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Note

Carbohydrate triflates: Introduction of substituents by double inversion at C-4″ of kanamycin A

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By the introduction of substituents with inversion of configuration at C-4″ of kanamycin A (**1**), some biologically interesting products, such as 4″-deoxy-4″-fluoro-4″-*epi*-kanamycin A (**17**), were obtained[1,2]. For tracing the effect of the substituent itself, modification with retention of configuration had also to be explored.

With regard to the complexity of the educt, we focussed our efforts on the stereospecific approach of "double inversion"; but, starting from 2′,3′,4′,2″,6″-penta-*O*-acetyl-4″-bromo-tetra-*N*-(*tert*-butoxycarbonyl)*-4″-deoxy-4″-*epi*-kanamycin A (**11**; ref. 2), all attempts at reinversion failed and resulted in regiospecific elimination, with formation of 2′,3′,4′,2″,6″-penta-*O*-acetyl-tetra-*N*-Boc-4″-deoxy-4″-eno-kanamycin A (**19**; ref. 2) only. Inasmuch as, in an earlier investigation[1], we had overcome similar difficulties by using trifluoromethanesulfonate (triflate) as the leaving group, the preparation of 4″-*epi*-kanamycin A protected at all positions** except O-4″ became of interest. Having at our disposal 2′,3′,4′,2″,6″-penta-*O*-acetyl-tetra-*N*-Boc-kanamycin A (**2**; ref 3), a method for the configurational inversion with direct formation of the corresponding *epi*-hydroxy compound (or, at least, allowing its selective liberation) had to be applied in order to avoid laborious de- and re-protection.

From the procedures described to effect such transformations, the sulfonate–potassium superoxide reaction of Corey *et al.*[5], as well as use of the trifluoroacetyl[6] and the chloroacetyl group[7], seemed, in our hands, to be unsuitable, as evidenced by the formation of numerous, polar by-products. Encouraging results were, however, obtained on application of the sulfonate–nitrite inversion-reaction[8,9] to carbohydrate triflates. Thus, for example[10], methyl 2,3,6-tri-*O*-acetyl-α-D-galactopyranoside was formed in 78% yield by treatment of methyl 2,3,6-tri-*O*-acetyl-4-

tert-Butoxycarbonyl is designated Boc.
**In the presence of *N*-Boc[3], or *N*-(ethoxycarbonyl) groups[4], the 5-hydroxyl group of the deoxystreptamine part does not react in simple esterification reactions.

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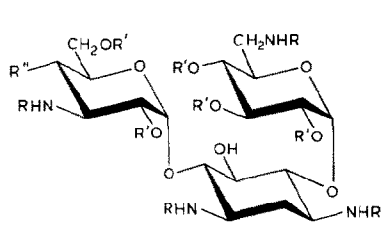
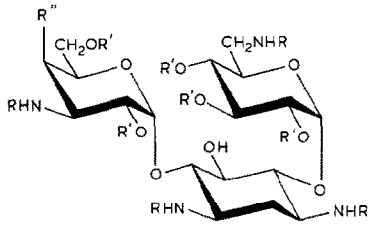


	R	R′	R″
1	H	H	OH
2	Boc	Ac	OH
3	Boc	Ac	OTf
4	Boc	Ac	Br
5	Boc	Ac	F
6	Boc	Ac	N_3
7	H	H	N_3
8	H	H	NH_2
9	H	H	Br
10	H	H	F

Boc = Me_3COCO

Tf = F_3CSO_2

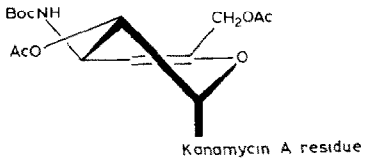


	R	R′	R″
11	Boc	Ac	Br
12	Boc	Ac	OH
13	Boc	Ac	OTf
14	H	H	N_3
15	H	H	NH_2
16	H	H	Br
17	H	H	F
18	H	H	OH

O-triflyl-α-D-glucopyranoside with sodium nitrite in *N,N*-dimethylformamide for 1 h at room temperature.

