Sequential Removal of Monosaccharides from the Reducing End of Oligosaccharides. 2. Fundamental Studies of a Reaction between Hydrazino Compounds and Sugars Having a Glycosyl Moiety on a Carbon Atom Adjacent to a Carbonyl Group

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Hydrazine and certain hydrazino derivatives react with sugars having a glycosidic substituent, where the glycosyl moiety is located on a carbon atom adjacent to an aldehyde or keto group of the aglycon, resulting in cleavage of the glycosidic linkage. The reaction proceeds whether the glycon is of the α or β configuration. The released glycosyl moiety, in the presence of excess hydrazino compound, reacts further to give a hydrazone derivative. Removal of the hydrazone group gives the reducing sugar derived from the glycon.

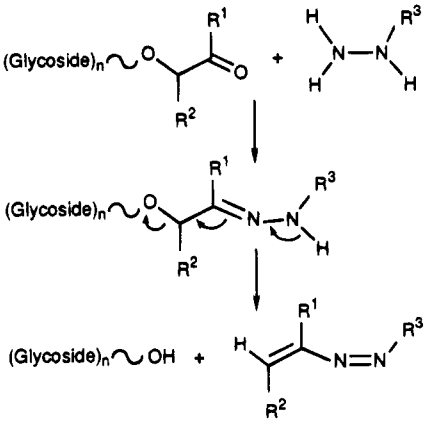
Introduction

Removal of monosaccharides from the nonreducing ends of oligosaccharides by glycosidases has been used for many years to prepare smaller structures. While such structures are often intended for preparative use, elucidation of an unknown requires permethylation analysis[1] before and after removal of a monomer to establish the particular hydroxyl group to which the released monomer was formerly linked. The process is subtractive; i.e., it requires analysis of all the methylated derivatives of the entire oligosaccharide, as any specific hydroxyl group could potentially be involved in a glycosidic linkage with the released monomer. Although the procedure can be performed repetitively, with subtractive analysis at each round, difficulties can ensue (for example, in an oligosaccharide comprised of just one monomer).

In the development of a methodology for the controlled sequential removal of monosaccharides from the reducing end of oligosaccharides, we reported a preliminary account of a reaction series which should be applicable to a variety of structures.[2] Such a procedure could be useful, both in preparative generation of oligosaccharides derived from the nonreducing portion(s) of larger molecules, and in structural elucidation. As a reducing monosaccharide can be selectively derivatized, it may be selectively identified regardless of other monomers present in the molecule. More importantly, linkage analysis may be restricted solely to a derivatized monomer present at the reducing end of an oligosaccharide, a task made simpler if that monosaccharide can first be identified unambiguously.

Any chemical procedure purporting to sequentially remove monosaccharides from the reducing end of oligosaccharides should give credence to the following through model studies. First, the method should remove the reducing monosaccharide in such a way that the next reducing monomer is recovered intact, without modifications such as epimerization at C-2 or enolization. For this reason, aqueous base treatments are not generally useful due to the well-known Lobry de Bruyn–Alberda van Ekenstein transformation.[3] Second, the procedure should be amenable to removal of a reducing sugar whether it is substituted at any particular hydroxyl group. Third, one round of the procedure should remove a single monomer, without continued degradation down the oligosaccharide chain, a consideration which further disfavors aqueous base treatment.[3] Fourth, the chemistry should be applicable to branch points. Obviously, reactions should be optimized to give high repetitive yields.

Scheme 1



Our procedure, in essence, involves two steps. First, an aldehyde or keto group is regioselectively generated next to the carbon atom involved in the glycosidic linkage at the reducing end of an oligosaccharide. Second, cleavage of the glycosidic bond is performed with a hydrazino compound. Herein we report fundamental studies of a reaction of hydrazine and some hydrazine derivatives with glycosides where the glycosyl moiety is attached to a carbon atom adjacent to an aldehyde or keto group in the aglycon (Scheme 1). Mechanistically, the reaction appears similar to reports by Wolfrom and colleagues[4] who demonstrated the elimination of a 2-*O*-acetyl group from acyclic, peracetylated phenylhydra-

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(1) For a review, see Lindberg, B.; Lönngren, J. *Methods Enzymol.* **1978**, *50*, 3.

(2) Bendiak, B.; Salyan, M. E.; Pantoja, M. *Tetrahedron Lett.* **1994**, *35*, 68, and references therein.

(3) Lobry de Bruyn, M. M. C. A.; Alberda van Ekenstein, W. *Recueil Travaux Chim. Pay-Bas* **1895**, *14*, 203. Aqueous base treatment of mono- and polysaccharides has been reviewed by Whistler, R. L., BeMiller, J. N. *Adv. Carbohydr. Chem.* **1958**, *13*, 289.

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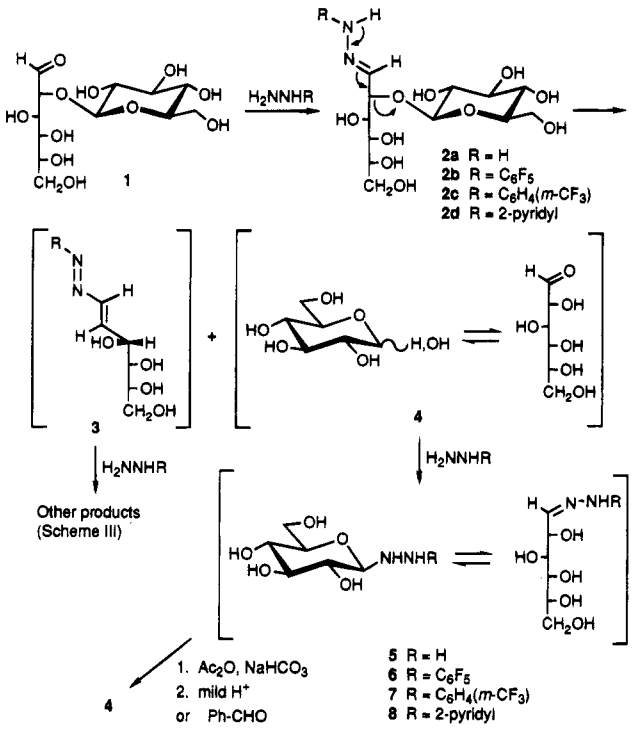
Scheme 2



zones of sugars, and Wharton et al.[5] who showed that hydrazine reacts with α,β-epoxy ketones to give allylic alcohols. Further studies of the removal of the hydrazone group and the highly regioselective preference of lead tetraacetate in the cold for oxidation of acyclic alditols as compared to cyclic glycopyranosides will be forthcoming in separate articles.

Results and Discussion

Studies with Hydrazine: Variation of the Nature and Anomericity of the Glycosyl Moiety and R¹ and R² (Scheme 1). The reaction between anhydrous hydrazine (N_2H_4) and 2-*O*-β-D-glucopyranosyl-D-glucose (**1**) at 55 °C gave rise to initial products as their hydrazones, which were chromatographically difficult to separate (Schemes 2 and 3). A great excess of N_2H_4 was used to prevent azine formation. Hydrazones were deprotected by N-acetylation/mild acid treatment[6] to give the respective carbonyl products which were separable. In addition to D-glucose **4**, derived from the glycon, the structures of four products were established which were derived from the reducing sugar, which suggests that the reaction proceeds through the hydrazone **2a** to give, at least in part, a reactive azoethylene intermediate **3**. The products 2,5-anhydro-D-mannose (**18**), 2,5-anhydro-D-glucose (**19**), and 2-(2-acetylhydrazino)-2-deoxy-D-mannose (**20**), generated via their respective hydrazones **9**, **13**, and **16** (Scheme 3), indicate intramolecular ring closure and N_2H_4 attack, respectively, on C-2, an electron-deficient site in **3**. A minor product, 2-deoxy-D-*arabino*-hexose **21** was also found in low yield, which arose from hydrazino compound **17**, presumably by reduction of the azoethylene **3**. The product **18** was proven by chemical synthesis[7] and by reduction with borohydride to the commercially available 2,5-anhydro-D-mannitol **22**. The 2-epimer of **18**, 2,5-anhydro-D-glucose (**19**), has had a long and elusive history,[8] and we thought it necessary, in order to make a claim that the azoethylene **3** may undergo ring closure at C-2 on either side of the double bond, to formally establish its structure. The compound was first reduced to 2,5-anhydro-D-glucitol (**23**). This molecule gave the same product, di-2-glyceryl ether **24** after periodate/borohydride treatment as derived from **22**, the reaction series having been used originally[7] to prove that **22** was, in fact, a 2,5-anhydro compound as opposed to possible 2,4- or 2,6-anhydro products. The product **20** was established based on its HRMS and ^{1}H-NMR decoupling pattern, giving two anomers and coupling constants indicative of a mannopyranose configuration.

Temperature dependence of the reaction between **1** and N_2H_4 was performed from 23−70 °C for 24 h. At 23 °C, only the starting disaccharide was recovered (after deprotection of the hydrazone). Increasing **4**, and **18**−**20** were observed, in variable yields, up to 70 °C; 55 °C was sufficient for complete cleavage of the disaccharide (Table 1). Product **21** was evidently in too low a concentration to be detected at lower temperatures, but was observed in low yields at temperatures of 55 °C and above. No D-mannose, the 2-epimer of **4**, was observable among the products. In control experiments where D-glucose was treated with N_2H_4, no noticable epimerization, enolization, or bis(hydrazone) formation occured up to 65 °C.

It should be noted that, with N_2H_4, the isolated products derived from the reducing sugar of the disaccharide did not add, in total yield, to the isolated monosaccharide derived from the glycon. It is not known whether this was due to isolation losses, or a result of other minor products derived from the reducing sugar (which were observed, but not fully characterized).

