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SYNTHESIS OF DIAZOMETHYL β-D-GALACTOPYRANOSYL AND β-D-GLUCOPYRANOSYL KETONES. POTENTIAL AFFINITY-LABELING REAGENTS FOR CARBOHYDRATE-BINDING PROTEINS*

ROBERT WALTER MYERS** AND YUAN CHUAN LEE

Department of Biology and The McCollum–Pratt Institute, The Johns Hopkins University, Baltimore, Maryland 21218 (U.S.A.)

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ABSTRACT

3,7-Anhydro-1-deoxy-1-diazo-D-*glycero*-L-*manno*-2-octulose (**6a**; diazomethyl β-D-galactopyranosyl ketone) and 3,7-anhydro-1-deoxy-1-diazo-D-*glycero*-D-*gulo*-2-octulose (**6b**; diazomethyl β-D-glucopyranosyl ketone) have been prepared. Readily available *C*-glycosyl compounds possessing the appropriate stereochemistry and hydroxyl-group protection, *viz.*, per-*O*-acetyl-2,6-anhydroheptononitriles and per-*O*-acetyl-2,6-anhydroheptonamides, were employed as precursors to per-*O*-acetyl-2,6-anhydroheptonic acids. These key intermediates were then converted into mixed carboxylic–carbonic acid anhydrides, and these caused to react with diazomethane, to give the corresponding per-*O*-acetyl-3,7-anhydro-1-deoxy-1-diazo-2-octuloses. Zemplén deacetylation gave, stereospecifically, the crystalline target-molecules in good overall yield. It is proposed that such *C*-glycosyl compounds as **6a** and **6b**, which possess the diazoacetyl functional group as their "aglycon", will be useful as enzyme-activated irreversible inhibitors (suicide substrates) of glycosidases, and as photoaffinity-labeling reagents and classical affinity-labeling reagents for carbohydrate-binding proteins.

INTRODUCTION

Despite significant advances, there remains a need for efficient and selective reagents capable of inactivating specific target glycosidases[1]. At the same time, there is considerable precedent for the use of substrate-derived diazoketones as affinity-labeling reagents[2]. Consideration of the mechanism by which these compounds function as enzyme-activated irreversible inhibitors[2], and the mechanism of glycohydrolase action[1,3], led us to propose that diazomethyl glycosyl ketones (*e.g.*,

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**Supported by NIH National Research Service Award GM 07231. Current address: Department of Biochemistry, Brandeis University, Waltham, MA, U.S.A.

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1a 6a R' = H (D-galacto) 1a,2a,4a,5a R' = OAc
1b 6b R' = H (D glucro) 1b,2b,4b,5b R' = OAc
3a,6a R' = O- (D galacto)
3b,6b R' = O- (D-gluco)

Scheme 1

compounds **6a** and **6b**, see Scheme 1) might serve as suicide substrates[4] of glycosidases. Thus, active site-mediated protonation of the diazo carbon atom of these substrate analogs by the corresponding glycohydrolases would generate an alkyldiazonium ion; this highly reactive species could then alkylate some active site nucleophile before dissociation occurred, thereby resulting in suicide inactivation of the enzyme. The diazoacetyl functional group can also be activated by photolysis and by certain transition-metal ions; accordingly, diazomethyl glycosyl ketones are of further interest as potential photoaffinity-labeling reagents[5] and classical affinity-labeling reagents[2] for carbohydrate-binding proteins (enzymes, lectins, antibodies, transport proteins, *etc.*).

We now report details of the synthesis of 3,7-anhydro-1-deoxy-1-diazo-D-*glycero*-L-*manno*-2-octulose (diazomethyl β-D-galactopyranosyl ketone, **6a**) and 3,7-anhydro-1-deoxy-1-diazo-D-*glycero*-D-*gulo*-2-octulose (diazomethyl β-D-glucopyranosyl ketone, **6b**). Preliminary accounts of this work have been presented[6]. Investigations by R. W. Myers, R. J. Gilson, M. M. Santoro, and J. N. BeMiller (unpublished results) have demonstrated that **6a** inactivates *Aspergillus oryzae* β-D-galactosidase *in vitro*, in the absence of light or transition-metal ions, in a time-dependent, pseudo-first-order process which exhibits saturation kinetics (*cf.* ref. 4). Similarly, **6b** inactivates sweet almond β-D-glucosidase *in vitro*.

RESULTS AND DISCUSSION

Compounds **6a** and **6b** were stereospecifically synthesized from the readily available *C*-glycosyl compounds 3,4,5,7-tetra-*O*-acetyl-2,6-anhydro-D-*glycero*-L-*manno*-heptononitrile[7] (**1a**) and 3,4,5,7-tetra-*O*-acetyl-2,6-anhydro-D-*glycero*-D-*gulo*-heptononitrile[7] (**1b**), respectively, according to the route depicted in Scheme 1. Conversion of these nitriles into the corresponding carboxylic acids (**4a** and **4b**) was accomplished by two routes. In the first, treatment of **1a** and **1b** with hydrogen bromide (~2.5 equiv.) in glacial acetic acid[8] was used to effect the controlled hydrolysis of the cyano functional group to a carbamoyl group, while leaving the acetoxy groups intact. The products, carboxamides **2a** (from **1a**) and **2b** (from **1b**) were obtained in 65 and 60% yield, respectively. Subsequent deamidation of these compounds with nitrogen dioxide (~3 equiv.) in dichloromethane[8,9] gave carboxylic acids **4a** and **4b** in 87 and 86% yield, respectively.

In the other approach to **4a** and **4b**, nitriles **1a** and **1b** were first converted into sodium carboxylates **3a** and **3b** in 91 and 89% yield, respectively, by deacetylation with methanolic sodium methoxide and subsequent treatment of the products with 1.1 equiv. of aqueous sodium hydroxide (a modification of the method reported by Helferich and Bettin[10] for the preparation of **3a**). Acetylation of **3a** and **3b** with acetic anhydride containing 1.1 equiv. of *p*-toluenesulfonic acid monohydrate[11] afforded carboxylic acids **4a** and **4b** in 93 and 94% yield, respectively. Lehmann and co-workers[12] independently synthesized **4a** from **1a** by a similar route in 55% overall yield.

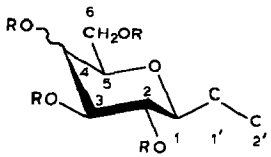

Fig. 1. Numbering system employed for discussion of results, including presentation of n.m.r. data, in order to facilitate comparison with *O*-aldohexopyranosyl compounds.

