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SYNTHETIC STUDIES ON AMINO-α-GLYCOSIDES OF AMINOCYCLITOLS. SYNTHESIS OF KANAMYCIN ANALOGUES

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ABSTRACT

A diastereoisomer of Kanamycin C has been synthesized by a modified Koenigs–Knorr reaction of 3,4,6-tri-*O*-acetyl-2-(2,4-dinitroanilino)-2-deoxy-α-D-glucopyranosyl bromide with 4-*O*-(3-acetamido-2,4,6-tri-*O*-benzyl-3-deoxy-α-D-glucopyranosyl)-*N*,*N*′-di[(benzyloxy)carbonyl]-2-deoxystreptamine. Several Kanamycin analogues were synthesized by a similar condensation reaction. Each of the condensed products was isolated as its crystalline tetra-*N*-acetyl derivative and was proved by n.m.r. spectroscopy in D_2O to have the α-configuration.

INTRODUCTION

For the total synthesis of some important carbohydrate antibiotics such as Kanamycins, Neomycins, Gentamicins, and others, a general synthetic procedure for the amino-α-glycopyranosides of the aminocyclitols is desired. Recently, several investigators[1–7] have demonstrated synthetic procedures for obtaining α-glycosides.

In the course of the total synthesis of Kanamycin-A[8–10], we attempted to synthesize amino-α-glucosides by a modified Koenigs–Knorr reaction by using 1-halo derivatives of 3-acetamido-3-deoxy-D-glucose and 6-acetamido-6-deoxy-D-glucose and, as aglycon, the isopropylidene acetal of 2-deoxystreptamine, in benzene or chloroform containing 10–20% *p*-dioxane. It was demonstrated that the condensation reaction gave high yields of α-glycosides, but no β-anomer.

We have applied the procedure for synthesis of paromamine and related compounds, and have established[11] that the procedure is useful for synthesis of various kinds of amino-α-glycosides. Furthermore, we have studied[12] the effect of solvent, the anchimeric assistance of an acetamido group, and the chemical properties of intermediates that accumulate in the condensation reaction with the 1-halo derivative of 3-acetamido-3-deoxy-D-glucose.

The present communication deals with a continuation of these studies: synthesis of four oligosaccharides† is described. The configurations of all of these amino-

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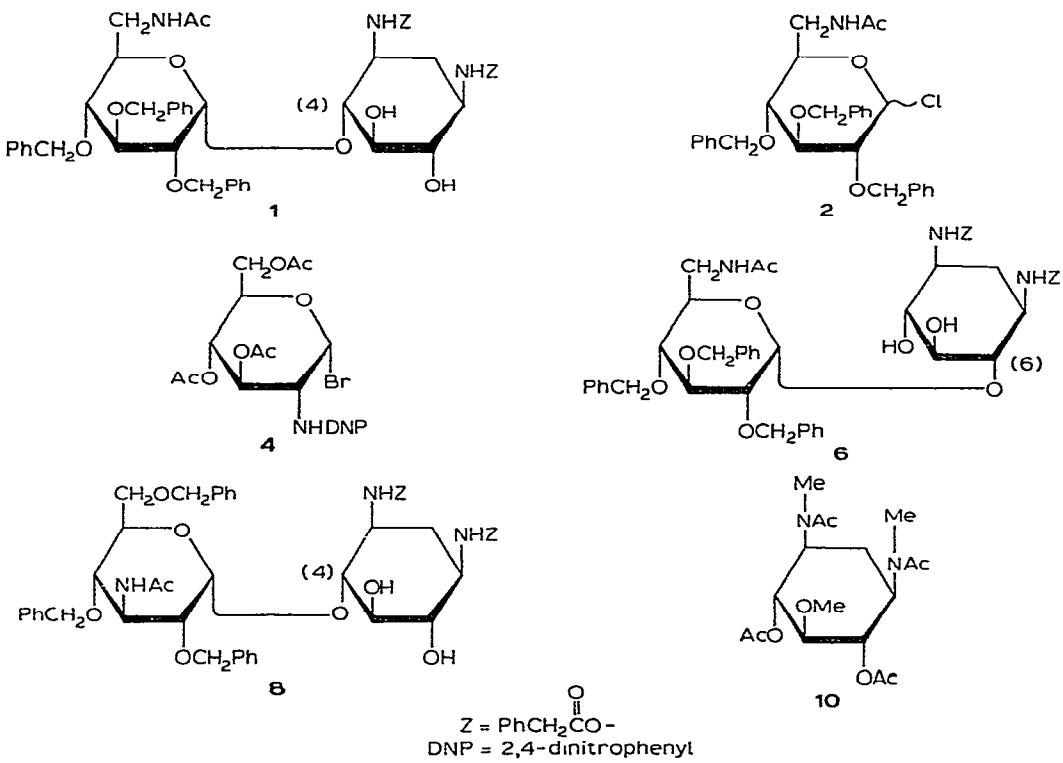
†Strictly, diglycosyl derivatives of a cyclitol.