Application of this procedure during 3 h to the kanamycin-A-4″-triflate **3** also led to the corresponding *epi*-hydroxy compound, *i.e.*, 2′,3′,4′,2″,6″-penta-*O*-acetyl-tetra-*N*-Boc-4″-*epi*-kanamycin A (**12**), in a yield of 83%.

In the re-inversion reactions applying azide, bromide, and fluoride, respectively, use was again made of the pronounced nucleofugality of the triflyloxy group. Nevertheless, starting from 4″-*epi*-triflate **13**, substitution was, in each case, accompanied by regiospecific elimination, affording **19**. (When the same conditions were applied to the 4″-triflate **3**, intramolecular attack of the neighboring *tert*-butoxycarbonyl group, to give a cyclic urethan, was a side reaction, as reported previously[1].)



19

Because **19** and the respective product of substitution (**4, 5,** or **6**) showed only very small differences in their chromatographic mobilities, the reaction mixtures were immediately treated with sodium methoxide (to effect *O*-deacetylation) and

then trifluoroacetic acid (to cause *N*-deprotection); the latter reagent rapidly decomposed the "glycal type" resulting from **19**. Thus, on treatment of 2′,3′,4′,2″,6″-penta-*O*-acetyl-tetra-*N*-Boc-4″-*O*-triflyl-4″-*epi*-kanamycin A (**13**) with sodium azide in *N,N*-dimethylformamide, and tetrabutylammonium bromide and fluoride (both in acetonitrile), respectively, followed by deprotection and ion-pair chromatography, 4″-azido-4″-deoxy- (**7**), 4″-bromo-4″-deoxy- (**9**), and 4″-deoxy-4″-fluoro-kanamycin A (**10**) were obtained; the yields, based on starting material **12**, were 48, 49, and 46%, respectively.

Hydrogenation of an aqueous solution of **7** in the presence of Raney nickel gave 4″-amino-4″-deoxykanamycin A (**8**, 93%).

The structural modifications present in compounds **7–10** were proved by ^{13}C-n.m.r. spectroscopy, which demonstrated the position of the substituents (by ^{13}C–^{19}F couplings in the spectrum of **10**: C-4″, ~180 Hz; C-3″, 18 Hz; C-6″, 7 Hz) and their steric orientation (chemical shift of C-3″, ~54–56 p.p.m.; products with inverted configuration at C-4″ generally show C-3″ signals between 51 and 52 p.p.m.); the nature of the substituents is evident from the substitution shifts observed, namely, azide, −7; amine, −19; bromide, −17; and fluoride, +20 p.p.m.

The results of antibacterial screening of compounds **7–10**, compared with kanamycin A (**1**), and of their 4″-*epi*-counterparts **14–18**, are collected in Table I. As far as may be judged from the strains tested, no dramatic influence arising from the configuration of C-4″ could be found; improvement of biological activity (if any) is effected only by the introduction of fluorine (although the basicity of the 3″-amino group is thus lowered considerably[11]).

TABLE I

MINIMUM INHIBITORY CONCENTRATION (μg/mL) OF 4″-MODIFIED KANAMYCINS **7–10** AND **14–18** IN COMPARISON TO KANAMYCIN A (**1**) (TRYPTICASE SOYA BROTH)

Organism	*Compound*									
	1	**18**	**7**	**14**	**8**	**15**	**9**	**16**	**10**	**17**
Staphylococcus aureus Δ 162	0.15	0.15	0.5	0.15	0.3	1.56	0.5	0.3	0.3	0.08
Staphylococcus aureus Δ 56	0.6	1.25	1.56	0.8	2.5	5	2.5	2.5	2.5	0.5
Streptococcus faecalis Δ76	10	5	10	5	25	25	10	2.5	10	2.5
Pseudomonas aeruginosa Δ 12	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
Escherichia coli Δ 120	2.5	4	10	5	25	25	50	10	10	2.5
Proteus mirabilis Δ 89	10	5	10	5	12.5	25	10	5	5	2.5

