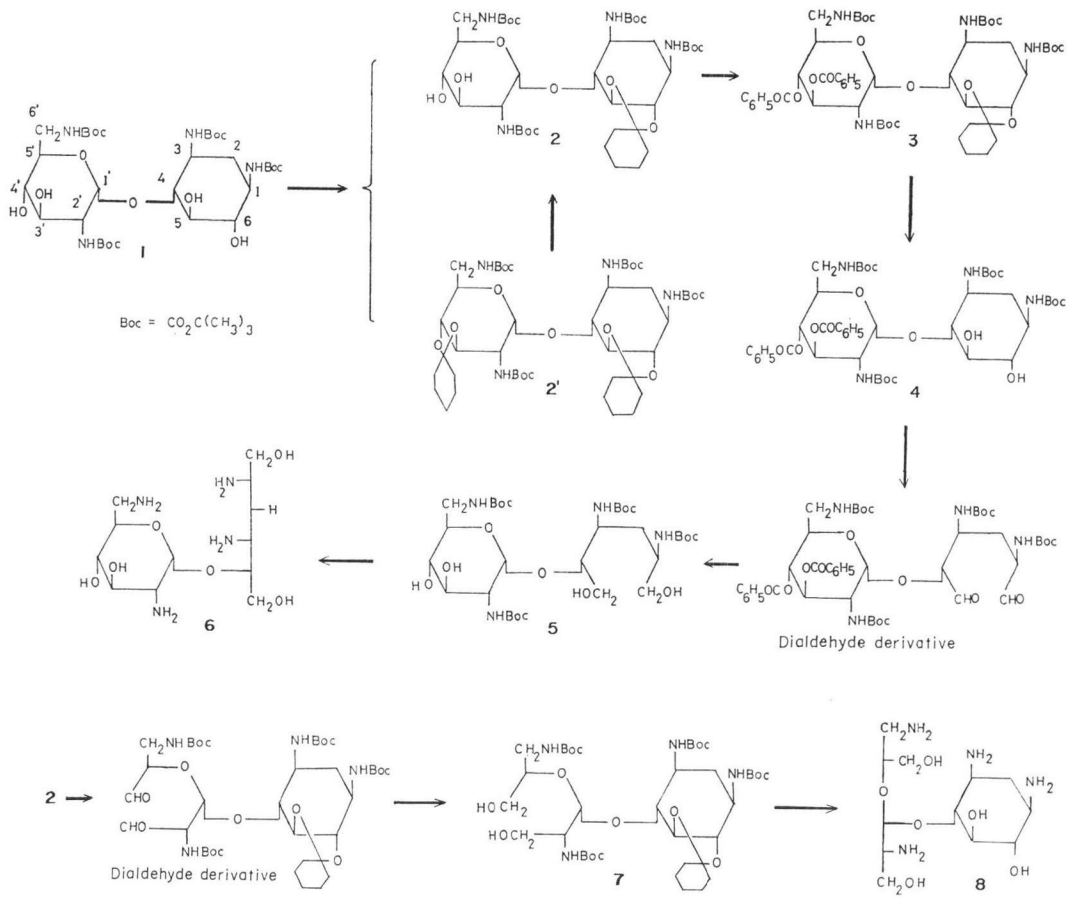
SYNTHESIS OF NOVEL AMINOGLYCOSIDE ANTIBIOTICS BY PERIODIC ACID OXIDATION OF NEAMINE

Sir:

The antibiotics GlAl[1] and sorbistin[2] exhibit broad-spectrum activity against resistant bacteria carrying R factor and resistant *Pseudomonas aeruginosa*. To obtain analogs of these compounds we have synthesized two aminoglycoside antibiotics with non-cyclitol aglycones by selective periodic acid oxidation of neamine.

