THE SYNTHESIS AND BIOLOGICAL ACTIVITIES OF SOME ANALOGS OF STREPTOZOTOCIN

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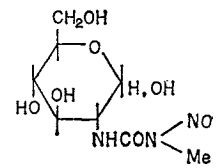
The α- and β-methyl glycosides (**IV** and **V**, respectively) of the antibiotic streptozotocin (**I**) have been synthesized. In addition, analogs involving epimeric changes at C_2 (**III**) and C_4 (**II**), and of two C_1 analogs, 3-β-D-glucopyranosyl-1-methyl-1-nitrosourea (**XXIII**) and the corresponding D-galactopyranosyl compound (**XXV**), together with their tetra-*O*-acetates (**XXII** and **XXIV**, respectively) have been prepared. An open-chain analog was obtained by the synthesis of 1-deoxy-1-(3-methyl-3-nitrosoureido)-D-glucitol (**XXIX**), but the 2-deoxy-D-glucitol derivative (**XXVII**) decomposed on attempted isolation. Epimerization at C_2 reduces the antibacterial activity markedly; all other changes made destroy it. All of the analogs show cytotoxic activity in the range of streptozotocin or higher, and all are devoid of diabetogenicity.

Streptozotocin is an antibiotic produced by *Streptomyces achromogenes* var. *streptozoticus*[1] and shown by degradation[2,3] and synthesis[3,4,5] to have the structure **I**. Streptozotocin possesses broad-spectrum antibacterial activity[1] and is active *in vivo*[6]. In addition, however, it displays marked anti-leukemic activity[7,8], and is a diabetogenic agent[9,10], being specifically toxic to the β-cells of the islets of LANGERHANS. It is currently under study in the therapy of malignant insulinomas[11,12].



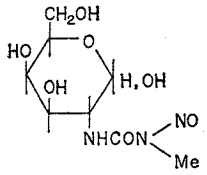
I

This multiplicity of activities made it of interest to prepare analogs with altered carbohydrate stereochemistry, modified substituents of the antibiotic itself, and transpositions of hydroxyl and nitrosoureido groups.

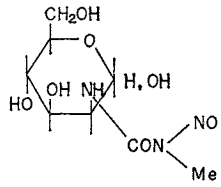
Prior to this investigation, it was known that 1,3,4,6-tetra-*O*-acetylstreptozotocin shows no antibacterial activity, and also that the replacement of the methyl group at N^3 of the nitrosourea by ethyl or *n*-butyl leads to antibacterially inactive products (Dr. R. R. HERR, The Upjohn Company; personal communication, 1967). However, while streptozotocin tetraacetate shows even greater inhibition of the growth of L-1210 cells than the antibiotic itself[13], the N^3-ethyl and butyl analogs and their tetraacetates show only slight activity (R. R. HERR, *vide supra*).

Since the completion of this work, two papers have appeared on the synthesis of analogs of streptozotocin, one on those derived from aminocyclitols[14] and the other on the methyl glycosides[15] of the antibiotic, the latter by a route analogous to that employed in the present work.

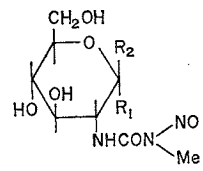
The first analogs investigated involved stereochemical modifications only. Using



II



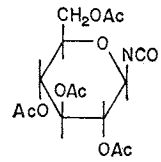
III



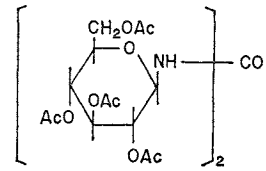
IV, R_1 = OMe, R_2 = H

V, R_1 = H, R_2 = OMe

the general method of urea formation and aqueous nitrosation of HESSLER and JAHNKE[5] without isolation of the intermediate, 4-*epi*-streptozotocin (II) was prepared from D-galactosamine, and 2-*epi*-streptozotocin (III) from D-mannosamine.



VI



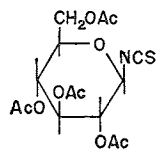
VII

Streptozotocin, as isolated from fermentation sources, varies widely in optical rotation from lot to lot, though all mutarotate to the same equilibrium value. The effect of glycoside formation and possible differences in activity between anomers was therefore examined. Details of the synthesis of the α- and β-methyl glycosides (**IV** and **V**, respectively) from the methyl N-carbobenzyloxy-D-glucosaminides[16] are given because of discrepancies in the physical constants between this and the literature report[15] and because the latter was restricted in scope to antileukemic activity.