The overall yields from per-*O*-acetylated glycosyl cyanides **1a** and **1b** to afford key intermediates **4a** and **4b** *via* the first method (*i.e.*, selective hydrolysis–deamidation) were 57 and 52%, respectively, whereas the second method (*i.e.*, deacetylation–alkaline hydrolysis–reacetylation) gave **4a** and **4b** in 85 and 84% yield, respectively. Thus, in terms of overall yield and experimental ease, the latter method is superior for the preparation of these compounds. However, as several per-*O*-acetyl-2,6-anhydroheptonamides are available directly by photoaddition of formamide to 1,5-anhydro-ald-1-hexenitol peracetates[13], the nitrogen dioxide deamidation reaction will be important for the preparation of an extended series of per-*O*-acetyl-2,6-anhydroheptonic acids, a potential recently realized[14].

Acylation of diazomethane to generate the desired diazoacetyl functional group required activation of the carboxyl group of **4a** and **4b**. Concern over the possibility of "anomerization"* of the activated glycosylcarboxylic acids (*i.e.*, epimerization at C-1; see Fig. 1 for the numbering system) prompted us to employ a mild procedure[16,17] for the synthesis of the diazoacetyl functional group. Thus, mixed carboxylic–carbonic acid anhydrides, formed from reaction of **4a** and **4b** with one equivalent each of 4-methylmorpholine and methyl chloroformate in tetrahydrofuran at −20°, were allowed to react, without purification, with an excess of diazomethane (2.2 equiv.) in diethyl ether at −20°, to give diazomethyl ketones **5a** and **5b** in 91 and 83% yield, respectively. These compounds were then uneventfully deacetylated with methanolic sodium methoxide, to give crystalline target-molecules **6a** and **6b** in 88 and 79% yield, respectively.

Preliminary experiments demonstrated the anticipated chemical stability of **6a** and **6b** (ref. 17). Thus, ^{13}C-n.m.r. spectra recorded for 0.25M solutions of **6a** and **6b** in unbuffered D_2O during an 8-h acquisition period showed no evidence of decomposition of the diazoacetyl functional group, even though the protium atom of the methine carbon atom of that group was fully exchanged for a deuterium atom. T.l.c. examination of the samples after ^{13}C-n.m.r. measurement revealed no detectable degradation. Moreover, a solution of **6a** in 0.1M sodium acetate buffer, pH 4.5, showed no change in optical absorbance at 280 nm during 24 h. Further studies demonstrated that **6a** and **6b** are stable during t.l.c. using 8:2:1 (v/v) ethyl acetate–

*Recent studies[15] have revealed that *C*-glycosyl compounds bearing unsaturated, electron-withdrawing functional groups attached directly to C-1 undergo both acid- and base-catalyzed epimerization at C-1 ("anomerization").

acetic acid–water as eluant and during chromatography on a column of silica gel employing 9:4:2 (v/v) ethyl acetate–isopropyl alcohol–water as eluant. Finally, samples of **6a** and **6b** were unchanged after storage for 15 months at room temperature in closed vials, in the absence of light, as determined by t.l.c. and m.p. criteria.

The structural assignments of *C*-glycosyl compounds **1–6** were established by chemical transformation, elemental analysis, vibrational or u.v.-visible spectroscopy, optical rotation correlations, and ^{13}C- and ^{1}H-n.m.r. spectroscopy. As a result of these studies, acetylated products **1a** (ref. 7), **2a, 4a,** and **5a** could be unequivocally assigned as *C*-β-D-galactopyranosyl compounds, and **1b** (ref. 7), **2b, 4b,** and **5b** as *C*-β-D-glucopyranosyl compounds, in the $^{4}C_{1}$(D) conformation.

The stereochemical assignments of deacetylation products **6a** and **3a** as *C*-β-D-galactopyranosyl compounds, and **6b** and **3b** as *C*-β-D-glucopyranosyl compounds, in the $^{4}C_{1}$(D) conformation, are based on the following arguments: (*1*) ^{13}C-n.m.r. data (see Table I) obtained for **6a** and **3a**, and for **6b** and **3b**, in D_2O demonstrated remarkable similarities; it may be noted that the relatively high-field values of δ (^{13}C) of C-1 (79.9–82.5 p.p.m.) confirm that these are *C*-aldohexopyranosyl compounds, whereas the relatively low-field values of δ (^{13}C) of C-3 (74.5–77.9 p.p.m.) and C-5 (79.1–80.4 p.p.m.) provide strong evidence for assigning an equatorial orientation to the C-1 substituent, *i.e.*, for the β-D- configuration[18] (for comparison, see the work of Lehmann and co-workers[12] and Fraser-Reid and co-workers[19]); (*2*) ^{1}H-n.m.r. data (300- and 400-MHz) (Table II) obtained for these compounds (see Experimental section) are consistent with the structures assigned; (*3*) the specific optical rotations of **6a** and **3a**, and of **6b** and **3b**, are quite similar (see Experimental section); the relatively "low" values of $[\alpha]_D^{25}$ may be noted; (*4*) recent studies by Fraser-Reid and co-workers[19], as well as Myers and BeMiller[14], demonstrated a strong thermodynamic preference for equatorially-oriented alkyl substituents at the "anomeric center" of *C*-aldohexopyranosyl compounds possessing acidic hydrogen atoms at either C-1′ (see ref. 19) or C-1 when these compounds are treated with strongly basic reagents (*i.e.*, *C*-β-D-aldohexopyranosyl compounds are the thermodynamic products of base-catalyzed "anomerization" reactions); (*5*) **6a/b** and **3a/b** were obtained in high yields from *C*-β-D-galacto- and -gluco-pyranosyl compounds **5a/b** and **1a/b**, respectively; (*6*) in particular, the conversion of **5a/b** into **6a/b** was rapid, *i.e.*, t.l.c. monitoring revealed that deacetylation was complete within 30 min under the prescribed conditions, and the chromatographic pattern remained unchanged for an additional 24 h; moreover, **6a** and **6b** crystallized within 30 and 60 min, respectively, from the reaction media; (*7*) **3a** and **3b** were converted into well-characterized **4a** and **4b**, respectively, in high yield under relatively mild conditions; and (*8*) the results of biochemical studies employing **6a** and **6b** (see Introduction) are consistent with the structures assigned.