The initial step of the synthesis, N-*t*-butoxycarbonylation[3], was successfully effected with O-*t*-butyl-S-4,6-dimethyl-2-pyrimidinyl-thiocarbonate (Boc-S)[4]. This reagent (162.6 g) was added to a solution of neamine (30 g) in aqueous dioxane (1: 2, 240 ml) and the solution was stirred at 60°C for 3 hours. Then the hot solution was poured into water (2 liters). The resulting precipitate was filtered and dried to give crude tetra-N-*t*-butoxycarbonylneamine (**1**, 49.75 g, 74%), which was purified by column chromatography on silica gel with methanol-chloroform (*1*: 30), $[\alpha]_D^{23}$ +47.6° (*c* 1.0, DMF), [Calcd. for $C_{32}H_{58}N_4O_{14}\cdot H_2O$: C 51.88, H 8.16, N 7.56; Found: C 51.75, H 8.02, N 7.09%]. **1** (5.00 g) was treated with 1, 1-dimethoxycyclohexane (6.75 ml) in DMF (33.8 ml), in the presence of anhydrous *p*-toluenesulfonic acid (1.08 g) at 55°C under reduced pressure (20 Torr), removing the generated methanol by coevaporation with solvent simultaneously[5]. After 30 minutes, the resulting solution contained three compounds as shown by tlc with methanol-chloroform (1: 25): 3′,4′;5,6-di-O-cyclohexylidene derivative (**2′**, Rf 0.64), 5,6-O-monocyclohexylidene derivative (**2**, Rf 0.21), and the starting material (**1**, Rf 0.05). To the solution, hydroxylamine hydrochloride (125 mg) in aqueous DMF (1: 1, 2 ml) was added and the mixture held at 40°C for 20 minutes. With this treatment, **2** became the major constituent and **2′** disappeared. After neutralization with triethylamine (2.0 ml), the solution was evaporated to a syrup and water was added. The resulting precipitate was filtered, washed with water and dried (4.43 g). Column chromatography on silica gel (250 g) with methanol-chloroform (1: 150) afforded a pale yellow powder (**2**) (2.77 g, 49%), $[\alpha]_D^{25}$ −0.2° (*c* 0.78, $CHCl_3$), [Calcd. $C_{38}H_{66}N_4O_{14}\cdot H_2O$: C 55.59, H 8.35, N 6.82; Found: C 55.65, H 8.07, N 6.61%]. A mixture of **2** (3.00 g) and benzoyl chloride (3.94 ml) in dry pyridine (46.5 ml) was stirred at 50°C for 1 hour. After addition of water (2.90 ml), the solution was concentrated to approximately 10 ml and to the concentrate chloroform (200 ml) was added. After stirring, the organic layer was washed successively with sodium hydrogen carbonate solution and water, then evaporated to yield the 3′,4′-di-O-benzoyl-5,6-O-monocyclohexylidene derivative (**3**, 3.79 g, quantitative). $[\alpha]_D^{20}$ +8.5° (*c* 0.30, $CHCl_3$), [Calcd. for $C_{52}H_{74}N_4O_{16}$: C 61.77, H 7.38, N 5.54; Found: C 62.25, H 7.15, N 5.06%]. A solution of **3** (3.78 g) in 70% aqueous acetic acid (3.2 ml) was heated at 60°C for 1 hour. The solution showed on tlc with methanol-chloroform (1: 25) only one product (Rf 0.21, *cf.* 3, Rf 0.70). The solution was evaporated with toluene to give a syrup (2.69 g). Column chromatography over silica gel (380 g) with methanol-chloroform (1: 120) afforded a white powder of the decyclohexylidenated derivative (**4**, 2.20 g, 63.4%), $[\alpha]_D^{25}$ +63.5° (*c* 1.02, $CHCl_3$), [Calcd. for $C_{46}H_{66}N_4O_{16}$: C 59.34, H 7.15, N 6.02; Found: C 59.07, H 7.10, N 5.87%]. Compound **4** (2.67 g) was dissolved in methanol (39.3 ml) and sodium metaperiodate[6] (2.78 g) was added to the solution with agitation. The solution was stirred at room temperature overnight[7,8]. After neutralization[9] with sodium hydrogen carbonate, the mixture was filtered and the filtrate was evaporated to a syrup. The residue obtained was triturated with several portions of ethanol (40 ml). To the solution was added sodium borohydride (1.05 g), and the mixture was stirred at room temperature overnight. After neutralization with 1 N hydrochloric acid (10 ml), the solution was evaporated to give a syrup, which was dissolved in chloroform. The solution was washed with water, dried over sodium sulfate and evaporated to give syrupy tetra-N-*t*-butoxycarbonyl-2,4-diamino-2,3,4-trideoxy-5-O-(2,6-diamino-2,6-deoxy-α-D-glucopyranosyl)-D-glucitol (**5**, 2.18 g, quantitative), $[\alpha]_D^{25}$ +8.6° (*c* 0.47, $CHCl_3$), [Calcd. for $C_{32}H_{60}N_4O_{14}\cdot H_2O$: C 51.74, H 8.41, N 7.54; Found: C 51.98, H 8.12, N 7.44%]. A solution of **5** (2.08 g) in 95% trifluoroacetic acid (25 ml) was kept at room temperature for 30 minutes. The solvent was removed by evaporation and the residue was dissolved in water (40 ml). The aqueous solution was neutralized with 2 N sodium