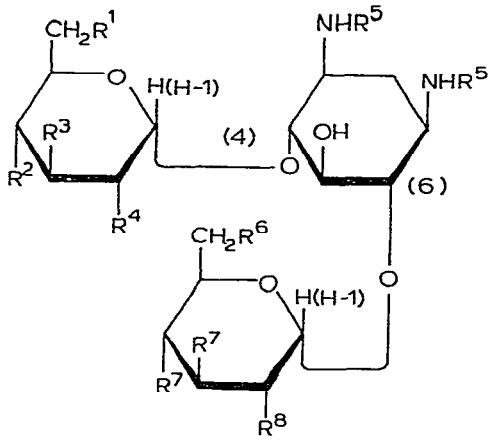
deoxy-α-glucosides have been determined by chemical procedures, and an empirical n.m.r.-spectral rule[13] has been used to determine the linkage positions of sugars to the 2-deoxystreptamine moiety in the carbohydrate antibiotics.

RESULTS AND DISCUSSION

Condensation of 4-*O*-(6-acetamido-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-glucopyranosyl)-*N*,*N*′-di[(benzyloxy)carbonyl]-2-deoxystreptamine (**1**) with 6-acetamido-2,3,4-tri-*O*-benzyl-6-deoxy-D-glucopyranosyl chloride[14] (**2**) (bearing a non-participating group at C-2) in chloroform–*p*-dioxane in the presence of silver carbonate and silver perchlorate gave in good yield a product having m.p.201–203° and $[\alpha]_D^{15}$ +29.5°. After removal of the protecting groups with sodium in liquid ammonia, *N*-acetylation afforded a tetra-*N*-acetyl derivative. The product was considered to have two α-linkages, as the anomeric-proton signals in D_2O, at τ 4.69 and 4.96, showed small spacings[15] (4.0 Hz for each). The linkage position of the aglycon with the sugar components was determined by an empirical rule[13], namely, that an anomeric proton of the sugar component α-linked at O-4 of *N*,*N*′-diacetyl-2-deoxystreptamine or *N*,*N*′-diacetylstreptamine resonates at lower field (τ 4.62–4.72) than it does (τ 4.86–4.95) when the substitutent is at O-6. Therefore, the tetra-*N*-acetyl derivative obtained



Form. 1



3a $R^1, R^6 = NHAc$, $R^2, R^3, R^4, R^7, R^8 = OCH_2Ph$, $R^5 = CO_2CH_2Ph$
3b $R^1, R^6 = NHAc$, $R^2, R^3, R^4, R^7, R^8 = OH$, $R^5 = Ac$
5a $R^1 = NHAc$, $R^2, R^3, R^4 = OCH_2Ph$, $R^5 = CO_2CH_2Ph$, $R^6, R^7 = OAc$, $R^8 = NHC_6H_3(NO_2)_2$
5b $R^1, R^8 = NHAc$, $R^2, R^3, R^4, R^6, R^7 = OH$, $R^5 = Ac$
7a $R^1, R^2, R^3 = OAc$, $R^4 = NHC_6H_3(NO_2)_2$, $R^5 = CO_2CH_2Ph$, $R^6 = NHAc$, $R^7, R^8 = OCH_2Ph$
7b $R^1, R^2, R^3, R^7, R^8 = OH$, $R^4, R^6 = NHAc$, $R^5 = Ac$
9a $R^1, R^2, R^4 = OCH_2Ph$, $R^3 = NHAc$, $R^5 = CO_2CH_2Ph$, $R^6, R^7 = OAc$, $R^8 = NHC_6H_3(NO_2)_2$
9b $R^1, R^2, R^4, R^6, R^7 = OH$, $R^3, R^8 = NHAc$, $R^5 = Ac$