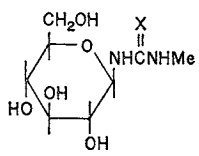
The effect of placing the N-nitrosoureido substituent at position 1 in glucose was examined. The early literature[17,18] records the acid-catalyzed condensation of glucose with N-methylurea to give "*d*-Glucosemonomethylureid", but attempts to repeat this reaction led to complex, intractable mixtures.

In contrast to the results of FISCHER[19], the reaction between acetobromoglucose and silver cyanate in xylene under reflux failed to yield the desired isocyanate (**VI**); the reaction gave the di-β-D-glucosylurea octa-*O*-acetate (**VII**) in good yield, identified by direct comparison with the product of acetylation of a commercial sample of "diglucosylurea"[20]. The data agree with those of MESSMER *et al.*[21] Sufficient moisture presumably was present in the reaction mixture in spite of efforts to prevent this, that some of the first-formed isocyanate **VI** hydrolyzed to the glucosylamine which then reacted with unhydrolyzed isocyanate.

The isothiocyanate[22] (**VIII**) was obtained by the reaction between acetobromoglucose and silver thiocyanate, and the addition of methylamine proceeded smoothly with concomitant deacetylation to give the thiourea (**IX**). Treatment of an aqueous solution of **IX** with mercuric oxide did not yield the expected urea (**X**) but gave a product shown by analysis to have a molecular formula of the urea minus one molecule of

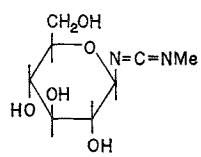


VIII

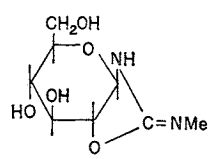


IX X=S

X X=O



XI

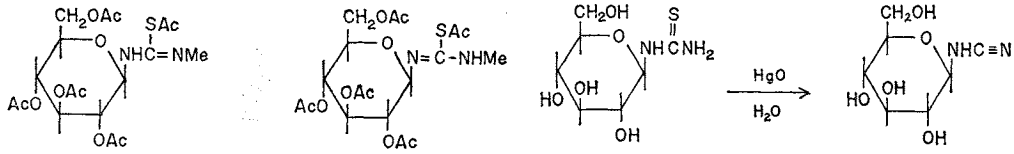


XII

water, confirmed by mass spectral evidence (M^+, *m/e* 218). The carbodiimide (**XI**) was ruled out by the lack of absorption in the 2150 cm^{-1} region of the IR spectrum; apparent amide I and amide II bands at 1665 and 1535 cm^{-1} could be attributable to the >C=N- and -NH-C= groupings of an isourea. The NMR spectrum showed four exchangeable hydrogens and $J_{1,2}$=9.5 Hz, requiring that H_1,H_2 be diaxial. Examination of molecular models shows that, with this requirement, only the C_2 hydroxyl can be involved, giving the *trans*-1,2-bicyclo isourea structure **XII**. The double bond is placed *exo*-cyclically in view of the singlet NMe peak at δ 2.78 in the NMR spectrum.

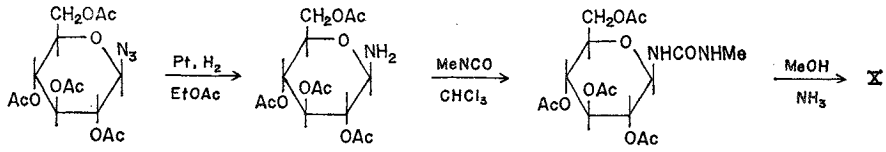
The attempted protection of the hydroxyls against participation during the mercuric oxide reaction by acetylation was found to be accompanied by S-acetylation, both double-bond isomers (**XIII** and **XIV**) being formed. Assignments of structure were based on NMR data (see Experimental).

FISCHER[19] reported the reaction between the analogous glucosylthiourea (**XV**) and mercuric oxide in water to give an ill-defined, unstable, amorphous product to which he assigned the cyanamide structure **XVI**.



XIII **XIV** **XV** **XVI**

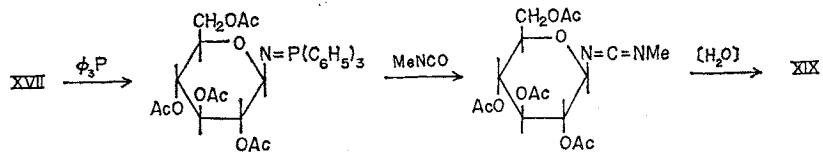
In view of the failure of the above methods to yield the desired urea (**X**), recourse was made to the method of BERTHO[23,24] of the reaction of acetobromoglucose with sodium azide to the β-azide (**XVII**) and reduction to the tetra-*O*-acetyl-β-D-glucosylamine (**XVIII**). Reaction with methyl isocyanate occurred readily to give the tetraacetyl urea (**XIX**), deacetylated with methanolic ammonia to the free glucosylurea (**X**).