Thus, target-molecules **6a** and **6b** were readily prepared in four steps from nitriles **1a** and **1b** in 68 and 55% overall yield, respectively, and in three steps from carboxamides **2a** and **2b** in 70 and 56% overall yield, respectively. Throughout this

TABLE I

^{13}C-N.M.R. DATA FOR **1–6**[a]

Compound	*Solvent*	*Chemical shift for atom in p.p.m.*[b]									
		C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*C-1'*	*C-2'*	C=*O*[c]	$COCH_3$
6a	Me_2SO-d_6[d]	82.8	68.4	74.2	68.4	79.3	60.6	192.4	54.0[e]	—	—
	D_2O	82.5	69.6	74.5	69.6	79.7	62.0	195.1	—	—	—
3a	D_2O	80.4	69.9	74.7	69.9	79.1	62.2	177.7	—	—	—
1a[f]	$CDCl_3$	66.8	66.0	70.8	66.7	75.4	61.2	114.3	—	168.8, 169.8, 169.9, 170.3	20.4, 20.4, 20.5, 20.6
2a	$CDCl_3$	76.4	67.4	71.5	66.5	74.4	61.7	169.5–170.5[g]	—	169.5, 169.9, 170.1, 170.5	20.7
4a	$CDCl_3$	76.0	67.3	71.5	66.5	74.4	61.7	170.0–170.8[g]	—	170.0, 170.3, 170.6, 170.8	20.6
5a	$CDCl_3$	80.0	67.3	71.5	66.5	74.5	61.6	189.5	54.0	169.8, 170.0, 170.0, 170.4	20.6
6b	Me_2SO-d_6[d,h]	81.9 (0.06)	71.7 (0.13)	77.7 (0.18)	69.6 (0.12)	80.9 (0.06)	61.0 (0.12)	192.1 (0.04)	54.1[e]	—	—
	D_2O	81.8	72.4	77.8	70.0	80.4	61.6	194.8	—	—	—
3b	D_2O	79.9	72.7	77.9	70.3	79.9	61.8	177.5	—	—	—
1b[f]	$CDCl_3$	66.4	68.9	72.8	67.2	76.8	61.3	114.1	—	168.7, 169.1, 170.0, 170.5	20.4, 20.5, 20.5, 20.6
2b	$CDCl_3$	75.7	69.3	73.3	68.1	75.7	62.0	169.5–170.8[g]	—	169.5, 169.7, 169.9, 170.1, 170.8	20.6
4b	$CDCl_3$	75.9	69.3	73.6	68.1	75.9	62.1	169.2–171.0[g]	—	169.2, 169.6, 170.4, 171.0	20.5
5b	$CDCl_3$	79.4	69.3	73.4	68.1	75.8	61.9	189.2	54.0	169.4, 169.6, 170.0, 170.5	20.5

[a]Compounds **1a/b** at 100 MHz; all others at 20 MHz; δ relative to internal standards: in D_2O, 1,4-dioxane (67.4 p.p.m.); in $CDCl_3$ and Me_2SO-d_6, Me_4Si. [b]See Fig. 1 for numbering system. [c]For **1a/b** and **5a/b**, $COCH_3$ resonances; for **2a/b** and **4a/b**, $COCH_3$ and C-1' resonances. [d]Chemical shift of OH form. [e]Signal lost upon addition of D_2O. [f]Assignments for these compounds based on selective ^{1}H-decoupling studies; all other acetylated compounds by analogy. [g]Resonance lies in the range given; exact value uncertain. [h]Numbers in parentheses = upfield displacement of resonance, in p.p.m., on deuterium exchange; these DIS values (see ref. 23) support signal assignments for **6b**; assignments for **6a** and **3a/b** by analogy to **6b** and by comparison to model compounds (see ref. 24).

TABLE II

^{1}H-N.M.R. DATA FOR **1a/b, 2a/b, 4a/b,** AND **5a/b**[a]

	Compound							
	1a[c]	**2a**	**4a**[d]	**5a**	**1b**[c]	**2b**	**4b**	**5b**
Atom[b]	*Chemical shift for atom in p.p.m. (peak multiplicity)*							
H-"aglycon"	—	5.64[e], 6.40[e] (s)	6.60[e] (s)	5.770 (s)	—	5.50[e], 6.38[e] (s)	6.48[e] (s)	5.734 (s)
H-1	4.298 (d)	3.87 (d)	4.05 (d)	3.876 (d)	4.330 (d)	3.90 (d)	4.06 (d)	3.896 (d)
H-2	5.544 (t)	5.39 (t)	5.44 (t)	5.329 (t)	5.318 (t)	4.94	4.97	5.131 (t)
H-3	5.013 (dd)	5.08 (dd)	5.11 (dd)	5.077 (dd)	5.181 (t)	to	to	5.255 (t)
H-4	5.442 (dd)	5.48 (dd)	5.46 (dd)	5.462 (dd)	5.106 (t)	5.33[f] (m)	5.36[f] (m)	5.070 (t)
H-5	3.950 (dt)	3.98	3.96	3.947 (dt)	3.722 (ddd)	3.76 (m)	3.78 (m)	3.721 (ddd)
H-6	4.128[f] (d)	to	to	4.090 (dd)	4.148 (dd)	~4.24[f] (m)	~4.25[f] (m)	4.180 (dd)
H-6′	4.128[f] (d)	4.29[f] (m)	4.24[f] (m)	4.199 (dd)	4.243 (dd)	~4.24[f] (m)	~4.25[f] (m)	4.259 (dd)
$COCH_3$	2.008 (s)	1.99 (s)	2.00 (s)	1.995 (s)	2.028 (s)	2.01 (s)	2.02 (s)	2.018 (s)
	2.071 (s)	2.06 (s)	2.06 (s)	2.068 (s)	2.038 (s)	2.04 (s)	2.04 (s)	2.045 (s)
	2.130 (s)	2.08 (s)	2.06 (s)	2.073 (s)	2.114 (s)	2.07 (s)	2.05 (s)	2.063 (s)
	2.195 (s)	2.16 (s)	2.17 (s)	2.167 (s)	2.114 (s)	2.10 (s)	2.10 (s)	2.111 (s)
Coupling	*Coupling constant (Hz)*							
H-1, H-2	10.1	9.6	9.7	10.0	10.1	9.7	9.7	9.8
H-2, H-3	10.2	9.8	9.8	10.1	~9.4	—	—	~9.5
H-3, H-4	3.3	3.2	3.3	3.4	~9.4	—	—	~9.5
H-4, H-5	1.1	0.9	0.9	1.1	~9.4	—	—	~9.5
H-5, H-6	6.0	—	—	5.8	2.3	—	—	2.3
H-5, H-6′	6.9	—	—	6.9	4.8	—	—	5.0
H-6, H-6′	—	—	—	11.4	12.8	—	—	12.4