Form. 2

from the condensed product should have the structure **3b**. To establish further the linkage-position of 6-amino-6-deoxy-D-glucose to the 2-deoxystreptamine moiety, compound **3a** was permethylated with methyl iodide[16]. Hydrolysis of the glycosidic linkages and subsequent acetylation gave *N,N'*-diacetyl-*N,N'*-dimethyl-4,6-di-*O*-acetyl-5-*O*-methyl-2-deoxystreptamine[10] (**10**), thus demonstrating that, in compound **3a**, two 6-amino-6-deoxy-D-glucose residues are linked at O-4 and O-6 of 2-deoxystreptamine.

Oligosaccharides related to Kanamycin were also synthesized in good yields by condensation of **1**, the diastereoisomer (**6**) of **1**, and 4-*O*-(3-acetamido-2,4,6-tri-*O*-benzyl-3-deoxy-α-D-glucopyranosyl)-*N,N'*-di[(benzyloxy)carbonyl]-2-deoxystreptamine (a diastereoisomer of "kanosaminide", **8**) with 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranosyl bromide[17] by a procedure similar to that used for compound **3a**.

The glycosides (**5a**, **7a**, and **9a**) obtained were converted into their tetra-*N*-acetyl derivatives (**5b**, **7b**, and **9b**) by removal of the protecting groups and subsequent *N*-acetylation. Their n.m.r. spectra in D_2O showed that they had the α-configuration, as the anomeric-proton signals were narrow doublets (J less than 4.0 Hz), and the sugars were linked to O-4 and O-6 of the 2-deoxystreptamine moiety, according to the

foregoing empirical rule. Each of the condensed products (**5a**, **7a**, and **9a**), after permethylation, followed by hydrolysis of the glycosidic linkages, and acetylation, gave **10**. This result attests to the reliability of the empirical n.m.r.-spectral rule for determining the linkage position of the aglycon with the sugar. In this modified Koenigs–Knorr reaction, it should be noted that aminodeoxy-α-glucosides were obtained in fairly high yields, but no β-anomer was isolated. By t.l.c. examination, the reaction was complete within 30 min at 30°.

Antibacterial activities of these synthetic amino-α-glucosides will be reported elsewhere.

EXPERIMENTAL

General. — Melting points were determined on a Yanagimoto micro melting-point apparatus and were uncorrected. Specific rotations were determined with a Yanagimoto polarimeter OR-50. N.m.r. spectra were recorded with a Varian A-60 spectrometer by using D_2O as solvent. I.r. spectra were recorded with a Jasco IRA-I spectrophotometer. Column chromatography was conducted on silicic acid (100 mesh; Mallinckrodt, St. Louis, U. S. A.).

