ANALOGS AND CONVERSION PRODUCTS OF STREPTOZOTOCIN

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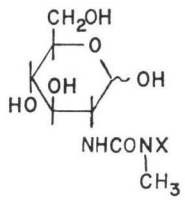
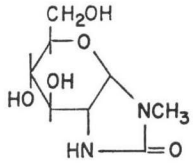
(Received for publication May 31, 1976)

Reduction of the nitroso group in streptozotocin (**1a**) has led to cyclized products rather than a semicarbazide (**1c**). Some analogs (**1e**, **9a**, **9b**, **9c** and **9d**) of streptozotocin in which the nitroso group was replaced by other groups have been prepared.

Streptozotocin (**1a**) is an antibiotic having antibacterial[1] and antileukemic[2] activity and activity against malignant insulinomas.[3] Because of the interesting biological properties of streptozotocin and the relative simplicity of its structure[4~9] a number of analogs have been prepared varying in the carbohydrate moiety, in the position of the nitrosourea group, in the N-3 alkyl group and in groups attached to the streptozotocin hydroxyl groups. However, all compounds having two substituents on N-3 retained the nitroso group. It appeared to be of interest to prepare analogs of streptozotocin in which the nitroso group would be replaced by other groups, preferably electron-rich group such as NO_2 (**1b**) but with no other changes.



1a : X = NO
1b : X = NO_2
1c : X = NH_2
1d : X = H
1e : X = C_6H_5



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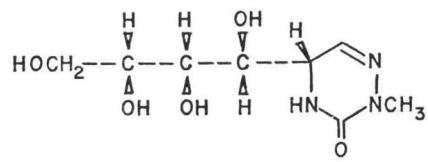
The first approach to analog synthesis was the reduction of the NO group in **1a** in an attempt to prepare **1c**. Treatment of streptozotocin with hydrogen under low pressure using either Pd–C or Pd-$BaSO_4$ catalyst gave a mixture of two products as indicated by tlc and an NMR spectrum which exhibited two resonances attributable to CH_3N groups. Crystallization of the amorphous mixture from alcohol usually led to a material (**2a**) which had been reported previously by HERR *et al.*[10] The identity was established by mp, ir, NMR, rotation and elemental analysis. However, in one experiment a second product (**2b**) was isolated. Slow evaporation of an aqueous solution of **2b** to a crystalline residue converted it to **2a**. A high resolution mass spectrum of **2b** established that it is isomeric with **2a**. The ir spectrum of **2b** shows functional groups similar to those in **2a** and combined with the ready conversion of **2b** to **2a** and the same composition suggests that the two compounds differ only in configuration at C-1. Structure **2** has been assigned to **2a** with no discussion of configuration at C-1.[10] Although the chemical shifts of the anomeric protons in **2a** and **2b** suggest the α and β configurations, respectively, the coupling constants of these protons indicate the reverse configurations.[11] BUNDLE and his coworkers[12] have reported the ^{13}C NMR spectra of the α and β isomers of methyl 2-deoxy-2-acetamide-β-D-glucopyranoside. Since the ^{13}C NMR spectrum of **2a** much more closely resembles that of the β-isomer, especially at C-3 and C-5 which give the significantly different chemical shifts in the two

isomers, this evidence again suggests the β-isomer for **2a**. The rotations of **2a** and **2b** are, respectively, $-22°$ and $+76°$ in H_2O which would be consistent with a β-configuration for **2a** and an α configuration for **2b** as the rotations of methyl β- and α-D-glycopyranoside are $-31.2°$ (H_2O) and $+158.2°$ (H_2O), respectively. While these data are not conclusive, the majority of the evidence favors assignment of the β-form to **2a** and of the α to **2b**. The formation of these products probably involves an initial reduction to **1d** followed by cyclization. That such a cyclization could occur was shown by conversion of **1d** obtained by the procedure of HESSLER and JAHNKE[18] to **2a** simply by crystallization from ethanol.