[a]Compounds **1a/b** and **5a/b** at 300 MHz, **2a/b** and **4a/b** at 80 MHz; in $CDCl_3$; δ relative to Me_4Si. [b]See Fig. 1 for numbering system. [c]See ref. 7. [d]In agreement with lit. 250 MHz, ^{1}H-n.m.r. (see ref. 12). [e]D_2O-exchangeable. [f]Spectrum of higher order.

study, emphasis was placed on the development of mild reaction conditions to effect the required transformations, in order to avoid epimerization at C-1 ("anomerization"). The resulting methodology should be readily adaptable to the preparation of isotopically labeled **6a** and **6b**. As the synthetic intermediates and the target-molecules were obtained in high yield, and in crystalline form without chromatographic purification, these syntheses have been conducted on a larger scale. Accordingly, considerable quantities of the diazomethyl glycosyl ketones **6a/b** and **5a/b** are now available both for biochemical studies and for further synthetic manipulations leading to other *C*-glycosyl compounds. In this regard, **6a/b** (and **5a/b**) have been converted into the corresponding 3,7-anhydro-1-chloro-1-deoxy-2-octuloses (chloromethyl β-D-galacto- and -gluco-pyranosyl ketones)[6], potential classical affinity-labeling reagents[20] for the corresponding specific carbohydrate-binding proteins.

In summary, reactions described here and elsewhere[6] lead to *C*-glycosyl-based, potential affinity-labeling reagents for carbohydrate-binding proteins. This methodology, coupled with the availability of the per-*O*-acetyl-2,6-anhydro-heptononitrile[7] and -heptonamide[13] derivatives of a number of naturally occurring aldohexoses, should provide access to an extended series of these reagents.

EXPERIMENTAL

Materials. — The following materials were obtained from the sources indicated and were used without further treatment: Diazald, 99%, and tetrahydrofuran, 99.5% (Aldrich Chem. Co.); molecular sieves type 4A (Davison Chemical); hydrogen bromide (Linde Division, Union Carbide); nitrogen dioxide (Matheson Gas Products); 2-methoxyethanol, Sequanal grade (Pierce Chem. Co.). All other compounds used in this study were of reagent grade. *p*-Toluenesulfonic acid monohydrate (J. T. Baker Chem. Co.) was recrystallized, and 4-methylmorpholine, 99%, and methyl chloroformate, 97% (Aldrich Chem. Co.) were redistilled prior to use. Solutions of diazomethane in alcohol-free diethyl ether were prepared from Diazald as described[21], and their concentrations were determined by using $\varepsilon = 7.2\text{M}^{-1}\,\text{cm}^{-1}$ at 410 nm[22].

General methods. — Where indicated, solvents were dried over molecular sieves type 4A. Conventional processing of organic solutions involved drying with anhydrous sodium sulfate, filtering, washing the solid with additional organic solvent, and evaporating the combined filtrates. All evaporations were conducted with a rotary evaporator under diminished pressure at 20–40°. T.l.c. was performed on layers (0.20 mm) of silica gel 60 F_{254} precoated on aluminum sheets (E. Merck), using the following solvent systems: (*A*) 1:1 (v/v) toluene–acetone, (*B*) 19:1 (v/v) chloroform–methanol, (*C*) 3:2:1 (v/v) ethyl acetate–acetic acid–water, (*D*) 5:5:1:3 (v/v) ethyl acetate–pyridine–acetic acid–water, (*E*) 2:1 (v/v) chloroform–methanol, (*F*) 9:4:2 (v/v) ethyl acetate–isopropyl alcohol–water, (*G*) 8:2:1 (v/v) ethyl acetate–acetic acid–water, (*H*) 1:1 (v/v) toluene–ethyl acetate, and (*I*) 100:1 (v/v)

chloroform–methanol. Components on t.l.c. plates were detected by u.v. irradiation and by spraying with 15% (v/v) sulfuric acid in 50% (v/v) aqueous ethanol followed by heating for several minutes at 140°. It was necessary to repeat the spraying with the detection reagent and the heating in order to visibilize some of the *C*-glycosyl compounds.

Elemental analyses were performed by Guelph Chem. Lab., Guelph, Ontario, Canada, or Galbraith Lab., Inc., Knoxville, TN, U.S.A. Instrumental analyses were conducted with the following equipment: Fisher–Johns melting-point apparatus, Perkin–Elmer 141 polarimeter, 599B infrared spectrophotometer, and 576 ultraviolet–visible recording spectrophotometer, Varian CFT-20 nuclear magnetic resonance spectrometer (20 MHz, broad-band-decoupled, for ^{13}C-n.m.r., and 80 MHz for ^{1}H-n.m.r. spectroscopy), Bruker WM-300 wide-bore n.m.r. spectrometer (300 MHz, ^{1}H-n.m.r. spectroscopy), and Varian XL-400 n.m.r. spectrometer (100 MHz for ^{13}C-n.m.r., and 400 MHz for ^{1}H-n.m.r. spectroscopy). Melting points reported are not corrected. ^{1}H-N.m.r. and ^{13}C-n.m.r.* chemical shifts for samples examined in $CDCl_3$ are reported in parts per million from an internal standard of tetramethylsilane; internal standards for samples recorded in other solvents are as indicated. Vicinal coupling constants reported are from a first-order analysis of the observed, spin–spin coupling pattern.

*3,4,5,7-Tetra-*O*-acetyl-2,6-anhydro-*D-glycero-L-manno-*heptonamide* (**2a**, *2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosylformamide*). — A suspension of 3,4,5,7-tetra-*O*-acetyl-2,6-anhydro-D-*glycero*-L-*manno*-heptononitrile[7] (**1a**, 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl cyanide; 7.15 g, 20.0 mmol) in ~20% (w/w) hydrogen bromide–glacial acetic acid (20 mL) was stirred in a closed vessel for 3 h at room temperature. The resulting solution was then poured into stirred ice and water (200 mL), which was immediately extracted with chloroform (2 × 200 mL). The extracts were combined, successively washed with (4°) saturated aqueous sodium hydrogencarbonate solution (2 × 100 mL) and (4°) water (100 mL), and then processed as described under general methods. Crystallization from chloroform (10 mL)–diethyl ether (40 mL) gave **2a** (4.87 g, 13.0 mmol; 65% yield) as heteromorphic crystals, homogeneous by t.l.c. (solvents *A* and *B*). Compound **2a** partially melted at 125–127°, recrystallized while on the hot stage of the apparatus, and fully melted at 138–140°.