*4,6-Di-*O*-(6-acetamido-2,3,4-tri-*O*-benzyl-6-deoxy-α-*D*-glucopyranosyl)-*N,N'*-di[(benzyloxy)carbonyl]-2-deoxystreptamine* (**3a**). — A mixture of 4-*O*-(6-acetamido-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-glucopyranosyl)-*N,N'*-di[(benzyloxy)carbonyl]-2-deoxystreptamine (**1**, 900 mg), silver carbonate (5 g), silver perchlorate (100 mg), drierite (10 g), abs. chloroform (50 ml), and abs. *p*-dioxane (10 ml) was stirred for 5 h at 30° in the dark. A solution (10 ml) of the halide (**2**, 2.0 g) in abs. chloroform was added, and stirring was continued for 45 min at 30°. The course of the reaction was monitored by t.l.c. After the reaction, insoluble material was filtered off, and the residue washed with dichloromethane. The filtrate and washings were combined, and evaporated *in vacuo* to a syrup, which was chromatographed on a column (2.0 cm in diameter) of silicic acid (50 g) with 10:1 chloroform–*p*-dioxane and later 40:1 chloroform–methanol as eluants. From fractions obtained with the latter eluant, 780 mg (57%) of **3a** was isolated. Recrystallization from ethanol gave needles having m.p. 201–203°, $[\alpha]_D^{15}$ +29.5° (*c* 0.5, chloroform); ν_{max}^{KBr} 3450, 3350 (OH, NH), 1690, 1540 [NH(CO)O], 1645, 1540 (NHAc), and 1030–1050 cm^{-1} (glycoside).

Anal. Calc. for $C_{80}H_{88}N_4O_{17}$: C, 69.75; H, 6.44; N, 4.07. Found: C, 70.20; H, 6.40; N, 4.32.

*4,6-Di-*O*-(6-acetamido-6-deoxy-α-*D*-glucopyranosyl)-*N,N'*-diacetyl-2-deoxystreptamine* (**3b**). — Compound **3a** (300 mg) and sodium (330 mg) were alternately added during 20 min at −60 to −70° to liquid ammonia (15 ml), with stirring. After the reaction, ammonium chloride (750 mg) was carefully added, and ammonia was evaporated off at room temperature. Water (20 ml) was added to the resulting solid. Insoluble material was filtered off and then washed with water. The filtrate and washings were combined and evaporated *in vacuo* to solid, which was acetylated with pyridine (10 ml) and acetic anhydride (5 ml) by heating for 2 h at 90°. To a solution of

the acetylated product in methanol (50 ml) a small amount of sodium methoxide was added. After 10 min at room temperature, the solution was evaporated *in vacuo*. The resultant solid was dissolved in water (50 ml) and the solution was deionized with Amberlite IR-120 and Amberlite IR-410 resins. The aqueous solution was evaporated *in vacuo* to give colorless crystals. Recrystallization from acetone gave needles having m.p. >250°, $[\alpha]_D^{18}$ +42° (*c* 0.5, water); n.m.r. data (D_2O): τ 4.69 (doublet, $J_{1,2}$ 4.0 Hz, H-1), 4.96 (doublet, $J_{1',2'}$ 4.0 Hz, H-1'), 7.98 and 8.01 (NAc).

Anal. Calc. for $C_{26}H_{44}N_4O_{15}$: C, 47.85; H, 6.80; N, 8.59. Found: C, 47.61; H, 7.10; N, 8.38.

4-O-(6-Acetamido-2,3,4-tri-O-benzyl-6-deoxy-α-D-glucopyranosyl)-6-O-[3,4,6-tri-O-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranosyl]-N,N'-di[(benzyloxy)-carbonyl]-2-deoxystreptamine (**5a**). — A mixture of **1** (900 mg), silver carbonate (5.0 g), silver perchlorate (100 mg), Drierite (10 g), abs. chloroform (50 ml), and abs. *p*-dioxane (10 ml) was stirred for 5 h at 30° in the dark. A solution in abs. chloroform (10 ml) of the halide **4** (2.0 g) was added, and stirring was continued for 45 min at 30°. Inorganic material was filtered off and the residue was washed with dichloromethane. The filtrate and washings were combined and evaporated *in vacuo* to a syrup, which was chromatographed on a column (2.0 cm in diameter) of silicic acid (50 g), with chloroform and 50:1 chloroform–methanol as eluants. From the latter eluant, 660 mg (49%) of **5a** was isolated as yellow crystals. Recrystallization from ethanol gave yellow needles, m.p. 255°, $[\alpha]_D^{25}$ +11° (*c* 0.5, chloroform); ν_{max}^{KBr} 3400–3500 (NH, OH), 1750, 1235 (OAc), 1710, 1530 [NH(CO)O], 1670, 1530 (NHAc), and 1020–1060 cm^{-1} (glycoside).

