Aminoglycoside antibiotics. The total synthesis of 5-deoxykanamycin A

GERRY KAVADIAS,[1] PIERRE DEXTRAZE, ROBERT MASSÉ, AND BERNARD BELLEAU

Bristol Laboratories of Canada, 100 Industrial Boulevard, Candiac, P.Q., Canada J5R 1J1

Received December 16, 1972

GERRY KAVADIAS, PIERRE DEXTRAZE, ROBERT MASSÉ, and BERNARD BELLEAU. Can. J. Chem. **56**, 2086 (1978).

Condensation of 1,6-*N*,*O*-carbonyl-3-*N*-ethoxycarbonyl-2,5-dideoxystreptamine (**7**) with 6-azido-2,3,4-tri-O-benzyl-6-deoxy-α-D-glucopyranosyl chloride by the Koenigs–Knorr reaction produced the α-glycoside **10**. The cyclic carbamate of **10** was opened with ethanol in the presence of sodium ethoxide and the resulting 4-*O*-(6-azido-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-glucopyranosyl)-*N*,*N*′-diethoxycarbonyl-2,5-dideoxystreptamine (**12**) was glycosidated with 3-acetamido-2,4,6-tri-*O*-benzyl-3-deoxy-α-D-glucopyranosyl chloride (**21**) to give the α,α-diglycoside **22**. Hydrogenation of the azido group and removal of the protective groups gave 5-deoxykanamycin A (**25**), an antibiotic with a potency about half that of kanamycin A.

GERRY KAVADIAS, PIERRE DEXTRAZE, ROBERT MASSÉ et BERNARD BELLEAU. Can. J. Chem. **56**, 2086 (1978).

La condensation de la *N*,*O*-carbonyl-1,6 *N*-éthoxycarbonyl-3 didéoxy-2,5 streptamine (**7**) avec le chlorure de l'azido-6 tri-*O*-benzyl-2,3,4 déoxy-6 α-D-glucopyrannosyle, en faisant appel à la réaction de Koenigs–Knorr, conduit à l'α glycoside **10**. On a ouvert le carbamate cyclique de **10** avec de l'éthanol en présence d'éthylate de sodium et la *O*-(azido-6 tri-*O*-benzyl-2,3,4 déoxy-6 α-D-glucopyrannosyl)-4 *N*,*N*′-diéthoxycarbonyl didéoxy-2,5 streptamine (**12**) qui en résulte a été soumise à une réaction de glycosidation avec le chlorure de l'acétamido-3 tri-*O*-benzyl-2,4,6 déoxy-3 α-D-glucopyrannosyle (**21**) pour conduire à l'α,α-diglycoside **22**. L'hydrogénation du groupe azido et l'enlèvement des groupes protecteurs conduit à la déoxy-5 kanamycine A (**25**), un antibiotique dont la puissance est à peu près égale à la moitié de celle de la kanamycine A.

[Traduit par le journal]

In recent years, extensive studies have been carried out on the modification of aminoglycoside antibiotics with the purpose of studying the structure–activity relationship and of producing new antibiotics that are effective against resistant bacteria (1).

Most of the modifications have been directed at the aminosugar part of the molecule and only a few studies have been reported on the relationship between the structural features of the aminocyclitol moiety and the activity of the antibiotics (2).

The present paper describes the synthesis of 5-deoxykanamycin A (**25**) by glucosidation of the readily available and suitably protected 2,5-dideoxystreptamines (3) **7** and **14** by the Koenigs–Knorr reaction and demonstrates the usefulness of **7** and **14** as key intermediates for the synthesis of other 5-deoxyaminocyclitol antibiotics. With the exception of the mutamicins 2 and 2a, no other 5-deoxyaminocyclitol antibiotics have been reported (4). The interesting antibacterial properties of mutamicin 2 was an added incentive in the pursuit of our goals.

In the synthesis of aminoglycosides of 2-deoxystreptamine by the Koenigs–Knorr reaction, mercuric cyanide and dioxane–benzene or dioxane–chloroform are commonly used as catalyst and solvent, respectively (5–13). The role of dioxane in assisting the formation of α-glycosides has been discussed (7). The low solubility of the protected 2-deoxy- and 2,5-dideoxystreptamines in dioxane–benzene solutions led us to examine the use of dimethylformamide as the solvent in this coupling reaction. To establish workable experimental conditions for the synthesis of 5-deoxykanamycin A using dimethylformamide as the solvent, the condensation of **7** and **14** with the readily available 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl chloride (**6**) (14) was examined first.

Reaction of **7** with **6** in DMF in the presence of mercuric cyanide at 65°C for 24 h gave, after purification on a silica column, 4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-1,6-*N*,*O*-carbonyl-3-*N*-ethoxycarbonyl-2,5-dideoxystreptamine (**8**) in 80% yield. The nmr signal for the anomeric proton in **8** was not clearly defined so that structure **8** could only be inferred. Removal of the protective benzyl groups by catalytic reduction of **8** in the presence of 10% palladium-on-carbon followed by opening of the carbamate ring of **9** with a catalytic amount of sodium in absolute ethanol gave 4-*O*-(α-D-glucopyranosyl)-*N*,*N*′-diethoxycarbonyl-2,5-dideoxystreptamine (**11**). The nmr spectrum of **11** in deuterium oxide showed a doublet at δ 5.08 with a coupling of 2.8 Hz, values which are characteristic of the

[1]Author to whom correspondence may be addressed.

