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Structural Studies on the Cyclic Carbamate Derivatives of Kanamycin A

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Treatment of tetrakis(*N*-benzyloxycarbonyl)-6″-*O*-tritylkanamycin A with sodium hydride in *N*,*N*-dimethylformamide, followed by chromatography, afforded 4′,6′ : 3″,4″-, and 4′,6′ : 2″,3″-bis(cyclic carbamate) and 3″,4″- and 2″,3″-mono(cyclic carbamate) (**2**, **3**, and others). The structures of the position isomers **2** and **3** were determined by the NMR spectra at 500 MHz of their *N*-tosyl-*O*-acetyl derivatives.

Simultaneous protection of vicinal trans-equatorial amino and hydroxyl groups of amino sugars is very advantageous in aminoglucoside synthesis. For this purpose we previously reported an efficient and facile procedure[1,2] involving formation of a cyclic carbamate; this procedure has been widely applied. In this paper, we describe the separation and structural determination of the isomeric carbamate derivatives of kanamycin A as an example of the carbamate formation of complex aminoglycosides.

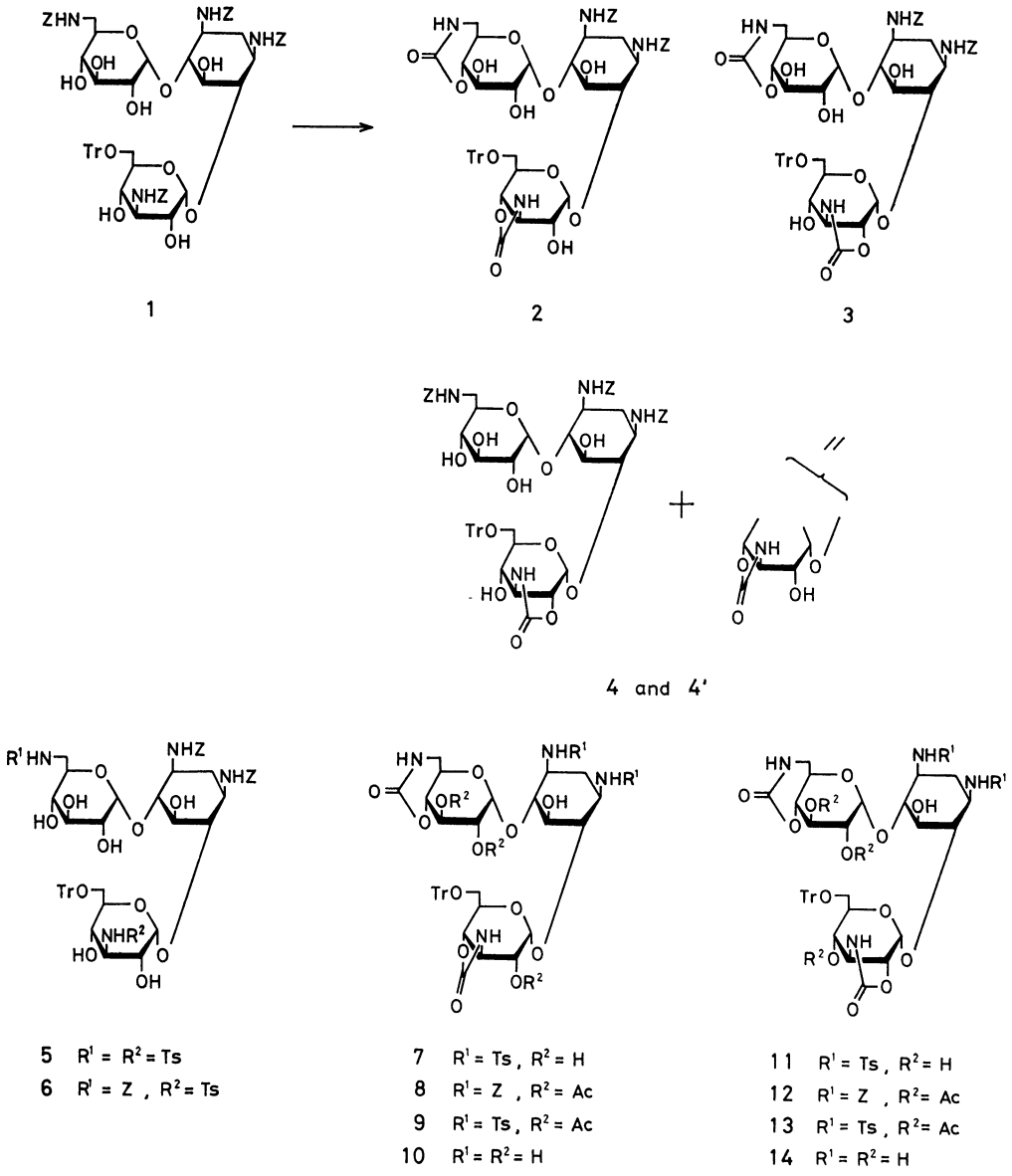
One method[2] for the preparation of cyclic carbamate is to treat an *N*-benzyloxycarbonyl derivative with sodium hydride in *N*,*N*-dimethylformamide (DMF). In the case of kanamycin A, 4′,6′ : 2″,3″-bis(cyclic carbamate) and/or 4′,6′ : 3″,4″-bis(cyclic carbamate) are expected to be formed by this treatment.