Anal. Calc. for $C_{69}H_{76}N_6O_{23}$: C, 61.05; H, 5.64; N, 6.19. Found: C, 60.98; H, 5.84; N, 6.40.

4-O-(6-Acetamido-6-deoxy-α-D-glucopyranosyl)-6-O-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-N,N'-diacetyl-2-deoxystreptamine (**5b**). — Removal of the protecting groups from **5a** (250 mg) was achieved with sodium (330 mg) in liquid ammonia (20 ml), in a manner similar to that used for compound **3b**; subsequent *N*-acetylation afforded 80 mg (67%) of **5a** as crystals. Recrystallization from acetone gave colorless needles having m.p. 210–220° dec., $[\alpha]_D^{25}$ +54° (*c* 0.5, water); n.m.r. data (D_2O): τ 6.70 (doublet, $J_{1,2}$ 3.5 Hz, H-1), 4.88 (doublet, $J_{1',2'}$ 4.0 Hz, H-1'), 7.97, 8.00, and 8.08 (NAc).

Anal. Calc. for $C_{26}H_{44}N_4O_{15}$: C, 47.85; H, 6.80; N, 8.59. Found: C, 47.53; H, 6.99; N, 8.38.

4-O-[3,4,6-Tri-O-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranosyl]-6-O-(6-acetamido-2,3,4-tri-O-benzyl-6-deoxy-α-D-glucopyranosyl)-N,N'-di[(benzyloxy)-carbonyl]-2-deoxystreptamine (**7a**). — The condensation reaction was performed by using 6-*O*-(6-acetamido-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-glucopyranosyl)-*N,N'*-di-[(benzyloxy)carbonyl]-2-deoxystreptamine (**6**, 900 mg), silver carbonate (5.0 g), silver perchlorate (100 mg), Drierite (10 g), and the halide **4** (2.0 g) in abs. chloroform (60 ml) and abs. *p*-dioxane (10 ml) in a manner similar to that used for compound **3a**. After similar processing, the resultant syrup was chromatographed on a column (2.0 cm in

diameter) of silicic acid (50 g) eluted with chloroform and later 50:1 chloroform–methanol. The compound **7a** (650 mg, 49%) was isolated from fractions obtained with the second eluant and was recrystallized from methanol to give yellow needles having m.p. 253° dec, $[\alpha]_D^{25}$ +7.5° (*c* 0.5, chloroform); ν_{max}^{KBr} 3400–3500 (NH, OH), 1750, 1220–1240 (OAc), 1710, 1510–1530 [NH(CO)O], 1670, 1510–1530 (NHAc), and 1020–1060 cm^{-1} (glycoside).

Anal. Calc. for $C_{69}H_{76}N_6O_{23}$: C, 61.05; H, 5.64; N, 6.19. Found: C, 61.01; H, 5.55; N, 6.31.

4-O-(2-Acetamido-2-deoxy-α-D-glucopyranosyl)-6-O-(6-acetamido-6-deoxy-α-D-glucopyranosyl)-N,N'-diacetyl-2-deoxystreptamine (**7b**). — Removal of protecting groups from **7a** (250 mg) with sodium (330 mg) in liquid ammonia (20 ml), as described for compound **5b**, and subsequent *N*-acetylation, afforded 100 mg (83%) of **7b**. Recrystallization from acetone gave colorless needles, m.p. 200–220° dec, $[\alpha]_D^{25}$ +53.5° (*c* 0.5, water); n.m.r. data (D_2O): τ 6.64 (doublet, $J_{1,2}$ 3.5 Hz, H-1), 6.92 (doublet, $J_{1',2'}$ 3.5 Hz, H-1'), 7.97, 8.00, and 8.08 (NAc).

