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A Synthesis of Streptozotocin Derivatives

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In connection with a previous paper,[1] biologically active streptozotocin derivatives have been prepared in our laboratory.

Streptozotocin is an antibiotic produced by *streptomyces achromogenes var. 128*, which shows wide antimicrobial and antitumor activities.[2-5] The structure has been established to be *N*-carbamyl-*N'*-methyl-*N'*-nitroso-D-glucosamine.[6-7]

In the present paper, a preparation of methyl glycosides of streptozotocin by a two-step reaction will be described. Methyl β-D-glucosaminide hydrochloride (III) was prepared by the method of Neuberger and Rivers,[8] and then it was treated with methyl isocyanate under the presence of silver carbonate to give methyl *N*-carbamyl-*N'*-methyl-β-D-glucosaminide (IV).

Nitrosation of IV was carried out with sodium nitrite in aqueous acetic acid to give methyl *N*-carbamyl-*N'*-methyl-*N'*-nitroso-β-D-glucosaminide (V) as needle crystals of mp 149°C (dec.) in 70% yield.

Starting from methyl *N*-carbobenzyloxy-α-D-glucosaminide (VII),[8] methyl *N*-carbamyl-*N'*-methyl-*N'*-nitroso-α-D-glucosaminide (X) of mp 133°C was obtained in 33% yield by an analogous reaction process.

A mixture of V and X (XV) was also prepared by an analogous process in 28% yield, beginning with methyl *N*-carbobenzyloxy-D-glucosaminide (XII).

The three compounds: V, X and XV, were fairly active against Ehrlich ascites tumor.

Experimental

A melting point was determined on a Mitamura Riken micro hot stage. A melting point marked with an asterisk was measured in a liquid bath and uncorrected. The infrared spectra were determined in potassium bromide discs. The proton magnetic resonance (pmr) spectra were measured at 60 MHz with a Varian A-60D instrument and the peak position was expressed in τ-values.

Methyl *N*-Carbobenzyloxy-β-D-glucosaminide (II). The compound was prepared by the method of Neuberger and Rivers[8] in a yield of 15.2% from *N*-carbobenzyloxy-D-glycosamine (I).[9] Mp 166—

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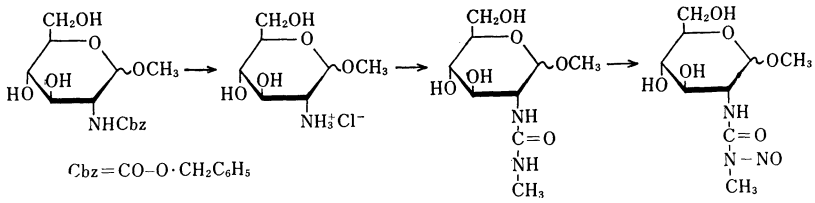

Cbz=CO-O·$CH_2C_6H_5$

β-Anomer			
II	III	IV	V
α-Anomer			
VII	VIII	IX	X
Mixture of α- and β-anomers			
XII	XIII	XIV	XV

167.5°C, $[\alpha]_D^{25}$ −22.8° (*c* 1.00, pyridine).

Methyl β-D-Glucosaminide hydrochloride (III). The compound was obtained from II by the method of Neuberger and Rivers[8] in 94.3% yield. Mp 188°C (dec.), $[\alpha]_D^{25}$ −24.0° (*c* 1.00, water). The product showed a single spot at R_f 0.48 in a paper chromatogram in a solvent system of *n*-butanol : pyridine : water (6 : 4 : 3). Lit,[8] mp 190°C, $[\alpha]_D$ −24°.

Methyl *N*-Carbamyl-*N'*-methyl-β-D-glucosaminide (IV). A mixture of III (800 mg) and methyl isocyanate (200 mg) in 65% aqueous acetonitrile (60 m*l*) was heated under reflux for 1 hr in the presence of silver carbonate (580 mg). The reaction mixture was filtered to remove an insoluble matter and the filtrate was evaporated under reduced pressure. The residue was digested in ethanol to give 492 mg of the product, mp 239.5 −241°C. From the mother liquor, 63 mg of the product was obtained. The total yield of the crude product was 64.0%. Recrystallization from ethanol gave an analytically pure sample, mp 239.5—241°C.

