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# Deoxygenation of Amino-Glycoside Antibiotics *via* Anhydro Intermediates. III.[1] New Synthesis of 3′-Deoxykanamycin A

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A new synthetic route has been exploited for the large-scale production of 3′-deoxykanamycin A. The key stage in the synthesis involves the formation of the 3′,4′-anhydro-3′-epi derivative (**9**) or 2′,3′-anhydro-3′-epi derivative (**10**) followed by conversion to the 3′-deoxy derivative by reduction with Raney-nickel or sodium borohydride. Compound (**9**) or (**10**) was prepared by the treatment of 2′,2″-di-*O*-benzoyl-3′-*O*-methylsulfonyl-tetra-*N*-ethoxycarbonylkanamycin A with sodium methoxide.

**Keywords**——kanamycin A; 3′-deoxykanamycin A; synthesis; deoxygenation; 2′,3′-anhydro-3′-epi derivative; 3′,4′-anhydro-3′-epi derivative

Tsuchiya reported a synthesis of 3′-deoxyamikacin[2] and described its antibacterial activity. The activity is reported to be better than that of amikacin, which has been widely used as a chemotherapeutic agent. In order to evaluate the chemotherapeutic activity of 3′-deoxyamikacin in detail, a new preparative method was needed for 3′-deoxykanamycin A (**12**),[2,3] an intermediate to 3′-deoxyamikacin.

We now present a novel synthesis of the title compound (**12**). It is known that direct $S_{N2}$ replacement of the 3-*O*-sulfonate of methyl α-D-glucopyranoside is difficult because a β-*trans*-axial effect of the anomeric group[4] is operative in this case. Thus, we sought some other deoxygenation method and found that the reduction of the anhydro intermediate derived from the 3′-*O*-sulfonate of kanamycin A is a method of choice for large-scale preparation of **12**.

In order to prepare 3′-*O*-sulfonate selectively, the other functional groups should be protected. The four amino groups of kanamycin A were reacted with ethoxycarbonyl chloride to obtain the tetra-*N*-ethoxycarbonylkanamycin A (**1**) in 86% yield. Then the 4″- and 6″-hydroxyl groups of **1** were protected selectively by the formation of the cyclohexylidene derivative (**2**) in the usual manner.[5] In the previous papers,[1,5] we reported that the 4′-hydroxyl group of 4″,6″-*O*-cyclohexylidene derivatives was least reactive toward benzoylation with benzoyl chloride–pyridine. Treatment of the 4″,6″-*O*-cyclohexylidene derivative (**2**) with benzoyl chloride (3.5 mol to **2**) in pyridine at 5°C afforded 2′,3′,2″-tri-*O*-benzoate (**3**) (70% yield) together with a small amount of a faster moving material on thin layer chromatography (TLC). In the case of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside,[6] selective benzoylation of the 2- and 3-hydroxyl groups with benzoyl chloride in pyridine has not been reported. We considered that selective benzoylation at the 2-position might be possible if an alkali-stable and bulky group existed at the 4-position. The tetrahydropyranyl group was presumed to be adequate for this purpose. Reaction of the tri-*O*-benzoate (**3**) with dihydropyran and *p*-toluenesulfonic acid in DMF at room temperature gave 4′-*O*-tetrahydropyranylate (**4**). Treatment of **4** with methanolic sodium methoxide gave the de-*O*-benzoylated derivative (**5**) in 54% yield, and this product was re-*O*-benzoylated selectively to provide the 2′,2″-di-*O*-benzoate (**6**) in 78% yield with benzoyl chloride. In this case, the reactivity of 3′-hydroxyl group was greatly reduced by steric hindrance due to the 4′-*O*-tetrahydropyranyl group, as was expected. Treatment of **6** with mesyl chloride (4 mol to **6**) in pyridine at 50°C gave the desired 3′-*O*-mesylate (**7**) in 60% yield. Then the 4′-*O*-tetrahydropyranyl and the 4″,6″-O-

