THE AMINOGLYCOSIDE ANTIBIOTICS

I. SYNTHESIS AND BIOLOGICAL EVALUATION OF AN ANALOG OF GENTAMICIN

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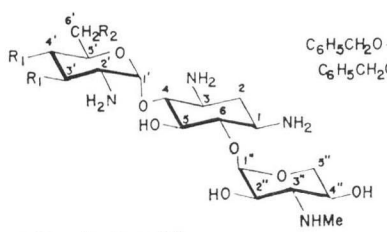
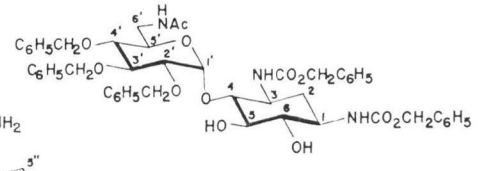
The synthesis of 2-deoxy-4-O-(2, 6-diamino-2, 3, 4, 6-tetradeoxy-α-D-erythrohexopyranosyl)-6-O-(3-deoxy-3-methylamino-α-D-xylopyranosyl)-D-streptamine **(1)**, an analog of gentamicin A, from dideoxyneamine and methyl 3-methylamino-3-deoxy-β-D-xylopyranoside is described. The product was characterized by its ^{13}C nmr spectrum and was found to exhibit broad spectrum antibacterial activity.

Recently, several studies have been published describing modifications of aminoglycosides designed to improve their antimicrobial activities, especially against bacteria with R-factor mediated resistance. This work has been reviewed by S. UMEZAWA[1], H. UMEZAWA[2] and PRICE[3]. As part of our program aimed at evaluating the antibacterial properties of pseudodisaccharides and pseudotrisaccharides derived from neamine, an analog (**1**) of gentamicin A (**2**)[4] was synthesized by attaching 3-deoxy-3-methylamino-D-xylose (gentosamine) to 3′, 4′-dideoxyneamine.



1 $R_1 = H$, $R_2 = NH_2$

2 $R_1 = R_2 = OH$



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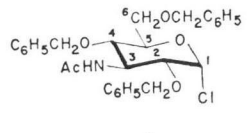

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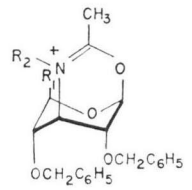

5 $R_1 = CH_2OCH_2C_6H_5$, $R_2 = H$

6 $R_1 = H$; $R_2 = CH_3$

In an analogous synthesis of kanamycin A, HASEGAWA *et al.*[5~7] reported the facile condensation of protected kanamine **3** with crystalline 3-acetamido-2, 4, 6-tri-O-benzyl-3-deoxy-α-D-glucopyranosyl chloride (**4**) using a modified KOENIGS-KNORR reaction with silver perchlorate-silver carbonate in benzene-dioxane to yield the corresponding O-6 α-glycoside in 41% yield. The presence of a non-parti-

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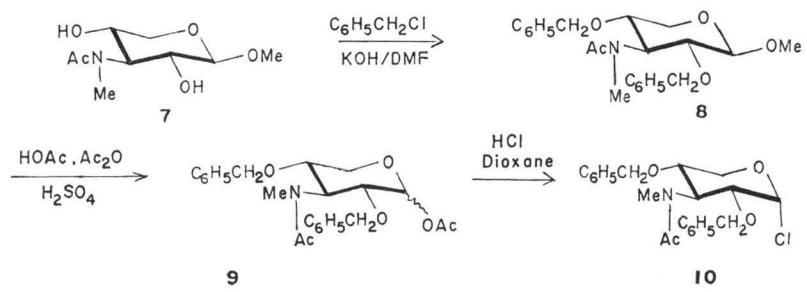
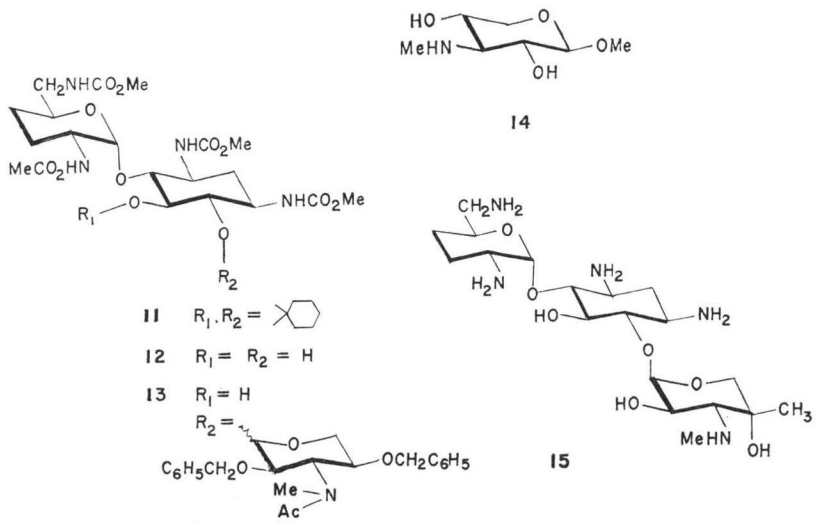
Scheme 1.