Reduction of streptozotocin with a zinc-acetic acid mixture gave a new product (**3**). Mass spectral and analytical data establish a molecular formula of $C_8H_{15}N_3O_5$. The ir spectrum has a strong band at 1660 cm^{-1}. A singlet at δ 3.15 in the ^1H NMR spectrum indicates a CH_3N group while a doublet at δ 7.05 can be assigned to the proton on the original C–1 on the basis of the ^1H NMR spectrum of the model compound acetaldehyde semicarbazone. The corresponding protons in the latter gave rise to two doublets, δ 6.66 and 7.38, arising from the *syn* and *anti* forms. The ^{13}C NMR spectrum has a downfield resonance at δ 155.0 arising from amide or urea carbonyl.[14] Five carbon atoms give rise to chemical shifts of δ 55.8~72.1 and these must be C–2 through C–6 of the original glucose skeleton.[14] The carbon atom of the methyl group has a chemical shift of δ 37.4. The only remaining carbon gives a signal at δ 141.1 and therefore cannot be anomeric but must be the original C–1 converted to a doubly bonded carbon as in **3**. All of these data are consistent with structure **3**. It seems probable that reduction of –NO to $-NH_2$ (**1c**) occurs followed by cyclization to C–1. Ring opening follows under the influence of acid as has been shown to occur in the conversion of **1a** to **4**.[15]



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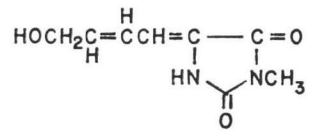

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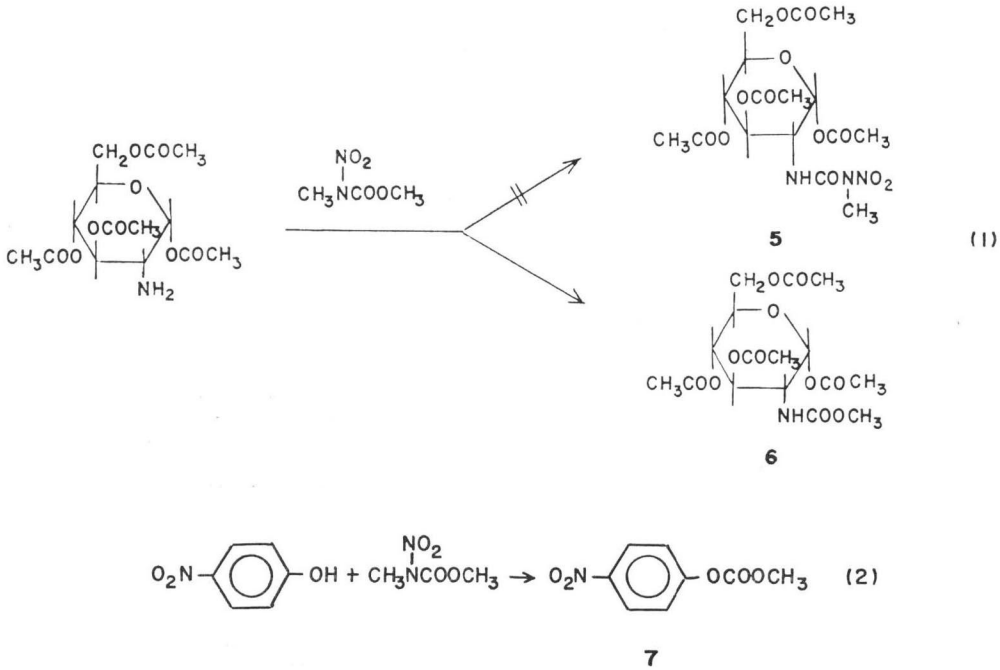
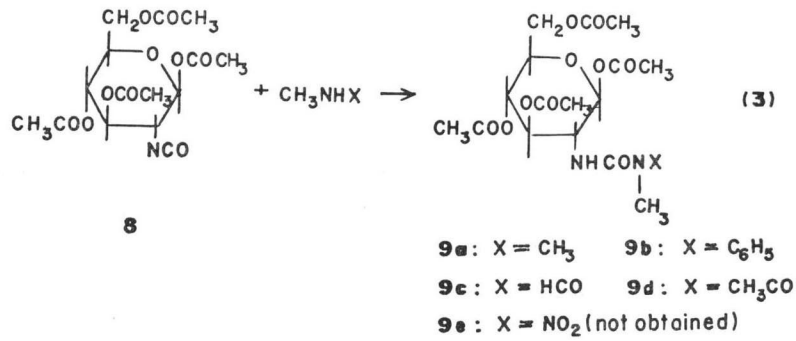
A second possible approach to the desired streptozotocin analogs would be acylation of a suitably protected glucosamine derivative having a free amino group by an appropriate acylating agent. In order to prepare an analog (**5**) having a nitro group in the original nitroso position acylation was attempted with methyl N-nitro-N-methylcarbamate (eq. 1). However, the carbomethoxy group was transferred rather than the $CH_3\overset{NO_2}{\overset{|}{N}}CO$ moiety and the product was **6**. The structure of the product is based on analysis, spectral data and synthesis from tetra-O-acetyl-2-deoxy-2-amino-α-D-glucopyranose and methyl chloroformate by the procedure of BROMUND and HERBST.[16] The compound **6** but with no assignment of stereochemistry at C–1 has been reported,[16] but the product obtained by us differed in melting point and rotation from that of BROMUND and HERBST. The anomeric hydrogen has a coupling constant (J=4 Hz) which suggests the α isomer.[17] It has been shown by MICHEEL, *et al.*[18] that tetra-O-acetyl-2-deoxy-2-amino-D-glucopyranose prepared according to the procedure of MOGGRIDGE and NEUBERGER[19] (as was that used in these experiments) is the α-anomer as shown below and the previously reported compound[16] is the β-isomer. Investigation of a simpler case of acylation with methyl N-nitro-N-methylcarbamate (eq. 2) also demonstrated carbomethoxy transfer.