Tetrakis(*N*-benzyloxycarbonyl)kanamycin A[3] gave several products which could not be separated[4] by chromatographic separation in pure states owing to their low solubility in common organic solvents. Similar treatment of tetrakis(*N*-benzyloxycarbonyl)-4″,6″-*O*-cyclohexylidenekanamycin A[3] did not improve the purification. In order to enhance the solubility of the products, we introduced a trityl group and prepared tetrakis(*N*-benzyloxycarbonyl)-6″-*O*-tritylkanamycin A (**1**). This compound was soluble in most common organic solvents. Treatment of **1** with sodium hydride in DMF, followed by separation of the products by column chromatography, gave two isomers of mono- (**4**,**4′**; 10% in total) and di-carbamates (**2** and **3**, 62% in total in the ratio of about 5 : 2). All products isolated showed the absorption peak at 1760 cm^{-1}, indicating the presence of a five-membered cyclic carbamate.[1] NMR spectra and elemental analysis showed that **2** and **3** had two benzyloxycarbonyl groups and **4** and **4′** had three.

In order to determine the structures of **2** and **3**, several derivatives were prepared. When **2** or **3** was hydrolyzed with a limited amount of barium hydroxide, the cyclic carbamate rings were cleaved selectively to give the same product, 1,3-bis(*N*-benzyloxycarbonyl)-6″-*O*-tritylkanamycin A. Tosylation of the product gave a di-*N*-tosyl derivative (**5**). Acidic hydrolysis (with 8 M aqueous HCl–EtOH=1 : 1 at 100 °C) of **5** followed by detection by TLC of the hydrolyzates showed a single ninhydrinpositive spot for 2-deoxystreptamine, but no spots corresponding to 6-amino-6-deoxy- and 3-amino-3-deoxy-D-glucose or their glucosides were observed. This indicates the location of the tosyl groups of **5** at 6′- and 3″-amino groups; it is therefore clear that the same amino groups in **2** and **3** are protected by the cyclic carbamates. This result was further supported by another experiment. Catalytic hydrogenolysis of the benzyloxycarbonyl groups of **2** and **3**, followed by tosylation of the products, gave 1,3-di-*N*-tosyl derivatives (**7** and **11**). On cleavage of the cyclic carbamates followed by acidic hydrolysis, those derivatives gave 3-amino-3-deoxy- and 6-amino-6-deoxy-D-glucose (detected by TLC), a result which supports the structures of **7** and **11**.