Chart 1.



hydroxide and passed through a column of Amberlite CG-50 resin (NH_4^+ form, 60 ml). The column was washed with 400 ml of water and then eluted stepwise with 450 ml of 0.1 N NH_4OH and 400 ml of 0.3 N NH_4OH. The eluates showing a major spot at Rf 0.37 on tlc ($CHCl_3$ - MeOH - conc. NH_4OH - H_2O in 1: 4: 2: 1, ninhydrin, neamine Rf 0.43) were combined, evaporated *in vacuo*, and lyophilized to give a colorless solid, 2,4-diamino-2,3,4-trideoxy-5-O-(2,6-diamino-2,6-deoxy-α-D-glucopyranosyl)-D-glucitol (**6**, 684 mg, 73.4%), $[\alpha]_D^{25}$ +127.4° (*c* 1.36, H_2O). The mass spectrum of **6** exhibited a peak at *m/e* 325 attributable to the $(M^+ + 1)$ ion[10]. NMR (D_2O): δ 5.14 ppm (1H, d, J=4 Hz, H-1′), 1.61~1.94 (2H, m, H-2). [Calcd. for $C_{12}H_{28}N_4O_6 \cdot H_2O \cdot \frac{1}{2}H_2CO_3$: C 40.21, H 8.37, N 15.00; Found: C 40.58, H 8.17, N 15.20%].

Compound **2** was treated with periodate as described above to give a tetra-N-*t*-butoxycarbonyl derivative consisting of 2-deoxystreptamine and a 2,6-diamino-2,6-dideoxy-D-glucose (2,6-AG) moiety which had been cleaved between C-3 and C-4, (**7**, 96.9%), $[\alpha]_D^{25}$ +7.2°(*c* 1.0, CH_3OH). [Calcd. for $C_{38}H_{68}N_4O_{14}$: C 56.41, H 8.51, N 6.96; Found: C 56.41, H 8.33, N 6.81%]. Compound **7** was treated as described above for **5** to give the deprotected 2-deoxystreptamine derivative (**8**, 21.6%). The eluates showing a major spot at Rf 0.21 on tlc (*n*-BuOH - EtOH - $CHCl_3$ - conc·NH_4OH in 4: 5: 2: 5, ninhydrin, neamine Rf 0.17) were combined. $[\alpha]_D^{27}$ +17.3° (*c* 0.72, H_2O). Mass $(M^+ + 1)$[10] 325; NMR (D_2O): δ 5.51 ppm (1H, J=1 Hz, H-1′), 2.56 (1H, d-t, J=12.0 and 4.0 Hz, H-2_{eq}), 1.98 (1H, q, J=12.0 Hz, H-2_{ax}). [Calcd. for $C_{12}H_{23}N_4O_6 \cdot \frac{1}{4}H_2O$: C 43.83, H 8.73, N 17.04; Found: C 43.82, H 8.47, N 16.79%].

Table 1. Antibacterial spectrum of products from neamine, **6** and **8**

Test organisms*	MIC** (mcg/ml)		
	6	**8**	neamine
Staph. aureus Rosenbach FDA 209-P JC-1	12.5	100	1.56
Staph. aureus Smith S-424	25	200	3.13
Staph. aureus No. 26	12.5	100	1.56
Staph. aureus ApO-1	12.5	100	1.56
Staph. aureus N-0089	50	>400	3.13
Staph. epidermidis ATCC 14990	0.78	1.56	0.39
Staph. epidermidis 109	6.25	3.13	100
Str. faecium ATCC 8043	0.39	0.78	50
B. subtilis ATCC 6633	6.25	12.5	3.13
B. anthracis No. 119	6.25	6.25	12.5
E. coli (M) Cast. & Chalm. NIHJ JC-2	>100	>400	12.5
E. coli No. 29	>100	>400	12.5
E. coli W 677 (A-20684)	50	100	3.13
E. coli JR 66/W 677 (A-20683)	>100	>400	>400
E. coli A-0001	>100	>400	50
E. coli A-0003	>100	>400	50
Sal. typhi O-901-W	50	>400	6.25
Sal. typhimurium LT-2	>100	>400	12.5
Ps. aeruginosa IAM-1007	50	200	200
V. parahaemolyticus K-5	12.5	50	12.5

* Tests conducted on MUELLER-HINTON agar
** Minimal inhibitory concentration

The antibacterial spectrum is shown in Table 1. The synthetic products are weakly bioactive, but may be useful as starting materials for the synthesis of improved antibiotics.

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