cipating group on the two position of glycosyl chlorides such as **4** is considered mandatory to prevent anchimeric assistance that would lead to formation of *trans*-(β)-glycosides[8,9]. Exclusive α-glycoside formation was observed by HASEGAWA, presumably assisted anchimerically by the 3-acetamido group *via* a ring-inverted intermediate such as **5**. In addition, the well documented,[10~12] selective coupling at the six position of the kanamine was observed.

To parallel this procedure, the corresponding di-O-benzyl chloride **10** was prepared as shown in Scheme 1 from the known methyl xyloside **7**[4,13]. Chloride **10** was expected to perform at least as well as **4** in the glycosylation reaction since, lacking the bulky C–5 substituent present in **4**, it should more readily undergo conversion to its potential bridged intermediate **6**. The prerequisite diol (**12**) was prepared by hydrolysis of the known protected 3′, 4′-dideoxyneamine (**11**).[14]

Treatment of **12** with an excess of **10** yielded, after chromatography, a mixture of pseudotrisaccharides (**13**) in 15% yield. The lower than expected yield can be attributed in part to the relative insolubility of diol **12** in the reaction medium since 65% was recovered on workup. Thus, the yield based on **12** consumed was 42%. The protected isomers could not be separated by chromatography, but they were readily separated by chromatographing them after removal of the blocking groups by hydrogenation and basic hydrolysis. The major product was the pseudotrisaccharide (**1**) having the desired new α-linkage as confirmed by the appearance in its nmr of two anomeric doublets with coupling constants of 4 Hz. The minor component displayed typical diaxial coupling of 8 Hz for its new anomeric proton



and was presumed to be the corresponding β-isomer. The product ratio was approximately 3: 1, indicating significantly less stereoselectivity for glycosylation with the xylosyl chloride **10** as compared to that reported for the glucosyl chloride **4** where no β-isomer was detected.[5~7]

Although there exists precedent in the literature[10~12] that would lead one to expect that selective glycosylation had occurred at the desired O-6 position of the 2-deoxystreptamine moiety (rather than the O-5 position), the lower than expected biological activity of **1** (see below) cast some doubt on this assignment. We therefore deemed it necessary to confirm the position of linkage of the 3-aminosugar to 2-deoxystreptamine. Recently, several papers have appeared describing the ^{13}C nmr (cmr) spectra of various aminoglycoside structures[15~20]. This work has demonstrated that protonation of an amino group results in an upfield shift of the resonances due to carbons beta to that amino group. The chemical shifts and their tentative assignments for **1** and the β-methyl glycoside **14** are listed in Table 1. In addition, Fig. 1 shows the pH profiles of the three 2-deoxystreptamine resonances of interest (C–4, C–5 and C–6) between 70 and 90 ppm. The two downfield peaks at 88 ppm (pH 12) result from carbons attached to electron withdrawing glycosyl oxygens whereas the upfield peak (75.2 ppm) results from a simple carbinol carbon. Since both downfield peaks shift on acidification, they are derived from carbons

Fig. 1. Change in chemical shifts (CMR spectra) with deuterium ion concentration (70 to 90 ppm only). Chemical shift in ppm from TMS

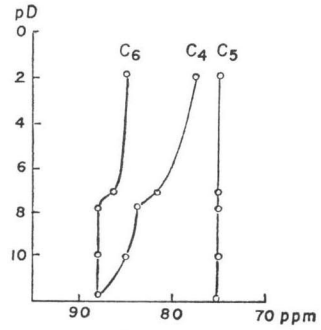


Table 1. The ^{13}C-chemical shifts of **1** and **14** at pH (pD) 2 and 12 and their differences, Δ.