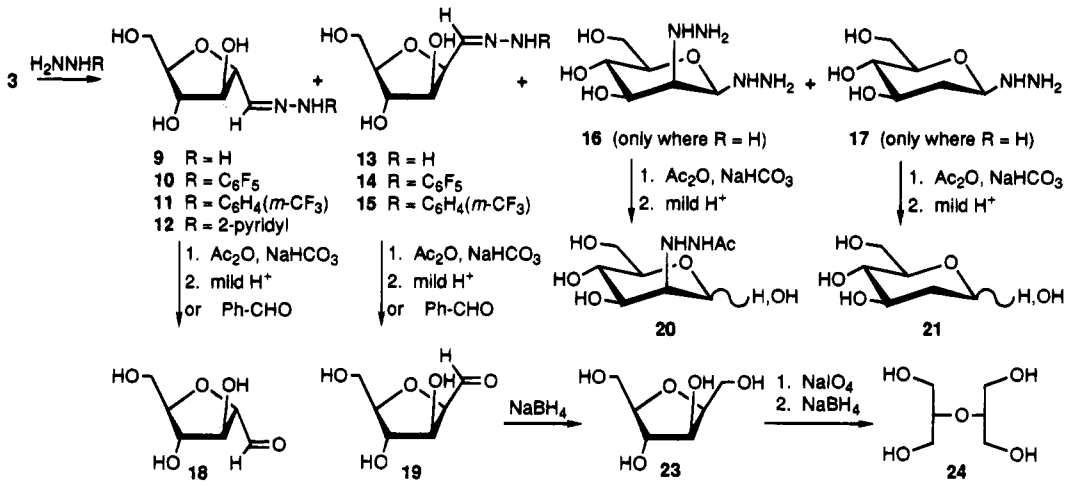
Treatment of 2-*O*-α-D-glucopyranosyl-D-glucose (**33**) with N_2H_4 at 50 °C for 24 h gave the same products, **4**, **18**, and **20** being the major components in addition to small amounts of starting compound and **19** after deprotection of the hydrazone. Product **21** was not present in detectable quantity. At 60−70 °C, **33** was absent from the products (Table 1). No D-mannose was found, nor was evidence for bis(hydrazone) formation or enolization of the released D-glucose.

Similarly, 2-*O*-α-D-mannopyranosyl-D-mannose (**34**), upon N_2H_4 treatment gave D-mannose (**35**) (Table 1) upon deprotection of the hydrazone. D-Glucose was not found among the products, but **18** and **20** were isolated. No effort was made to attempt to isolate or analyze for the presence of **19**, due to the relatively small amount of starting material, but **19**, if present, was not an abundant product. The presence of the same products (**18** and **20**)

(4) (a) Wolfrom, M. L.; Fraenkel, G.; Lineback, D. R.; Komitsky, F., Jr. *J. Org. Chem.* **1964**, *29*, 457. (b) El Khadem, H.; Wolfrom, M. L.; El Shafei, Z. M.; El Ashry, S. H. *Carbohydr. Res.* **1967**, *4*, 225.
(5) Wharton, P. S.; Bohlen, D. H. *J. Org. Chem.* **1961**, *26*, 3615.
(6) Bendiak, B.; Cumming, D. A. *Carbohydr. Res.* **1985**, *144*, 1.
(7) Bera, B. C.; Foster, A. B.; Stacey, M. *J. Chem. Soc.* **1956**, 4531.

(8) Claims for 2,5-anhydro-D-glucose (**19**) initiated with a report for "epichitose" upon treatment of D-mannosamine hydrochloride with HgO (Levene, P. A. *J. Biol. Chem.* **1919**, *39*, 69). We repeated this procedure, but the crystalline product obtained was not **19** and it was neither reducible with $NaBH_4$ nor reactive with N_2H_4 at 55 °C for 8 h. Also, the mother liquor did not contain any **19** as examined by ^{1}H-NMR. In a later claim to its synthesis (Defaye, J. *Bull. Soc. Chim. Fr.* **1967**, 1101), NMR of the compound was not performed and the structure of the ring form (i.e., 2,4, 2,5, or 2,6 anhydro) was not unambiguously demonstrated. A similar reaction performed with the same compounds (Horton, D.; Magbanua, L. G.; Tronchet, J. M. J. *Chem. Ind.* **1966**, 1718) gave a 2-*S*-ethyl-2-thioaldose as the major product. Later, treatment of 2-amino-2-deoxy-D-mannose with nitrous acid gave D-glucose as the major product (Horton, D.; Philips, K. D.; Defaye, J. *Carbohydr. Res.* **1972**, *21*, 417), although the same treatment of 2-amino-2-deoxy-D-glucose gives 2,5-anhydro-D-mannose.[7]

Scheme 3



derived from the reducing mannose as were derived from the reducing glucose upon treatment of **1** and **34** strongly suggests the common azoethylene derivative **3** is an intermediate in the reaction, which evidently reacts further to give, after deprotection of the hydrazone, **18**, and **20**.

The reaction between N_2H_4 and 3-*O*-α-D-glucopyranosyl-D-fructose (**25**) was also examined (Scheme 4). Two major products were isolated after removal of the hydrazone; chromatography gave D-glucose and another component which was chromatographically unusual in that it was interconvertable among at least five forms, which gave separable HPLC peaks, but gave identical ^{1}H-NMR spectra. Each peak, upon rechromatography, gave the same equilibrium mixture of additional peaks. The compound was identified as 3-deoxy-D-*erythro*-2-hexosulose (**29**). ^{1}H-NMR of **29** indicated at least six interconvertible species, as evidenced by decoupling the pairs of gem-coupled H-3 protons for individual forms. MS (both ESMS and FABMS) data was inconclusive, apparently due to hydrate and/or hemiacetal formation with alcoholic solvent at one or both of the carbonyl groups. Hence, **29** was synthesized by a published method,[9] which gave the same (complex) ^{1}H-NMR spectrum. To obain MS data, **29** was converted to the bis(benzoylhydrazone) **30**,[9] which gave good HRMS data, but ^{1}H-NMR spectra showed broad signals, which were attributed to molecular interconversions on the same time scale as single NMR accumulations. Therefore, for formal proof of structure, **29** was converted, via the bis(phenylhydrazone) **31** to 3-deoxy-D-*erythro*-hexose phenylosotriazole **32**, which yielded straightforward ^{1}H-NMR and HRMS data. Bis(phenylhydrazones) are known to mutarotate, and **31** also failed to give unambiguous ^{1}H-NMR data.[10] The product **29** was derived from the reducing fructose and is postulated to result from tautomerization of the initial azoethylene product (**27a** through **27c**), resulting in the bis(hydrazone) **28** as the abundant stable product. Compound **29** was obtained in 71% yield.

Treatment of the additional compounds **38**, **44**, **49**, **54**, **55**, and **62** with N_2H_4 also gave high yields of the reducing saccharides derived from the glycon of the glycoside, which were characterized (Table 1). However, the molecules arising from the aglycon, in these cases, were not characterized in detail. No evidence was found for epimerization, enolization, or bis(hydrazone) formation of the reducing sugars derived from the glycon of these compounds.

Variation of the R^3 Group (Scheme 1). Using the model disaccharide **1**, a number of R^3 groups were examined under various conditions to assess their utility in the overall reaction series to remove a monosaccharide from the reducing end of an oligosaccharide. Although the results were largely disappointing and the vast majority of hydrazino derivatives proved unsatisfactory for high yields in the reaction series, some salient observations are worthy of note.

Alkylhydrazines, such as methylhydrazine, ethylhydrazine, propylhydrazine and *tert*-butylhydrazine did react according to Scheme 1, but were unsatisfactory overall because a complex series of side products arose under conditions required for the reaction to go to completion. Similarly, acylhydrazides and aroylhydrazides, such as acetohydrazide or benzoylhydrazide, sulfonylhydrazides, such as (*p*-tolylsulfonyl)hydrazine, and semicarbazide, thiosemicarbazide, and carbohydrazide, were either insufficiently reactive or only reacted at temperatures high enough to result in significant side products. A large number of arylhydrazines were tested; these readily underwent the reaction in Scheme 1, at relatively low temperatures, but the released glycosyl group reacted more rapidly with additional arylhydrazine to give the well-studied bis(arylhydrazone) derivatives.[11] Therefore, most arylhydrazines are not satisfactory in the overall reaction series, as the chirality at C-2 of the released glycosyl moiety is lost.

However, among the arylhydrazines tested were three which did not give bis(arylhydrazone) formation, epimerization, or enolization under conditions in which the reaction in Scheme 1 occurred and did not give bis(arylhydrazones) with D-glucose in separate experiments. These were (pentafluorophenyl)hydrazine, [3-(trifluoro-

(9) El Khadem, H.; Horton, D.; Meshreki, M. H.; Nashed, M. A. *Carbohydr. Res.* **1971**, *17*, 183. *Carbohydr. Res.* **1972**, *22*, 381.

(10) Positive identifications of bis(phenylhydrazones) of sugars is restricted because they melt with decomposition and mutarotate; conversion to the phenylosotriazoles gives sharp melting points and no mutarotation (Richtmyer, N. K., *Methods Carbohydr. Chem.* **1963**, *2*, 127 and 132). We observed that ^{1}H-NMR spectra of bis(phenylhydrazones) can be complex due to more than one acyclic and/or cyclic forms, which vary in abundance depending upon equilibration time before and temperature during acquisition.