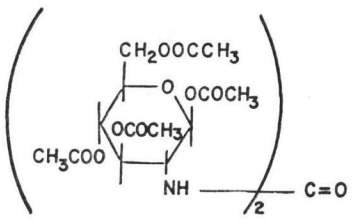
The next and successful approach to analog synthesis was the reaction of tetra-O-acetyl-2-deoxy-2-isocyanato-β-D-glucopyranose (**8**) with compounds of the type CH_3NHX (eq. 3). Compound **8** was prepared by the method of BERGMANN and ZERVAS[20] which has been shown to give the β-isomer as indicated.[18] Four analogs (**9a**, **9b**, **9c** and **9d**) were prepared by this procedure. In the cases of **9a** and **9b**



reaction was carried out in solvents, but in the other two cases the reactants were heated together at 85°C with no solvent. The products were identified by the usual spectral techniques and analyses. Elemental analyses of some of these compounds were not as close to theoretical values as desired and so were supplemented by high resolution mass spectrometry. Attempted conversion of compounds of type **9** to type **1** by ammonolysis failed when $X=CH_3CO$ but succeeded with $X=C_6H_5$ (**9b**——→**1e**). No conditions were found which would remove the O-acetyl groups from **9d** without removing the N-acetyl group.

Reaction of methyl nitramine with **8** did not proceed by eq. 3 to give **9e** but gave instead the β-

isomer of **6** and an approximately equimolar amount of bis-1,3-(tetra-O-acetyl-β-D-glucosyl-2) urea (**10**). The product isomeric with **6** had the same mp and rotation as did the carbomethoxy derivative of tetra-O-acetyl-β-glucopyranose reported by BROMUND and HERBST.[16] The isomer of **6** was synthesized by reaction of **8** with methanol. The ^{1}H NMR spectrum was consistent with the β-isomer in that the coupling constant of the anomeric proton had a value of 10 Hz.