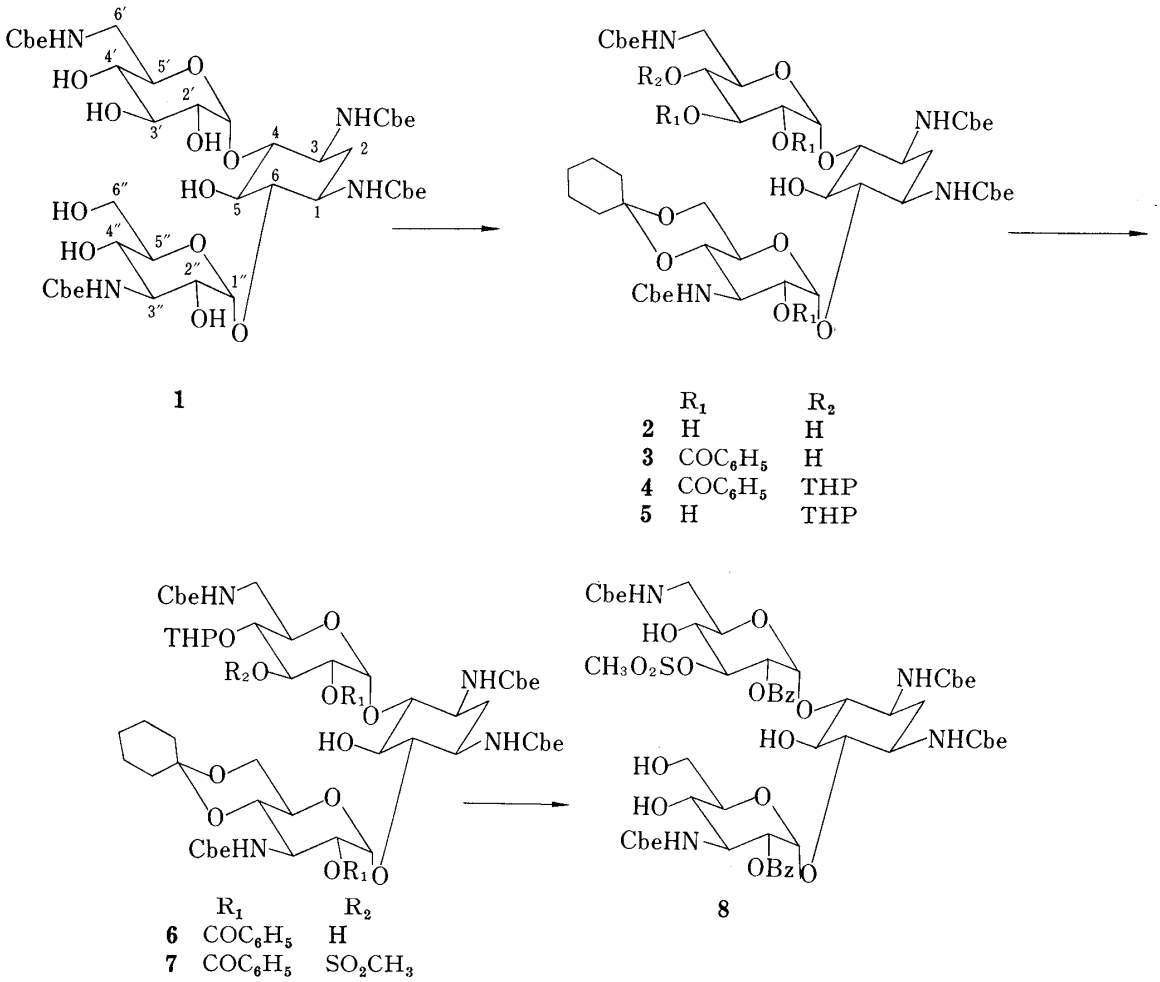


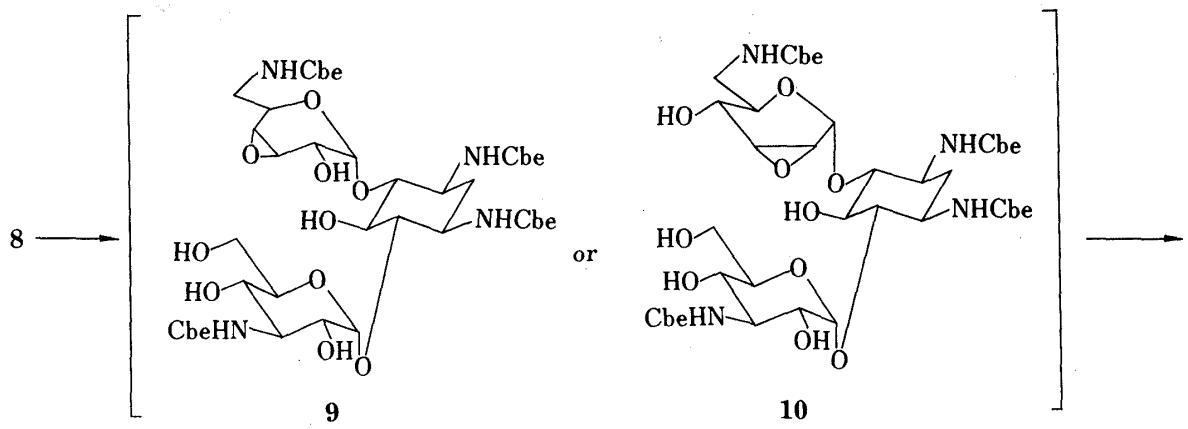

Chart 1

cyclohexylidene groups of **7** were removed by treatment with 1% trifluoroacetic acid in methanol at 50°C afford **8**. $^1$H-Nuclear magnetic resonance (NMR) spectrum of **8** showed the presence of one mesyl and two benzoyl groups.

Treatment of **8** in methanolic sodium methoxide gave a product that showed one spot on TLC although both the 3′,4′-anhydro-3′-epi derivative (**9**) and the 2′,3′-anhydro-3′-epi derivative (**10**) were expected to be produced. It is known that catalytic hydrogenation of methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-alloside[7] and 3′,4′-anhydro-4″,6″-*O*-cyclohexylidene-3′-epi-penta-*N*-ethoxycarbonylkanamycin B[8] with Raney-nickel gives only methyl 3-deoxy-α-D-glucopyranoside and 4″,6″-*O*-cyclohexylidene-3′-deoxy-penta-*N*-ethoxycarbonylkanamycin B, respectively. Therefore, hydrogenation of the above anhydro derivative (**9**) or (**10**) with Raney-nickel was carried out in the hope of preferential formation of the 3′-deoxy derivative. Actually, only 3′-deoxy-tetra-*N*-ethoxycarbonylkanamycin A (**11**) was produced, as was expected.

Treatment of **9** or **10** with sodium borohydride, which has no selectivity in the reduction of epoxides in general, again afforded only the 3′-deoxy derivative (**11**). The reason for this can be considered to be as follows: the structures of the anhydro derivatives (**9**) and (**10**) could be represented as I and II, and III and IV, respectively, as shown in Chart 3.

The conformers (II) and (IV) should be more stable because hydrogen bonding[9] between neighboring hydroxyl groups and the anhydro oxygen atom is present in these conformers. Attack of a hydride should take place at the C-3′ position of both II and IV to afford the 3′-deoxy derivative.