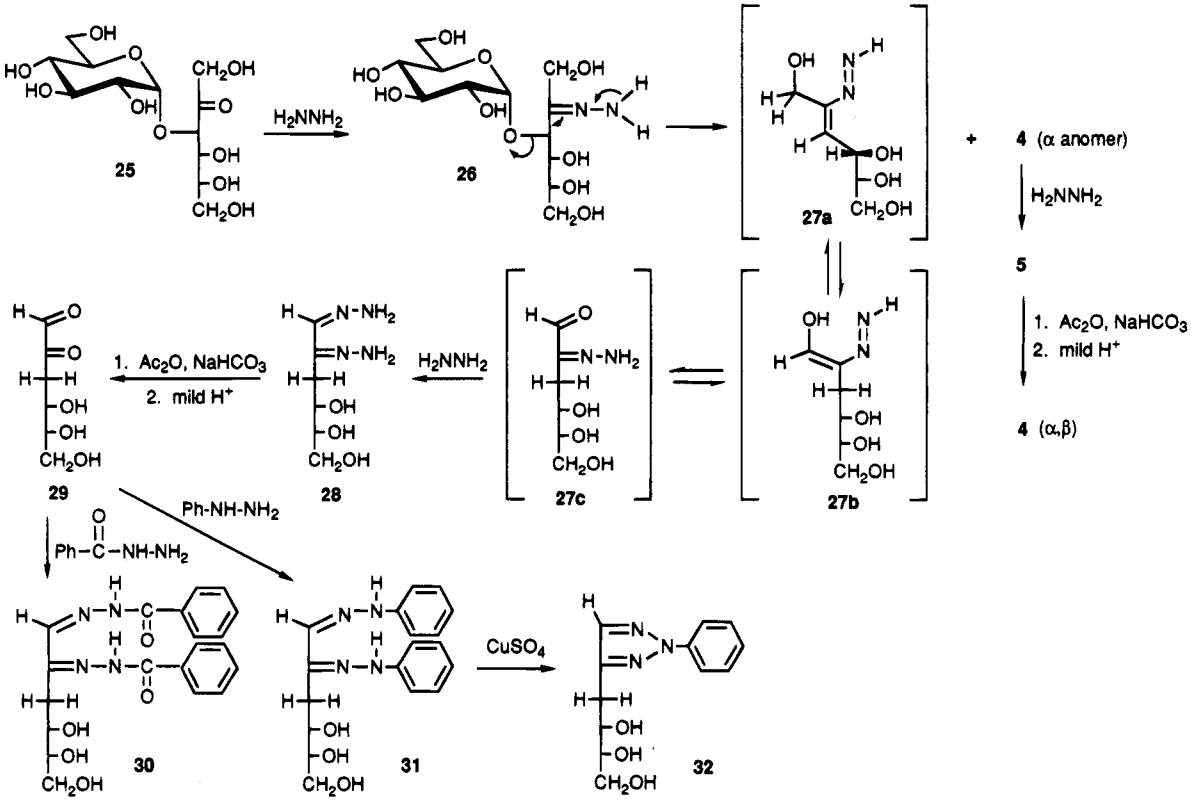
(11) (a) Fischer, E. *Ber.* **1884**, *17*, 579. (b) Reviewed by Mester, L.; El Khadem, H. S. *The Carbohydrates, Chemistry and Biochemistry*; Pigman, W., Horton, D., Eds.; Academic Press: New York, 1980; Vol. IB, p 929.

Table 1. Reaction of Hydrazine with Sugars Having a Glycosidic Substituent on a Carbon Atom Adjacent to a Carbonyl Group[a]

starting compound	product from glycon	% yield	conditions (time, °C)
2-*O*-β-D-glucopyranosyl-D-glucose (**1**)	D-glucose (**4**)	97	24 h, 55
2-*O*-α-D-glucopyranosyl-D-glucose (**33**)	D-glucose (**4**)	95	24 h, 65
2-*O*-α-D-mannopyranosyl-D-mannose (**34**)	D-mannose (**35**)	91	48 h, 70
3-*O*-α-D-glucopyranosyl-D-fructose (**25**)	D-glucose (**4**)	98	24 h, 55
4-acetamido-4-deoxy-2-*O*-(β-D-galactopyranosyl)-L-xylose (**38**)	D-galactose (**41**)	93	24 h, 60
3-acetamido-3-deoxy-2-*O*-(β-D-galactopyranosyl)-L-threose (**44**)	D-galactose (**41**)	99	24 h, 60
2-*O*-α-D-glucopyranosyl-glycolaldehyde (**49**)	D-glucose (**4**)	86	48 h, 70
2-*O*-α-D-glucopyranosyl-L-threose (**54**)	D-glucose (**4**)	92	48 h, 65
2-*O*-α-D-glucopyranosyl-D-arabinose (**55**)	D-glucose (**4**)	94	48 h, 65
2-*O*-(6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-galactopyranosyl)-D-erythrose (**62**)	6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-D-galactose (**63**)	97	24 h, 60
3-*O*-α-D-glucopyranosyl-D-*arabino*-2-hexosulose bis(phenylhydrazone) (**67**)	D-glucose (**4**)	87	48 h, 65
3-*O*-β-D-galactopyranosyl-D-*erythro*-2-pentosulose bis(phenylhydrazone) (**70**)	D-galactose (**41**)	89	48 h, 65

[a] Yields are those of the isolated reducing saccharide product derived from the glycon, following release of the hydrazone group(s).

Scheme 4



methyl)phenyl]hydrazine, and 2-hydrazinopyridine. In preliminary experiments, (pentafluorophenyl)hydrazine and **1** were combined at lower temperatures, which resulted in the disaccharide pentafluorophenylhydrazone **2b**, with minor cleavage of the glycosidic linkage. At 90 °C in the presence of NaOAc, (pentafluorophenyl)hydrazine reacted quantitatively with **1**, giving **4**, **6**, **10**, and **14**, (Schemes 2 and 3); structures of **6** and **10** were established through chemical synthesis. The structure of **14** was established through deprotection to give **19**. Of interest, it was observed, during the synthesis of **6**, that excess (pentafluorophenyl)hydrazine did not give the hydrazone product quantitatively from **4**; the reaction reaches a thermodynamic equilibrium which is reversible in water at 90 °C. However, chromatographically pure **6** is stable and isolable from water at room temperature. Hence, treatment of **1** with (pentafluorophenyl)hydrazine to give **4** directly in low yields results from incomplete conversion of **4**, the primary product, to the arylhydrazone **6**.

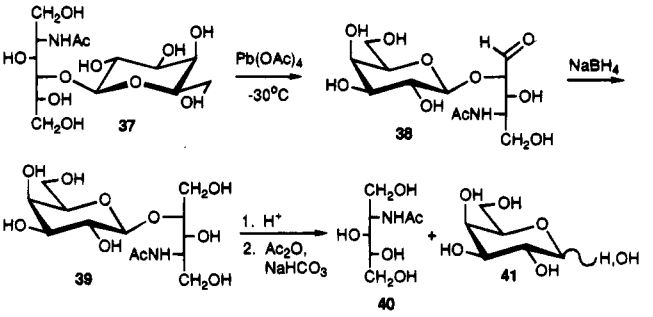
Similarly, [3-(trifluoromethyl)phenyl]hydrazine reacted with **1** to give **7**, **11**, and **15** (Schemes 2 and 3); the structures of **7** and **11** were established through separate chemical syntheses. However, in this case, **4** was not observed among the products. D-Glucose reacted with excess [3-(trifluoromethyl)phenyl]hydrazine to give the hydrazone product **7** in 69% isolated yield, although the reaction yield was essentially quantitative, as indicated by ^{1}H-NMR of the reaction mixture; the bis((3-trifluoromethyl)phenylhydrazone) was not formed.

In addition, 2-hydrazinopyridine reacted with **1** to give products, but it was not possible in our hands to chromatographically purify them, there being considerable smearing. An additional complication was that 2-hydrazinopyridine, although volatile enough to vacuum

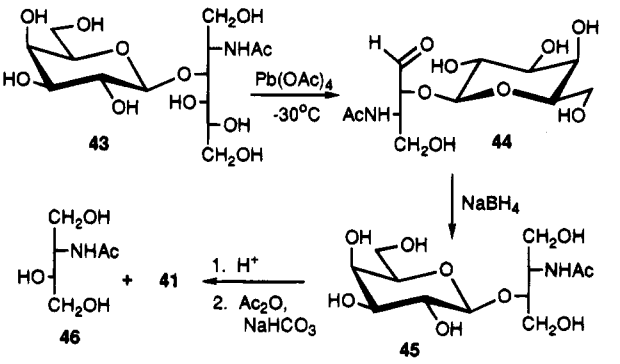
distil to a clear liquid, tended to form orange-reddish, aryl impurities on its own over time, which were not volatile and were difficult to separate chromatographically. However, direct ^{1}H-NMR analysis of reaction products, after volatilization of the 2-hydrazinopyridine, showed the following. First, treatment of **1**, **4**, and **18** at room temperature showed complete loss of the signals corresponding to the starting sugars; decoupling experiments showed, in all three cases, that the sugars were converted to the 2-pyridylhydrazones (**2d**, **8**, and **12**, respectively; **8** and **12** were separately prepared using a butanol extraction step to remove minor aryl impurities). Second, heating **1** at 90 °C with 2-hydrazinopyridine: water, 1:1 gave, in high reaction yields, ^{1}H-NMR signals corresponding to the products D-glucose (2-pyridylhydrazone) **8**, and 2,5-anhydro-D-mannose (2-pyridylhydrazone) **12** (Schemes 2 and 3), which were positively identified with decoupling experiments as compared to synthetic **8** and **12**.