The structures of the position isomers **2** and **3** were clarified by high-resolution NMR spectra of their tri-*O*-acetyl derivatives. Acetylation of **2** and **3** in the usual manner gave the tri-*O*-acetyl derivatives (**8** and **12**). In the NMR spectra of **12** at 200 MHz, H-2′ appeared as a quartet at δ 5.35, (J=3.5 and 10 Hz), and, H-3′ and H-4″ appeared as triplets at δ 6.27 (J=10 Hz) and 5.76 (J=9 Hz). The assignments were made from their shift and J values and suggested that the five-membered cyclic carbamate of **12** is formed between the 3″-amino and 2″-hydroxyl groups; therefore, **8** was deduced to have the 3″,4″-carbamate structure. These results were further verified by the NMR spectra at 500 MHz of **9** and **13**, which were obtained from **8** and **12** by catalytic hydrogenolysis followed by *N*-tosylation. In the NMR spectrum of **9**, H-2′ and H-2″ appeared as quartets at δ 5.30 and 5.51, respectively, and H-3′ appeared as a triplet at 5.80. In the NMR spectrum of **13**, H-2′ appeared as a quartet at δ 5.30 and H-3′ and H-4″ appeared as triplets at 6.04 and 5.69. These assignments were confirmed by the decoupling method. These results clearly showed that **9** and **13**, and consequently **2** and **3**, have the 3″,4″-*N*,*O*- and 3″,2″-*N*,*O*-carbonyl structure, respectively. It is noteworthy that the yield of the 3″,4″-*N*,*O*-carbonyl derivative (**2**, 38%) dominates over that of 3″,2″-derivative (**3**, 15%) in contrast to the results for the 3″,2″-*N*,*O*-carbonyl structure given by Kumar *et al.*[4] Debenzyloxycarbonyl derivatives (**10** and **14**) could not be obtained in pure state from **2** and **3**, but they will be usable as key intermediates for derivations of the 1- and 3-amino groups of kanamycin A.

Similarly, the minor products **4** and **4′** were determined to be monocarbamates. The mixture of **4** and **4′** was subjected to sequential mild alkaline hydrolysis and *N*-tosylation to afford 3″-*N*-tosyl derivative (**6**). Acid hydrolyzate of **6** showed, on TLC, two ninhydrin-positive spots of 2-deoxystreptamine and 6-amino-6-deoxy-D-glucose, suggesting that cyclic carbamate was formed at the 3″-amino group. When the mixture of **4** and **4′** was hydrogenated and then tosylated, two products (**15** and **16**) were obtained in a ratio of 2.4 : 1.



NMR spectra and elemental analysis of **15** and **16** showed that each has three tosyl groups. Decarbamation of **15** and **16** with barium hydroxide, followed by acidic hydrolysis, gave 3-amino-3-deoxy-D-glucose as the sole ninhydrin-positive product (checked by TLC), showing that **4** (and **4'**) is 3″,2″- or 3″,4″-cyclic carbamate. The position of the cyclic carbamate in **4** and **4'** remains unsolved.

Experimental

^{1}H-NMR spectra were recorded at 90, 200, and 500 MHz with Varian EM-390, Varian XL-200, and Bruker WM-500 spectrometers, respectively. TLC was performed on Wakogel B-5 and E. Merck silica gel with sulfuric acid spray for detection and on microcrystalline Avicel SF (Funakoshi Co.) with the spray of 0.5% ninhydrin in pyridine. In the latter case, the solvent system of 1-butanol–pyridine–water–acetic acid (6 : 4 : 3 : 1) was used as developer. For column chromatography, silica gel (Wakogel C-200) was used. On the description of reprecipitation, substance was dissolved in the first-cited solvent, and precipitated by adding the last-cited solvent.

*1,3,6′,3″-Tetrakis(*N*-benzyloxycarbonyl)-6″-*O*-tritylkanamycin A (**1**).* To a solution of tetrakis(*N*-benzyloxycarbonyl)-kanamycin A[3] (3.00 g) in dry pyridine (30 ml), trityl chloride (4.14 g, 2 mol equivalents for the starting material) was added and the solution was kept at room temperature for 18 h. The solution showed, on TLC with chloroform–ethanol (15 : 1), a major spot at R_f 0.3 (**1**). After addition of water (3 ml), the solution was poured into an ice-cold aqueous saturated sodium hydrogencarbonate (300 ml) with stirring. The mixture was extracted with chloroform (100 ml×4) and the chloroform solution was washed with aqueous sodium hydrogencarbonate and concentrated with several additions of toluene to give a pale yellow solid (6.76 g). The solid was chromatographed over silica gel with benzene as the developer to remove triphenylmethanol, then with ethyl acetate–chloroform (7 : 1) to elute **1**, 2.98 g (80%). The solid was reprecipitated from chloroform–hexane, $[\alpha]_D^{25}$ +64° (*c* 1, dioxane).