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These compounds were tested *in vitro* against L1210, a leukemia cell line. The results are given in Table 1 and are expressed as μg/ml inhibiting growth of the cells by 50% and 90%. As can be seen they are about as active as streptozotocin tetraacetate. *In vivo* tests against P388 leukemia in mice showed that all were inactive.

Table 1. Cytotoxicity.

Compound	ID_{50}	ID_{90}
Streptozotocin tetraacetate	7.0	20
1d	51	142
9a	22	80
9b	25	135
9c	4.0	>10
9d	10	35

Experimental

Catalytic Reduction of Streptozotocin.

A solution of 2.65 g (0.01 mole) of streptozotocin in 50 ml of 95% EtOH was added to a mixture of 1 g of prereduced 10% Pd–C and 100 ml of 95% EtOH. The mixture was shaken for 5 hours at an initial pressure of 45 psi. In less than an hour about 0.01 mole of hydrogen had been absorbed and very little more reacted. Catalyst was removed by filtration, and the filtrate was concentrated to dryness under reduced pressure. Both **2a** and **2b** were prepared by the same procedure but in different experiments.

2a: One gram of the above residue was dissolved in EtOH, and the solution was filtered. After several days refrigeration at −15°C 340 mg of crystals precipitated. Recrystallization gave a mp of 171°C (lit.[10] 177~178°): $[\alpha]_D$ −22° (*c* 1, H_2O) (lit.[10] −21°); Rf (silica gel; EtOH-MeOH; 9: 1), 0.48; ir (Nujol) 3260, 3090, 1660 and 1500 cm^{-1}; ^{1}H NMR (D_2O) δ 2.82 (s, 3H, CH_3N), δ 3.55~4.20 (m, 6H, CHO and CHN), δ 4.70 (s, exchangeable), δ 5.67 (d, 1H, anomeric, J=6 Hz); ^{13}C NMR (D_2O) δ 163.47 (C=O), δ 92.77 (C-1), δ 79.68 (C-5), δ 76.07 (C-3), δ 69.93 (C-4), δ 64.89 (C-2) and δ 62.39 (C-6, off resonance spectrum).

Anal. Calcd. for $C_8H_{14}N_2O_5$: N, 12.84
Found: N, 12.94

2b: The total residue was recrystallized from EtOH giving two fractions. Recrystallization from EtOH of the combined materials gave 770 mg, mp 131~132°C; $[\alpha]_D$ +76° (*c* 1, H_2O); ir (Nujol) 3270, 1680 and 1505 cm^{-1}; uv (H_2O) only end absorption; ^{1}H NMR (D_2O) δ 2.78 (s, 3H, CH_3N), δ 3.5~3.95 (m, 6H, CHO and CHN), δ 4.65 (s, 6–7H, exchangeable), δ 5.04 (d, 1H, anomeric, J=2.2 Hz); mass spectrum *m/e* 218.0880 (calcd. for $C_8H_{14}N_2O_5$, 218.0902).

Anal. Calcd. for $C_8H_{14}N_2O_5$: N, 12.84.
Found: N, 12.03.

Synthesis of **2a** from 2-Deoxy-2-amino-D-glucopyranose.

The procedure of HESSLER and JAHNKE[18] was used to synthesize 8.1 g of **1d** starting with 7.16 g of 2-deoxy-2-amino-D-glycopyranose and 2.8 ml of methyl isocyanate. Two grams was dissolved in hot

ethanol, and the solution was cooled giving a first fraction of 0.50 g, mp 165~170°C, and a second fraction of 0.21 g, mp 172.5~175°C, ir ,rotation and ^{1}H NMR the same as previously obtained material.

Anal. Calcd. for $C_8H_{14}N_2O_5$: N, 12.84.
Found: N, 12.69.

Zinc-Acetic Acid Reduction of Streptozotocin (**3**).