Anal. Calc. for $C_{26}H_{44}N_4O_{15}$: C, 47.85; H, 6.80; N, 8.59. Found: C, 47.83; H, 6.89; N, 8.71.

4-O-(3-Acetamido-2,4,6-tri-O-benzyl-3-deoxy-α-D-glucopyranosyl)-6-O-[3,4,6-tri-O-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranosyl]-N,N'-di[(benzyloxy)carbonyl]-2-deoxystreptamine (**9a**). — The condensation reaction was effected with 4-*O*-(3-acetamido-2,4,6-tri-*O*-benzyl-3-deoxy-α-D-glucopyranosyl)-*N,N'*-di[(benzyloxy)carbonyl]-2-deoxystreptamine[9] (**8**, 300 mg), silver carbonate (1.0 g), silver perchlorate (50 mg), Drierite (1 g), and the halide **4** (600 mg) in abs. chloroform (30 ml) and abs. *p*-dioxane (10 ml) as described for compound **3a**. After similar processing, the syrup obtained was chromatographed on silicic acid (25 g, column 1.5 cm in diameter) with chloroform and 50:1 chloroform–methanol as eluant. From the latter eluant, 180 mg (40%) of **9a** was obtained as crystals. Recrystallization from methanol gave colorless needles having m.p. >250°, $[\alpha]_D^{28}$ +28° (*c* 1.0, chloroform); ν_{max}^{Nujol} 3300–3500 (NH, OH), 1750, 1220–1230 (OAc), 1700, 1550 [NH(CO)O], 1650, 1550 (NHAc), and 1030–1050 cm^{-1} (glycoside).

Anal. Calc. for $C_{69}H_{76}N_6O_{23}$: C, 61.05; H, 5.64; N, 6.19. Found: C, 60.97; H, 5.75; N, 6.42.

4-O-(3-Acetamido-3-deoxy-α-D-glucopyranosyl)-6-O-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-N,N'-diacetyl-2-deoxystreptamine (**9b**). — Removal of the protecting groups from **9a** (300 mg) with sodium (500 mg) in liquid ammonia (30 ml) as described for compound **5b**, and subsequent *N*-acetylation, afforded 85 mg (58%) of **9b**, m.p. >270°, $[\alpha]_D^{30}$ +106° (*c* 0.1, water); n.m.r. data (D_2O): τ 4.72 (doublet, $J_{1,2}$ 2.5 Hz, H-1), 4.88 (doublet, $J_{1',2'}$ 2.0 Hz, H-1'), 7.95 and 8.10 (NAc).

Anal. Calc. for $C_{26}H_{44}N_4O_{15}$: C, 47.85; H, 6.80; N, 8.59. Found: C, 47.58; H, 6.69; N, 8.35.

N,N'-Diacetyl-2-deoxy-4,6-di-O-acetyl-N,N'-dimethyl-5-O-methylstreptamine (**10**). — To a stirred solution of **3a** (200 mg) in abs. *N,N*-dimethylformamide (8 ml) and methyl iodide (1.5 ml), was added freshly prepared silver oxide in four 900-mg

portions at intervals of 20 min. After stirring for 12 h at room temperature, the silver salts were filtered off and washed with *N,N*-dimethylformamide. The combined filtrate and washings were evaporated *in vacuo*, and the residue was extracted with chloroform and evaporated to dryness.

The resulting syrup was dissolved in *p*-dioxane (10 ml) and 5% aqueous HCl (50 ml). The solution was heated for 90 min at 90–100°, and then evaporated *in vacuo*. The residue was acetylated with pyridine (5 ml) and acetic anhydride (2 ml), and the acetylated product was purified by column chromatography on silicic acid with 20:1 chloroform–methanol as eluant. The crystalline product obtained was recrystallized from ethanol giving needles of **10**; yield 15 mg (28%), m.p. 233–234°. A mixed m.p. with an authentic sample[10] was not depressed, and the i.r. spectra of the two samples were identical. Compound **10** was also obtained from **5a**, **7a**, and **9a** in 25–40% yields by the same procedure as just described.

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