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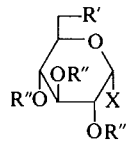
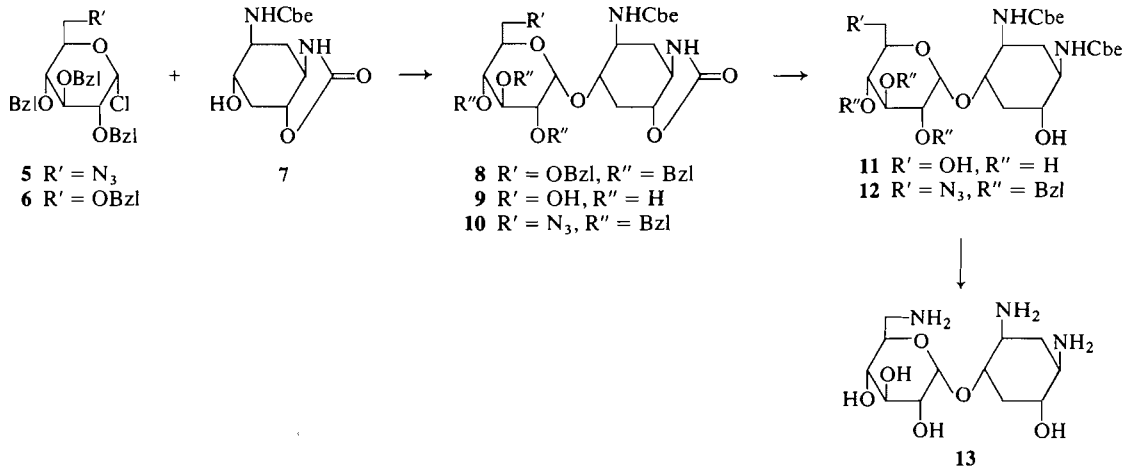


1 R′ = N_3, R″ = Ac, X = OCH_3
2 R′ = N_3, R″ = H, X = OCH_3
3 R′ = N_3, R″ = Bzl, X = OCH_3
4 R′ = N_3, R″ = Bzl, X = OH
5 R′ = N_3, R″ = Bzl, X = Cl
6 R′ = OBzl, R″ = Bzl, X = Cl



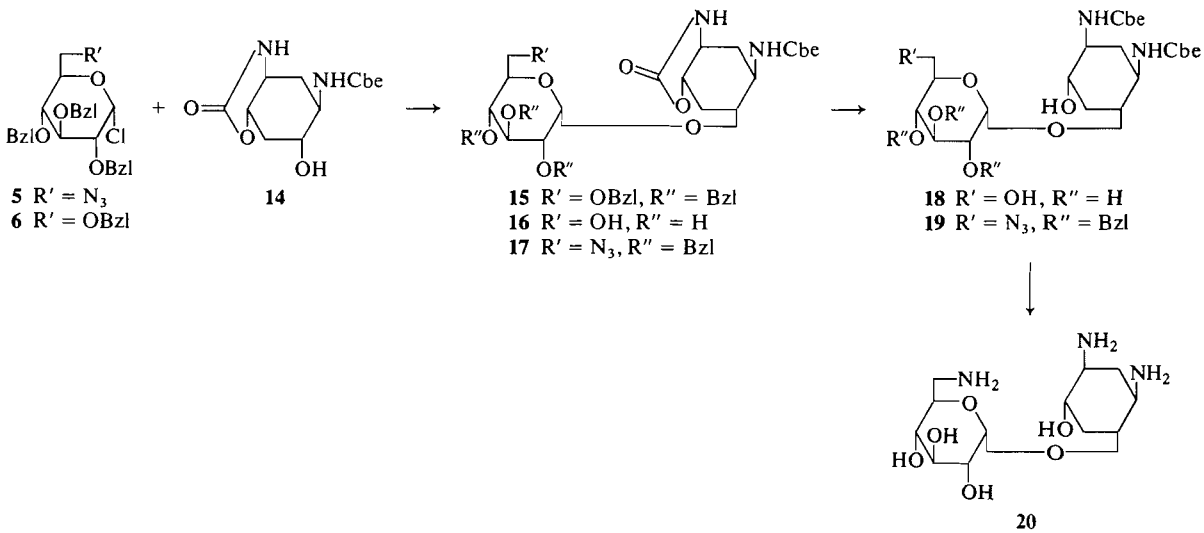
5 R′ = N_3
6 R′ = OBzl

7

8 R′ = OBzl, R″ = Bzl
9 R′ = OH, R″ = H
10 R′ = N_3, R″ = Bzl

11 R′ = OH, R″ = H
12 R′ = N_3, R″ = Bzl

13



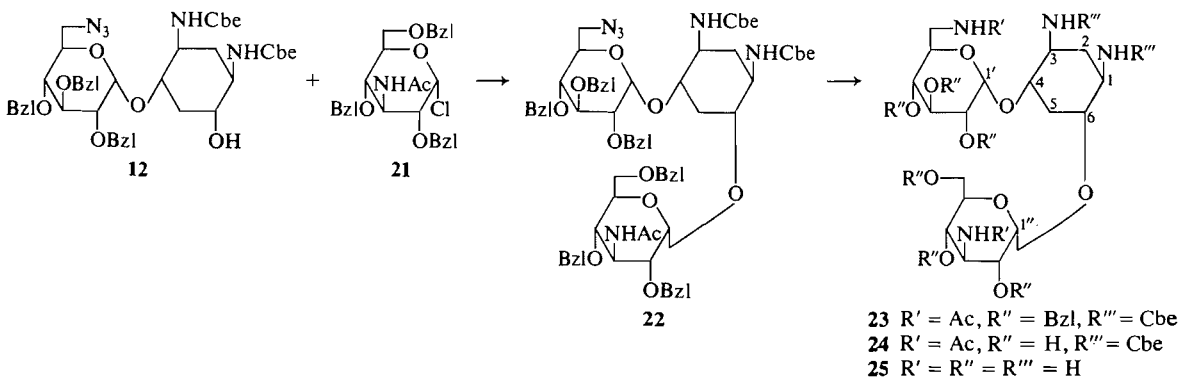
5 R′ = N_3
6 R′ = OBzl

14

15 R′ = OBzl, R″ = Bzl
16 R′ = OH, R″ = H
17 R′ = N_3, R″ = Bzl

18 R′ = OH, R″ = H
19 R′ = N_3, R″ = Bzl

20



12

21

22

23 R′ = Ac, R″ = Bzl, R‴ = Cbe
24 R′ = Ac, R″ = H, R‴ = Cbe
25 R′ = R″ = R‴ = H

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expected signal for the anomeric hydrogen of α-D-glucopyranosides.