XVII **XVIII** **XIX**

The physical constants of **X** agreed with those reported[17,18] indicating that the direct condensation of glucose and N-methylurea had given the β-pyranosyl product.

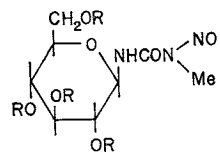
Alternatively, use can be made of the STAUDINGER reaction[25] to go from the azide (**XVII**) to the phosphineimine (**XX**)[21]; this reacted readily with methyl isocyanate to



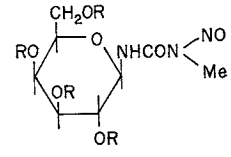
XX **XXI**

give the carbodiimide **XXI**, which underwent hydration on silica to the urea (**XIX**).

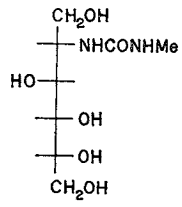
Nitrosation of the tetraacetylurea (**XIX**) in pyridine with nitrosyl chloride gave the acetylated N-nitrosourea **XXII**, while aqueous nitrosation of **X** gave the unacetylated analog **XXIII**. By an analogous series of reactions starting with acetobromogalactose, the galacto-analogs **XXIV** and **XXV** were obtained.



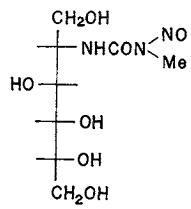
XXII R = Ac
XXIII R = H



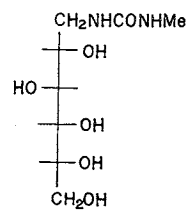
XXIV R = Ac
XXV R = H



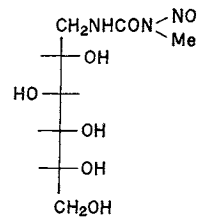
XXVI



XXVII



XXVIII



XXIX

The urea **XXVI** was obtained readily from D-glucosaminol. However, the nitrosated product **XXVII**, a reduced, open-chain analog of streptozotocin itself, decomposed during isolation. In the similar series from D-glucamine, nitrosation of the urea **XXVIII** gave the desired N-nitrosourea **XXIX**.

Activities

Antibacterial: Inversion of the 4-hydroxyl group of streptozotocin leads to complete loss of antibacterial activity against the test organism *Proteus vulgaris*, as does methyl glycoside formation in both anomeric configurations. The open-chain analog **XXIX** equally is devoid of activity, as are the glucosyl (**XXIII**) and galactosyl (**XXV**) analogs and their tetraacetates (**XXII** and **XXIV**, respectively). Inversion of configuration at C_2 is less crucial, the mannosamine analog **III** showing activity against *P. vulgaris*, but only to the extent of 5~10% of that of streptozotocin.

Diabetogenic: None of the compounds described here shows diabetogenicity in the rat at a level of 65 mg/kg, at which concentration streptozotocin shows a four-fold elevation of blood-sugar concentration.

Cytotoxic: The cytotoxic activities against L-1210 cells in culture of the analogs described are summarized in Table 1. Structural variations make it possible to separate the cytotoxic activity from the diabetogenicity and antibacte-

Table 1. Cytotoxic activity of streptozotocin, its derivatives and analogs.

Compound	ID_{50} *in vitro* μg/ml*
Streptozotocin, I	84
Streptozotocin α-Me glycoside, IV	47
Streptozotocin β-Me glycoside, V	80
2-*epi*-Streptozotocin, III	62
4-*epi*-Streptozotocin, II	75
β-Glucosyl analog, **XXIII**	55
β-Galactosyl analog, **XXV**	76
Glucamine analog, **XXIX**	51
Streptozotocin tetraacetate	10.5
β-Glucosyl analog tetraacetate, **XXII**	18
β-Galactosyl analog tetraacetate, **XXIV**	28

* The dose necessary to cause 50% inhibition of the growth of L-1210 cells. Cells and drug were inoculated into tubes on day 0 and cell numbers were counted on day 3.

rial activity. All of the analogs show activity at least equal to streptozotocin; interestingly, the α-methyl glycoside **IV** is twice as active as the β-anomer **V**. As with streptozotocin tetraacetate, the tetra-*O*-acetates **XXII** and **XXIV** show enhanced activity. The carbohydrate moiety appears to act as a carrier for the N^3-methyl-N^3-nitrosourea group. The anti-leukemic activity of simple methyl nitrosoureas is well documented[26].