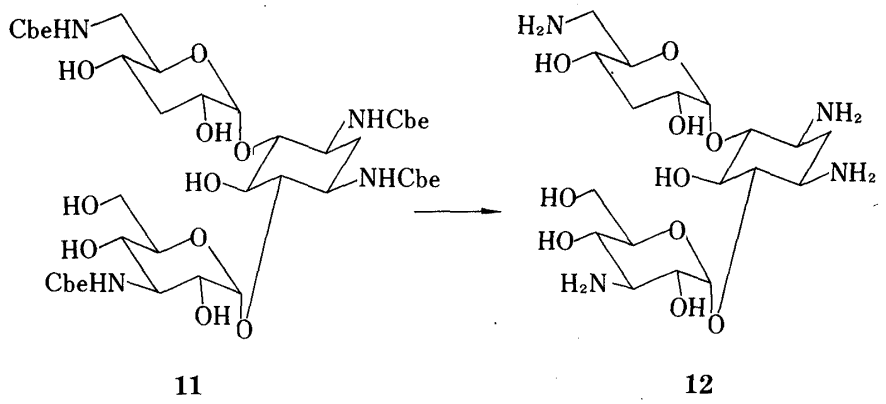




Chart 2

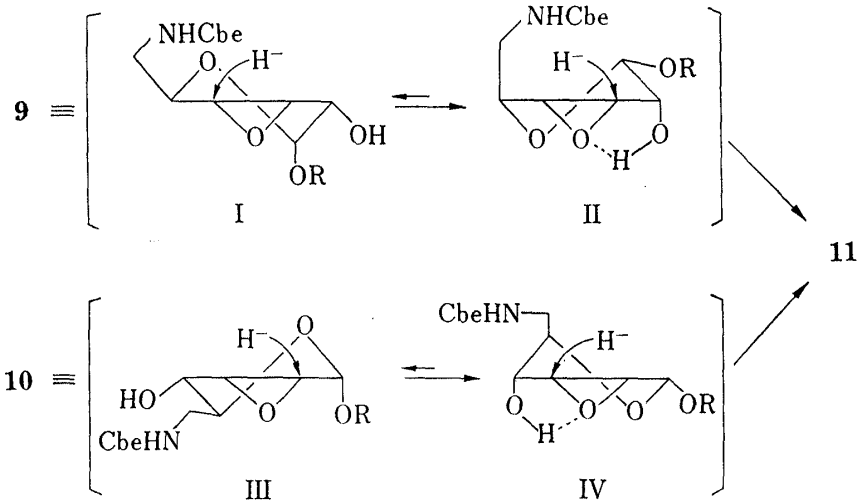



Chart 3

Deprotection of **11** with aqueous alkali gave 3′-deoxykanamycin A (**12**), the structure of which was established by comparison of $^1$H-NMR, TLC and biological activity results with those of an authentic sample. The preparative method for **12** was thus established.

## 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*-ethoxycarbonylkanamycin A** (**1**)——Ethoxycarbonyl chloride (69 ml) was added dropwise to a solution of kanamycin A·$H_2SO_4$ (40 g) in 2 M sodium hydroxide (400 ml) and methanol (200 ml) at 5°C, and the resulting solution was stirred for 4 h. The resulting precipitate was filtered off, washed with water and dried (45.7 g, 86%); mp 266—267°C (dec.), $[\alpha]_D^{25}$ +48.3° ($c$=0.5, DMF). *Anal.* Calcd for $C_{30}H_{52}N_4O_{19}$: C, 46.62; H, 6.80; N, 7.25. Found: C, 46.38; H, 6.80; N, 7.10.

**2′,3′,2″-Tri-*O*-benzoyl-4″,6″-*O*-cyclohexylidene-tetra-*N*-ethoxycarbonylkanamycin A** (**3**)——*p*-Toluenesulfonic acid hydrate (130 mg) and 1,1-dimethoxycyclohexane (4.2 ml) were added to a solution of **1** (1.8 g) in *N*,*N*-dimethylformamide (36 ml), and the mixture was allowed to stand at room temperature overnight. After addition of triethylamine (0.5 ml), the solution was concentrated to one-third in volume, and dissolved in dry pyridine (20 ml). Benzoyl chloride (0.85 ml) was added dropwise to this solution at 5°C. After addition of water (0.5 ml), the solution was concentrated to a syrup and water was added to this. The resulting precipitate was filtered off, washed with water and dried (2.44 g). Column chromatography over silica gel (60 g) with chloroform–methanol (35: 1) afforded **3** as a white powder (1.9 g, 70%); mp 185—195°C, $[\alpha]_D^{25}$ +109.4° ($c$=1.0, $CHCl_3$). *Anal.* Calcd for $C_{57}H_{72}N_4O_{22}$: C, 58.74; H, 6.24; N, 4.81. Found: C, 58.47; H, 6.30; N, 4.67.