Condensation of 3,4-*N*,*O*-carbonyl-1-*N*-ethoxycarbonyl-2,5-dideoxystreptamine (**14**) with **6** as described above for the synthesis of **8** afforded 6-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-3,4-*N*,*O*-carbonyl-1-*N*-ethoxycarbonyl-2,5-dideoxystreptamine (**15**) as crystals, mp 188–190°C (75.5% yield). To establish the anomeric configuration of this compound, the benzyl protective groups were removed by hydrogenation and the cyclic carbamate opened with sodium in ethanol to give 6-*O*-(α-D-glucopyranosyl)-*N*,*N*′-diethoxycarbonyl-2,5-dideoxystreptamine (**18**) as a crystalline solid, mp 218–220°C, in 84% yield based on **15**. The nmr spectrum of **18** clearly supported the α-glucosidic structure since it exhibited a doublet at δ 5.02 with a coupling of 3.5 Hz for the anomeric hydrogen.

The synthesis of 6-azido-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-glucopyranosyl chloride (**5**) has been reported (15) and the advantages afforded by this intermediate in glycosidation reactions by comparison with the acylamino or aryloxycarbonylamino derivatives of the glycosyl halides have been discussed (8). In this synthesis, 6-azido-2,3,4,-tri-*O*-benzyl-6-deoxy-D-glucose (**4**) (the precursor of **5**) was obtained from the methylglucoside **3** by acid hydrolysis with hydrochloric acid in acetic acid. In our hands, the hydrolysis of **3** by the above method gave poor yields (10–15%) of **4**, the major product being a polar compound with R_f 0 (tlc). The hydrolysis of **3** to **4** was best accomplished by heating **3** in aqueous dioxane in the presence of *p*-toluenesulfonic acid (38% yield). The reaction was slow (5 days) but the only other major component of the reaction mixture was the starting material **3**.

Condensation of 1,6-*N*,*O*-carbonyl-3-*N*-ethoxycarbonyl-2,5-dideoxystreptamine (**7**) with **5** in DMF in the presence of mercuric cyanide at 80–85°C for 24 h produced, after purification on a silica column, 4-*O*-(6-azido-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-glucopyranosyl)-1,6-*N*,*O*-carbonyl-3-*N*-ethoxycarbonyl-2,5-dideoxystreptamine (**10**) in 53.5% yield. The cyclic carbamate of **10** was opened by heating in boiling ethanol in the presence of a catalytic amount of sodium to give 4-*O*-(6-azido-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-glucopyranosyl)-*N*,*N*′-diethoxycarbonyl-2,5-dideoxystreptamine (**12**), mp 167–168°C, in 70% yield. Compound **12** was hydrogenated over palladium hydroxide whereupon the azido group was reduced and the benzyl protecting groups removed. The product was then heated in boiling aqueous barium hydroxide to give 4-*O*-(6-amino-6-deoxy-α-D-glucopyranosyl)-2,5-dideoxystreptamine (**13**), mp 235–237°C with decomposition beginning at 220°C; $[\alpha]_D^{25}$ +83.6°, $[\alpha]_{436}^{25}$ +168°; $\Delta[M]_{TACu}^{16}$ +767.

The observed positive $\Delta[M]_{TACu}^{16}$ for compound **13** shows that it is the 4-isomer and therefore establishes the absolute configuration of **7** as was previously reported (3).

Condensation of **14** with the 6-azido sugar **5** afforded in 55% yield, 6-*O*-(6-azido-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-glucopyranosyl)-3,4-*N*,*O*-carbonyl-1-*N*-ethoxycarbonyl-2,5-dideoxystreptamine (**17**), mp 150–151°C. Reaction of **17** with a catalytic amount of sodium in ethanol gave compound **19**, mp 201–203°C, in 80% yield. The protective groups of **19** were removed as described above for the synthesis of **13**, to give 6-*O*-(6-amino-6-deoxy-α-D-glucopyranosyl)-2,5-dideoxystreptamine (**20**), mp 240–244°C (dec.); $[\alpha]_D^{25}$ +58.3, $\Delta[M]_{TACu}$ −444. The negative $\Delta[M]_{TACu}$ for **20** indicates that it is the 6-isomer and this confirms the assigned absolute configuration of **14** (3).

Condensation of 4-*O*-(6-azido-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-glucopyranosyl)-*N*,*N*′-diethoxycarbonyl-2,5-dideoxystreptamine (**12**) with **21** (17) gave crystalline 4-*O*-(6-azido-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-glucopyranosyl)-6-*O*-(3-acetamido-2,4,6-tri-*O*-benzyl-3-deoxy-α-D-glucopyranosyl)-*N*,*N*′-diethoxycarbonyl-2,5-dideoxystreptamine (**22**), in 70% yield. The azido group of **22** was reduced catalytically and the resulting amino group acetylated to give 4-*O*-(6-acetamido-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-glucopyranosyl)-6-*O*-(3-acetamido-2,4,6-tri-*O*-benzyl-3-deoxy-α-D-glucopyranosyl)-*N*,*N*′-diethoxycarbonyl-2,5-dideoxystreptamine (**23**), mp 210–213°C, in 67% yield. Debenzylation of **23** in the usual manner produced crystalline **24**. The nmr spectrum of the latter showed the signals for the anomeric hydrogens, H-1″ and H-1′, at δ 4.98 and 5.06 with a coupling of 3.5 Hz indicating that both amino sugar units are in the α configuration. Removal of the protective groups of **24** by hydrolysis with aqueous barium hydroxide afforded the desired 5-deoxykanamycin A (**25**). 5-Deoxykanamycin A showed the same spectrum of antibiotic activity as kanamycin A but about half the potency of the latter. Its antibiotic properties are summarized in Table 1.

Experimental

The melting points were determined on a Mel-Temp melting point apparatus and are not corrected. Optical rotations were measured with a Perkin–Elmer Model 141 polarimeter. Nuclear magnetic resonance spectra were taken with a Varian A-60A spectrometer. Tetramethylsilane (for solution other than deuterium oxide) and sodium 4,4-dimethyl-4-silapentane-1-sulfonate (for the solutions of deuterium oxide) were used as internal standards. Thin-layer chromatography (tlc) was carried out on microscope slides coated with silica gel and the spots were visualized with perchloric acid.