The antileukemic activity of these analogs *in vivo* will be reported elsewhere by Dr. B. K. BHUYAN of these laboratories.

Experimental Section

Melting points were determined on a Gallenkamp (England) capillary melting point apparatus, using a thermometer calibrated for stem exposure. Thin-layer chromatography was run on 2″×8″ Uniplates[27] coated with silica gel GF (250 μ) using the solvent system quoted (parts by volume). Zones were detected by spraying with LEMIEUX[28] reagent or with 50 % aqueous H_2SO_4 followed by heating at 100°C; the N-nitroso derivatives could also be detected by their strong fluorescence under UV irradiation. Brinkmann silica gel (0.05~0.20 mm) for chromatography[29] was used for column chromatography. Solvents were removed on a rotating evaporator at 40°C/7 mm. Specific rotations were determined in a 2 dm cell with a Bellingham and Stanley (England) polarimeter. IR spectra were measured with a Perkin-Elmer 421 grating spectrometer, using Nujol mulls. UV spectra were measured in EtOH with a Cary 15 recording spectrometer. NMR spectra were measured at 60 MHz with a Varian A 60 A spectrometer; chemical shifts are given on the δ scale, and spectra were measured in $CDCl_3$ with TMS as the internal standard. For the purposes of this work, spectra in D_2O were calibrated against the HOD peak at δ 4.67. Mass spectra were measured on an Atlas CH-4 at 70 ev.

4-*epi*-Streptozotocin (2-deoxy-2-(3-methyl-3-nitrosoureido)-D-galactopyranose) (II).

D-Galactosamine·HCl (1.0 g) in H_2O (5 ml) was neutralized with $NaHCO_3$ (390 mg), Et_2O (2.5 ml) added, and the mixture cooled in ice/MeOH. MeNCO (265 mg, 1 equiv.) was added, the mixture stirred for 30 minutes, and $NaNO_2$ (320 mg, 1 equiv.) added. This solution was allowed to drop during 20 minutes into aqueous H_2SO_4 (2 N, 2.32 ml, 1 equiv.) cooled in ice/MeOH. Volatile material was removed from the yellow solution which then was diluted with water and lyophilized. Chromatography (1 MeOH : 3 $CHCl_3$) gave material which separated from MeOH as pale yellow needles (157 mg), m.p. 109~110 °C (dec.), $[\alpha]_D$ +69° (*c* 0.89, H_2O).

Anal. Calcd. for $C_8H_{15}N_3O_7$: C 36.23, H 5.70, N 15.84
Found: C 36.33, H 5.90, N 16.10

2-*epi*-Streptozotocin (2-deoxy-2-(3-methyl-3-nitrosoureido)-D-mannopyranose) (III).

By a sequence entirely analogous to that above, D-mannosamine·HCl (1.0 g) yielded III as a hygroscopic pale yellow amorphous solid (280 mg), $[\alpha]_D$+1° (*c* 0.71, H_2O).

Anal. Calcd. for $C_8H_{15}N_3O_7$: C 36.23, H 5.70, N 15.84
Found: C 36.52, H 5.65, N 15.84

Anomeric Methyl N-benzyloxycarbonyl-D-glucosaminides.

This mixture was obtained by the method of NEUBERGER and PITT RIVERS[16], but it was separated by chromatography (1 MeOH : 7 $CHCl_3$), the α-anomer moving faster than the β-anomer. At room temperature after 160 hours, the ratio of $\alpha:\beta$ is 5.9 : 1; after 82 hours, the ratio is 1 : 2.

α-Anomer: *Anal.* Calcd. for $C_{15}H_{21}NO_7$: C 55.04, H 6.47, N 4.28
Found: C 55.22, H 6.31, N 4.35
m.p. 163.5~164°C (PrOH), $[\alpha]_D$+92° (*c* 0.78, H_2O)
lit[15]. m.p. 156~158°C, $[\alpha]_D$+92.6° (pyridine)
lit[16]. m.p. 154~155°C, $[\alpha]_D$+80° (pyridine).