Oxidation of Saccharide Alditols. Within the context of a reaction series for removal of a monosaccharide from the reducing end of oligosaccharides, the studies reported herein raised the need to restrict a chemistry to the reducing monosaccharide such that an aldehyde or keto function could be introduced on a carbon atom adjacent to the glycosidic linkage. Our efforts led to examination of several reactions with potential,[12] but oxidations in the cold with lead tetraacetate and periodate were most generally applicable, after reduction of the reducing sugar with $NaBH_4$ to give the alditol.[13] A number of binary and ternary solvent systems were systematically examined at low temperatures. Among them, the use of acetic acid:acetone:water (various ratios, to −78 °C),[14] and acetic acid:DMSO (1:1, to −65 °C) were suitable with lead tetraacetate, and DMF:water (1:1, to −20 °C) with periodic acid. Lead tetraacetate was only poorly soluble in the acetic acid:acetone:water mixture, which was dependent on solvent ratio and temperature, and this was normally a heterogeneous system. However, $Pb(OAc)_4$ was soluble to 50 mM in the DMSO:acetic acid mixture and this solution could be used for pseudo-first order rate studies.[15] Periodic acid in DMF:water was also fully soluble.

Scheme 5



Scheme 6



Oxidation of the disaccharitol **37** with lead tetraacetate at −30 °C gave **38** in 92% isolated yield (Scheme 5). It was evident from the ^{1}H-NMR of this molecule that the reducing monomer exists primarily in the cyclic α and β pyranose hemiacetals. To formally establish that oxidation of the glycon had not occurred, the product was reduced to give **39**, which showed a mass increase of 2 amu. Hydrolysis/*N*-reacetylation resulted in **40** and **41**, thereby establishing a clear preference of lead tetraacetate for the C-5,C-6 diol of the alditol in **37**.

The product **44** was isolated in 77% yield from **43** by lead tetraacetate oxidation (Scheme 6). In this case, a two-carbon portion of the alditol was removed, either by direct oxidation between C-4 and C-5 or by a two-step series from the C-6 end. Reduction of **44** to **45**, and hydrolysis/*N*-reacetylation gave the isolated products **41** and **46**, confirming the site of oxidation.

Interestingly, 6-*O*-α-D-glucopyranosyl-D-glucitol (**48**) was converted to 2-*O*-α-D-glucopyranosyl-glycolaldehyde (**49**) in 82% isolated yield by lead tetraacetate oxidation at −30 °C. The product, as observed by ^{1}H-NMR, exists primarily as a hydrate, due to the upfield position (δ 5.22) of the signal assigned to the glycolaldehyde H-1. Reduction gave α-D-glucopyranosyl-ethanediol (**50**), which gave isolated **4** and ethylene glycol after hydrolysis.[16]

Oxidation of 3-*O*-α-D-glucopyranosyl-D-glucitol (**53**) with lead tetraacetate showed a major product **54** (below,

(12) These included, besides the bis(arylhydrazone) reactions reported herein, "descent of the series" reactions (a) Fletcher, H. G., Jr.; Diehl, H. W.; Hudson, C. S. *J. Am. Chem. Soc.* **1950**, *72*, 4546. (b) Whistler, R. L.; Schweiger, R. *J. Am. Chem. Soc.* **1959**, *81*, 5190. (c) Whistler, R. L.; Yagi, K. *J. Org. Chem.* **1961**, *26*, 1050. (d) Weygand, F.; Löwenfeld, R. *Chem. Ber.* **1950**, *83*, 559, and Amadori rearrangements (a) Kuhn, R.; Weygand, F. *Ber.* **1937**, *70*, 769. (b) Micheel, F.; Schleppinghoff, B. *Chem. Ber.* **1956**, *89*, 1702. (c) Reviewed by Paulsen, H.; Pflughaupt, K.-W. *The Carbohydrates, Chemistry and Biochemistry*; Pigman, W., Horton, D., Eds.; Academic Press: New York, 1980; Vol. IB, p 881.

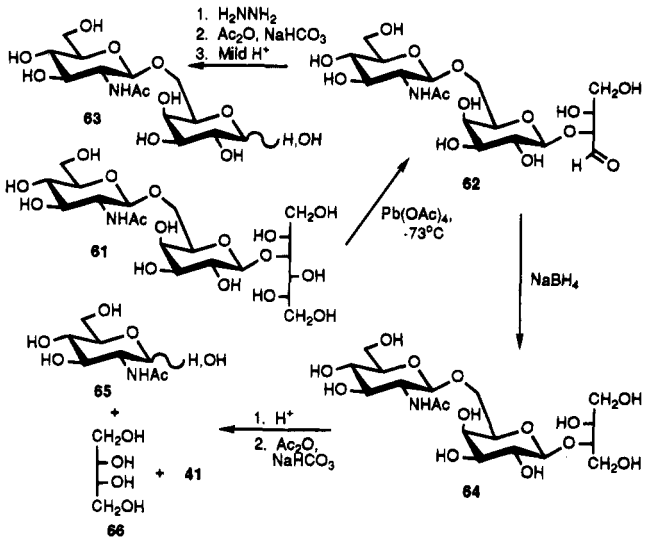
(13) Under the presumption that both these reagents undergo a planar, or nearly planar cyclic transition state, it was suspected that lower reaction temperatures should accentuate rate differences between alditols and pyranosides, due to ring puckering of the latter. Lead tetracetate has been observed, at or near room temperature, to highly favor alditol over glycopyranoside oxidation, having been used in the preparation of various 2-*O*-glycosyl-glycerols [(a) Charlson, A. J.; Gorin, P. A. J.; Perlin, A. S. *Methods Carbohydr. Chem.* **1962**, *1*, 419, and references therein, (b) Parrish, F. W.; Perlin, A. S.; Reese, E. T. *Can. J. Chem.* **1960**, *38*, 2094]. However, we have found that lead tetraacetate oxidizes alditols rapidly at temperatures 50−85 °C below room temperature; it has therefore been used over the years at much higher temperatures than are actually required for alditol oxidation.

(14) Appreciable water (Baer, E.; Grosheintz, J. M.; Fischer, H. O. L. *J. Am. Chem. Soc.* **1939**, *61*, 2607) may be used as a cosolvent with acetic acid in the cold. Lead tetraacetate added to saturation to a solution of acetic acid/water, 60/40 (v/v) at −20 °C does not result in lead oxide formation. Acetone may be added in varying proportion to lower the freezing point; solutions of acetic acid:water:acetone (3:2:2) to −30 °C, (3:2:4) to −40 °C, (3:2:7) to −50 °C, (3:2:10) to −60 °C, or (3:2:14) to −78 °C, may be used without lead oxide formation, with lead tetraacetate added to the solution after cooling.

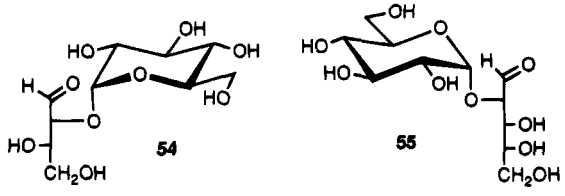
(15) We have had difficulty in assigning numbers to these comparative rates because they were so high, but data comparing 2-acetamido-2-deoxy-D-glucitol and 2-acetamido-2-deoxy-D-galactitol with their respective α- and β-*O*-methyl glycopyranosides gave measured values well above 10^3, probably above 10^4, at −60 °C in this solvent mixture.

(16) Oxidation of other 6-substituted alditols under these conditions gave a similar result, the 2-*O*-glycosyl-glycolaldehyde being the primary product, which will be presented elsewhere. The observation that lead tetraacetate oxidation of an unbroken series of vicinal diols in a polyol proceeds rapidly irrespective of the stereochemical configuration has been reported (Hockett, R. C.; Dienes, M. T.; Fletcher, H. G., Jr.; Ramsdem, H. E. *J. Am. Chem. Soc.* **1944**, *66*, 467).

Scheme 7



84% isolated) and a minor product **55** (below, 9% isolated), indicating that oxidation proceeded with a preference for the C-4 to C-6 portion of the alditol rather than the C-1, C-2 vicinal diol. Products were firmly estab-



lished by reduction, hydrolysis, and isolation of the respective alditol and **4**. Interestingly, the preference of lead tetraacetate for oxidation of the 4-substituted glucitol **61** (Scheme 7) was for the C-1 to C-3 portion of the alditol rather than the C-5, C-6 vicinal diol, giving **62** (93%). While it is possible that lead tetraacetate has a preference for an *internal* vicinal diol of polyalcohols, the conditions were different for these specific reactions. Practically, however, for the complete reaction series, the "end" at which the 3- or 4-substituted alditols were oxidized made little difference, as both products contained an aldehyde group adjacent to the carbon involved in the glycosidic linkage, and both were susceptible to the same reaction according to Scheme 1.

Treatment of 3-*O*-(Glycosyl)-2-aldosuloses and Their Bis(phenylhydrazones) 67 and 70 with Hydrazine. Preliminary experiments demonstrated that the 3-*O*-glycosyl-2-aldosuloses derived from **67** and **70** (Scheme 8) underwent a similar reaction with N_2H_4 to the other molecules having a glycosyl substituent on a carbon atom adjacent to an aldehyde or keto function. However, it was later found that **67** and **70** could be directly treated with excess N_2H_4, releasing the glycon in good yield without the need to deprotect the bis(phenylhydrazones), which is presented in Table 1. Presumably, **67** and **70** are converted from bis(phenylhydrazones) to their bis(hydrazones) upon N_2H_4 treatment, and these react according to Scheme 1, resulting in the reducing sugar derived from the glycon in 85–90% yields.