**2′,3′,2″-Tri-*O*-benzoyl-4″,6″-*O*-cyclohexylidene-4′-*O*-tetrahydropyranyl-tetra-*N*-ethoxycarbonylkanamycin A** (**4**)——*p*-Toluenesulfonic acid hydrate (60 mg) and 2,3-dihydropyran (1.7 ml) were added to a solution of **3** (1.85 g) in *N*,*N*-dimethylformamide (15 ml), and the mixture was allowed to stand for 2 h at room temperature. After addition of triethylamine (0.3 ml), the solution was concentrated to a syrup and water was added to this. The resulting precipitate was filtered off, washed with water and dried (1.9 g). Column chromatography over silica gel (50 g) with chloroform–acetone (7: 1) afforded **4** as a white powder (1.5 g, 75%). $^1$H-NMR ($CDCl_3$): 7.3—8.2 (15H, m, $C_6H_5$). *Anal.* Calcd for $C_{62}H_{80}N_4O_{23}$: C, 60.54; H, 6.32; N, 4.38. Found: C, 60.19; H, 6.65; N, 4.03.

**4″,6″-*O*-Cyclohexylidene-4′-*O*-tetrahydropyranyl-tetra-*N*-ethoxycarbonylkanamycin A** (**5**)——A solution of **4** (1.35 g) in methanol (30 ml) was treated with sodium methoxide (0.4 g) and the solution was stirred for 1 h at room temperature. After neutralization with concd. hydrochloric acid at 0°C, the resulting solution was concentrated to a syrup and chloroform (100 ml) was added to this. The resulting solution was washed twice with 30 ml of water and dried ($MgSO_4$). The solvent was removed by evaporation and the residue was adsorbed on a silica gel column (33 g). The column was developed with chloroform–methanol (20: 1) to give **5** (0.54 g, 54%). *Anal.* Calcd for $C_{41}H_{68}N_4O_{20}$: C, 52.54; H, 7.33; N, 5.98. Found: C, 52.15; H, 7.20; N, 5.62.

**2′,2″-Di-*O*-benzoyl-4″,6″-*O*-cyclohexylidene-4′-*O*-tetrahydropyranyl-tetra-*N*-ethoxycarbonylkanamycin A** (**6**)——Benzoyl chloride (1.74 ml) was added dropwise to a solution of **5** (4.0 g) in pyridine (60 ml) at 5°C. After addition of water (0.3 ml), the solution was concentrated to a syrup and ethyl acetate (200 ml) was added to this. The resulting solution was washed with satd. sodium bicarbonate and water. After being dried with magnesium sulfate, the solution was concentrated to give a powder (4.75 g). Column chromatography on silica gel (120 g) with chloroform–acetone (5: 1) afforded **6** as a white powder (3.3 g, 68%); $^1$H-NMR ($CDCl_3$): 7.3—8.2 (10H, m, $C_6H_5$). *Anal.* Calcd for $C_{55}H_{76}N_4O_{22}$: C, 57.67; H, 6.70; N, 4.89. Found: C, 57.40; H, 6.72; N, 4.57.