A mixture of 1 g of streptozotocin, 1 g of zinc dust and 25 ml of glacial acetic acid was stirred at room temperature for 16 hours. The mixture was filtered, and the filtrate was allowed to evaporate to dryness in a current of air at room temperature, wt. 0.87 g. Recrystallization from a mixture of EtOH–H_2O at −15°C gave 0.31 g, mp 173~177°C. Further recrystallization from water raised the mp to 180°C; ir (Nujol) 3190, 1660, 1310, 1115, 1090, 1050, 1030, 960, 920, 900, 875, 850 and 745 cm^{-1}; uv (H_2O) max 255 nm (ε 2470); ^{1}H NMR (D_2O) δ 3.23 (s, 3H, CH_3N), δ 3.4~4.4 (m, 6H, CHO and CHN), δ 4.7 (s, exchangeable), δ 7.05 (d, 1H, CH=, J=2.5 Hz); ^{13}C NMR (D_2O) δ 37.36 (CH_3N), δ 55.77 (CHN), δ 64.12 (CH_2O), δ 70.91, 71.81 and 72.13 (CHO), δ 141.06 (CH=), δ 154.99 (C=O); mass spectrum *m/e* (M^++1) 234.11014 (calcd. for $C_8H_{15}N_3O_5$+H, 234.1090).

Anal. Calcd. for $C_8H_{15}N_3O_5$: C, 41.20; H, 6.49; N, 18.02.
Found: C, 41.22; H, 5.91; N, 18.59.

Tetra-O-acetyl-N-carbomethoxy-2-deoxy-2-amino-α-D-glucopyranose (**6**).

A. From Methyl N-nitro-N-methylcarbamate: A solution of 210 mg (1.55 mmoles) of methyl N-nitro-N-methylcarbamate and 1.4 g (4 mmoles) of tera-O-acetyl-2-deoxy-2-amino-α-D-glucopyranose (MOGGRIDGE and NEUBERGER[19]) in 5 ml of dry DMSO was allowed to stand at room temperature for 6 days. The solution was diluted with 30 ml of water and extracted with three 25-ml portions of $CHCl_3$. The solvent was removed from the combined extracts by evaporation under reduced pressure, and the residue was chromatographed on 50 g of silica gel in cyclohexane-ethyl acetate-ethanol (5:4:1) and collecting one hundred and forty-seven 10-ml fractions. On the basis of a weight analysis fractions 6~14 were combined as pool 1 and fractions 16~29 were combined as pool 2. The two pools were evaporated under reduced pressure. The material from pool 2 was identified as starting glucosamine. Pool 1 gave 0.61 g which was recrystallized from ethyl acetate-Skellysolve B to give 268 mg, mp 105~107°C; $[\alpha]_D$+95.5° (*c* 2, $CHCl_3$); ir (Nujol) 3200 (NH), 1745 (CH_3CO), 1690 (OCON) and 1555 cm^{-1} (amide II); ^{1}H NMR ($CDCl_3$) δ 2.05, 2.09 and 2.15 (3s, 12H, CH_3CO), δ 3.65 (s, 3H, CH_3O), δ 3.8~5.16 (m, 7H, CHO and CHN, NH), δ 6.18 (d, 1H, anomeric, J=4 Hz).

Anal. Calcd. for $C_{16}H_{23}NO_{11}$: C, 47.41; H, 5.72; N, 3.46.
Found: C, 47.19; H, 5.64; N, 4.10.

B. From Methyl Chloroformate: This was done by the procedure of BROMUND and HERBST[16] except using tetra-O-acetyl-2-deoxy-2-amino-glucopyranose prepared by the method of MOGGRIDGE and NEUBERGER.[19] The yield of **6**, mp 103~106°C, was 35%. Recrystallization as in (A) gave mp 105~106°C; $[\alpha]_D$+98° (*c* 2, $CHCl_3$); ir and ^{1}H NMR identical with those of the product in (A).

Anal. Calcd. for $C_{16}H_{23}NO_{11}$: C, 47.41; H, 5.72; N, 3.46.
Found: C, 47.22; H, 5.70; N, 3.34.