Mechanistic Considerations. The mechanism presented in Scheme 1 appears to be the most reasonable based on the products isolated from the many reactions reported herein. The proposed azoethylene intermediate is reactive and undergoes attack at C-2 on either side of the double bond, giving rise, from **1**, to the epimeric 2,5-anhydro products **18** and **19**. When N_2H_4 was used, **20** and **21** were also found, presumably by attack of the solvent at C-2 for **20** and by reduction, possibly via diimide, for **21**. The same initial mechanism may be invoked for the products derived from **25**, **67**, and **70**. Another reasonable mechanism which might be considered is the possibility of a direct S_N2 displacement at C-2 *activated* by a hydrazone at C-1, if epimerization at C-2 could occur prior to the displacement. Three lines of evidence argue against this mechanism, at least as the sole pathway. First, product **21** would not have been found (although it was found in low yield). Second, conditions for treatment of **1** with (pentafluorophenyl)-hydrazine were near neutrality (pH 7.1),[17] and epimerization would be unlikely to occur in this case. Third, (phenylazo)ethylenes have been reported which are stable, derived from peracetylated sugar phenylhydrazones.[4] Solvolysis can probably be excluded as a possible mechanism, as the above products would not be expected in a direct solvolytic reaction.

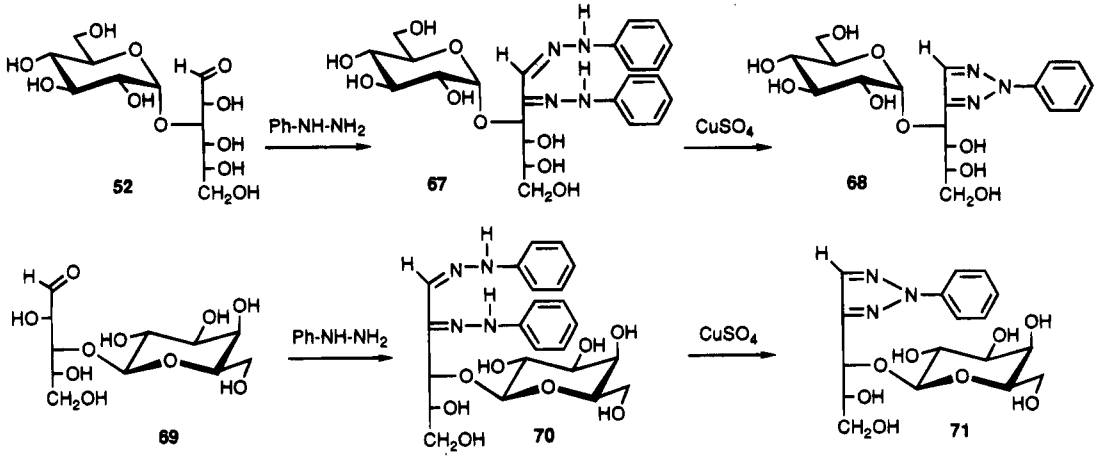
Conclusions

The model studies in this paper form the basis of a reaction series permitting the removal of a monosaccharide from the reducing end of an oligosaccharide, a process which may be applied repetitively if so desired. Two key steps are employed. The first is a regioselective modification of the monosaccharide at the reducing end so that an aldehyde or keto group is introduced on a carbon atom adjacent to the glycosidic linkage. The second is a cleavage of the glycosidic bond with N_2H_4 or certain substituted hydrazino derivatives. With regard to the glycosidic cleavage step, evidence presented herein indicates (1) that the reaction proceeds whether the glycosidic linkage is α or β; (2) that the released glycon can be recovered in high yields without epimerization or enolization after a deprotection step; (3) that the reaction also proceeds with a 3-*O*-glycosyl-2-aldosulose, and does not require deblocking of a bis(arylhydrazone) derivative prior to N_2H_4 treatment; and (4) that the reaction probably proceeds via the azoethylene intermediate (Scheme 1), based upon the products observed in a number of reactions. Another observation of note is that most arylhydrazines are not useful in the reaction series; although they react according to Scheme 1, they react more rapidly with the released glycon group to form the well-known bis(arylhydrazone) ["arylosazone"] derivatives. Relevant to this specific point is that N_2H_4 reacts with the 3-*O*-glycosyl-2-keto compound **25** to give **4** and **29**, yet phenylhydrazine has been reported to react with **25** to give the 3-*O*-glycosyl bis(phenylhydrazone) derivative,[10] a result which we have repeated and confirmed.

With regard to the oxidation of saccharide-alditols, results demonstrate (1) that lead tetraacetate is capable of oxidizing vicinal diols within acyclic polyalcohols at temperatures 50–85 °C lower than conditions normally employed, (2) that lead tetraacetate oxidation below −30 °C is operationally confined to exocyclic vicinal diols; we have not observed oxidation of pyranosides under the conditions described, (3) that 3, 4, or 6-substituted hexitols are oxidized so that an aldehyde is generated

(17) (Pentafluorophenyl)hydrazine is actually slightly acidic, a saturated solution in water having a pH of 6.4. With sodium acetate, as used for reactions herein, the pH was 7.1.

Scheme 8



52 67 68 69 70 71

adjacent to a carbon involved in the glycosidic linkage. Hence, the capability of removal of reducing-end sugars having a 2, 3, 4, or 6 substituent has been demonstrated in this paper for the overall reaction series, and (4) that the solvent systems so described for lead tetraacetate oxidation tolerate compounds of varying polarity, or the polarity may be modified significantly in the acetic acid/water/acetone system; oxidations in the cold need not be restricted to carbohydrates, but may be performed to effect highly regioselective oxidations of vicinal diols within a variety of organic compounds. Additionally, on the basis of our trials to date, the acetic acid/water/acetone mixture is best for preparative procedures, as removal of DMSO in the alternate system is laborious on a large scale. The acetic acid/DMSO system, however, is a homogenous mixture which may be used analytically.

In addition to oxidations with diol-cleavage reagents, we examined alternate methods for generation of a carbonyl group on a carbon adjacent to the glycosidic linkage, including bis(phenylhydrazone) formation, "descent of the series" reactions, Amadori rearrangements, and combinations thereof.[10-12] Although yields were lower, many of these reactions were discovered when crystallization was the only route to purification and were optimized to give highest isolated yields, but not necessarily examined under conditions which would drive reactions to completion to give highest reaction yields. Also, in the light of more recent chromatographic advances, many reagents which did not previously result in crystalline products could actually give higher reaction yields. Based upon the ability of hydrazine or certain hydrazino derivatives to cleave a glycosidic linkage as shown in Scheme 1, further improvements are warranted for those degradations which can be regiospecifically restricted to the reducing monosaccharide of an oligosaccharide, to enable a carbonyl group to be introduced on a carbon atom adjacent to the glycosidic linkage. Reopening studies of "descent of the series" reactions as applied to oligosaccharides seems worthwhile.

While many additional saccharides need to be examined, it would appear, on the basis of our investigations up to this point, that the reaction series should be applicable to a variety of oligosaccharides. We have successfully achieved the complete sequential degradation of two tetrasaccharides, the details of which will be forthcoming.

Experimental Section

General Methods. ^{1}H-NMR spectra were accumulated at 500 MHz. Chemical shifts in D_2O are reported relative to internal acetone (Ace., δ 2.225) or sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS, δ 0.00). Chemical shifts of exchanged samples in DMSO-d_6–2% D_2O are reported relative to tetramethylsilane (TMS, δ 0.00). Assignments, where made, were through decoupling experiments. FABMS was performed on a JEOL HX-110 double-focusing magnetic sector instrument at 10 kV. For HRFABMS, peak matching was performed using standards within 30 amu of samples. Mass measurement evaluation was determined for 187 samples of masses varying from 132 to over 1000 amu; 99% of values were within ±7.48 ppm, by mass, of the calculated values (±2.57 σ, where σ = 2.91 ppm). Electrospray (ES) MS was performed on a few samples not amenable to FABMS, on a triple quadrupole spectrometer to 1 amu. Chromatography was performed with an HPLC equipped with a UV detector and a differential refractometer connected in series. Unless otherwise stated, "standard HPLC conditions" refer to the use of a Waters Glyco-Pak N column[18] (7.8 × 300 mm), chromatographed isocratically with 85/15 acetonitrile (MeCN)/water at 1.0 mL/min, monitoring at 200 nm on the UV detector with simultaneous RI detection. Compounds **36**, **42**, **47**, **52**, and **60** (below) from Sigma were purified by HPLC on the Glycopak N column prior to use. Concentration of samples from solvents, unless otherwise mentioned, was by rotary evaporation at 35 °C. Small-scale reactions used 1.5–2.0 mL glass vials having Teflon-lined cap septa (Pierce), unless otherwise stated. Dowex AG50W used in all experiments refers to X8, 100–200 mesh, and Dowex AG1 refers to X8, 100–200 mesh; unless otherwise mentioned, columns were washed with 20–30 volumes of water immediately before use.