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TABLE 1. Minimum inhibitory concentration of 5-deoxykanamycin A against various microorganisms (μg/ml)

Organism	Minimum inhibitory concentration
S. pneum. 9585	125
S. pyogenes 9604[a,b]	125
S. aureus 9537	2
S. aureus 9606	4
S. aureus 20240	>125
E. coli 9632[b]	8
E. coli 21218[b]	>125
E. coli 20895[b]	8
E. coli 20732[b]	125
E. coli 20665[b]	>125
E. coli 20683[b]	>125
E. cloacae 9656	8
E. cloacae 20364[b]	>125
E. cloacae 21006[b]	>125
K. pneum. 9977	1
P. mirab. 9900	32
P. rettgeri 9637	1
P. rettgeri 21207[b]	16
P. stuartii 21210	4
P. stuartii 20894[b]	32
S. marc. 20019	32
S. marc. 20460	>125
P. aerug. 9843A	>125
P. aerug. 20653	>125
P. aerug. 20741	>125
P. aerug. 20717	>125
P. aerug. 20601	>125
P. aerug. 21509	>125

[a]45% antibiotic assay broth, 50% Mueller–Hinton broth, and 5% serum. All others are Mueller–Hinton broth.
[b]10^{-3} organism dilution. All others are 10^{-4} organism dilution.

*Methyl 6-Azido-6-deoxy-2,3,4-tri-*O*-acetyl-α-*D*-glucopyranoside (1)*

This compound was prepared by the method described by Castro *et al.* (18), mp 102–103°C (lit. (19) mp 103°C).

*Methyl 6-Azido-6-deoxy-α-*D*-glucopyranoside (2)*

A solution of 68.2 g methyl 6-azido-6-deoxy-2,3,4-tri-*O*-acetyl-α-D-glucopyranoside in 600 ml dry methanol to which 150 mg sodium hydride had been added (300 mg of 50% NaH–oil suspension washed with benzene), was stirred at room temperature for 45 min and then neutralized with carbon dioxide and the mixture filtered. Evaporation of the filtrate gave 43.3 g of **2** as a syrup; $[\alpha]_D^{22}$ +122° (*c* 1, H_2O) (lit. (15) $[\alpha]_D^{22}$ +122° (*c* 1, H_2O)).

*Methyl 6-Azido-2,3,4-tri-*O*-benzyl-6-deoxy-α-*D*-glucopyranoside (3)*

This compound was prepared from methyl 6-azido-6-deoxy-α-D-glucopyranoside by reaction with benzyl chloride in DMF in the presence of potassium hydroxide according to the published method (15).

*6-Azido-2,3,4-tri-*O*-benzyl-6-deoxy-*D*-glucose (4)*

A solution of 53.5 g (107.3 mmol) of **3** and 81.5 g (430 mmol) *p*-toluenesulfonic acid monohydrate in 430 ml of 70% dioxane–water was heated under reflux for 5 days. After cooling to room temperature, the reaction mixture was poured onto water (500 ml) and the mixture extracted with chloroform (3 × 200 ml). The combined extracts were washed successively with water, aqueous sodium bicarbonate, and water and after drying over sodium sulfate, it was evaporated to dryness. The dark-brown residue showed on tlc (silica, 10% ether–benzene) three spots with R_f values of 0.58 (compound **3**), 0.25 (compound **4**), and 0. The crude product was chromatographed on silica gel (activity II), (300 g, 20 cm × 6.1 cm id) and eluted with 2% ether–benzene. The effluent was collected in 50 ml fractions. Fractions 1–12 were combined and evaporated to dryness to give 23.5 g (44% recovery) of unreacted starting material. Fractions 13–18 gave, after evaporation, 5.0 g of a mixture of starting material **3** and product **4**. The column was then washed with 25% ether–benzene to give 19.3 g (38%) of **4** as a syrup (a mixture of the two anomers) which solidified upon standing at room temperature. This material was used as such in the next reaction.

*6-Azido-2,3,4-tri-*O*-benzyl-6-deoxy-α-*D*-glucopyranosyl Chloride (5)*

This compound was prepared as described by Tagaki *et al.* (15) by reacting **4** with thionyl chloride.

*4-*O*-(2,3,4,6-Tetra-*O*-benzyl-α-*D*-glucopyranosyl)-1,6-*N,*O*-carbonyl-3-*N*-ethoxycarbonyl-2,5-dideoxystreptamine (8)*

To a flame-dried and nitrogen-purged 50-ml three-neck flask fitted with condenser, mechanical stirrer, and nitrogen inlet and outlet tubes was added 2.16 g (8.8 mmol) of **7** (3), 7.3 g of anhydrous calcium sulfate, and 12 ml of dry DMF and the mixture heated to 65°C while stirring under a nitrogen stream. After 1 h, 5.7 g of mercuric cyanide was added together with a solution of 5.5 g (9.8 mmol) of 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl chloride (**6**) (14) in 15 ml of DMF. The mixture was stirred at 65°C for 27 h and after cooling to room temperature, it was diluted with chloroform (50 ml), filtered, and the solids washed with chloroform (3 × 20 ml). The combined filtrate and washings were washed with water, dried, and evaporated to dryness. The residue was chromatographed on a silica column (240 g, 55 cm × 3.3 cm id) and eluted with MEK–benzene (1:4) to give 5.4 g (80%) of **8** as a homogeneous syrup (tlc on silica, 2% MeOH in $CHCl_3$ as solvent). This material was used as such in the next reaction. A sample was crystallized from isopropyl alcohol – diisopropyl ether and then recrystallized from isopropyl alcohol to give the analytical sample, mp 172–173°C; $[\alpha]_D^{25}$ +38.2 (*c* 1.2, $CHCl_3$); ir (KBr): 1760 (cyclic carbamate) 1700 and 1530 cm^{-1} ($NHCO_2Et$). *Anal.* calcd. for $C_{44}H_{50}N_2O_{10}$: C 68.91, H 6.57, N 3.65; found: C 69.02, H 6.63, N 3.72.