Carbon atom	Compound **1**			Compound **14**		
	pH 12	pH 2	Δ	pH 12	pH 2	Δ
1	51.4	50.5				
2	36.6	28.6	8.0			
3	50.7	49.5				
4	88.0	77.6	10.4			
5	75.2	75.2				
6	88.0	84.5	3.5			
1′	(102.1)	95.7	6.4			
2′	50.4	49.4				
3′	27.0	21.3	5.7			
4′	28.3	26.2				
5′	(70.9)	68.8	2.1			
6′	45.9	43.4				
1″	(100.7)	101.5		105.3	104.8	
2″	(71.5)	67.1	4.4	71.6	68.5	3.1
3″	(63.1)	63.4		65.7	64.8	
4″	68.7	64.3	4.4	68.3	65.0	3.3
5″	(62.8)	61.5		66.9	66.7	
NMe	34.2	30.5	3.7	34.1	31.4	2.7
OMe				57.7	58.2	

Chemical shifts in ppm from TMS. Shifts in parentheses were too close to be assigned unequivocally.

Table 2. *In vitro* antibacterial activity of **1** and the gentamicin C complex measured as their sulfate salts. Agar dilution method, Penassay seed agar, pH 8.

Test organism	Minimum inhibitory concentration mcg/ml	
	1	Gentamicin C complex
Staphylococcus aureus HH 127	3.1	0.8
Staphylococcus aureus SK & F 23990	1.6	0.8
Staphylococcus aureus Villaluz (M. R.) SK & F 70399	1.6	0.8
Streptococcus faecalis HH 34358	50	6.3
Escherichia coli SK & F 12140	3.1	0.4
Escherichia coli HH 33779	6.3	0.8
Klebsiella pneumoniae SK & F 4200	0.8	0.2
Klebsiella pneumoniae SK & F 1200	3.1	0.4
Salmonella paratyphi ATCC 12176	3.1	0.4
Shigella paradysenteriae HH 117	12.5	1.6
Pseudomonas aeruginosa HH 63	1.6	0.4
Serratia marcescens ATCC 13880	25	0.8
Proteus morganii 179	1.6	0.4
Enterobacter aerogenes ATCC 13048	3.1	0.8
Enterobacter cloacae HH 31254	1.6	0.4

beta to amino groups, *i. e.* C–4 and C–6, whereas the single upfield peak does not shift and is therefore derived from the carbon that is not beta to an amino group, namely C–5. Since the glycosidic linkage originally present in the starting neamine is at the four position, the new glycoside must be attached to the six position as expected.

In vitro antibacterial testing of **1** (Table 2) showed it to have bioactivity about one-quarter to one-eighth that of the gentamicin C complex. Since **1** differs from gentamicin C_{1a} (**15**) only in its 3-amino-sugar moiety, the observed activity variations can be attributed to this structural factor.* Thus, the attachment of gentosamine seems to offer no biological advantage over the gentamicin sugar garosamine.

Experimental Section

Column chromatography was carried out on J. T. Baker silica gel (60~200 mesh). Proton nuclear magnetic resonance spectra (nmr) were run on a Varian T–60 instrument using internal or external TMS as standard. Mass spectra were obtained with a Perkin-Elmer RMU–6 or a Varian CH–5 instrument. Carbon magnetic resonance (cmr) spectra were recorded on a Varian CFT–20 instrument and calibrated with an internal (~5%) dioxane standard set at 67.4 ppm. Samples for cmr spectra were decarbonated by passage through a short column of Amberlite IRA–400 (OH^-) resin and lyophilized. All manipulations, including neutralizations with 38% DCl, were performed in a CO_2-free nitrogen atmosphere. The pD's were measured with pHydrion papers (Micro Essential Laboratories, Brooklyn, New York) and are uncorrected for D_2O.