Found: C, 65.35; H, 5.96; N, 4.39%. Calcd for $C_{69}H_{74}N_4O_{19}$: C, 65.60; H, 5.90; N, 4.44%.

*Reaction of **1** with Sodium Hydride in DMF (Formation of **2**, **3**, **4**, and **4'**).* To an ice-cold solution of **1** (20.1 g) in dry

DMF (200 ml), 50% oily sodium hydride (5.02 g, 6.6 mol equivalents for **1**) was added under nitrogen atmosphere and the mixture was stirred at 5 °C for 13 h whereupon a slurry resulted. On TLC with chloroform–ethanol (7 : 1), the slurry showed four spots: R_f 0.36 (trace), 0.30 (**4**, minor), 0.24 (**3**, minor), and 0.21 (**2**, major). The slurry was poured into an ice-cold 1% acetic acid solution (1.6 l) and the mixture was extracted with chloroform (0.5 l×4). The chloroform solution was washed with aqueous sodium hydrogencarbonate and water, dried over sodium sulfate and concentrated with several additions of xylene to give a syrup, which was washed with ether. The resulting pale brown solid (16.9 g) was charged on a silica gel column and eluted with chloroform-ethanol (8 : 1). From the earlier fractions, a mixture of 1,3,6′-tris(*N*-benzyloxycarbonyl)-3″,2″-*N,O*-carbonyl-6″-*O*-tritylkanamycin A (**4**) and its 3″,4″-*N,O*-carbonyl isomer (**4′**) were obtained, 1.79 g (10%). This mixture was reprecipitated from chloroform–ether, $[\alpha]_D^{25}$ +60° (*c* 1, dioxane); IR (KBr): 1760 (five-membered cyclic carbamate), 1700, 1520 cm^{-1}.

Found: C, 64.45; H, 5.77; N, 4.81%. Calcd for $C_{62}H_{66}N_4O_{18}$: C, 64.46; H, 5.76; N, 4.85%.

From the middle fractions, a solid of 1,3-bis(*N*-benzyloxycarbonyl)-6′,4′: 3″, 2″-di-*N,O*-carbonyl-6″-*O*-tritylkanamycin A (**3**), 2.56 g (15%), was obtained. Since the solid was slightly contaminated with **2**, **4**, and **4′**, it was purified by column chromatography and then reprecipitated from dioxane–water, $[\alpha]_D^{25}$ +46° (*c* 1, dioxane); IR (KBr): 1760 (five-membered cyclic carbamate), 1700, 1510 cm^{-1}.

Found: C, 62.21; H, 5.47; N, 5.15%. Calcd for $C_{55}H_{58}N_4O_{17}\cdot H_2O$: C, 62.02; H, 5.68; N, 5.26%.

From the last fractions, a solid of 1,3-bis(*N*-benzyloxycarbonyl)-6′,4′: 3″,4″-di-*N,O*-carbonyl-6″-*O*-tritylkanamycin A (**2**), 6.33 g (38%), was obtained. Since the solid was slightly contaminated with **3**, it was further purified by column chromatography and then reprecipitated from chloroform–ether, $[\alpha]_D^{25}$ +55° (*c* 1, dioxane); IR (KBr): 1760, 1700, 1510 cm^{-1}.

Found: C, 63.32; H, 5.71; N, 5.33%. Calcd for $C_{55}H_{58}N_4O_{17}$: C, 63.09; H, 5.58; N, 5.35%.

From the fractions between the middle and the last fractions, an additional mixture of **2** and **3** (1.50 g, 9%) was obtained.