*4-*O*-(α-*D*-Glucopyranosyl)-*N,N'*-diethoxycarbonyl-2,5-dideoxystreptamine (11)*

A solution of 4.46 g (5.8 mmol) of **8** in ethanol (220 ml) was hydrogenated over 10% Pd-on-carbon (0.9 g) under a hydrogen pressure of 3 cm of mercury at room temperature for 48 h. The catalyst was removed, washed with ethanol (2 × 30 ml), and the combined filtrate and washings evaporated to give 2.22 g (94%) of **9** as a syrup. Thin-layer chromatography on silica (chloroform–methanol (3:1)) showed a single spot with R_f 0.39.

Compound **9** (2.22 g) was dissolved in absolute ethanol (115 ml) in which 50 mg of sodium hydride had been dissolved (55% suspension in oil) and the solution heated under reflux for 3 h. After cooling to room temperature, it was neutralized with a cation exchange resin and evaporated to dryness to give crystalline **11**, mp 273–275°C (EtOH), $[\alpha]_D^{25}$ +81.0° (*c*, 0.2, H_2O); nmr spectrum at 80 MHz showed δ: 1.24 and 4.08, (triplet and quartet for N—CO_2Et, J = 7.1 Hz), 5.08 (d, 1, H-1, J = 2.8 Hz). *Anal.* calcd. for $C_{18}H_{32}N_2O_{11}$: C 47.78, H 7.13, N 6.19; found: C 47.65, H 7.11, N 6.35.

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6-O-(2,3,4,6,Tetra-O-benzyl-α-D-glucopyranosyl)-3,4-N,O-carbonyl-1-N-ethoxycarbonyl-2,5-dideoxystreptamine (15)

A quantity of 1.8 g (7.3 mmol) of 3,4-*N,O*-carbonyl-1-*N*-ethoxycarbonyl-2,5-dideoxystreptamine (**14**) (3) was reacted with 5.2 g (9.3 mmol) of **6** in DMF (25 ml) in the presence of mercuric cyanide and anhydrous calcium sulfate as described above for the synthesis of **8**. After work-up, the crude syrupy product crystallized upon exposure to ether. The crystalline product was filtered off to give 3.91 g (70%) of **15**, mp 188–190°C. Thin-layer chromatography on silica (2% methanol in chloroform) showed a single spot with R_f 0.24. The mother liquor was evaporated to dryness and chromatographed on a column of silica (60 g, 12 cm × 3.1 cm id) using MEK–benzene 1:2 as the eluent. The fractions containing **15** were combined and evaporated to dryness to give 470 mg which on trituration with ether gave 300 mg of crystalline **15**, mp 190–192°C. This fraction was combined with the first crop above to give a total of 4.21 g (75.5%). Recrystallization from ethanol gave the analytical sample, mp 190–192°C; $[\alpha]_D^{25}$ +19.9 (*c*, 1.4, $CHCl_3$); ir (KBr): 1770 (cyclic carbamate), 1700 cm^{-1} ($NHCO_2Et$). *Anal.* calcd. for $C_{44}H_{50}N_2O_{10}$: C 68.91, H 6.57, N 3.65; found: C 68.84, H 6.60, N 3.72.

6-O-(α-D-Glucopyranosyl)-3,4-N,O-carbonyl-1-N-ethoxycarbonyl-2,5-dideoxystreptamine (16)

A mixture of 4.0 g (5.2 mmol) of **15** in ethanol (400 ml) was hydrogenated over 0.8 g of 10% Pd/C as described for the synthesis of **11** above. Removal of the ethanol solvent by evaporation gave crystalline **16**. Recrystallization from ethanol gave 2.08 g (88.5%) of crystalline **16** containing **1** mol of EtOH of crystallization, mp 242–243°C; $[\alpha]_D^{25}$ +45.4 (*c*, 2.0, H_2O); ir (KBr): 3320, 1745, 1705, 1690, and 1543 cm^{-1}; nmr (D_2O) δ: 5.02 (d, one proton, H-1, $J_{1,2}$ = 3.5 Hz). *Anal.* calcd. for $C_{18}H_{20}N_2O_{10}\cdot C_2H_5OH$: C 47.78, H 7.13, N 6.19; found: C 47.87, H 7.17, N 6.34.

6-O-(α-D-Glucopyranosyl)-N,N′-diethoxycarbonyl-2,5-dideoxystreptamine (18)

Compound **16** (1.0 g, 2.46 mmol) in absolute ethanol (30 ml) in which a catalytic amount of sodium hydride had been dissolved was heated under reflux for 3 h and the product isolated as described above for the synthesis of **11** to give compound **18** in 95% yield, mp 218–220°C (EtOH); $[\alpha]_D^{25}$ +67.0 (*c*, 0.75, H_2O); ir (KBr): 1695 and 1540 cm^{-1} ($NHCO_2Et$): nmr (D_2O) δ: 5.02 (d, one proton, H-1, $J_{1,2}$ = 3.5 Hz). *Anal.* calcd. for $C_{18}H_{32}N_2O_{11}$: C 47.78, H 7.13, N 6.19; found: C 47.55, H 7.14, N 6.11.

4-O-(6-Azido-2,3,4-tri-O-benzyl-6-deoxy-α-D-glucopyranosyl)-1,6-N,O-carbonyl-3-N-ethoxycarbonyl-2,5-dideoxystreptamine (10)