Quantitation. Most compounds described herein were isolated as syrups, which, after lyophilization, contained water in variable quantities which was difficult to completely remove. Therefore, quantitation of hexoses or glycosidically-linked hexoses was performed using the anthrone reagent,[19] which was accurate to within ±2%, with the following caveats. The reaction gives different color yields for different hexoses, so the same hexose was used as a standard as was found in individual glycosides. Aldehydes (glycolaldehyde, glyceraldehyde) interfered with the reagent, hence structures containing fragments of alditols (i.e. **44**, **49**, and **55**) needed to be reduced

(18) Bendiak, B.; Orr, J.; Brockhausen, I.; Vella, G.; Phoebe, C. *Anal. Biochem.* **1988**, *175*, 96. Glyco-Pak N is a non-silica-based, hydroxylated polymeric material having a polar surface. We have used the same column for a five-year period, with little change in retention times. It gives essentially quantitative recoveries for monosaccharides, as determined by the anthrone reagent. Column flow should never exceed 1.0 mL/min, particulates should be filtered, and column life can be prolonged by an occasional run of MeCN/water from 95/15 to 0/100 over 3–4 h.

(19) Spiro, R. G. *Methods Enzymol.* **1966**, *8*, 3.

prior to quantitation. Where structures were not amenable to analysis by the anthrone reagent (i.e. **14**, **18**, **32**, **51**), the mass of the compound dried as much as possible was determined. For analysis of completion of reductions, the ferricyanide method[20] was used. It gives highly reproducible replicates, but does not give the same color yield for different aldehydes. By comparison of the reducing substance in a given sugar sample with 25- to 100-fold more of the product from the same sample, reduction yields could be quantitated to within 0.1%.

Reductions. Up to 100 μmol of reducing sugar was dissolved in 1.0 mL of water, and 1.0 mmol of $NaBH_4$ was added. After 24 h, 4.0 mL of water followed by 1.0 mL of 2.0 M acetic acid were added. After 1 h, the solution was passed through a 5.0 mL column of Dowex AG50W (H^+ form), followed by 4 × 5.0 mL washes with water. The eluate was concentrated to near-dryness and concentrated 5× to dryness (35 °C) with 5−10 mL of 1% acetic acid in methanol and then 3× with methanol. Final products were taken up in water for quantitative analyses.

Reaction of 2-*O*-β-D-Glucopyranosyl-D-glucose (1) with Hydrazine. The disaccharide **1** (141 μmol, Koch-Light or Serva) was dissolved in 10 mL of anhydrous N_2H_4 (Pierce or Aldrich) in a glass vessel under Ar. After heating to 55 °C for 24 h, N_2H_4 was removed in vacuo (<100 mTorr) to give a clear residue (*Caution: have some water precondensed in the trap*). For conversion of hydrazones to carbonyl compounds, the sample was dissolved in saturated aqueous $NaHCO_3$ (10 mL), followed immediately by addition of 0.5 mL of acetic anhydride (Ac_2O), dissolved by gentle swirling. After 10 min, a second 0.5 mL of Ac_2O was added similarly, and after a further 50 min, the sample diluted with 40 mL of water and passed immediately through a 50 mL column of Dowex AG50W (H^+ form). After washing with 250 mL of water in stages, the eluate was concentrated to a stiff residue and taken up in 5.0 mL of water; 5.0 mL of 0.2 M HCl was added, and the sample warmed at 35 °C for 1 h to convert the acetohydrazide derivative to the reducing sugar.[6] It was diluted with 15 mL of water and immediately passed through two columns in tandem: Dowex AG50W (H^+ form, 25 mL) and then Dowex AG1 (OAc form, 25 mL). After an additional 250 mL water wash, eluate was concentrated and a sample examined by ^{1}H-NMR, which showed none of the starting compound **1**, but gave signals corresponding to **4** and other products. No signals corresponding to the C-2 epimer, D-mannose, were observed. The mixture was concentrated to dryness, taken up in 0.75 mL of water and diluted with 3.0 mL of MeCN, and products were separated in batches by HPLC, first under standard conditions. The major RI-detectable product at 58 min was pure D-glucose (**4**, 137 μmol, 97%); ^{1}H-NMR, identical to authentic compound. Other fractions had one major component, but were not pure, as judged by ^{1}H-NMR; fractions at 27, 34, 37, and 47 min, respectively, were primarily **19**, **21**, **18**, and **20**. Compound **18**, 2,5-anhydro-D-mannose, was purified by rechromatography under standard conditions, which gave 27 μmol (19%), as the hydrate (characterization data in supporting information). Synthesis of **18** was performed by the action of nitrous acid on glucosamine hydrochloride,[7] which gave a product with an identical ^{1}H-NMR. Reduction of **18** (11.1 μmol) was carried out according to the general procedure on one-fourth scale, which gave 2,5-anhydromannitol **22** (11 μmol, 99%); ^{1}H-NMR identical to an authentic sample (Sigma). FABMS (3-nitrobenzyl alcohol [3-NBA] or glycerol, matrices) *m/z* 165 $(M + H)^+$, and, with NaOAc added, 187 $(M + Na)^+$. HRFABMS calcd for $C_6H_{13}O_5$ $(M + H)^+$ 165.0763, found 165.0764. Product **21**, 2-deoxy-D-*arabino*-hexose, was purified by HPLC on a second (polymeric) column of Shodex DC-613, 6 × 150 mm, chromatographed at 0.6 mL/min with MeCN/water, 91/9. The compound eluted between 25−40 min, and was concentrated giving **21** (2.4 μmol, 1.7%); ^{1}H-NMR, identical to an authentic sample (Sigma). ESMS *m/z* 187 $(M + Na)^+$, 203 $(M + K)^+$. Product **20**, 2-(2-acetylhydrazino)-2-deoxy-D-mannose, was purified on the Shodex DC-613 column, as for **21**, but using MeCN/water, 90/10. An interconvertable pair of peaks, at 115 and 139 min, gave identical ^{1}H-NMR spectra, and were anomers of **20**. Yield 23.3 μmol, 17%; ^{1}H-NMR (D_2O−Ace., 36 °C) δ 5.24 (d, α H-1, J = 1.2 Hz) 4.95 (d, β H-1, J < 1.0 Hz), 4.00 (dd, J = 4.6, 9.5 Hz), 3.88 (dd, J = 2.2, −12.3 Hz), 3.85 (dd, J = 1.7, −11.6 Hz) 3.79 (m), 3.72 (dd, J = 5.8, −12.2 Hz), 3.64 (dd, J = 9.5, 9.5 Hz) 3.49 (dd, J = 9.5, 9.5 Hz), 3.35 (ddd, J = 2.3, 5.9, 9.6 Hz), 3.19 [dd, overlapping H-2 signals, J = 1.2, 4.6 Hz for major (α) form], 1.98 and 1.96 (both s, N-Ac methyl protons). FABMS (3-NBA, matrix) *m/z* 237 $(M + H)^+$, and, with added NaOAc, 259 $(M + Na)^+$. HRFABMS calcd for $C_8H_{16}N_2O_6Na$ $(M + Na)^+$ 259.0906, found 259.0913. Product **19**, 2,5-anhydro-D-glucose, was obtained in pure form by rechromatography under standard conditions. Yield 8.9 μmol, primarily hydrate (6.3%). This compound was also prepared in pure form by deprotection of **14**; its characterization data and proof of structure through conversion to **23** and **24** are described below.

Reaction of 3-*O*-α-D-Glucopyranosyl-D-fructose (25) with Hydrazine. Compound **25**, (150 μmol, Sigma) was dissolved in 10 mL of anhydrous N_2H_4 under Ar. Further treatment was identical to that described for the reaction between **1** and N_2H_4. The product mixture was examined by ^{1}H-NMR, which showed none of the starting compound, but gave signals characteristic of **4**, some singlets in the anomeric region, and upfield gem-coupled signals characteristic of deoxy sugar(s). No signals corresponding to D-mannose were observed. The product was dissolved in 0.75 mL of water, 3.0 mL of MeCN was added, and compounds were separated in batches by HPLC under standard conditions. The major RI-detectable product at 57 min was pure D-glucose (**4**, 147 μmol, 98%); ^{1}H-NMR identical to authentic compound. A broad set of peaks, each having the same ^{1}H-NMR spectrum, eluted from 23−45 min; rechromatography of a single peak gave the same series of HPLC peaks, which was due to interconversions of the suspected product, 3-deoxy-D-*erythro*-2-hexosulose (**29**, as the hydrate, 107 μmol, 71%); ^{1}H-NMR (D_2O−Ace., 36 °C, spectrum in supporting information shown without Ace.) showed multiple cyclic forms and possible hydrates δ 5.06 (s), 4.94 (s), 4.93 (s), 4.89 (s), 4.81 (s), 4.79 (s), 4.774 (s), 4.768 (s), 4.49 (ddd, J = 2.3, 7.4, ~1.5 Hz), 4.46 (ddd, J = 2.5, 7.6, ~1.0 Hz), 4.40 (ddd, J = 6.1, 7.3, 7.3 Hz), 4.27 (ddd, J = 4.3, 4.3, 7.7 Hz), 4.16 (m), 4.10 (m), 4.00 (dd, J = −12.5, ~1.0 Hz), 3.95 (m), 3.90 (m), 3.85 (m), 3.76 (m), 3.69 (m), 3.63 (dd, J = 5.3, −12.3 Hz), 3.55 (m), 3.49 (m), 2.73 (dd, J = 7.5, −14.1 Hz), 2.67 (dd, J = 7.4, −14.0 Hz), 2.49 (J = 7.5, −14.4 Hz), 2.35 (J = 5.0, −13.1 Hz), 2.26 (dd, J = 7.2, −13.7 Hz), 2.22 (dd, J = 7.3, −13.8 Hz), 2.18 (m), 2.13 (m), 2.00 (m), 1.92 (m), 1.83 (m, tightly coupled gem pair), 1.74 (dd, J = 11.5, −13.0 Hz), 1.69 (dd, J ~ 1.0, −14.1 Hz), 1.54 (dd, J = ~1.5, −14.0 Hz). ESMS *m/z* 203 [hydrate, $(M + Na)^+$], 219 [hydrate, $(M + K)^+$], and, with methanol, 217 [methyl hemiacetal, $(M + Na)^+$], and 233 [methyl hemiacetal, $(M + K)^+$]. Due to the unusual nature of compound **29**, it was synthesized as described earlier via the bis(benzoylhydrazone) **30** using the improved method of El Khadem et al., [9] which gave **29**, having the same ^{1}H-NMR spectrum. Despite matching spectra, derivatives **30**, **31**, and **32** were also prepared (experimental detail and characterization data in supporting information).