Methyl 2, 4-di-O-benzyl-3-deoxy-3-(N-methylacetamido)-β-D-xylopyranoside (**8**)

A mixture of methyl 3-(N-methylacetamido)-β-D-xylopyranoside,[4,13] **7** (22 g, 0.1 mole), DMF (100 ml), finely ground potassium hydroxide (70 g, 1.25 mole) and finely ground Drierite (70 g) was stirred at room temperature for 30 minutes after which benzyl chloride (90 ml, 0.65 mole) was added

* The test organisms used lack R factors which produce kanamycin acetyltransferase [AAC (6′)][2,3] and thus the *in vitro* activities observed for the gentamicin C complex should be equivalent to those of gentamicin C_{1a}[21].

dropwise with cooling to maintain a temperature below 70°C. The mixture was maintained at 65°C with stirring for an additional 2 hours, cooled to room temperature and filtered through Celite. The solids were washed with DMF and the combined filtrates concentrated *in vacuo* (0.1 mm). The residue was partitioned between water and ether, the ether phases washed with water, dried (Na_2SO_4) and concentrated. The crude product was purified by column chromatography on silica gel (ethyl acetate-petroleum ether gradient, 0 ~100%) to yield **8** (33.5 g, 85%) as a viscous, thermally labile oil, $[\alpha]_D^{25}-11°$ (*c* 1.0, $CHCl_3$); MS (M^+) *m/e* 399.

Calcd. for $C_{23}H_{29}NO_5 \cdot 1/4\ H_2O$: C, 68.38; H, 7.36; N, 3.47%.
Found: C, 68.04; H, 7.47; N, 3.39%.
Exact mass: Calcd. $C_{23}H_{29}NO_5$, *m/e* 399.2045.
Found: *m/e* 399.2063.

1-O-Acetyl-2, 4-di-O-benzyl-3-deoxy-3-(N-methylacetamido)-D-xylopyranosides (**9**)

A solution of methyl glycoside **8** (19 g, 0.05 mole) in a mixture of glacial acetic acid (100 ml) and acetic anhydride (40 ml) was cooled in an ice bath and concentrated sulfuric acid (4 ml) was added. The solution was allowed to warm to room temperature and after 3 hours was poured into sodium bicarbonate-ice water and extracted with chloroform. The organic phase was washed with aqueous sodium bicarbonate until neutralized, dried (Na_2SO_4) and concentrated *in vacuo* to give **9** (20 g, 98%), contaminated with acetic anhydride but suitable for the next reaction.

For analysis, a sample was purified by preparative thin-layer chromatography (TLC) on silica gel (ethyl acetate - hexane, 3: 7) to give an anomeric mixture of **9** as a thermally labile, viscous oil, $[\alpha]_D^{25}+45.9°$ (*c* 1.0, $CHCl_3$). NMR ($CDCl_3$): δ 6.31 (1/2 H, 2d, J=3.5 Hz, restricted conformers of α-anomer), 5.56 (1/2 H, d, J=8 Hz, β-anomer).

Calcd. $C_{24}H_{29}NO_6 \cdot 1/4\ H_2O$: C, 66.73; H, 6.88; N, 3.24%.
Found: C, 66.74; H, 6.96; N, 3.07%.
Exact mass: Calcd. $C_{24}H_{29}NO_6$, *m/e* 427.1995.
Found: *m/e* 427.2007.

2, 4-Di-O-benzyl-3-deoxy-3-(N-methylacetamido)-α-D-xylopyranosyl chloride (**10**)

A sealed solution of **9** (18 g, 0.042 mole) in dry (distilled from lithium aluminum hydride) dioxane (800 ml) containing dry HCl (43 g) was stored in the dark at room temperature for one week. The solution was concentrated *in vacuo* to dryness and evaporated several times with dry toluene. The residue was dissolved in dry toluene, filtered, and concentrated to give the unstable chloride **10** (18 g, 94%) as a tan, viscous oil; NMR, ($CDCl_3$): δ 6.2 (2d, J=3 Hz, restricted conformers of α-halide).