A flame-dried, nitrogen purged, three-neck flask (100 ml) equipped with a condenser, mechanical stirrer, and nitrogen inlet and outlet tubes was charged with 2.44 g (10 mmol) of (**7**) (3) and 15 ml dry DMF. Under a slow stream of nitrogen, 5.5 g anhydrous calcium sulfate was added and the mixture stirred at room temperature for 30 min followed by heating to 80°C for 15 min. A solution of 5.1 g (10.3 mmol) of 6-azido-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-glucopyranosyl chloride (**5**) in 5 ml dry DMF was then added, followed by the addition of 6.3 g of mercuric cyanide. The mixture was heated at 80–85°C under nitrogen with stirring for 17 h. Fresh mercuric cyanide, 3.0 g was added and heating continued for 3 h. After cooling to room temperature, the reaction mixture was diluted with chloroform (150 ml) and filtered. The solids were washed with chloroform (2 × 10 ml) and the combined filtrate and washings were washed with water (5 × 100 ml), dried, and evaporated to dryness to give a dark brown sticky residue. On tlc (silica, 5% MeOH–$CHCl_3$) it showed five spots with R_f 0, 0.28 (major, compound **10**), 0.38, 0.5, and 0.92, respectively. The crude product was dissolved in 20 ml chloroform and the solution added dropwise with stirring to 175 ml of petroleum ether (bp 40–60°C). The petroleum ether phase was decanted and the residue dissolved in $CHCl_3$ (20 ml) and the latter solution again added to petroleum ether (175 ml). The petroleum ether phase was again decanted and combined with the preceding petroleum ether phase. The residue, 4.1 g, showed on tlc one major spot with R_f 0.28 (compound **10**) and two minor ones with R_f 0 and 0.38, respectively. The petroleum ether phase showed on tlc a small spot for **10** (R_f 0.28) and the spots with R_f 0.38, 0.5, and 0.92 and upon standing at room temperature overnight it deposited 200 mg of compound **10**. The combined yield of **10** was 4.3 g. This material was further purified by passing through a column of silica (activity II) (45 g, 6.5 cm × 4.3 cm id) using 10% methanol in chloroform as the eluent (250 ml). This treatment removed the impurity with R_f 0. The eluent was evaporated to dryness to give 3.75 g (53.5%) of a brown syrup which on tlc showed one major spot with R_f 0.28 (compound **10**) and a trace of a compound with R_f 0.38. This material was used as such in the next reaction.

An analytical sample was obtained by crystallization from ether, mp 75–85°C; $[\alpha]_D^{25}$ +87.4 (*c*, 1.0, $CHCl_3$); ir ($CHCl_3$): 2110 (N_3), 1760 (cyclic carbamate), 1710 and 1520 cm^{-1} (N—CO_2Et). *Anal.* calcd. for $C_{37}H_{43}N_5O_9$: C 63.32, H 6.18, N 9.98; found: C 63.35, H 6.14, N 9.83.

4-O-(6-Azido-2,3,4-tri-O-benzyl-6-deoxy-α-D-glucopyranosyl)-N,N′-diethoxycarbonyl-2,5-dideoxystreptamine (12)

A solution of 3.75 g of the preceding crude **10** in 30 ml absolute ethanol in which 20 mg NaH (55% in oil) had been dissolved was heated under reflux for 3 h with exclusion of moisture. After cooling to room temperature, acetone (35 ml) was added to dissolve the solids and the solution neutralized by passing through a column containing 4.0 g Amberlite IR-120 (H^+ form, washed with water and EtOH). The neutral effluent was evaporated to dryness to give a residue which on tlc (silica; benzene–MEK, 2:1) showed one major spot with R_f 0.39 (compound **12**) and four minor ones with R_f 0, 0.72, 0.89, and 1.0. It was chromatographed through a column packed with 100 g of silica (activity II) (14.5 cm × 4.0 cm id) and the product eluted with benzene–MEK (2:1). Fractions of 20 ml were collected and the material eluted in tubes 6–20. The combined elutes were evaporated, the residue dissolved in 20 ml of boiling ethanol and the mixture filtered. The filtrate was evaporated to dryness to give 2.8 g (70%) of crystalline compound **12**. Recrystallization from ethyl acetate (10 ml) – petroleum ether (20 ml) gave 2.4 g, mp 167–168°C; $[\alpha]_D^{25}$ +77.4° (*c*, 1.0, $CHCl_3$); ir ($CHCl_3$): 2110 (N_3), 1705 and 1520 cm^{-1} (N—CO_2Et). *Anal.* calcd. for $C_{39}H_{49}N_5O_{10}$: C 62.63, H 6.60, N 9.36; found: C 62.28, H 6.54, N 9.04.

4-O-(6-Amino-6-deoxy-α-D-glucopyranosyl)-2,5-dideoxystreptamine (13)

A mixture of 251 mg of **12** in methanol was hydrogenated in a Paar apparatus over 20% $Pd(OH)_2$-on-carbon (200 mg) at room temperature and at an initial hydrogen pressure of 45 psi. After 16 h, the mixture was filtered through a Celite bed and the cake washed with methanol (25 ml). The combined filtrate and washings were evaporated to dryness to give 4-*O*-(6-amino-6-deoxy-α-D-glucopyranosyl)-*N,N′*-diethoxycarbonyl-2,5-dideoxystreptamine as a foam. Thin-layer chromatography on silica with MeOH–$CHCl_3$–NH_4OH (4:1:1) as the solvent showed a single spot (ninhydrin spray). The material was dissolved in water (10 ml), barium hydroxide (1.2 g) was added, and the solution heated at reflux under nitrogen for 22 h. The mixture was diluted with water (20 ml) and the excess barium hydroxide neutralized by passing

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carbon dioxide through the solution. The resulting mixture was heated to boiling and filtered through a bed of Celite and the solids washed with boiling water (2 × 20 ml). The filtrate and the washings were combined and acidified with 1 *N* sulfuric acid to precipitate barium. The mixture was filtered through Celite, the filtrate neutralized with an anion exchange resin (Rexyn 201, OH^- form), and the solution evaporated to dryness to give a syrupy residue which solidified on treatment with methanol. Thin-layer chromatography on silica using $CHCl_3$—MeOH—NH_4OH—H_2O (1:4:2:1) as solvent showed one major spot with R_f 0.40 (ninhydrin spray) and some impurities with lower R_f values. This material was absorbed on a cation exchange resin (Rexyn 102, NH_4^+ form, 3 ml) and the column was first washed with water (50 ml) and then with 0.2 *N* aqueous ammonium hydroxide which after evaporation gave **13** (51 mg, 50%) as a solid, mp 235–237°C (it foamed and decomposed); $[\alpha]_D^{25}$ +83.6 (*c* 0.30, H_2O), $[\alpha]_{436}^{25}$ +168° (*c*, 0.30, H_2O), $[\alpha]_{436}^{25}$ +418 (*c*, 0.35, TACu), $\Delta[M]_{TACu}$ +767.

