickel (2 g). After removal of the catalyst, sodium methoxide (150 mg) was added and the solution kept at room temperature for 1 h. After neutralization with 1 N hydrochloric acid, the solution was concentrated to dryness and the residue was triturated with water. The resulting precipitate was filtered off, washed with water and dried (720 mg). Column chromatography on silica gel (15 g) with chloroform–acetone (2: 1) gave **14** as a white powder (510 mg, 69%); mp 204—215°C (dec.), $[\alpha]_D^{25}$ +72° (c=0.75, CH_3OH). *Anal.* Calcd for $C_{44}H_{76}N_4O_{18}$: C, 55.67; H, 8.09; N, 5.90. Found: C, 55.25; H, 8.00; N, 5.64.

4′-Deoxykanamycin A (15)——A solution of **14** (220 mg) in 95% trifluoroacetic acid (2 ml) was kept at room temperature for 10 min. The solvent was removed by evaporation and the residue was dissolved in water (4 ml). The aqueous solution was neutralized with 1 M sodium hydroxide and charged onto a column of Amberlite CG-50 (NH_4^+ form, 3 ml). The column was washed with water and developed with 0.3 M ammonium hydroxide. The eluate containing **15** was evaporated to dryness to give a colorless solid (85 mg, 78%).

This compound was confirmed to be identical with an authentic sample of 4′-deoxykanamycin A in all respects including biological activity.

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References and Notes

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