EXPERIMENTAL

General. — Melting points (Tottoli) are uncorrected. Optical rotations were measured with a Perkin–Elmer 141 polarimeter. T.l.c. was performed on silica gel 60 F_{254} precoated aluminum sheets (Merck 5554) using 1:2 (v/v) ethyl acetate–toluene (Solvent *A*) for *N*/*O*-protected and 1:2:2 (v/v/v) chloroform–methanol–25% ammonia (Solvent *B*) for unprotected derivatives, respectively. Column chromatography[12] was accomplished on silica gel 60, 230–400 mesh (Merck 9385), eluted with 1:1 (v/v) ethyl acetate–toluene. Ion-pair chromatography was performed on Amberlite CG 50 (NH_4^+) resin, using 0.1–0.3M ammonia as the eluant. ^{13}C-N.m.r. spectra for solutions in deuterium oxide were recorded at 22.62 MHz with a Bruker WH-90 DS instrument in the pulsed, Fourier-transform, proton-decoupled mode, using 1,4-dioxane (67.4 p.p.m.) as the internal standard; resolution, 1.471 Hz per data point; shifts upon acidification with DCl (pD 1), in parentheses; data obtained from the corresponding 4″-*epi*-compounds[1,2] (related to the same standard) are given in square brackets.

2′,3′,4′,2″,6″-Penta-O-*acetyl-tetra*-N-(tert-*butoxycarbonyl*)-*4″*-epi-*kanamycin A* (**12**). — To a solution of compound[3] **2** (6.0 g, 5.48 mmol) in 19:1 dichloromethane–pyridine (120 mL) was added a mixture of trifluoromethanesulfonic anhydride (1.1 mL, 6.70 mmol) and dichloromethane (10 mL) at −5°. After 1 h at this temperature, t.l.c. showed quantitative formation of triflate **3** (R_F 0.72; for **2**, 0.52; *A*). The mixture was rapidly washed successively with cold M hydrochloric acid (100 mL) and saturated, aqueous hydrogencarbonate (100 mL), dried (sodium sulfate), and evaporated at <35°, to give syrupy **3**. Its solution in *N,N*-dimethylformamide (70 mL) was immediately treated with sodium nitrite (3.0 g, 43.5 mmol) for 3 h (t.l.c.) at room temperature, the suspension filtered, and the filtrate evaporated, and compound **12** was isolated by chromatography; yield, 5.0 g (83.3%); m.p. 140–142°, $[\alpha]_D^{20}$ +84.9° (*c* 1.1, chloroform), R_F 0.52 (*A*). Complete deprotection of **12** gave 4″-*epi*-kanamycin A[1] (**18**), identified by its ^{13}C-n.m.r. spectrum.

4″-Azido-4″-deoxykanamycin A (**7**). — By reaction of **12** (3.3 g, 3 mmol) with triflic anhydride (0.6 mL, 3.66 mmol), as described for the synthesis of **3**, triflate **13** (R_F 0.72) was formed. Treatment of its solution in *N,N*-dimethylformamide (70 mL) with sodium azide (1.95 g, 30 mmol) at room temperature led within 3 h to a mixture of **6** and **19** (R_F 0.56 for both). After filtration, and evaporation of the filtrate, the resulting brown residue was mixed with ethyl acetate (50 mL), the suspension filtered through a short column of silica gel (25 mL), and the filtrate evaporated to dryness. A solution of the residue in 0.01M methanolic sodium methoxide (50 mL) was kept for 30 min at room temperature, made neutral with Amberlite IR 120 (H^+) resin (15 mL), the suspension filtered, the filtrate evaporated, and the residue treated with trifluoroacetic acid (12 mL) until gas evolution ceased (5 min).