*6-O-(6-Azido-2,3,4-tri-O-benzyl-6-deoxy-α-D-glucopyranosyl)-3,4-N,O-carbonyl-1-N-ethoxycarbonyl-2,5-dideoxystreptamine (**17**)*

Compound **14** (2.70 g, 11.14 mmol) was reacted with 5.96 g (12.1 mmol) of 6-azido-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-glucopyranosyl chloride (**5**) in dry DMF (23 ml) at 83–85°C, in the presence of mercuric cyanide and calcium sulfate as described above for the synthesis of **10**. After the reaction was completed (20 h) the reaction mixture was cooled to room temperature, diluted with chloroform (150 ml), and filtered. The solids were washed with chloroform (3 × 20 ml) and after combining the washings with the filtrate, the solution was washed with water, dried and evaporated to dryness to give 6.54 g of a brown syrup. Thin-layer chromatography on silica using 5% methanol in chloroform as solvent showed two spots with R_f 0.33 (major, compound **17**) and 0.61 (minor). The crude product was chromatographed on a column of silica (activity III) (150 g, 20 cm × 3.8 cm id) using MEK–benzene (1:4) mixture as the eluent. Fractions of 10 ml were collected and fractions 4–15 contained the compound with R_f 0.61 (silica, 5% MeOH–$CHCl_3$). They were combined and evaporated to dryness to give 1.4 g of a syrup which consisted of 6-azido-2,3,4-tri-*O*-benzyl-6-deoxy-D-glucose (**4**). The fractions 20–98 were combined and evaporated to dryness to give 4.29 g (54.8%) of compound **17** as a syrup. It was crystallized from ethanol–ether to give crystalline **17**, mp 150–151°C; $[\alpha]_D^{25}$ +33.0 (*c*, 1.0, $CHCl_3$); ir (Nujol): 3340 (NH), 2110 (N_3), 1750 (cyclic carbamate) 1710 cm^{-1} (*N*-carbethoxy). *Anal.* calcd. for $C_{37}H_{43}N_5O_9$: C 63.32, H 6.18, N 9.98; found: C 63.35, H 6.15, N 10.16.

*6-O-(6-Azido-2,3,4-tri-O-benzyl-6-deoxy-α-D-glucopyranosyl)-N,N′-diethoxycarbonyl-2,5-dideoxystreptamine (**19**)*

A solution of 2.22 g (3.2 mmol) of **17** in dry ethanol (50 ml) in which 20 mg of sodium hydride (55% oil suspension) had been dissolved was heated under reflux for 3 h. After cooling to room temperature, it was neutralized by treatment with Amberlite IR 120 (H^+ form), the resin filtered off, and the filtrate evaporated to dryness to give 2.4 g (100%) of solid material which on tlc (silica, 5% MeOH–$CHCl_3$) showed one major spot with R_f 0.30 (compound **19**) and traces of a compound with R_f 0.12. Recrystallization from ethanol–ethylacetate (5:1) gave 1.92 g (80.6%) of **19**, mp 201-203°C; $[\alpha]_D^{25}$ +40.2 (*c*, 1.45, $CHCl_3$); ir (Nujol): 3310 (NH, OH), 2110 (N_3), 1690 and 1540 cm^{-1} (*N*-carbethoxy). *Anal.* calcd. for $C_{39}N_{49}N_5O_{10}$: C 62.63, H 6.60, N 9.36; found: C 62.50, H 6.51, N 9.48.

*6-O-(6-Amino-6-deoxy-α-D-glucopyranosyl)-2,5-dideoxystreptamine (**20**)*

A solution of 1.10 g of **19** in methanol (30 ml) was hydrogenated over 1 g of 20% $Pd(OH)_2$-on-carbon as described for the preparation of **13** above. The product was hydrolyzed with aqueous barium hydroxide and purified as described above (compound **13**) to give 252 mg of **20** as an amorphous solid, mp 240–244°C (dec.); $[\alpha]_D^{25}$ +58.3 (*c*, 0.75, H_2O), $[\alpha]_{436}^{25}$ +114 (*c*, 0.75, H_2O), $[\alpha]_{436}^{25}$ −30.8 (*c*, 0.51, in TACu), $\Delta[M]_{TACu}$ −444.5.

*4-O-(6-Azido-2,3,4-tri-O-benzyl-6-deoxy-α-D-glucopyranosyl)-6-O-(3-acetamido-2,4,6-tri-O-benzyl-3-deoxy-α-D-glucopyranosyl)-N,N′-diethoxycarbonyl-2,5-dideoxystreptamine (**22**)*

A flame-dried nitrogen purged 50 ml two-neck flask fitted with mechanical stirrer, condenser, and nitrogen inlet and outlet tubes was charged with 2.99 g (4 mmol) of compound **12** and 10 ml dry DMF and the mixture stirred at room temperature under nitrogen until dissolution was complete. Anhydrous calcium sulfate (3.0 g) was then added and the mixture stirred at room temperature for 30 min. To this was added 1.3 g (4.0 mmol) of 3-acetamido-2,4,6-tri-*O*-benzyl-3-deoxy-α-D-glucopyranosyl chloride (**21**) followed by 3.0 g of powdered mercuric cyanide. The mixture was heated at 80°C while stirring under nitrogen. After 9 h, 1.31 g of **21** and 1.5 g $Hg(CN)_2$ were added, the mixture stirred again at 80°C for 12 h after which time 1.0 g of **21** and 1.0 g $Hg(CN)_2$ were again added. After stirring for 22 h at 80°C, the mixture was cooled to room temperature, diluted with $CHCl_3$ (150 ml), filtered, and the solids washed with $CHCl_3$ (2 × 10 ml). The combined filtrate and washings were washed with water (5 × 150 ml), dried, and evaporated to dryness. Thin-layer chromatography (alumina, 2% MeOH–$CHCl_3$) showed one major spot R_f 0.51 (compound **22**) and three minor ones with R_f values of 0.17, 0.27, and 0.76. The sticky residue on treatment with ethyl acetate – ether crystallized. It was chromatographed through a column packed with alumina (activity II) (150 g) (21.5 cm × 2.8 cm id) and the product eluted in 20 ml fractions with 1% MeOH–$CHCl_3$. Tubes 6–17 were combined and after evaporation gave 4.7 g of a colorless sticky residue which on tlc showed one major spot for **22** and a minor one with R_f 0.76. It was treated with boiling ethyl acetate (40 ml) and the mixture filtered to remove some insoluble solids (220 mg). The filtrate was evaporated to dryness, dissolved into 15 ml of boiling ethyl acetate, and the solution diluted with 30 ml ether. After standing at room temperature overnight, the crystalline product **22** was collected to give 3.40 g (70%), mp 178–188°C. *Anal.* calcd. for $C_{68}H_{80}N_6O_{15}$: C 66.87, H 6.60, N 6.88; found: C 67.28, H 6.67, N 6.57.