Methyl *p*-Nitrophenyl Carbamate (**7**).

A solution of 0.26 g (2 mmoles) of methyl N-nitro-N-methylcarbamate, 1.35 g (10 mmoles) of *p*-nitrophenol and 3 drops of $(C_2H_5)_3N$ in 30 ml of benzene was refluxed with slow distillation of benzene until about 40 ml had been removed. The benzene was replenished from time to time. After the reaction mixture had stood at room temperature for 24 hours, it was filtered. The filtrate was washed with two 10-ml portions of saturated $NaHCO_3$ solution and two 10-ml portions of water. Evaporation *in vacuo* gave 357 mg. Two recrystallizations from EtOH gave 167 mg, mp 111~112°C (lit.[21] 111~112°C); ir (Nujol) 1755 (C=O); ^{1}H NMR ($CDCl_3$) δ 3.96 (s, 3H, CH_3O), δ 7.24 (d, 2H, J=9.5 Hz, aromatic), δ 8.16 (d, 2H, J=9.5 Hz, aromatic).

Anal. Calcd. for $C_8H_7NO_5$: C, 48.74; H, 3.66; N, 7.11.
Found: C, 48.75; H, 3.66; N, 6.86.

Tetra-O-acetyl-2-deoxy-2-(3-dimethylureido)-β-D-glucopyranose (**9a**).

A mixture of 1.12 g (3 mmoles) of **8** and 15 ml of dry ether was shaken until no more solid dis-

solved. This was followed by addition of 0.22 ml (0.15 g; 3.3 mmoles) of $(CH_3)_2NH$. The mixture was allowed to stand at room temperature for 5 hours and filtered. The yield was 0.89 g, mp 145°C (dec.). Recrystallization from acetone-Skellysolve B at −15°C gave 150 mg, mp 157~160°C (dec.); ir (Nujol) 3230 (NH), 1740 (CH_3COO) and 1640 (NCON) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.0, 2.06 and 2.12 (3 s, 12H, CH_3CO), δ 2.82 (s, 6H, $(CH_3)_2N$), δ 3.95~4.5 and 4.76~5.30 (m, 7H, CHO, CHN and NH), δ 5.70 (d, 1H, anomeric H, J=10.5 Hz).

Anal. Calcd. for $C_{17}H_{26}N_2O_{10}$: C, 48.80; H, 6.26; N, 6.70.
Found: C, 48.39; H, 6.26; N, 6.53.

Tetra-O-acetyl-2-deoxy-2-(3-methyl-3-phenylureido)-β-D-glucopyranose (**9b**).

A solution of 15 g (0.04 mole) of **8** in 270 ml of dry $CHCl_3$ was stirred while adding dropwise a solution of 5.35 g (0.05 mole) of N-methylaniline in 270 ml of $CHCl_3$. The mixture was heated under reflux for 6 hours and evaporated under reduced pressure. The residue was dissolved in benzene and treated twice with charcoal. The solvent was removed by evaporation under reduced pressure, and the residue was recrystallized twice from benzene, yield 15.6 g (81%) mp 61°C; ir (Nujol) 3450 (NH), 1735 (CH_3COO) and 1650 (NCON) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.96, 2.05 and 2.15 (3s, 12H, CH_3COO), δ 3.25 (s, 3H, CH_3N), δ 4.0~4.36 and 5.0~5.2 (m, 7H, CHO, CHN and NH), δ 5.66 (d, 1H, anomeric, J=8 Hz), δ 7.0~7.5 (m, 5H, aromatic); mass spectrum *m/e* 480.1740 (calcd. for $C_{22}H_{28}N_2O_{10}$, 480.1743).

Anal. Calcd. for $C_{22}H_{28}N_2O_{10}$: C, 55.05; H, 5.88; N, 5.84.
Found: C, 54.52; H, 5.97; N, 5.69.

2-Deoxy-2-(3-methyl-3-phenylureido)-β-D-glucopyranose (**1d**).