*1,3-Bis(*N*-benzyloxycarbonyl)-6′,3″-di-*N*-tosyl-6″-*O*-tritylkanamycin A (**5**).* *a) From **2**:* To a solution of **2** (101 mg) in aqueous acetone (1 : 10, 5.5 ml), $Ba(OH)_2\cdot 8H_2O$ (37 mg) was added; the mixture was then stirred at 60 °C for 26 h. More $Ba(OH)_2\cdot 8H_2O$ (15 mg) was added and the mixture was stirred for an additional 3 h. The resultant mixture showed, on TLC with chloroform–methanol–8% ammonia (1 : 1 : 1, lower layer), spots of R_f 0.36 (trace), 0.22 (trace), 0.13 (major), and 0.05 (slight). After introduction of carbon dioxide, the mixture was filtered and the solid was washed thoroughly with dioxane. The filtrate and the washings were combined and concentrated to give a solid, 94 mg. To an ice-cold solution of the solid (94 mg) in aqueous dioxane (1 : 6, 2.5 ml), anhydrous sodium carbonate (24 mg) and tosyl chloride (42 mg) were added and the mixture was stirred at 5 °C for 15 h. On TLC with chloroform–ethanol (15 : 1), the mixture showed spots of R_f 0.39 (trace), 0.34 (trace), 0.20 (**5**, major), and 0.05 (slight). The mixture was poured into ice–water and the precipitate was filtered, washed with water and ether, and dried. The solid (94 mg) was chromatographed over silica gel with chloroform–ethanol (15 : 1). Concentration of the fractions containing only **5** gave a colorless solid, 42 mg (32%), $[\alpha]_D^{25}$ +60° (*c* 1, chloroform); IR (KBr): 1710, 1520, 1320 (ν_{as} SO_2), 1150(ν_s SO_2) cm^{-1}; ^{1}H-NMR (pyridine-d_5): δ 2.10 and 2.12 (each 3H s, CH_3 of Ts).

Found: C, 61.52; H, 5.77; N, 4.18; S, 4.98%. Calcd for $C_{67}H_{74}N_4O_{19}S_2$: C, 61.74; H, 5.72; N, 4.30; S, 4.92%.

*b) From **3**:* Compound **3** (58 mg) was treated as described above to give a solid of **5**, 23 mg (31%), $[\alpha]_D^{25}$ +63° (*c* 1, chloroform). The IR and ^{1}H-NMR spectra were superimposable with those of **5** obtained in *a)*.

*1,3,6′-Tris(*N*-benzyloxycarbonyl)-3″-N-tosyl-6″-O-tritylkanamycin A (**6**).* To an solution of a mixture of **4** and **4′** (99 mg) in aqueous dioxane (2 : 3, 5 ml), $Ba(OH)_2\cdot 8H_2O$ (28 mg) was added and the mixture was stirred at 60 °C for 14 h. The resultant mixture was then treated as described for **5** to give a colorless solid, 92 mg. The solid was then treated with tosyl chloride (19 mg) and anhydrous sodium carbonate (11 mg) as described for **5** to give a solid of **6**, 50 mg (46%), $[\alpha]_D^{25}$ +53° (*c* 0.8, chloroform); IR (KBr): 1710, 1520, 1320 (ν_{as} SO_2), 1150(ν_s SO_2) cm^{-1}; ^{1}H-NMR (pyridine-d_5): δ 2.11 (3H s, CH_3 of Ts).

Found: C, 63.37; H, 5.94; N, 4.16; S, 2.28%. Calcd for $C_{68}H_{74}N_4O_{19}S_1$: C, 63.64; H, 5.81; N, 4.37; S, 2.50%.

*6′,4′ : 3″,4″-Di-*N,O*-carbonyl-1,3-di-N-tosyl-6″-O-tritylkanamycin A (**7**).* To a solution of **2** (53 mg) in aqueous dioxane (1 : 5, 3 ml), 0.2 M aqueous hydrochloric acid was added until the pH became 4—5; the solution was then hydrogenated in the presence of palladium black at room temperature for 1.5 h. During the reaction, pH was maintained at 4—5 by occasional additions of diluted hydrochloric acid. Palladium black was removed by filtration. To the ice-cold filtrate, anhydrous sodium carbonate (20 mg) and tosyl chloride (22 mg) were added and the mixture was stirred at 5 °C for 15 h. The reaction mixture showed, on TLC with chloroform–ethanol (7 : 1), a major spot at R_f 0.2 (**7**). Concentration to a small volume followed by addition of water gave a solid, which was washed with water and ether, and dried. The solid (46 mg) was chromatographed over silica gel with the same solvent system to give **7**, 42 mg (76%), $[\alpha]_D^{25}$ −3° (*c* 1, dioxane); IR (KBr): 1760, 1700, 1330, 1160 cm^{-1}. The absorption peak for amide II (≈1510 cm^{-1}) disappeared; ^{1}H-NMR (pyridine-d_5): δ 2.31 (6H s, CH_3 of Ts).