Recrystallization of **2a** (0.94 g, 2.50 mmol) from chloroform (1.5 mL)–diethyl ether (6 mL) gave an analytical sample (0.78 g, 2.08 mmol; 83% yield); m.p. 138–140°, $[\alpha]_D^{25}$ +30.9° (*c* 1.03, $CHCl_3$); R_F 0.41 (solvent *A*) and 0.33 (solvent *B*); $\nu_{max}^{CHCl_3}$ 3510, 3400, 1700, and 1570 cm^{-1} ($CONH_2$). Alternatively, **2a** can be recrystallized to high purity, in excellent yield, from 95% ethanol.

Anal. Calc. for $C_{15}H_{21}NO_{10}$ (375.19): C, 47.97; H, 5.64; N, 3.73. Found: C, 47.94; H, 5.55; N, 3.74.

*3,4,5,7-Tetra-*O*-acetyl-2,6-anhydro-*D-glycero-D-gulo-*heptonamide* (**2b**,

*With some exceptions, n.m.r. data are presented in Tables I and II.

2,3,4,6-tetra-O-*acetyl*-β-D-*glucopyranosylformamide*). — Compound **2b** was prepared from 3,4,5,7-tetra-*O*-acetyl-2,6-anhydro-D-*glycero*-D-*gulo*-heptononitrile[7] (**1b**, 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl cyanide; 7.15 g, 20.0 mmol) by the method described for the synthesis of **2a** from **1a**. Crystallization of the resulting product from chloroform (10 mL)–diethyl ether (40 mL) gave **2b** (4.50 g, 12.0 mmol; 60% yield) as heteromorphic crystals, homogeneous by t.l.c. (solvents *A* and *B*). Compound **2b** partially melted at 112–114°, recrystallized while on the hot stage of the apparatus, and fully melted at 146–147°.

Recrystallization of **2b** (0.38 g, 1.01 mmol) from chloroform (1 mL)–diethyl ether (5 mL) gave an analytical sample (0.28 g, 0.75 mmol; 74% yield); m.p. 146–148°, $[\alpha]_D^{25}$ +17.1° (*c* 1.02, $CHCl_3$); R_F 0.40 (solvent *A*) and 0.33 (solvent *B*); $\nu_{max}^{CHCl_3}$ 3505, 3395, 1700, and 1576 cm^{-1} ($CONH_2$). Alternatively, **2b** can be recrystallized to high purity, with excellent recovery, from 95% ethanol; however, **2b** so prepared has m.p. 162–164°.

Anal. Calc. for $C_{15}H_{21}NO_{10}$ (375.19): C, 47.97; H, 5.64; N, 3.73. Found: C, 47.98; H, 5.57; N, 3.75.

Sodium 2,6-anhydro-D-glycero-L-manno-*heptonate* (**3a**, *sodium* β-D-*galactopyranosylformate*). — A suspension of nitrile **1a** (35.7 g, 100 mmol; prepared according to ref. 7) in dry methanol (400 mL) was mixed with 4.0M methanolic sodium methoxide (142 μL) and stirred for 4 h at room temperature in a stoppered, 1-L, round-bottomed flask. The resulting solution was then evaporated to a solvent-free foam which was dissolved in water (380 mL) and mixed with 6.0M aqueous sodium hydroxide (18.2 mL); the solution was gently refluxed for 4 h. At that time, the solution was treated with Norit A (0.58 g), cooled to room temperature, brought to pH 7 with Dowex 50W-X8 (H^+) cation-exchange resin (200–400 mesh), and filtered through Celite, which was then washed extensively with water. Concentration of the combined filtrates to ~50 mL gave crystalline **3a** as the monohydrate (22.6 g, 91.1 mmol; 91% yield), homogeneous by t.l.c. (solvents *C* and *D*). When slowly heated, compound **3a** was stable up to 253°, at which point it began to decompose.

Recrystallization of **3a** (1.60 g, 6.45 mmol) from water (6 mL) gave an analytical sample of **3a** monohydrate (1.17 g, 4.72 mmol; 73% yield); slow decomposition above 253°; $[\alpha]_D^{25}$ +51.8° (*c* 1.03, H_2O) {lit.[10] $[\alpha]_D^{20}$ +51.8° (*c* 5, H_2O)}; R_F 0.26 (solvent *C*) and 0.25 (solvent *D*); ^{1}H-n.m.r. data* (300 MHz; Me_2SO-d_6; internal standard, Me_4Si): δ 3.172 (d, 1 H, $J_{1,2}$ 9.7 Hz, H-1), 3.275 (dd, 1 H, $J_{2,3}$ 9.2, $J_{3,4}$ 3.2 Hz, H-3), 3.354–3.473 (m, 4 H, H-2,5,6,6'), 3.562 [d, ~1 H, $J_{3,4}$ 3.2 Hz ($J_{4,5}$ <1 Hz?), H-4], 3.587–3.652 (m, O*H*-2 tentative), 4.503 (d, 1 H, *J* 4.7 Hz, O*H*-4 tentative), 4.762 (d, 1 H, *J* 5.9 Hz, O*H*-3 tentative), and 5.263 (t, 1 H, *J* ~6 Hz, O*H*-6); ^{1}H-n.m.r. data [300 MHz; D_2O; internal standard, sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS)]: δ 3.645 (d, 1 H, $J_{1,2}$ 9.8 Hz, H-1),

*Data for H-1–H-6,6' from sample previously exchanged with D_2O; data for O*H* (and, where applicable, COC*H*N_2) from nonexchanged sample.

3.667 (dd, 1 H, $J_{2,3}$ 9.2, $J_{3,4}$ 3.2 Hz, H-3), 3.669–3.843 (m, 4 H, H-2,5,6,6'), and 3.948 (bd, 1 H, $J_{3,4}$ 3.2, $J_{4,5}$ ~1.0 Hz, H-4).

Anal. Calc. for $C_7H_{11}NaO_7 \cdot H_2O$ (248.10): C, 33.85; H, 5.28. Found: C, 33.90; H, 5.44.

Sodium 2,6-anhydro-D-glycero-D-gulo-*heptonate* (**3b**, *sodium β*-D-*gluco-pyranosylformate*). — Compound **3b** was prepared from nitrile **1b** (8.93 g, 25.0 mmol; prepared according to ref. 7) by the method described for the synthesis of **3a** from **1a**. Crystallization of the resulting product from water (15 mL)–methanol (90 mL) gave **3b** (5.10 g, 22.2 mmol; 89% yield), homogeneous by t.l.c. (solvents *C* and *D*). When slowly heated, compound **3b** was stable up to 260°, at which point it began to decompose.