Twelve grams of **9b** was dissolved in 1.2 liters of dry methanol containing 5% NH_3. The solution was allowed to stand at room temperature for 19 hours. The solvent and ammonia were removed by evaporation under reduced pressure at room temperature. The residue (19.2 g) was chromatographed on 450 g of silica using ethyl acetate - methanol - Skellysolve B (5: 1: 4) and collecting three hundred and fifty-two 20-ml fractions. The solvent mixture was changed to a ratio of 10: 3: 7 and 105 fractions were collected. On the basis of a weight analysis fractions 170~215 were combined as pool 1 and fractions 354~457 as pool 2. Evaporation of pool 1 under reduced pressure gave a product which was identified as acetamide by NMR. Evaporation of pool 2 under reduced pressure gave 4.75 g of residue which was chromatographed on 215 g of silica using ethyl acetate - methanol - Skellysolve B (10: 3: 7). In each chromatography the material was dried down out of methanol onto silica and added to the top of the column. A total of one hundred and forty-seven 20-ml fractions were collected. Fractions 81~120 were combined (wt. analysis) and evaporated under reduced pressure, yield 3.80 g (48%), indefinite mp but homogeneous by tlc (EtOAc - CH_3OH - Skellysolve B; 5: 3: 2); ir (Nujol) 3450 (OH/NH) and 1655 (NCON) cm^{-1}; uv (EtOH) max 238 nm (ε 10,550); ^1H NMR (d_6-DMSO) δ 3.18 (s, 3H, CH_3N), δ 4.95 (m, 1H, anomeric), δ 7.37 (s, 5H, aromatic); mass spectrum *m/e* (4 TMS) 600.2852 (calcd. for $C_{26}H_{52}Si_4N_2O_6$, 600.2902).

Anal. Calcd. for $C_{14}H_{20}N_2O_6$: C, 53.84; H, 6.45; N, 8.97.
Found: C, 53.05; H, 6.75; N, 8.68.

Tetra-O-acetyl-2-deoxy-2-(3-methyl-3-formyluredio)-β-D-glucopyranose (**9c**).

A mixture of 2.24 g (6 mmoles) of **8** and 0.41 g (7 mmoles) of N-methylformamide was heated at 85~90°C for 24 hours. The product was chromatographed three times on silica (about 50 g/g) using $CHCl_3$ - MeOH (9: 2) and collecting the first material which came off until it began to be contaminated by following material. This was followed by tlc ($CHCl_3$ - MeOH; 95: 5). Yield 0.46 g (17.8%), indefinite mp but homogeneous by tlc; ir (Nujol) 3280 (NH), 1750 (CH_3COO), 1705 (HCON) and 1680 (NCON) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.95~2.12 (4s, 12H, CH_3CO), δ 3.05 (s, 3H, CH_3N), δ 3.65~4.32 and 4.92~5.55 (m, 6H, CHO and CHN), δ 6.01 (d, 1H, anomeric, J=7.5 Hz), δ 8.48 (s, 1H, HCO), δ 8.80 (d, 1H, NH); mass spectrum *m/e* 389.1174 ($M^+ - CH_3CO$) (calcd. for $C_{15}H_{21}N_2O_{10}$, 389.1195).

Anal. Calcd. for $C_{17}H_{24}NO_{11}$: C, 47.21; H, 5.60; N, 6.48.
Found: C, 47.83; H, 5.74; N, 6.16.

Tetra-O-acetyl-2-deoxy-2-(3-methyl-3-acetylureido)-β-D-glucopyranose (**9d**).