Found: C, 43.36; H, 7.48; N, 11.52%. Calcd for $C_9H_{18}N_2O_6$: C, 43.19; H, 7.25; N, 11.20%.

Methyl *N*-Carbamyl-*N'*-methyl-*N'*-nitroso-β-D-glucosaminide (V). To a solution of IV (456 mg) in 23% aqueous acetic acid (13 m*l*), 4.3 m*l* of 0.5 M sodium nitrite solution was added dropwise in 20 min under ice cooling. The reaction solution was settled overnight in a refrigerator, and then was treated with Amberlite IR-120(H^+) to remove a sodium ion. After the resin was removed by filtration, the filtrate was freeze-dried to give a crude product. Recrystallization from ethanol afforded 354 mg (69.7%) of needle crystals: mp 149°C (dec.), mp* 118°C (dec.). $[\alpha]_D^{25}$ −23.7° (*c* 0.5, water). IR: 1720(C=O), 1550(NH), 1490(N-NO) and 875 cm^{-1} (β-anomeric C-H).[10]

Found: C, 39.06; H, 6.23; N, 14.87%. Calcd for $C_9H_{17}N_3O_7$: C, 38.71; H, 6.09; N, 15.05%.

Methyl 3,4,6-Tri-*O*-acetyl-*N*-carbamyl-*N'*-methyl-*N'*-nitroso-β-D-glucosaminide (VI). A 140 mg sample of V was acetylated with 2 m*l* of acetic anhydride in 3 m*l* of pyridine at room temperature overnight. The mixture was evaporated under reduced pressure and the residue was recrystallized from aqueous ethanol to give 152 mg (74.9%) of pale yellow crystals, mp 96—99°C. IR: 1745(OAc), 1710(C=O), 1540(NH), 1480(N-NO) and 900 cm^{-1} (β-anomeric C-H).[10]

Pmr (in $CDCl_3$): τ 5.16 (one proton doublet, $J_{1,2}$=8 Hz, H_1); 6.38 (O-CH_3)[11]; 6.75 (*N*-CH_3); 7.79, 7.85, 7.89 (singlets, three protons, three protons and three protons, OAc).

Found: C, 44.98; H, 5.86; N, 10.08%. Calcd for $C_{15}H_{23}N_3O_{10}$: C, 44.44; H, 5.72; N, 10.37%.

Methyl *N*-Carbobenzyloxy-α-D-glucosaminide (VII). The compound was prepared by the method of Neuberger and Rivers[8] in a yield of 79.0% from I. Mp 156—158°C, $[\alpha]_D^{25}$ +92.6° (c 2.4, pyridine). Lit,[8] mp 154—155°C, $[\alpha]_D$ +80° (pyridine).

Methyl *N*-Carbamyl-*N'*-methyl-α-D-glucosaminide (IX). A solution of VII (1.0 g) in ethanol (15 m*l*) was hydrogenated with 0.3 m*l* of 12 N hydrochloric acid under the presence of 0.2 g of palladium black at 3.4 kg/cm^2 of hydrogen stream for 21 hr. After the catalyst was removed by filtration, the filtrate was evaporated to give a crude product (735 mg) of methyl α-D-glucosaminide hydrochloride (VIII), which showed a single spot on a paper chromatogram at R_f 0.54 in a solvent system of *n*-butanol : pyridine : water (6 : 4 : 3). R_f of D-glucosamine hydrochloride was 0.41 in the same solvent system. The product was used in a successive synthesis without a further purification.

A mixture of VIII (735 mg), methyl isocyanate (200 mg) and 65% aqueous acetonitrile (45 m*l*) was heated under reflux for 45 min in the presence of silver carbonate (532 mg). After an insoluble matter was removed by filtration, the filtrate was evaporated under reduced pressure. The residue was recrystallized from ethanol to give 494 mg (64.7%) of the product, mp 189—191°C. Recrystallization from *n*-propanol gave crystals melting at 194.5—196°C. $[\alpha]_D^{23}$ +97.5° (*c* 1.0, water).