Recrystallization of **3b** (0.75 g, 3.26 mmol) from water (3.2 mL)–methanol (20 mL) gave an analytical sample (0.63 g, 2.74 mmol; 84% yield); slow decomposition above 260°; $[\alpha]_D^{25}$ +25.4° (*c* 1.02, H_2O); R_F 0.30 (solvent *C*) and 0.27 (solvent *D*); ^{1}H-n.m.r. data* (300 MHz; Me_2SO-d_6; internal standard, Me_4Si): δ 2.965 (t, 1 H, *J* ~9.0 Hz, identity unknown, H-2, 3, or 4, tentative), 3.052–3.180 (m, 3 H), 3.220 (d, 1 H, $J_{1,2}$ 9.4 Hz, H-1), 3.342–3.405 (m, >2 H; H-6, O*H*-2, and HOD tentative), 3.734 (dd, 1 H, $J_{5,6'}$ 2.3, $J_{6,6'}$ 11.4 Hz, H-6' tentative), 4.984 (d, 1 H, *J* 4.1 Hz, O*H*), 5.032 (d, 1 H, *J* 4.7 Hz, O*H*), and 5.283 (t, 1 H, O*H*-6); ^{1}H-n.m.r. data (300 MHz; D_2O; internal standard, DSS): δ 3.407–3.556 (m, 4 H, H-2,3,4,5), 3.709 (dd, 1 H, $J_{1,2}$ 9.5 Hz, H-1), 3.704–3.756 (m, 1 H, H-5 tentative), and 3.892 (dd, 1 H, $J_{5,6'}$ ~2.0, $J_{6,6'}$ 12.3 Hz, H-6').

Anal. Calc. for $C_7H_{11}NaO_7$ (230.08): C, 36.51; H, 4.81. Found: C, 36.42; H, 5.01.

3,4,5,7-Tetra-O-*acetyl-2,6-anhydro*-D-glycero-L-manno-*heptonic acid* (**4a**, *2,3,4,6-tetra*-O-*acetyl-β*-D-*galactopyranosylformic acid*). — *Method a.* To a stirred solution of carboxamide **2a** (3.75 g, 10.0 mmol) in dry dichloromethane (43 mL) was cautiously added a −20° solution of 4.6M nitrogen dioxide in dichloromethane (6.54 mL). After 3 h at room temperature, the solution was extracted with (4°) saturated aqueous sodium hydrogencarbonate (150 mL) and discarded. The alkaline aqueous extract was acidified to pH 1.2 with 2.0M aqueous sulfuric acid, and extracted with chloroform (3 × 75 mL). The chloroform extracts were combined, and then processed as described under general methods. Crystallization from toluene (40 mL) gave **4a** (3.53 g, 9.38 mmol; 94% yield), m.p. 133–138°. Recrystallization by dissolution in chloroform, evaporation, and addition of toluene (40 mL) gave **4a** (3.26 g, 8.66 mmol; 87% yield); m.p. 136–139°; homogeneous by t.l.c. (solvents *A*, *E*, *F*, and *G*).

Recrystallization of **4a** (0.94 g, 2.50 mmol) by dissolution in chloroform, evaporation, and addition of diethyl ether (5 mL) gave an analytical sample (0.55 g, 1.46 mmol; 58% yield); m.p. 136–138° (lit.[12] m.p. 136°), $[\alpha]_D^{25}$ +16.6° (*c* 0.99, $CHCl_3$) {lit.[12] $[\alpha]_{578}^{22}$ +17.9° (*c* 1.0, $CHCl_3$)}; R_F 0.28 (solvent *E*), 0.33 (solvent *F*), and 0.60 (solvent *G*); $\nu_{max}^{CHCl_3}$ 3460, 3320–2840, 1750, 1370, and 1240 cm^{-1} (COOH).

*See footnote on p. 152.

Anal. Calc. for $C_{15}H_{20}O_{11}$ (376.18): C, 47.85; H, 5.35. Found: C, 47.83; H, 5.24.

Method b. Sodium carboxylate **3a** (2.48 g, 10.0 mmol) was added portionwise to a stirred solution of *p*-toluenesulfonic acid monohydrate (2.09 g, 11 mmol) in acetic anhydride (9.0 mL, 95.4 mmol) during 45 min at 0°. After an additional 45 min at 0°, the mixture was brought to room temperature and stirred for 24 h, with protection from atmospheric moisture. At that time, the mixture was cautiously added to a vigorously stirred, saturated aqueous sodium hydrogencarbonate solution (200 mL) at 4° and the resulting solution was stirred for several minutes to hydrolyze the excess of acetic anhydride. The alkaline aqueous solution was then washed with chloroform (50 mL), the chloroform wash discarded, and the aqueous solution acidified to pH 1.2 with 2.0M aqueous sulfuric acid, and then extracted with chloroform (3 × 75 mL). The extracts were combined, and processed as described under general methods. Crystallization from toluene (40 mL) gave **4a** (3.50 g, 9.30 mmol; 93% yield); m.p. 136–139°; homogeneous by t.l.c. (solvents *A*, *E*, *F*, and *G*).

Recrystallization of **4a** (0.94 g, 2.50 mmol) by dissolution in chloroform, evaporation, and addition of diethyl ether (5 mL) gave an analytical sample of the compound (0.65 g, 1.73 mmol; 69% yield); m.p. 136–138° (alone or in admixture with **4a** prepared by Method *a*). Samples of **4a** prepared by methods *a* and *b* were also identical by t.l.c. (solvents *A*, *E*, *F*, and *G*) and 80-MHz, ^{1}H-n.m.r.-spectral criteria. Subsequent experiments have shown that **4a** can be recrystallized to a comparable level of purity, but in higher yield, by dissolution in chloroform, evaporation, and subsequent addition of toluene, and from acetone–water, which affords **4a** as a monohydrate.

Anal. Calc. for $C_{15}H_{20}O_{11}$ (376.18): C, 47.85; H, 5.35. Found: C, 47.84; H, 5.23.