Found: C, 58.33; H, 5.50; N, 4.88; S, 5.72%. Calcd for $C_{33}H_{58}N_4O_{17}S_2$: C, 58.55; H, 5.38; N, 5.15; S, 5.90%.

*2′,3′,2″-Tri-*O*-acetyl-1,3-bis(*N*-benzyloxycarbonyl)-6′,4′ : 3″,4″-di-*N,O*-carbonyl-6″-O-tritylkanamycin A (**8**).* To a solution of **2** (327 mg) in dry pyridine (6 ml), acetic anhydride (0.4 ml) was added and the solution was kept at room temperature for 25 h. The solution showed, on TLC with chloroform–ethanol (7 : 1), a single spot of **8** (R_f 0.43). After addition of water (0.4 ml), the solution was concentrated *in vacuo* and the chloroform solution of the concentrate was washed with aqueous potassium hydrogensulfate and water, dried (Na_2SO_4), and concentrated to give a colorless solid 332 mg (91%), $[\alpha]_D^{25}$ +80° (*c* 1, dioxane); ^{1}H-NMR (pyridine-d_5): δ 1.73, 2.00, 2.23 (each 3H s, Ac).

Found: C, 62.52; H, 5.60; N, 4.61%. Calcd for $C_{61}H_{64}N_4O_{20}$: C, 62.45; H, 5.50; N, 4.78%.

*2′,3′,2″-Tri-*O*-acetyl-6′,4′ : 3,″4″-di-*N,O*-carbonyl-1,3-di-N-tosyl-6″-O-tritylkanamycin A (**9**).* To a solution of **8** (228 mg) in aqueous dioxane (1 : 10, 11 ml), 1 M hydrochloridc acid was added until it became weakly acidic (pH≈4); the solution was then hydrogenated in the presence of palladium black for 2 h. During the reaction, pH was maintained at ≈4 by occasional additions of 1 M hydrochloric acid. The solution was filtered and water (3 ml) was added. To this solution, anhydrous sodium carbonate (70 mg) and tosyl chloride (91 mg) were added, and the mixture was treated as described for **7** to give a solid of **9** (after chromatography with chloroform–ethanol=15 : 1), 144 mg (61%), $[\alpha]_D^{25}$ +73° (*c* 1, dioxane); IR (KBr): 1760 (broad), 1330, 1160 cm^{-1}; ^{1}H-NMR (pyridine-d_5) (at 500 MHz): δ 1.61, 2.01, 2.28, 2.32,

2.38 (each 3H s, Ac and CH_3 of Ts), 3.31 (1H t, $J_{5',6'a}=J_{6'a,6'b}=10$ Hz, H-6'a), 3.41 (1H dd, $J_{5'',6''a}=6$ Hz, $J_{6''a,6''b}=10.5$ Hz, H-6''a), 3.49 (1H dd, $J_{5'',6''b}=3$ Hz, $J_{6''a,6''b}=10.5$ Hz, H-6''b), 3.76 (1H, ddd, $J_{6'b,NH}=4.5$ Hz, $J_{5',6'b}=6.5$ Hz, $J_{6'a,6'b}=10$ Hz, H-6'b), 4.34 (1H, t, $J_{3',4'}=J_{4',5'}=10$ Hz, H-4'), 4.96 (1H, dt, $J_{4',5'}=J_{5',6'a}=10$ Hz, $J_{5,6'b}=6.5$ Hz, H-5'), 5.300 (1H, m, H-5''), 5.51 (1H, dd, $J_{1'',2''}=3.5$ Hz, $J_{2'',3''}=10.5$ Hz, H-2''), 5.302 (1H, dd, $J_{1',2'}=4$ Hz, $J_{2',3'}=10$ Hz, H-2'), 5.80 (1H, t, $J_{2',3'}=J_{3',4'}=10$ Hz, H-3'), 6.33 (1H, d, $J_{1',2'}=4$ Hz, H-1'), 6.47 (1H, d, $J_{1'',2''}=3.5$ Hz, H-1''). The signals assignable to H-5'' cculd only be discerned after resolution enhancement.