*4-O-(6-Acetamido-2,3,4-tri-O-benzyl-6-deoxy-α-D-glucopyranosyl)-6-O-(3-acetamido-2,4,6-tri-O-benzyl-3-deoxy-α-D-glucopyranosyl)-N,N′-diethoxycarbonyl-2,5-dideoxystreptamine (**23**)*

A solution of 3.0 g (2.46 mmol) of **22** in 100 ml of 90% EtOH–water was hydrogenated in a Paar apparatus over Raney nickel at room temperature and at an initial hydrogen pressure of 55 psi. After 3 h, the reaction mixture was filtered and the catalyst washed with EtOH. The combined filtrate and washings were evaporated to dryness to give 2.77 g (94.5%) of a syrup which solidified. Infrared spectroscopy confirmed that hydrogenation was complete (absence of azido band). Thin-layer chromatography on silica (10% MeOH–$CHCl_3$) showed one major spot with R_f 0.43 and four minor ones. The material was dissolved in dry pyridine (10 ml) and acetic anhydride (1.5 ml) was added. After standing at room temperature for 1 h, water (0.5 ml) was added, the solution diluted

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with $CHCl_3$ (150 ml), and washed first with water (3 × 100 ml), then with 5% aqueous HCl (100 ml), and finally with water (100 ml). After drying and removal of the solvent by evaporation, the sticky residue was treated with boiling ethylacetate (25 ml) and the crystalline product **23** collected to give 1.95 g (67%), mp 210–213°C. Recrystallization from EtOH produced an analytical sample; mp 217–218°C. *Anal.* calcd. for $C_{70}H_{84}N_4O_{16}$: C 67.96, H 6.84, N 4.53; found: C 68.09, H 6.90, N 4.57.

4-O-(6-Acetamido-6-deoxy-α-D-glucopyranosyl)-6-O-(3-acetamido-3-deoxy-α-D-glucopyranosyl)-N,N′-*diethoxycarbonyl-2,5-dideoxystreptamine* (**24**)

A solution of 700 mg of compound **23** in 80 ml 95% ethanol–water was hydrogenated over 100 mg of Pd/C (10%) at room temperature and at atmospheric pressure for 24 h. The reaction mixture was filtered and the catalyst washed with boiling 70% EtOH–water (4 × 15 ml). The combined filtrate and washings were evaporated to dryness to give 360 mg of crystalline **24**; mp 260–262°C (dec.). The nmr spectrum at 100 MHz in D_2O showed δ: 1.22 and 1.24 (two triplets for CH_3 of $NHCO_2Et$), 4.10 and 4.12 (two quartets for CH_2 of $NHCO_2Et$), 2.03 (s, 6H, NHAc), 4.98 (d, 1, H-1″, $J_{1'',2''}$ = 3.5 Hz) 5.06 (d, 1, H-1′, $J_{1',2'}$ = 3.5 Hz). *Anal.* calcd. for $C_{28}H_{48}N_4O_{16}$: C 48.27, H 6.94, N 8.04; found: C 47.53, H 6.84, N 7.64.

5-Deoxykanamycin A (**25**)

A solution of 360 mg (0.515 mmol) of **24** in 20 ml of 1 *N* aqueous barium hydroxide was heated at reflux for 24 h while excluding CO_2. It was then diluted with 30 ml water, neutralized with CO_2, heated to boiling, filtered, and the solids washed with boiling water (3 × 30 ml). The filtrate and washings were combined, 5 ml of 1 *N* H_2SO_4 added to precipitate barium, and the solids removed by filtration. The filtrate was passed through a column containing 10 g Rexyn 201 (strongly basic resin in the OH^- form) to remove the acids (H_2SO_4 and AcOH) and the effluent evaporated to dryness to give 200 mg of sticky residue. Thin-layer chromatography on silica (H_2O–MeOH–NH_4OH–$CHCl_3$, 1:4:2:1, ninhydrin spray) showed two spots with R_f 0.19 (major, compound **25**) and R_f 0.49 (probably the cyclic urea). The material was chromatographed using a column packed with Rexyn 102 (NH_4^+ form) (0.9 cm id × 10.2 cm). The column was washed first with water (100 ml), then with 1 *N* ammonium hydroxide (300 ml). The effluent containing compound **25** was evaporated to dryness, the residue dissolved in water and the solution treated with an anion exchange resin (Rexyn 201, OH^- form) to remove some ammonium carbonate. After evaporation of the resin filtrate to dryness, the residue was treated with ethanol and the crystalline material filtered to give 140 mg of **25**, mp 250–255°C (dec.) (decomposition started at 160°C); $[\alpha]_D^{25}$ +101.8 (*c*, 0.33, water). The nmr spectrum at 100 MHz in D_2O showed δ: 5.17 (d, 1, H-1″, $J_{1'',2''}$ = 3.75 Hz), 5.22 (d, 1, H-1′, $J_{1',2'}$ = 3.75 Hz). *Anal.* calcd. for $C_{18}H_{36}N_4O_{10}$: C 46.15, H 7.74; found: C 45.95, H 7.67.

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