β-Anomer: *Anal.* Calcd. for $C_{15}H_{21}NO_7$: C 55.04, H 6.47, N 4.28
Found: C 54.87, H 6.61, N 4.43
m.p. 173.5~174°C (H_2O), $[\alpha]_D$−25° (*c* 0.74, H_2O)
lit[15]. m.p. 166~167.5°C, $[\alpha]_D$−22.8° (pyridine)
lit[16]. m.p. 166~168°C, $[\alpha]_D$−38° (pyridine)

Methyl 2-deoxy-2-(3-methylureido)-α-D-glucopyranoside.

The α-anomer of the benzyloxycarbonyl compound (5.0 g) in EtOH (200 ml) was hydrogenolyzed overnight in the presence of Pd/C (10 %, 500 mg) under 50 p.s.i. H_2. Tlc (1 MeOH : 7 $CHCl_3$) showed the absence of starting material and the formation of product at the origin. Filtration from catalyst and removal of solvent gave a syrup (3.23 g) which was dissolved in H_2O (12.5 ml), Et_2O (7 ml) added, followed by MeNCO (0.96 g, 1.1 equivs.) and the mixture was stirred overnight. No amine remained (tlc, 1 MeOH : 3 $CHCl_3$), and a new zone of higher Rf was present. Removal of the solvent and crystallization from MeOH gave the urea (2.43 g), m.p. 194~196°C, $[\alpha]_D$+129° (*c* 0.88, DMF) Lit[15]. m.p. 194.5~196°C, $[\alpha]_D$+97.5° (H_2O).

Anal. Calcd. for $C_9H_{18}N_2O_6$: C 43.19, H 7.25, N 11.20, OCH_3 12.40
Found: C 42.90, H 7.28, N 11.32, OCH_3 12.38

Methyl 2-deoxy-2-(3-methyl-3-nitrosoureido)-α-D-glucopyranoside (IV).

Nitrosation of the α-anomeric urea (1.29 g) in aqueous solution as described for the earlier analogs resulted in the precipitation of a solid; (IV) crystallized from MeOH-Et_2O as pale yellow needles, m.p. 175~185°C (dec.), $[\alpha]_D$+117° (*c* 0.63, H_2O). Lit[15]. m.p. 129~133°C, $[\alpha]_D$+107° (H_2O).

Anal. Calcd. for $C_9H_{17}N_3O_7$: C 38.71, H 6.14, N 15.05, OCH_3 11.11
Found: C 38.62, H 6.02, N 15.39, OCH_3 11.26

Methyl 2-deoxy-2-(3-methylureido)-β-D-glucopyranoside.

Hydrogenolysis of the β-anomeric benzyloxycarbonyl compound (3.58 g) and formation of the urea were conducted as above, yielding the crystalline product (1.83 g) from MeOH, m.p. 244~245°C, $[\alpha]_D$−52°, (*c* 0.64, DMF). Lit[15]. m.p. 239.5~241°C.

Anal. Calcd. for $C_9H_{18}N_2O_6$: C 43.19, H 7.25, N 11.20, OCH_3 12.40
Found: C 42.93, H 6.89, N 11.33, OCH_3 12.41

Methyl 2-deoxy-2-(3-methyl-3-nitrosoureido)-β-D-glucopyranoside (V).

Nitrosation, conducted as usual, of the urea (1.59 g) gave (V) from H_2O, m.p. 185~193°C (dec.), $[\alpha]_D$−4°, (*c* 0.57, DMF). Lit[15] m.p. 149°C (dec.), $[\alpha]_D$−23.7° (H_2O).

Anal. Calcd. for $C_9H_{17}N_3O_7$: C 38.71, H 6.14, N 15.05, OCH_3 11.11
Found: C 38.50, H 6.03, N 15.38, OCH_3 11.10

N,N'-*bis*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)urea (VII).

Reaction between α-acetobromoglucose (19.15 g) in xylene (distilled from Na, 1500 ml) and AgNCO (dried at 100°C/high vac., 25.0 g), gave a syrup which showed extremely weak absorption at 2280 cm^{-1} (possibly -N=C=O): chromatography (1 Me_2CO : 1 Skellysolve B[30]) gave a colorless solid which separated from Me_2CO-Skellysolve B in needles, m.p. 159.5~161°C, $[\alpha]_D$−5° (*c* 0.88, $CHCl_3$).