**2′,2″-Di-*O*-benzoyl-4″,6″-*O*-cyclohexylidene-3′-*O*-methylsulfonyl-4′-*O*-tetrahydropyranyl-tetra-*N*-ethoxycarbonylkanamycin A** (**7**)——Methylsulfonyl chloride (0.25 ml) was added to a solution of **6** (0.92 g) in pyridine (15 ml) and the solution was stirred at 50°C for 1 h. After addition of water (0.1 ml), the solution was concentrated to a syrup and water was added to this. The resulting precipitate was filtered off, washed with water and dried (0.92 g). Column chromatography over silica gel (25 g) with chloroform–acetone (6: 1) afforded **7** as a white powder (590 mg, 60%); $^1$H-NMR ($CDCl_3$): 2.83 (1.5H, s, $SO_2CH_3$), 3.10 (1.5H, s, $SO_2CH_3$). *Anal.* Calcd for $C_{56}H_{78}N_4O_{24}S$: C, 54.98; H, 6.44; N, 4.58; S, 2.62. Found: C, 54.55; H, 6.27; N, 4.58; S, 2.88.

**2′,2″-Di-*O*-benzoyl-3′-*O*-methylsulfonyl-tetra-*N*-ethoxycarbonylkanamycin A** (**8**)——A solution of **7** (500 mg) and trifluoroacetic acid (0.1 ml) in methanol (10 ml) was kept at 50°C for 2 h, then concentrated to dryness *in vacuo*. Column chromatography over silica gel (12 g) with chloroform–methanol (18: 1) afforded **8** as a white powder (273 mg, 63%); mp 180—189°C (dec.), $[\alpha]_D^{25}$ +106.7° ($c$=0.8, $CH_3OH$); $^1$H-NMR ($CDCl_3$): 3.05 (3H, s, $SO_2CH_3$), 7.3—8.2 (10H, m, $C_6H_5$). *Anal.* Calcd for $C_{45}H_{62}N_4O_{23}S$: C, 51.03; H, 5.91; N, 5.29; S, 3.02. Found: C, 50.57; H, 5.87; N, 5.12; S, 3.43.

**Anhydro formation and 3′-Deoxy-tetra-*N*-ethoxycarbonylkanamycin A** (**11**)——i) With Raney-nickel: Sodium methoxide (200 mg) was added to a solution of **8** (500 mg) in methanol (15 ml), and the solution was kept at room temperature for 3 h. The solution containing anhydro derivative (**9**) or (**10**) was hydrogenated with Raney-nickel (1 g) under 2 atm pressure for 5 h. After removal of the catalyst, the solution was neutralized with concd. hydrochloric acid. The solvent was removed by evaporation and the residue was triturated with cold water. The resulting precipitate was filtered off, washed with cold water and dried (310 mg). Reprecipitation from hot water afforded **11** as a white powder (232 mg, 65%); mp 260—280°C (dec.), $[\alpha]_D^{25}$ +92.1° ($c$=0.9, DMF). *Anal.* Calcd for $C_{30}H_{52}N_4O_{18}$: C, 47.60; H, 6.94; N, 7.40. Found: C, 47.27; H, 6.99; N, 7.04.

ii) With Sodium Borohydride: Sodium methoxide (200 mg) was added to a solution of **8** (500 mg) in methanol (15 ml), and the solution was kept at room temperature for 3 h. After neutralization with 2 M hydrochloric acid, the solution was concentrated to a syrup and diglyme (10 ml) was added. A mixture of sodium borohydride (220 mg) and the solution was stirred at 65°C for 8 h. After neutralization with 2 M hydrochloric acid, the solution was concentrated to a syrup, which was treated with cold water and then dried. Reprecipitation from hot water afforded **11** as a white powder (143 mg, 40%).

**3′-Deoxykanamycin A (12)**——A stirred mixture of **11** (200 mg) and $Ba(OH)_2 \cdot 8H_2O$ (400 mg) in water (5 ml) was heated under reflux for 4 h. The mixture was neutralized with dry ice, and filtered to remove the inorganic salt. The filtrate was charged onto a column of Amberlite CG-50 ($NH_4^+$ form, 5 ml). After being washed with water, the column was developed with 0.3 M ammonium hydroxide. The eluate containing **12** was evaporated to dryness to give a colorless solid (89 mg, 71%).

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

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