Irradiation at δ 5.30 (H-2' and 5'') collapsed the quartet of H-6''a to a doublet, the quartet of H-6''b to a doublet, the triplet of H-3' to a doublet, and the doublet of H-1' to a singlet. Irradiation at δ 5.51 (H-2'') collapsed the doublet of H-1'' to a singlet. Irradiation at δ 6.33 (H-1') collapsed the quartet of H-2' to a doublet. Irradiation at δ 6.47 (H-1'') collapsed the quartet of H-2'' to a doublet.

Found: C, 58.02; H, 5.51; N, 4.35; S, 4.84%. Calcd for $C_{59}H_{64}N_4O_{20}S_2$: C, 58.41; H, 5.32; N, 4.62; S, 5.29%.

6',4'-3'',4''-Di-N,O-*carbonyl-6''*-O-*tritylkanamycin A* (**10**). Compound **2** (550 mg) dissolved in ethanol–acetic acid (97 : 3, 28 ml) was hydrogenated with palladium black as described before. Filtration followed by concentration gave a solid which was thoroughly washed with ether to give **10**, 366 mg. The solid was slightly contaminated with an impurity of R_f 0.39 (on TLC developed with the lower layer of chloroform–methanol–20% acetic acid=5 : 7 : 5; *cf* **10**, 0.33). IR (KBr): 1760 (five-membered cyclic carbamate), 1700 cm^{-1} (six-membered cyclic carbamate). No peak for amide II (near 1520 cm^{-1}).[4]

6',4'-3'',2''-Di-N,O-*carbonyl-1,3-di*-N-*tosyl-6''*-O-*tritylkanamycin A* (**11**). Compound **3** (55 mg) was hydrogenated and tosylated as described for **7**. The crude solid (46 mg, 81%) obtained was chromatographed over silica gel with chloroform–ethanol (7 : 1) to give pure **11**, 34 mg $[\alpha]_D^{25}$ −1° (*c* 1, dioxane); Ir (KBr): 1760, 1700, 1330, 1160 cm^{-1}; ^{1}H-NMR (pyridine-d_5): δ 2.30 (6H s, CH_3 of Ts).

Found: C, 58.84; H, 5.52; N, 5.05; S, 5.72%. Calcd for $C_{53}H_{58}N_4O_{17}S_2$: C, 58.55; H, 5.38; N, 5.15; S, 5.90%.

2',3',4''-Tri-O-*acetyl-1,3-bis*(N-*benzyloxycarbonyl*)-*6',4'-3'',2''-di*-N,O-*carbonyl-6''*-O-*tritylkanamycin A* (**12**). Compound **3** (331 mg) was acetylated as described for **8** to give a solid of **12**, 344 mg (93%), $[\alpha]_D^{25}$ +87° (*c* 1, dioxane); ^{1}H-NMR (pyridine-d_5) (at 200 MHz): δ 1.60, 1.77, 1.99 (each 3H s, Ac), 5.35 (1H, dd, $J_{1',2'}=3.5$ Hz, $J_{2',3'}=10$ Hz H-2'), 5.43 (2H, AB q, $C_6H_5C\underline{H}_2OCO$, $J=12$ Hz), 5.53 (2H s, C_6H_5-$C\underline{H}_2OCO$), 5.76 (1H, t, $J=9$ Hz, H-4''), 6.06 (1H, broad s, H-1''), 6.27 (1H t, $J=10$ Hz, H-3'), 6.31 (1H, d, $J=3.5$ Hz, H-1').

Found: C, 62.21, H, 5.52; N, 4.56%. Calcd for $C_{61}H_{64}N_4O_{20}$: C, 62.45; H, 5.50; N, 4.78%.