By addition of abs. ethyl ether (100 mL), a precipitate was obtained; this was dissolved in water (20 mL), and the solution treated with Dowex-1 X-1 (OH^-)

resin (15 mL); **7** was isolated by ion-pair chromatography; yield, 0.74 g (48.4%); $[\alpha]_D^{20}$ +128.7° (*c* 0.78, water); R_F 0.72 (*B*); ^{13}C-n.m.r.: δ 53.6 (54.0) C-3″ and 63.1 (58.6) C-4″ [for **14**: δ 52.1 (51.7) C-3″ and 64.5 (60.1) C-4″].

Anal. Calc. for $C_{18}H_{35}N_7O_{10}$ (509.5): C, 42.43; H, 6.92; N, 19.24. Found: C, 41.94; H, 6.78; N. 19.02.

4-″Amino-4″-deoxykanamycin A (**8**). — A solution of **7** (0.51 g, 1 mmol) in 1:1 ethanol–water (10 mL) was hydrogenated in the presence of Raney nickel (0.5 g) at 0.4 MPa. After the reaction was complete (5 h, t.l.c.), the catalyst was filtered off, and the filtrate evaporated, to yield **8** as a white, amorphous powder; yield, 0.45 g (93.1%); $[\alpha]_D^{20}$ +130.4° (*c* 0.96, water); R_F 0.48 (*B*); ^{13}C-n.m.r.: δ 55.0 (53.3) C-3″ and 51.1 (50.4) C-4″ [for **15**: δ 52.1 (51.6) C-3″ and 52.1 (50.8) C-4″].

Anal. Calc. for $C_{18}H_{37}N_5O_{10}$ (483.5): C, 44.71; H, 7.71; N, 14.48. Found: C, 44.47; H, 7.24; N. 13.98.

4-″Bromo-4″-deoxykanamycin A (**9**). — Treatment of triflate **13**, prepared from **12** (3.3 g, 3 mmol) as already described, with tetrabutylammonium bromide (5.0 g, 16.2 mmol) in acetonitrile (70 mL) for 0.5 h at room temperature gave a mixture of **4** and **19**, from which **9** was obtained by applying the deprotection and purification procedure described for the synthesis of **7**; yield, 0.81 g (49.3%); $[\alpha]_D^{20}$ +103.4° (*c* 0.62, water); R_F 0.69 (*B*); ^{13}C-n.m.r.: δ 56.3 (56.7) C-3″ and 53.1 (44.4) C-4″ [for **16**: δ 51.5 (52.1) C-3″ and 60.6 (52.1) C-4″].

Anal. Calc. for $C_{18}H_{35}BrN_4O_{10}$ (547.4): C, 39.50; H, 6.44; Br, 14.60; N, 10.23. Found: C, 39.11; H, 6.17; Br, 14.33; N, 9.88.

4-″Deoxy-4″-fluorokanamycin A (**10**). — Compound **10** was prepared *via* **5** as described for **9**, but using tetrabutylammonium fluoride* (3.8 g, 12.5 mmol; predried for 48 h at 120° *in vacuo*) in a reaction time of 4 h; yield, 0.67 g (45.9%); $[\alpha]_D^{18}$ +110.4° (*c* 0.9, water); R_F 0.58 (*B*); ^{13}C-n.m.r.: δ C-2″ n.d. (68.9/7.35 Hz), C-3″ 54.1/17.65 Hz (54.5/17.65 Hz), C-4″ 90.8/178.0 Hz (86.2/182.4 Hz), and C-6″ 61.1/0 Hz (60.8/0 Hz) [for **17**: δ C-3″ 51.0/17.65 Hz (52.1/17.6 Hz), C-4″ 91.5/176.5 Hz (88.3/179.4 Hz), and C-6″ 60.6/5.88 Hz (60.8/5.89 Hz)].

Anal. Calc. for $C_{18}H_{35}FN_4O_{10}$ (486.5): C, 44.44; H, 7.25; N, 11.52. Found: C, 44.13; H, 7.07; N, 11.23.

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*According to Sharma and Fry[13], the product obtained by drying tetrabutylammonium fluoride trihydrate consists mainly of tetrabutylammonium hydrogendifluoride.

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