Anal. Calcd. for $C_{29}H_{40}N_2O_{19}$: C 48.33, H 5.60, N 3.89
Found: C 48.68, H 5.31, N 4.04

Acetylation (Ac_2O-pyridine) of 1,3-di-β-D-glucosylurea[20] gave a product indistinguishable from the above. [Lit[21]. m.p. 162°C, $[\alpha]_D$−3.6° ($CHCl_3$)].

3-β-D-Glucopyranosyl-1-methylthiourea (IX).

β-D-Glucopyranosyl isothiocyanate tetraacetate[22] (VIII, 8.0 g) was allowed to stand overnight in MeOH saturated at 0°C with $MeNH_2$. Removal of the solvent and crystallization of the residue from H_2O-EtOH gave (IX) as colorless plates (4.08 g), m.p. 229~230°C, $[\alpha]_D$−40° (*c* 0.88, H_2O).

Anal. Calcd. for $C_8H_{16}N_2O_5S$: C 38.08, H 6.39, N 11.11, S 12.71
Found: C 38.23, H 6.37, N 10.94, S 12.56

N,O²-[(Methylimino)methylene]-β-D-glucopyranosylamine (**XII**).

A solution of **IX** (2.0 g) in H_2O (50 ml) was stirred overnight with HgO (yellow, 8.26 g), filtered from the black solid, and the filtrate taken to dryness. The product (**XII**) crystallized from EtOH, m.p. 173~174°C (dec.), $[\alpha]_D$+133° (*c* 0.79, H_2O).

Anal. Calcd. for $C_8H_{14}N_2O_5$: C 44.03, H 6.47, N 12.84
Found: C 44.06, H 6.68, N 12.44

Acetylation of **IX**.

Acetylation of **IX** (3.17 g) in acetic anhydride-pyridine gave a syrup showing two products by tlc (1 EtOAc : 1 cyclohexane) of Rf 0.14 and 0.23, which were separated on a column (same system) and gave product A (Rf 0.23, 2.43 g) and product B (Rf 0.14, 2.80 g). Product A crystallized as needles from EtOH, m.p. 163.5~165°C, $[\alpha]_D$+6° (*c* 0.93, $CHCl_3$).

Anal. Calcd. for $C_{18}H_{26}N_2O_{10}S$: C 46.74, H 5.67, N 6.06, S 6.93
Found: C 46.61, H 5.56, N 6.08, S 6.80

and was assigned structure **XIII** on the basis of NMR data (δ 11.90, broad doublet, NH; δ 3.7, s, NMe; δ 2.38, s, SAc); UV λ_{max}. 233 (ε 11,350), 275 nm (ε 16,700). M^+, *m/e* 462. Product B crystallized as platelets from EtOAc-Skellysolve B, m.p. 131~132°C, $[\alpha]_D$+155° (*c* 0.60, $CHCl_3$).

Anal. Calcd. for $C_{18}H_{26}N_2O_{10}S$: C 46.74, H 5.67, N 6.06, S 6.93
Found: C 46.68, H 5.82, N 5.95, S 6.77

UVλ_{max}. 225 (ε 5,500), 271 (ε 9,750), 348 nm (ε 71), and was assigned structure **XIV** (NMR, δ 8.25, broad, NH; δ 3.18, d, NHMe; δ 2.38, s, SAc).

3-β-D-Glucopyranosyl-1-methylurea tetra-*O*-acetate (**XIX**).

The acetylated glucosyl azide[23] (**XVII**, 5.17 g) was reduced in EtOAc to the tetraacetylglucosylamine; solvent removal gave a solid showing no $-N_3$ absorption at 2120 cm^{-1}, but $-NH_2$ absorption at 3380, 3300, and 3200 cm^{-1}. This product in $CHCl_3$ (75 ml) was left overnight with MeNCO (3 ml). Solvent removal and crystallization from EtOAc gave **XIX** as needles (4.04 g), m.p. 194.5~195°C, $[\alpha]_D$+1° (*c* 0.61, $CHCl_3$).

Anal. Calcd. for $C_{16}H_{24}N_2O_{10}$: C 47.52, H 5.98, N 6.93
Found: C 47.68, H 6.07, N 6.93

3-β-D-Glucopyranosyl-1-methyl-1-nitrosourea tetra-*O*-acetate (**XXII**).