Reaction of 2-*O*-α-D-Glucopyranosyl-D-glucose (33) with Hydrazine. The disaccharide **33** (14.6 μmol, Koch-Light) was dissolved in 1.0 mL of N_2H_4 in a vial under Ar. After heating to 65 °C for 24 h, N_2H_4 was removed in vacuo in a Speed-Vac rotary concentrator (Savant). The sample was transferred in a 1.0 mL total volume of saturated $NaHCO_3$ to a larger tube. *N*-Acetylation was carried out as described above for the reaction of **1**, except 50 μL aliquots of Ac_2O were used, the sample was diluted with 5 mL of water, and a 5.0 mL column of Dowex AG50W resin was used, followed by 5 × 5.0 mL washes with water. The eluate was concentrated and dissolved in 1.0 mL water, and 1.0 mL of 0.2 M HCl was added, followed by incubation at 35 °C for 1 h, to deprotect the acetohydrazide derivatives. The solution was diluted with 5.0 mL of water and immediately passed through two columns of Dowex ion exchange resins as described above for treatment of **1**, except these were 5 mL in volume, and columns were washed with 6 × 5.0 mL water. After concentration, the

(20) Park, J. T.; Johnson, M. J. *J. Biol. Chem.* **1949**, *181*, 149.

product mixture was examined by ^{1}H-NMR, which showed signals corresponding to **4** and other products, with negligible **33** present. No signals corresponding to D-mannose were observed. HPLC was performed under standard conditions, which gave **4** (13.9 μmol, 95%), **18** (2.3 μmol, 16%), and **20** (1.3 μmol, 9%) as isolated products (^{1}H-NMR as described for reaction of **1**). A small amount of (~2–3%) of **19** was observed by ^{1}H-NMR of one fraction, but was only ~70% pure.

Reaction of 2-*O*-α-D-Mannopyranosyl-D-mannose (34) with Hydrazine. The disaccharide **34** (10.0 μmol, V-Labs) and 1.0 mL N_2H_4 were heated under Ar to 70 °C for 48 h and concentrated in vacuo, and additional methods were performed as described above for compound **33** prior to HPLC. The mixture of products was examined by ^{1}H-NMR, which showed D-mannose (**35**) and other products, with a small amount (<5%) of the starting material **34**. No signals corresponding to D-glucose **4** were seen. HPLC was performed under standard conditions, which gave **35** (9.1 μmol, 91%) at an elution time of 53 min; ^{1}H-NMR identical to authentic **35**. Compounds **18** (1.4 μmol, 14%) and **20** (~1.0 μmol, 10%) were also isolated (^{1}H-NMR spectra as described for reaction of **1**).

Reaction of the Saccharides 38, 44, 49, 54, 55, and 62 with Hydrazine. These structures were prepared by lead tetraacetate oxidation of saccharide-alditols as described below. They were treated according to the protocol described for compound **33**, above, with modifications listed below for each compound as follows: quantity of starting compound; volume of N_2H_4 used; HPLC retention time of isolated glycon product; glycon product, characterization methods and data. HPLC was performed under standard conditions except for product **63**, which was modified to 79/21, MeCN/water. For these compounds, only the product derived from the glycon was isolated by HPLC, characterized by ^{1}H-NMR (and MS when required), and quantitated (products, yields and temperatures and times of N_2H_4 treatment are presented in Table 1); structures of molecules arising from the aglycon for these compounds were not further characterized. For 4-acetamido-4-deoxy-2-*O*-(β-D-galactopyranosyl)-L-xylose (**38**): 5.0 μmol; 0.5 mL; biphasic pair of peaks at 57 and 61 min; D-galactose **41** (no evidence among products for the C-2 epimer D-talose by NMR); ^{1}H-NMR, identical to authentic **41**. For 3-acetamido-3-deoxy-2-*O*-(β-D-galactopyranosyl)-L-threose (**44**): 5.0 μmol; 0.5 mL; biphasic pair of peaks at 58 and 62 min; D-galactose **41** (no evidence among products for D-talose by NMR); ^{1}H-NMR identical to authentic compound. For 2-*O*-α-D-glucopyranosyl-glycolaldehyde (**49**): 5.0 μmol; 0.5 mL; 57 min; D-glucose **4**; ^{1}H-NMR identical to authentic compound. For 2-*O*-α-D-glucopyranosyl-L-threose (**54**): 5.0 μmol; 1.0 mL; 57 min; D-glucose **4**; ^{1}H-NMR identical to authentic compound. For 2-*O*-α-D-glucopyranosyl-D-arabinose (**55**): 2.5 μmol; 1.0 mL; 58 min; D-glucose **4**; ^{1}H-NMR, identical to authentic compound. For the trisaccharide 2-*O*-[6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-galactopyranosyl]-D-erythrose **62**: 5.0 μmol; 1.0 mL; 44 min; 6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-D-galactose **63**; ^{1}H-NMR, identical to authentic compound (Sigma). FABMS (thioglycerol + glycerol matrix) *m*/*z* 382 $(M - H)^-$.

Reaction of the 3-Substituted Bis(phenylhydrazone) Derivatives 67 and 70 with Hydrazine. To 10.0 μmol of 3-*O*-α-D-glucopyranosyl-D-*arabino*-2-hexosulose bis(phenylhydrazone) (**67**) was added 1.0 mL N_2H_4, the sample heated under Ar at 65 °C for 48 h, and concentrated on the Speed-Vac to an orange-brown residue. Saturated $NaHCO_3$ (333 μL) was added, followed by 300 μL of ethanol and the sample gently agitated for about 12 h to dissolve the residue. This was transferred to a larger tube, followed by two 333 μL washes of the vial with saturated $NaHCO_3$. Ac_2O (50 μL) was added, followed by gentle swirling to dissolve. After 10 min, another 50 μL was added and the sample kept at room temp for another 50 min. Further treatments were performed as described for compound **33**, for purification of products and deprotection of the acetohydrazide derivative, except in the mild hydrolytic deprotection step, the volume was increased 3-fold to 6.0 mL to accommodate the increased buffering capacity of the phenylhydrazine. A sample of product was examined by ^{1}H-NMR, which showed signals corresponding to D-glucose **4** and other products, with no evidence of the starting compound. HPLC performed under standard conditions gave pure **4** (8.7 μmol, 87%) at 58 min; ^{1}H-NMR identical to authentic compound. The compound 3-*O*-β-D-galactopyranosyl-D-*erythro*-2-pentosulose bis(phenylhydrazone) (**70**) (10.0 μmol) was treated identically to **67**; product derived from the glycon was isolated by HPLC under standard conditions, giving D-galactose (8.9 μmol, 89%) as an interconverting pair of peaks at 58 and 62 min; ^{1}H-NMR identical to authentic compound; no D-talose was observed in the total product mixture by NMR.

Reaction of 2-*O*-β-D-Glucopyranosyl-D-glucose (1) with (Pentafluorophenyl)hydrazine. The disaccharide **1** (50 μmol), (pentafluorophenyl)hydrazine (500 μmol, Aldrich, used without further purification), NaOAc (300 μmol), and 1.5 mL of water were mixed prior to heating under N_2 to 90 °C for 48 h, with occasional shaking. While still warm, 10% of the sample was removed for ^{1}H-NMR analysis. The remaining 90% was transferred to a larger tube with repetitive water washes to give a 7.0 mL volume, which was extracted 3× with 5.0 mL of 1-butanol. Pooled butanol phases were concentrated, taken up in 5.0 mL of MeCN/water, 85/15, and HPLC was performed under standard conditions in batches. Two major fractions were found at 10–12 min and 14–16 min. That at 14–16 min (biphasic) was D-glucose pentafluorophenylhydrazone (**6**) (25.8 μmol, 52%, accounting for the 10% aliquot removed; some **6**, NaOAc, and **10** and **14**, characterized below, remained in the water phase). Characterization data is presented below for synthetic **6**. The fraction at 10–12 min was a mixture; it was rechromatographed under modified conditions of 95/5, MeCN/water which gave two major fractions. A peak at 21 min was 2,5-anhydro-D-mannose pentafluorophenylhydrazone (**10**) (10.5 μmol, 21%, accounting for 10% removed). Characterization data is presented below for synthetic **10**. A peak at 18.5 min was 2,5-anhydro-D-glucose pentafluorophenylhydrazone **14** (9.4 μmol, 19%, accounting for 10% removal). ^{1}H-NMR (D_2O–DSS, 27 °C) δ 7.39 (d, 1H, J = 6.0 Hz), 4.64 (dd, 1H, J = 4.6, 6.0 Hz), 4.30 (dd, 1H, J = 2.9, 4.6 Hz), 4.10 (dd, 1H, J = 2.9, 4.2 Hz), 3.93 (ddd, 1H, J = 3.8, 4.2, 5.9 Hz), 3.80 (dd, 1H, J = 3.8, −12.3 Hz), 3.73 (dd, 1H, J = 5.9, −12.3 Hz). FABMS (3-NBA, matrix) *m*/*z* 343 $(M+H)^+$, and, with NaOAc added, 365 $(M + Na)^+$. HRFABMS calcd for $C_{12}H_{11}N_2O_4F_5Na$ $(M + Na)^+$ 365.0537, found 365.0537. Proof of structure of **14** through conversion to **19**, **23**, and **24** is described below.