*3,4,5,7-Tetra-*O*-acetyl-2,6-anhydro-*D-glycero-D-gulo-*heptonic acid* (**4b**, *2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosylformic acid*). — *Method a.* Compound **4b** was prepared from carboxamide **2b** (3.75 g, 10.0 mmol) by the method described for the synthesis of **4a** from **2a** (method *a*). Crystallization of the resulting product from diethyl ether (25 mL) gave **4b** (3.88 g, 8.62 mmol; 86% yield) containing one mol.-equiv. of diethyl ether of crystallization. Solvent of crystallization was removed by heating under vacuum for 24 h at 60°, to give **4b** (3.24 g, 8.61 mmol; 86% yield); m.p. 138–140°; homogeneous by t.l.c. (solvents *E*, *F*, and *G*).

Recrystallization of **4b** (0.75 g, 2.00 mmol) by dissolution in dichloromethane, evaporation, and addition of diethyl ether (4 mL) gave an analytical sample (0.51 g, 1.36 mmol; 68% yield); m.p. 140–142°, $[\alpha]_D^{25}$ +1.7° (*c* 1.04, $CHCl_3$); R_F 0.28 (solvent *E*), 0.32 (solvent *F*), and 0.58 (solvent *G*); $\nu_{max}^{CHCl_3}$ 3475, 3320–2840, 1752, 1370, and 1235 cm^{-1} (COOH). Subsequent experiments have shown that **4b** can be crystallized and recrystallized to a comparable level of purity, with higher yield, by dissolution in chloroform, evaporation, and subsequent addition of toluene, and from acetone–water, which provides **4b** as a monohydrate.

Anal. Calc. for $C_{15}H_{20}O_{11}$ (376.18): C, 47.85; H, 5.35. Found: C, 47.89; H, 5.25.

Method b. Compound **4b** was prepared from sodium carboxylate **3b** (2.30 g, 10.0 mmol) by the method described for the synthesis of **4a** from **3a** (method *b*). Crystallization of the resulting product from toluene (40 mL) gave **4b** (3.53 g, 9.38 mmol; 94% yield); m.p. 140–142°; homogeneous by t.l.c. (solvents *A*, *E*, *F*, and *G*).

Recrystallization of **4b** (1.01 g, 2.69 mmol) by dissolution in chloroform, evaporation, and addition of toluene (9 mL) gave the compound (0.96 g, 2.57 mmol; 95% yield); m.p. 142–144° (alone or in admixture with **4b** prepared by method *a*). Samples of **4b** prepared by methods *a* and *b* were also identical by t.l.c. (solvents *A*, *E*, *F*, and *G*) and 80-MHz, ^{1}H-n.m.r.-spectral criteria.

*4,5,6,8-Tetra-*O*-acetyl-3,7-anhydro-1-deoxy-1-diazo-*D-glycero-L-manno-*2-octulose* (**5a**, *diazomethyl 2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl ketone*). — To a stirred solution of carboxylic acid **4a** (3.76 g, 10.0 mmol) and 4-methylmorpholine (1.1 mL, 10.0 mmol) in dry tetrahydrofuran (50 mL), cooled to −20°, was added methyl chloroformate (0.77 mL, 10.0 mmol). After 15 min, the mixture was filtered, and the solid was washed with dry tetrahydrofuran (100 mL) cooled to −20°. To the combined filtrates at −20° was added, all at once, with stirring, a freshly prepared solution of 0.38M diazomethane in diethyl ether (57.1 mL, 22 mmol of CH_2N_2) cooled to −20°. After 30 min, the temperature was raised to 4°, and the mixture stirred for an additional 3 h. At that time, the solution was evaporated to a syrup, which was dissolved in chloroform (100 mL). The solution was extracted with water (3 × 20 mL) and then processed as described under general methods. Crystallization of the resulting syrup from diethyl ether (20 mL) gave **5a** (3.66 g, 9.15 mmol; 91% yield) as yellow crystals, m.p. 138–140°, of high purity by t.l.c. (solvent *H*; **5a** is readily detected by u.v. irradiation).

Recrystallization of **5a** (0.80 g, 2.00 mmol) from tetrahydrofuran (4.0 mL) gave an analytical sample (0.46 g, 1.15 mmol; 57% yield); m.p. 140–142°, $[\alpha]_D^{25}$ +22.9° (*c* 1.08, $CHCl_3$); R_F 0.40 (solvent *H*) and 0.26 (solvent *I*); $\nu_{max}^{CHCl_3}$ 2118 and 1642 cm^{-1} ($COCHN_2$). Alternatively, **5a** can be recrystallized from ethanol or chloroform–diethyl ether.

Anal. Calc. for $C_{16}H_{20}N_2O_{10}$ (400.20): C, 47.98; H, 5.03; N, 7.00. Found: C, 48.15; H, 5.12; N, 6.93.

*4,5,6,8-Tetra-*O*-acetyl-3,7-anhydro-1-deoxy-1-diazo-*D-glycero-D-gulo-*2-octulose* (**5b**, *diazomethyl 2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl ketone*). — Compound **5b** was prepared from carboxylic acid **4b** (1.88 g, 5.00 mmol) by the method described for the synthesis of **5a** from **4a**. Crystallization of the syrupy product from diethyl ether (10 mL) gave **5b** (1.66 g, 4.15 mmol; 83% yield) as yellow crystals, m.p. 120–122°, of high purity by t.l.c. (solvent *H*; **5b** is readily detected by u.v. irradiation).

Recrystallization of **5b** (0.80 g, 2.00 mmol) from tetrahydrofuran (4 mL) gave an analytical sample (0.48 g, 1.20 mmol; 60% yield); m.p. 121–123°, $[\alpha]_D^{25}$ +0.3° (*c* 1.05, $CHCl_3$) and $[\alpha]_{436}^{25}$ −55.7° (*c* 1.05, $CHCl_3$); R_F 0.39 (solvent *H*) and 0.26

(solvent *I*); $\nu_{max}^{CHCl_3}$ 2118 and 1642 cm^{-1} ($COCHN_2$). Alternatively, **5b** can be recrystallized from ethanol or chloroform–diethyl ether.

Anal. Calc. for $C_{16}H_{20}N_2O_{10}$ (400.20): C, 47.98; H, 5.03; N, 7.00. Found: C, 47.74; H, 5.09; N, 6.86.

3,7-Anhydro-1-deoxy-1-diazo-D-glycero-L-manno-*2-octulose* (**6a**, *diazomethyl β-*D-*galactopyranosyl ketone*). — A suspension of diazomethyl ketone **5a** (1.00 g, 2.50 mmol) in dry methanol (10 mL) was mixed with 4.0M methanolic sodium methoxide (40 μL), and then stirred at room temperature in the dark; within 15 min, a solution was obtained, and after 30 min, crystalline **6a** formed. After 24 h, the suspension was filtered, and the solid was washed with dry methanol, to give **6a** (0.51 g, 2.20 mmol; 88% yield) as yellow crystals, m.p. 158–160° (with evolution of nitrogen), homogeneous by t.l.c. (solvents *F* and *G*; **6a** is readily detected by u.v. irradiation).