A solution of NOCl (3.63 g) in Ac_2O (15 ml) at 0°C was added dropwise to **XIX** (5.0 g) in pyridine (50 ml) also at 0°C and in an atmosphere of N_2, with stirring. After 20 minutes at room temperature, the yellow-orange solution was poured into ice-water, precipitating a red gum which was extracted thoroughly with $CHCl_3$, the extract washed with ice-cold HCl (*N*),H_2O, sat. aqueous $NaHC_3O$, H_2O, and dried (Na_2SO_4). Solvent removal gave a syrup which was chromatographed (1 Me_2CO : 1 Skellysolve B) to give a yellow solid (5.57 g); **XXII** separated from Me_2CO-Skellysolve B in pale yellow needles (3.94 g), m.p. 89~90°C, $[\alpha]_D$−8° (*c* 0.98, $CHCl_3$).

Anal. Calcd. for $C_{16}H_{23}N_3O_{11}$: C 44.34, H 5.35, N 9.70
Found: C 44.28, H 5.25, N 9.48

3-β-D-Glucopyranosyl-1-methylurea (**X**).

The tetraacetate (**XIX**, 4.23 g) was deacetylated in saturated methanolic ammonia at 0°C; solvent removal gave a syrup which crystallized from EtOH giving **X** (1.95 g) as needles, m.p. 213~215°C (dec.), $[\alpha]_D$−30° (*c* 0.32, H_2O)

Anal. Calcd. for $C_8H_{16}N_2O_6$: C 40.67, H 6.83, N 11.86
Found: C 40.87, H 6.83, N 11.64

Lit[17]. m.p. 216°C (dec.), $[\alpha]_D$−30.3° (H_2O); Lit[18]. m.p. 215°C (dec.), $[\alpha]_D$−31.8° (H_2O).

3-β-D-Glucopyranosyl-1-methyl-1-nitrosourea (**XXIII**).

The urea (**X**, 1.0 g) was nitrosated in aqueous solution by the standard procedure, and the product isolated by lyophilization. Chromatography (1 MeOH : 1 $CHCl_3$), followed by crystallization from MeOH, gave **XXIII** as pale yellow prisms, m.p. 177~180°C (dec.),

$[\alpha]_D$ −12° (*c* 0.87, H_2O).

Anal. Calcd. for $C_8H_{15}N_3O_7$: C 36.23, H 5.70, N 15.84
Found: C 36.42, H 5.95, N 15.88

2,3,4,6-Tetra-*O*-acetylglucosylamine triphenylphosphineimine (**XX**).

To a solution of the tetraacetylglucosyl azide (**XVII**, 5.0 g) in anhydrous Et_2O (100 ml) was added dropwise a solution of ϕ_3P (3.51 g, 1 equiv.) in Et_2O (50 ml) with stirring; N_2 was evolved. After 16 hours, crystalline material was removed, washed with Et_2O, and dried (4.08 g), m.p. 132~134°C. Recrystallization from ϕH–Skellysolve B gave **XX**, m.p. 133~134°C, $[\alpha]_D$ −18° (*c* 0.65, $CHCl_3$). UVλ_{max} 223 sh. (ε, 24,200), 254 sh. (ε, 2,900), 260.5 (ε, 3,050), 267 (ε, 3,150), 273 nm (ε, 2,550). Lit[21]. m.p. 136°C, $[\alpha]_D$ −18.2° (dioxane).

Anal. Calcd. for $C_{32}H_{34}NO_9P$: C 63.25, H 5.64, N 2.31, P 5.10
Found: C 63.51, H 5.69, N 2.30, P 5.16

Reaction of **XX** with MeNCO.

A solution of the phosphineimine (4.07 g) and MeNCO (382 mg, 1 equiv.) in ϕH (25 ml) was heated under reflux overnight, solvent removed, the residue dissolved in warm EtOAc, diluted with Skellysolve B, and the solid ($\phi_3P\rightarrow O$, 960 mg) removed by filtration. Removal of the solvent gave a semi-solid residue, showing strong absorption at 2160 cm^{-1} of the carbodiimide **XXI** but still containing $\phi_3P\rightarrow O$ (by tlc, 1 Me_2CO: 1 Skellysolve B, Rf of the carbodiimide, 0.54). Chromatography (1 Me_2CO: 2 Skellysolve B) yielded a product eluted much later than anticipated; the material obtained showed an Rf of 0.26 (1 Me_2CO: 1 Skellysolve B). It crystallized from EtOAc–Skellysolve B to give a product (1.23 g) shown (m.p., mixed m.p., tlc, IR) to be the ureido-derivative **XIX**, the carbodiimide having undergone hydration on the silica.

3-β-D-Galactopyranosyl-1-methylurea tetra-*O*-acetate.