Found: C, 43.18; H, 7.39; N, 10.91%. Calcd for $C_9H_{18}N_2O_6$: C, 43.19; H, 7.25; N, 11.20%.

Methyl *N*-Carbamyl-*N'*-methyl-*N'*-nitroso-α-D-glucosaminide (X). A 400 mg portion of IX was treated with nitrous acid as described in the preparation of V to give 214 mg (50.5%) of crystals. Mp 129—133°C, mp* 89°C (dec.). $[\alpha]_D^{25}$ +107° (*c* 0.5, water). IR: 1705(C=O), 1540(NH), 1485(N-NO) and 850 cm^{-1} (α-anomeric C-H).[10]

Found: C, 38.81; H, 6.31; N, 14.78%. Calcd for $C_9H_{17}N_3O_7$: C, 38.71; H, 6.09; N, 15.05%.

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***N'*-nitroso-α-D-glucosaminide (XI).** A 102 mg portion of X was acetylated analogously as described in the preparation of VI and the reaction mixture was poured into ice and water to give 116 mg (78.3%) of crystals, mp 114—115°C. Recrystallization from ethanol afforded pale yellow crystals melting at 115.5°C. IR: 1750(OAc), 1715(C=O), 1530(NH), 1480(N–NO) and 870 cm^{-1} (α-anomeric C–H).[10] PMR (in $CDCl_3$): τ 5.01 (one proton doublet, $J_{1,2}$=3.7 Hz, H_1); 6.45 (O–CH_3); 6.72 (N–CH_3); 7.77, 7.85, 7.91 (singlets, three protons, three protons and three protons, OAc).

Found: C, 45.04; H, 5.87; N, 10.23%. Calcd for $C_{15}H_{23}N_3O_{10}$: C, 44.44; H, 5.72; N, 10.37%.

Methyl *N*-Carbobenzyloxy-D-glucosaminide (XII). A mixture of I (1.78 g) in methanol (150 m*l*) and dry Amberlite IR-120(H^+) (18 m*l*) was heated under reflux for 2.5 hr. After the resin was removed by filtration, the filtrate was evaporated under reduced pressure. The residue was crystallized from ethanol to give 1.11 g (60.0%) of the product, mp 149—153°C, $[\alpha]_D^{25}$ +33.0° (*c* 2.5, pyridine). The compound consisted of 48% of α-anomer and 52% of β-anomer.

Methyl D-Glucosaminide hydrochloride (XIII). A 400 mg sample of XII was treated analogously as described above to give 162 mg (57.8%) of the product, mp 147—157°C (dec.). The product showed two spots at R_f 0.53 and 0.48 in a paper chromatogram in a solvent system of *n*-butanol : pyridine : water (6 : 4 : 3).

Methyl *N*-Carbamyl-*N'*-methyl-D-glucosaminide (XIV). A 400 mg portion of XIII was treated with methyl isocyanate as described above to give 240 mg (55.3%) of the product, mp 233—235°C. Recrystallization from aqueous ethanol afforded an analytically pure sample, mp 234.5—236°C.

Found: C, 43.39; H, 7.23; N, 11.30%. Calcd for $C_9H_{18}N_2O_6$: C, 43.19; H, 7.25; N, 11.20%.

Methyl *N*-Carbamyl-*N'*-methyl-*N'*-nitroso-D-glucosaminide (XV). A 180 mg sample of XIV was treated with nitrous acid as described above to give 172 mg (88.9%) of the crude product. Recrystallization from ethanol afforded needle crystals, mp 117—121°C (dec.), mp* 59°C (dec.). $[\alpha]_D^{23}$ −19.6° (*c* 0.5, water). The product consisted of 97% of β-anomer, since the β-anomer crystallized preferentially.

Found: C, 39.26; H, 6.26; N, 14.74%. Calcd for $C_9H_{17}N_3O_7$: C, 38.71; H, 6.09; N, 15.05%.

Antitumor activity. A detail of the antitumor activities of V, X and XV against Ehrlich mouse ascites tumor will be reported elsewhere.

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