Recrystallization of **6a** (0.47 g, 2.00 mmol) from methanol (30 mL) gave an analytical sample (0.34 g, 1.46 mmol; 72% yield); m.p. 158–160° (with evolution of nitrogen), $[\alpha]_D^{25}$ +52.6° (*c* 1.01, H_2O); R_F 0.31 (solvent *F*) and 0.21 (solvent *G*); λ_{H_2O} at 255 nm (ε 7000), 281 (ε 12,635), and 350 (ε 43$M^{-1}.cm^{-1}$); ^{1}H-n.m.r. data* (400 MHz; Me_2SO-d_6; internal standard, Me_2SO center, 2.505 p.p.m.): δ 3.290 (dd, 1 H, $J_{2,3}$ 8.9, $J_{3,4}$ 3.2 Hz, H-3), 3.378 (t, 1 H, $J_{5,6} = J_{5,6'} = \sim$6.1 Hz, H-5 tentative), 3.466–3.570 (m, 4 H, H-1,2,6,6′) [3.492 (d, ~1 H, $J_{1,2}$ 8.6 Hz, H-1 tentative) and 3.549 (t, ~1 H, $J_{1,2} = J_{2,3} = \sim$8.6 Hz, H-2 tentative)], 3.664 [d, 1 H, $J_{3,4}$ 3.1 Hz ($J_{4,5}$ <1 Hz?), H-4], 4.440 (d, 1 H, *J* 4.6 Hz, O*H*-4), 4.602 (t, 1 H, *J* 5.5 Hz, O*H*-6), 4.812 (d, 1 H, *J* 5.8 Hz, O*H*-3 tentative), 4.898 (d, 1 H, *J* 5.2 Hz, O*H*-2 tentative), and 6.125 (s, 1 H, D_2O-exchangeable, $COCHN_2$); ^{1}H-n.m.r. data (400 MHz; D_2O; internal standard, HOD, 4.800 p.p.m.): δ 3.665 (dd, 1 H, $J_{2,3}$ 9.1, $J_{3,4}$ 3.3 Hz, H-3), 3.687–3.811 (m, 5 H, H-1,2,5,6,6′), 3.970 [d, 1 H, $J_{3,4}$ 3.3 Hz ($J_{4,5}$ <1 Hz?), H-4], and 6.141 (s, <0.1 H, $COCHN_2$).

Anal. Calc. for $C_8H_{12}N_2O_6$ (232.12): C, 41.36; H, 5.21; N, 12.07. Found: C, 41.57; H, 5.41; N, 11.97.

3,7-Anhydro-1-deoxy-1-diazo-D-glycero-D-gulo-*2-octulose* (**6b**, *diazomethyl β-*D-*glucopyranosyl ketone*). — A suspension of diazomethyl ketone **5b** (1.00 g, 2.50 mmol) in dry methanol (5 mL) was mixed with 4.0M methanolic sodium methoxide (40 μL), and then stirred at room temperature in the dark; within 5 min, a solution was obtained, and by 60 min, crystalline **6b** formed. After 3 h, the solution was cooled to −20°. After an additional 21 h at −20°, the mixture was filtered, and the solid was washed with a small volume of (−20°) dry methanol, to give **6b** (0.39 g, 1.68 mmol; 67% yield) as yellow crystals, m.p. 146–148° (with evolution of nitrogen), homogeneous by t.l.c. (solvent *F*; **6b** is readily detected by u.v. irradiation). The mother liquor was evaporated, the residue treated with boiling dry ethanol (6 mL), the suspension filtered, and the filtrate slowly cooled to −20°, to give, following addition of a seed crystal, a second crop of **6b** (0.068 g, 0.29 mmol;

*See footnote on p. 152

12% yield), m.p. 145–148° (with evolution of nitrogen), of high purity by t.l.c. (solvent *F*). The total yield of **6b** was 79%.

Recrystallization of **6b** (0.205 g, 0.88 mmol) from ethanol (6.6 mL) gave an analytical sample (0.135 g, 0.58 mmol; 66% yield); m.p. 146–148° (with evolution of nitrogen), $[\alpha]_D^{25}$ −9.3° (*c* 1.01, H_2O); R_F 0.39 (solvent *F*) and 0.26 (solvent *G*); λ_{H_2O} at 255 nm (ε 8555), 281 (ε 14,725), and 350 (ε 42 M^{-1} cm^{-1}); ^{1}H-n.m.r.* (400 MHz; Me_2SO-d_6; internal standard, Me_2SO center, 2.503 p.p.m.): δ 3.044 (t, 1 H, *J* 8.9 Hz, identity unknown, H-2, 3, or 4 tentative), 3.112–3.238 (m, 3 H), 3.402 (dd, 1 H, $J_{5,6}$ 6.0, $J_{6,6'}$ 12.1 Hz, H-6), 3.559 (d, 1 H, $J_{1,2}$ 8.9 Hz, H-1), 3.660 (dd, 1 H, $J_{5,6'}$ 1.5 Hz, H-6'), 4.520 (t, 1 H, *J* 5.8 Hz, O*H*-6), 4.982 (d, 1 H, *J* 5.2 Hz, O*H*), 5.043 (d, 1 H, *J* 4.9 Hz, O*H*), 5.056 (d, 1 H, *J* 6.1 Hz, O*H*), and 6.216 (s, 1 H, D_2O-exchangeable, COC*H*N_2); ^{1}H-n.m.r. data (400 MHz; D_2O; internal standard, HOD, 4.800 p.p.m.): δ 3.423–3.524 (m, 4 H), 3.730 (dd, 1 H, $J_{5,6}$ 4.9, $J_{6,6'}$ 12.5 Hz, H-6), 3.867–3.901 (m, 2 H), [3.878 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1 tentative) and 3.884 (bd, 1 H, $J_{5,6'}$ <2.0 Hz?, H-6' tentative)], and 6.106 (s, ≪1 H, COC*H*N_2).

Anal. Calc. for $C_8H_{12}N_2O_6$ (232.12): C, 41.36; H, 5.21; N, 12.07. Found: C, 41.51; H, 5.24; N, 12.01.

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