2',3',4''-Tri-O-*acetyl-6',4'-3'',2''-di*-N,O-*carbonyl-1,3-di*-N-*tosyl-6''*-O-*tritylkanamycin A* (**13**). Compound **12** (246 mg) was hydrogenated and tosylated as described for **9**. The crude solid (183 mg, 72%) was chromatographed over silica gel with chloroform–ethanol (15 : 1) to give pure **13**, 173 mg (68%), $[\alpha]_D^{25}$ +66° (*c* 1, dioxane); IR (KBr): 1750 (broad), 1330, 1160 cm^{-1}; ^{1}H-NMR (pyridine-d_5) (at 500 MHz): δ 1.61, 1.78, 2.01 (each 3H, s, Ac); 2.32, 2.36 (each 3H, s, CH_3 of Ts), 3.13 (1H, dd, $J_{5'',6''a}=4$ Hz, $J_{6''a,6''b}=10.5$ Hz, H-6''a), 3.369 (1H, dd, $J_{5'',6''b}=2$ Hz, $J_{6''a,6''b}=10.5$ Hz, H-6''b), 3.374 (1H t, $J_{5',6'a}=J_{6'a,6'b}=10$ Hz, H-6'a), 4.41 (1H, t, $J_{3',4'}=J_{4',5'}=10$ Hz, H-4'), 4.86 (1H, ddd, $J_{5'',6''b}=2$ Hz, $J_{5'',6''a}=4$ Hz, $J_{4'',5''}=10$ Hz, H-5''), 5.22 (1H, dt, $J_{5',6'b}=6.5$ Hz, $J_{5',6'a}=J_{4',5'}=10$ Hz, H-5'), 5.30 (1H, dd, $J_{1',2'}=4$ Hz, $J_{2',3'}=10$ Hz, H-2'), 5.69 (1H, t, $J_{3'',4''}=J_{4'',5''}=10$ H-4''), 6.04 (1H, t, $J_{2',3'}=J_{3',4'}=10$ Hz, H-3'), 6.14 (1H, d, $J_{1',2'}=4$ Hz, H-1'), 6.49 (1H, d, $J_{1'',2''}=2$ Hz, H-1'').

Irradiation at δ 4.86 (H-5'') collapsed the quartet of H-6''a to a doublet, the quartet of H-6''b to a doublet, and the triplet of H-4'' to a doublet. Irradiation at δ 5.22 (H-5') collapsed the triplet of H-4' to a doublet. Irradiation at δ 5.30 (H-2') collapsed the triplet of H-3' to a doublet and the doublet of H-1' to a singlet. Irradiation at δ 5.69 (H-4'') collapsed the octet of H-5'' to a quartet.

Found: C, 58.13; H, 5.38; N, 4.35; S, 5.13%. Calcd for $C_{59}H_{64}N_4O_{20}S_2$: C, 58.41; H, 5.32; N, 4.62; S, 5.29%.

6',4'-3'',2''-Di-N,O-*carbonyl-6''*-O-*tritylkanamycin A* (**14**). Compound **3** (105 mg) was hydrogenated as described for **10** to give a solid **14** (75 mg). The solid was slightly contaminated with an impurity of R_f 0.39. (TLC with the lower layer of chloroform–methanol–20% acetic acid=5 : 7 : 5; *cf.* **14**, 0.33); IR (KBr): 1760, 1700 cm^{-1}. No peak for amide II was observed.

*3'',2''-(and 3'',4''-)*N,O-*Carbonyl-1,3,6'-tri*-N-*tosyl-6''*-O-*tritylkanamycin A (A Mixture of* **15** *and* **16**). A mixture of **4** and **4'** (526 mg) was hydrogenated and tosylated as described for **7**. The reaction mixture showed, on TLC with chloroform–ethanol (7 : 1), spots of R_f 0.46 (trace), 0.34 (**15**, major), and 0.23 (**16**, minor). Concentration gave a syrup, which was washed with water and ether, and dried to give a solid. It was chromatographed over silica gel with chloroform–ethanol (12 : 1) to give **15**, 234 mg (42%) and **16**, 97 mg (18%).

15: $[\alpha]_D^{25}$ +32° (*c* 1, dioxane); IR (KBr): 1760, 1320, 1160 cm^{-1}; ^{1}H-NMR (dioxane-d_8–D_2O=9 : 1): δ 2.43 (6H, s, CH_3 of Ts), 2.47 (3H, s, CH_3 of Ts).

Found: C, 58.02; H, 5.59; N, 4.41; S, 7.69%. Calcd for $C_{59}H_{66}O_{18}S_3$: C, 58.31; H, 5.47; N, 4.61; S, 7.91%.

16: $[\alpha]_D^{25}$ +16° (*c* 1, dioxane); IR (KBr): 1760, 1320, 1160 cm^{-1}; ^{1}H-NMR (dioxane-d_8–D_2O=9 : 1): δ 2.42 (3H, s, CH_3 of Ts), 2.49 (6H, s, CH_3 of Ts).

Found: C, 58.04; H, 5.71; N, 4.34; S, 7.61%.

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