A mixture of 6.72 g (18 mmoles) of **8** and 1.56 g (21 mmoles) of N-methylacetamide was heated at 85~90°C for 24 hours. The residue was dissolved in EtOH and allowed to stand overnight. The precipitate was removed by filtration, and the filtrate was evaporated to dryness under reduced pressure. The residue was chromatographed on 360 g of silica using $CHCl_3$ - EtOAc (1: 1) and collecting one hundred and fifty 20-ml fractions. The second weight maximum (fractions 80~140) was pooled and evaporated under reduced pressure. Crystallization from a mixture of benzene-Skellysolve B gave 3.7 g (46%), mp 61~67°C, homogeneous by tlc, Rf 0.44 ($CHCl_3$ - EtOAc; 1: 1); ir (Nujol) 3130 (NH), 1745 (CH_3COO) and 1680 (amide and urea CO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.97~2.06 (3s, 12H, CH_3COO), δ 2.20 (s, 3H, CH_3CON), δ 3.25 (s, 3H, CH_3N), δ 3.65~4.30 and 4.82~5.60 (m, 6H, CHO and CHN), δ 5.95 (d, 1H, anomeric, J=8 Hz), δ 9.53 (d, 1H, NH, J=9.5 Hz); mass spectrum *m/e* 403.1338 (M^+-CH_3CO) (calcd. for $C_{16}H_{23}N_2O_{11}$, 403.1352).

Anal. Calcd. for $C_{18}H_{26}N_2O_{11}$: C, 48.43; H, 5.87; N, 6.28.
Found: C, 48.00; H, 6.13; N, 5.80.

Tetra-O-acetyl-N-carbomethoxy-2-deoxy-2-amino-β-D-glucopyranose.

A. From Methylnitramine: A mixture of 1.12 g (3 mmoles) of **8** and 270 mg (3.5 mmoles) of methylnitramine was heated at 85~90°C for 18 hours. The product was dissolved in EtOH and cooled to give 447 mg of a crystalline product, mp 230°C. This was identified by mp, analysis and spectral data as 1,3-bis(tetra-O-acetyl-β-D-glucosyl-2) urea (**10**).[22] Evaporation of the filtrate gave 459 mg of residue which was crystallized twice from benzene-Skellysolve B, yield 277 mg, mp 144~145°C (lit.[16] 148~149.5°C), homogeneous in tlc, Rf 0.43 ($CHCl_3$ - MeOH; 95: 5); $[\alpha]_D$ +23.7° (*c* 2, $CHCl_3$); ir (Nujol) 3250 (NH), 1740 (CH_3COO), 1690 (NHCO) and 1530 (amide II) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.05, 2.10 and 2.22 (3s, 12H, CH_3COO), δ 3.60 (s, 3H, CH_3O), δ 3.65~4.30 and 4.98~5.42 (m, 7H, CHO, CHN and NH), δ 5.66 (d, 1H, anomeric, J=10 Hz); mass spectrum *m/e* 362.1116 (M^+-CH_3CO) (calcd. for $C_{14}H_{20}NO_{10}$, 362.1087).

Anal. Calcd. for $C_{16}H_{23}NO_{11}$: C, 47.41; H, 5.72; N, 3.46.
Found: C, 47.69; H, 5.74; N, 3.57.

B. From Methanol: A mixture of 500 mg of **8** and 5 ml of anhydrous methanol was heated to boiling. The solution was refrigerated and filtered. The filtrate was evaporated under reduced pressure. The residue was chromatographed on 25 g of silica in $CHCl_3$ - MeOH (98: 2) collecting 5-ml fractions. Fractions 16~25 were combined (wt. analysis) and evaporated under reduced pressure, and the residue was recrystallized twice from a mixture of ethyl acetate-Skellysolve B, yield 90 mg, mp 145~147°C; $[\alpha]_D$ +20.9° (*c* 2, $CHCl_3$); ir, NMR and Rf as in (A).

Anal. Found: C, 47.37; H, 5.74; N, 3.09.

Acknowledgement

We wish to thank Dr. A. D. ARGOUDELIS for suggestions and consultations, Miss SANDRA KUENTZEL for *in vitro* assays, Dr. GARY L. NEIL for *in vivo* assays and Dr. LUBOMIR BACZYNSKYJ for assistance with the mass spectra. This work was supported in part by Contract NO1-CM-23709 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

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