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl azide[22] (10.09 g) was reduced in EtOAc to the amine by the method of BERTHO and MEIER[24], the solvent removed, the residual solid dissolved in $CHCl_3$ (200 ml), MeNCO (6.16 g) added, and the solution allowed to stand for 3 days. Removal of the solvent and crystallization from EtOAc gave robust needles (6.9 g), m.p. 222~223°C, $[\alpha]_D$ +23° (*c* 0.77, $CHCl_3$).

Anal. Calcd. for $C_{16}H_{24}N_2O_{10}$: C 47.52, H 5.98, N 6.93
Found: C 47.64, H 5.93, N 7.17

3-β-D-Galactopyranosyl-1-methyl-1-nitrosourea tetra-*O*-acetate (**XXIV**).

Nitrosation of the tetraacetyl urea (5.0 g) (NOCl–pyridine) as for the gluco-analog, followed by chromatography (1 Me_2CO : 1 Skellysolve B), gave **XXIV** (2.57 g) from MeOH, m.p. 138~139°C (dec.), $[\alpha]_D$ +7° (*c* 0.90 $CHCl_3$).

Anal. Calcd. for $C_{16}H_{23}N_3O_{11}$: C 44.34, H 5.35, N 9.70
Found: C 44.65, H 5.45, N 9.80

3-β-D-Galactopyranosyl-1-methylurea.

Ammonolysis of the tetraacetyl urea (18.24 g) gave the galactosyl urea (8.35 g), Rf 0.32 in 1 MeOH : 1 $CHCl_3$, as a monohydrate from aqueous ethanol, m.p. 210~211.5°C (dec.), $[\alpha]_D$ 0° (*c* 0.67, H_2O).

Anal. Calcd. for $C_8H_{16}N_2O_6\cdot H_2O$: C 37.79, H 7.13, N 11.02, H_2O 7.09
Found: C 37.84, H 7.35, N 11.23, H_2O 7.08

3-β-D-Galactopyranosyl-1-methyl-1-nitrosourea (**XXV**).

Aqueous nitrosation of the galactosyl urea (1.0 g) by the usual method, followed by chromatography (1 MeOH : 1 $CHCl_3$) gave a solid, Rf 0.64 in 1 MeOH : 1 $CHCl_3$, (810 mg) obtained as pale yellow needles (MeOH, 0°C), gradually decomposing at 165~180°C, $[\alpha]_D$ +23° (*c* 0.63, H_2O).

Anal. Calcd. for $C_8H_{15}N_3O_7$: C 36.23, H 5.70, N 15.84
Found: C 36.46, H 6.08, N 15.76

2-Deoxy-2-(3-methylureido)-D-glucitol (**XXVI**).

Treatment of D-glucosaminol[31] (15.6 g) with MeNCO under the usual conditions gave

the urea **XXVI** (8.88 g), m.p. 150.5~152.5°C from H_2O, $[\alpha]_D$ −1° (*c* 1.01, H_2O).

Anal. Calcd. for $C_8H_{18}N_2O_6$: C 40.33, H 7.61, N 11.76
Found: C 40.47, H 7.71, N 11.84

While nitrosation in aqueous solution under the usual conditions appeared to proceed satisfactorily to **XXVII** [tlc, 1 MeOH : 1 $CHCl_3$, disappearance of urea (Rf 0.37), new zone, UV absorbing, Rf 0.57] decomposition with, apparently, N_2 evolution occurred during attempted isolation.

1-Deoxy-1-(3-methylureido)-D-glucitol (**XXVIII**).

D-Glucamine[32] (1.0 g) reacted with MeNCO under the usual conditions to give the urea **XXVIII** (900 mg), m.p. 125~127°C from H_2O, $[\alpha]_D$ −11° (*c* 0.81, H_2O).

Anal. Calcd. for $C_8H_{18}N_2O_6$: C 40.33, H 7.61, N 11.76
Found: C 40.12, H 7.79, N 11.93

1-Deoxy-1-(3-methyl-3-nitrosoureido)-D-glucitol (**XXIX**).

The nitrosoureido compound (3.19 g) separated from the reaction mixture on nitrosation of the urea (**XXVIII**, 4.0 g) under the standard conditions; **XXIX** was obtained as pale yellow platelets (2.19 g) from H_2O, m.p. 95~95.5°C (dec.), $[\alpha]_D$ −10° (*c* 0.92, H_2O).

Anal. Calcd. for $C_8H_{17}N_3O_7$: C 35.95, H 6.41, N 15.72
Found: C 36.15, H 6.54, N 15.78

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