2-Deoxy-N, N′-bis-(methoxycarbonyl)-4-O-[2, 3, 4, 6-tetradeoxy-2, 6-bis-[(methoxycarbonyl)amino]-α-D-erythrohexopyranosyl]-D-streptamine (**12**)

A mixture of 5, 6-cyclohexylidene-2-deoxy-N, N′-bis-(methoxycarbonyl)-4-O-[2, 3, 4, 6-tetradeoxy-2, 6-bis [(methoxycarbonyl)amino]-α-D-erythrohexopyranosyl]-D-streptamine[14], **11** (20 g 0.033 mole), methanol (100 ml) and 3 N aqueous hydrochloric acid (20 ml) was stirred at room temperature for 3 hours, neutralized with aqueous sodium bicarbonate and concentrated *in vacuo*. The residue was triturated with a minimum volume of water and recrystallized from isopropanol - ethanol with separation of insoluble salts to yield **12** (11.8 g, 70%), mp 222~225°C $[\alpha]_D^{25}+51.3°$ (*c* 1.0, MeOH).

Anal: Calcd. for $C_{20}H_{34}N_4O_{12}$: C, 45.97; H, 6.56; N, 10.72%.
Found: C, 46.25; H, 6.54; N, 10.65%.

2-Deoxy-N, N′-bis(methoxycarbonyl)-4-O-[2, 3, 4, 6-tetradeoxy-2, 6-bis-[(methoxycarbonyl)amino]-α-D-erythrohexopyranosyl]-6-O-[2, 4-di-O-benzyl-3-(N-methylacetamido)-α and β-D-xylopyranosyl]-D-streptamine (**13**)

A mixture of diol **12** (11.4 g, 0.02 mole) and crushed Drierite (40 g) in dry THF (400 ml) and dry methylene chloride (100 ml) was stirred for 3 hours with exclusion of moisture and a solution of chloride **10** (17.1 g, 0.04 mole) in dry methylene chloride (50 ml) was added. Stirring was continued for 2 hours at which point silver carbonate (18 g) and silver perchlorate (400 mg) were added. The mixture was stirred for 4 days at room temperature with exclusion of moisture and light, after which it was filtered and concentrated *in vacuo*. The residue was partitioned between methylene chloride and aqueous sodium

bicarbonate, the organic phases combined, dried (Na_2SO_4) and concentrated *in vacuo*. The carbohydrate byproducts were removed from the residue by precipitation from 1:1 ether-petroleum ether (600 ml) to give a crude product (11 g) containing **13** and unreacted **12**. Column chromatography on silica gel with toluene - methanol (98:2) yielded **13** (2.9 g, 15% based on **12**, 42% based on **12** consumed).

Anal: Calcd. $C_{42}H_{59}N_5O_{16}$: C, 56.68; H, 6.68; N, 7.86%.
Found: C, 56.35; H, 6.49; N, 7.49%.

2-Deoxy-4-O-(2, 6-diamino-2, 3, 4, 6-tetradeoxy-α-D-erythrohexopyranosyl)-6-O-(3-deoxy-3-methylamino-α-D-xylopyranosyl)-D-streptamine (**1**)

The mixture of isomers **13** (2.2 g, 2.5 mmole) was hydrogenated over 10% palladium on charcoal in ethanol and the product (1.5 g) was refluxed overnight in water (50 ml) containing barium hydroxide octahydrate (6 g). The suspension was neutralized at 100°C with carbon dioxide, filtered and the precipitate washed with hot distilled water. The filtrate was concentrated, neutralized to pH 7 with dilute sulfuric acid, filtered, and absorbed on a column of IRC-50 $(NH_4)^+$ resin. Elution with an ammonia gradient (0.5~1 M) yielded a mixture of α- and β-isomers which were separated by rechromatography on silica gel using the lower phase of chloroform-methanol-ammonium hydroxide (17%), 2:1:1. The β-isomer (40 mg) was eluted first followed by fractions containing the major component which were concentrated, neutralized to pH 3.5 with sulfuric acid and added to an excess of methanol. The resulting precipitate was dissolved in water and lyophilized to give **1** (180 mg, 10% based on **13**), $[\alpha]_D^{25}+88.3°$ (*c* 0.5, H_2O). NMR (D_2O): δ 6.0 (1H, d, J=4 Hz), 5.2 (1H, d, J=4 Hz), 2.9 (3H, s), MS: *m/e* 436 $(M+H)^+$.

Anal: Calcd. for $C_{18}H_{37}N_5O_7 \cdot 2.5H_2SO_4 \cdot 2H_2O$: C, 30.16; H, 6.46; N, 9.77; $SO_4^=$, 33.50%.
Found: C, 29.99; H, 6.85; N, 9.90; $SO_4^=$, 33.62%.

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