Reaction of 2-*O*-β-D-Glucopyranosyl-D-glucose (1) with [*m*-(Trifluoromethyl)phenyl]hydrazine. The disaccharide **1** (50 μmol), NaOAc (300 μmol), 1.5 mL of water, and freshly vacuum-distilled [*m*-(trifluoromethyl)phenyl]hydrazine (47 μL, 53 mg, 300 μmol, Aldrich) were mixed and heated in a vial under N_2 to 90 °C for 48 h, with occasional shaking. While still warm, 10% was removed for ^{1}H-NMR analysis. The remaining contents were transferred, with an additional 5 mL of water, to a larger tube, frozen, and lyophilized to remove excess arylhydrazine. The product was taken up in 7.0 mL of water and extracted three times with 5.0 mL of 1-butanol. Pooled butanol phases were concentrated and dissolved in 5.0 mL of 90/10 MeCN/water. HPLC was performed under modified standard conditions using 92/8, MeCN/water. Major peaks were observed at 15.5, 16.5, 21, and 26 min. Peaks at 21 and 26 min were interconvertible and were D-glucose[*m*-(trifluoromethyl)phenyl]hydrazone **7** (open-chain and cyclized forms, 33.8 μmol, 68%, accounting for the 10% aliquot removed). Spectral characteristics are described below for synthetic **7**. Peaks at 15.5 and 16.5 min partially overlapped and were rechromatographed at 95/5 MeCN/water, which gave complete separation of two compounds. A peak at 22.5 min was 2,5-anhydro-D-mannose *m*-(trifluoromethyl)phenylhydrazone (**11**) (16.3 μmol, 33%, accounting for the 10% aliquot removed). Characterization data are described below for synthetic **11**. A peak at 20.5 min was 2,5-anhydro-D-glucose *m*-(trifluoromethyl)phenylhydrazone (**15**) (9.7 μmol, 19%, accounting for the 10% aliquot removed). ^{1}H-NMR (D_2O–DSS, 36 °C) δ 7.31 (d, 1H, J = 6.3 Hz, sugar H-1), 4.71 (dd, 1H, J = 4.6, 6.4 Hz), 4.30 (dd, 1H, J = 2.7, 4.5 Hz), 4.13 (dd, 1H, J = 2.8, 4.2 Hz), 3.94 (ddd, 1H, J = 3.8, 4.2, 5.8 Hz), 3.82 (dd, 1H, J = 3.8, −12.1 Hz), 3.76 (dd, 1H, J = 5.8, −12.1 Hz), 7.45 (dd,

1H, aryl, J = 8.0, 8.0 Hz), 7.37 (broad s, 1H, aryl), 7.21 (d, 1H, aryl, J = 7.9 Hz), 7.22 (d, 1H, aryl, $J \sim 9$ Hz); downfield aryl protons showed barely discernable 4-bond couplings (~1 Hz). FABMS (3-NBA, matrix) m/z 321 $(M + H)^+$, and, with NaOAc added, 343 $(M + Na)^+$. HRFABMS calcd for $C_{13}H_{16}N_2O_4F_3$ $(M + H)^+$ 321.1062, found 321.1058.

Reaction of 2-*O*-β-D-Glucopyranosyl-D-glucose (1) with 2-Hydrazinopyridine. Preliminary trials at different temperatures showed that 2-hydrazinopyridine reacted readily with **1** at room temperature, but at higher temperatures, the 2-pyridylhydrazone **2d** gave rise to other products. To 10 μmol of **1** in a 200 μL vial was added 150 μL of a solution containing 1/1 (v/v) 2-hydrazinopyridine (freshly vacuum distilled, liquid when warm) and water, under N_2. After mixing, the solution was heated to 90 °C for 48 h. It turned orange-red, but in a control experiment without sugar, the same color appeared. After dilution with 1.0 mL of water excess 2-hydrazinopyridine was removed by lyophilization. Chromatographic separation of products was not possible, as these hydrazone derivatives smeared extensively. ^{1}H-NMR (D_2O–DSS) of the product mixture showed signals and coupling constants similar to products obtained by treatment of **1** with (pentafluorophenyl)-hydrazine and [*m*-(trifluoromethyl)phenyl]hydrazine (**8** and **12**), with none of the starting material (**1** or **2d**) present. As the products were not separable, syntheses of **8** and **12** were performed independently (below) to ascertain that the chemical shifts, coupling constants, and appropriate decouplings observed in the mixture were attributable to **8** and **12**. The estimated reaction yield was >85%.

D-Glucose Pentafluorophenylhydrazone (6) and 2,5-Anhydro-D-mannose Pentafluorophenylhydrazone (10). For **6**, 100 μmol of D-glucose, 300 μmol of NaOAc, and 400 μmol of (pentafluorophenyl)hydrazine were added to a vial. For **10**, 50 μmol of 2,5-anhydro-D-mannose hydrate,[7] 300 μmol of NaOAc, and 250 μmol of (pentafluorophenyl)hydrazine were similarly mixed. To each was added 1.0 mL of water, and samples were capped under N_2 and mixed prior to heating to 70 °C for 32 h. Samples were transferred in 5.0 mL of water to larger tubes and extracted twice with 5.0 mL of 1-butanol, and the butanol phases were pooled and concentrated. Products were dissolved in 5 mL of 80/20, MeCN/water, and HPLC was performed in batches under standard conditions. Product from D-glucose eluted at 15 min (biphasic), giving **6**, which was concentrated and lyophilized to give a white solid (79 μmol, 79%, hygroscopic). The open-chain hydrazone and more abundant (~95%) cyclic β-glycopyranosylhydrazine forms were found. ^{1}H-NMR (D_2O–DSS, 36 °C), major form, δ 4.19 (d, 1H, J = 9.0), 3.88 (dd, 1H, J = 1.7, −12.2 Hz), 3.71 (dd, 1H, J = 5.3, −12.2 Hz), 3.47 (dd, 1H, J = 8.8, 8.8 Hz), 3.39 (m, 2H), 3.27 (dd, 1H, J = 8.9, 8.9 Hz). Minor form, δ 7.36 (d, J = 6.7 Hz), 4.39 (dd, J = 6.6, 6.6 Hz), 3.95 (dd), 3.82 (dd), 3.77 (m), 3.64 (m). FABMS (3-NBA, matrix) m/z 361 $(M + H)^+$, and, with NaOAc added, 383 $(M + Na)^+$. HRFABMS calcd for $C_{12}H_{14}N_2O_5F_5$ $(M + H)^+$ 361.0823, found 361.0822. Product from 2,5-anhydro-D-mannose eluted at 11 min; it was concentrated and lyophilized to give a cream-colored solid, **10** (21 μmol, 42%). ^{1}H-NMR (D_2O–DSS, 36 °C), two forms observed (presumed hydrazone isomers), major (>95%) δ 7.38 (d, 1H, J = 6.2 Hz, H-1), 4.39 (dd, 1H, J = 6.3, 6.3 Hz), 4.23 (dd, 1H, J = 6.1, 6.1 Hz), 4.11 (dd, 1H, J = 6.0, 6.0 Hz), 3.99 (ddd, 1H, J = 3.3, 5.6, 6.0 Hz), 3.78 (dd, 1H, J = 3.3, −12.4 Hz), 3.71 (dd, 1H, J = 5.6, −12.4 Hz). Minor form, δ 6.90 (d, H-1). FABMS (3-NBA, matrix) m/z 343 $(M + H)^+$, and, with NaOAc added, 365 $(M + Na)^+$. HRFABMS calcd for $C_{12}H_{11}N_2O_4F_5Na$ $(M + Na)^+$ 365.0537, found 365.0537.

D-Glucose *m*-(Trifluoromethyl)phenylhydrazone (7) and 2,5-Anhydro-D-mannose *m*-(Trifluoromethyl)phenylhydrazone (11). For **7**, D-glucose (50 μmol), NaOAc (300 μmol), and 1.0 mL of water were mixed; freshly vacuum distilled [*m*-(trifluoromethyl)phenyl]hydrazine (320 μmol, Aldrich) was added. For **11**, 2,5-anhydro-D-mannose hydrate[7] (50 μmol) was treated identically. Vials were capped under N_2, heated to 70 °C for 18 h, transferred to a larger tube in about 5 mL of water, and lyophilized to remove excess [*m*-(trifluoromethyl)phenyl]hydrazine. They were taken up in 5.0 mL of water and extracted 2× with 5 mL of 1-butanol, the butanol phases were concentrated, and HPLC was performed under standard conditions. The product from D-glucose eluted at 13–15.5 min and was concentrated and lyophilized to give **7** (34.5 μmol, 69%). ^{1}H-NMR (D_2O–DSS, 36 °C) showed open-chain, and β- and α-glycopyranosylhydrazine forms, in a ratio of ~35:60:5, respectively. Major (β) form δ 4.13 (d, J = 9.0 Hz, H-1), 3.92 (dd, J = 1.0, −12.4 Hz), 3.72 (dd, some virtual coupling, J = 5.6, −12.5 Hz), 3.49 (dd, some virtual coupling, J = 9.0, 9.0 Hz), 3.39 (m, tight coupling), 3.35 (dd, J = 9.0, 9.0 Hz). Open-chain form δ 7.30 (d, J = 6.0 Hz, H-1), 4.45 (dd, J = 6.0, 7.1 Hz), 3.99 (dd, J = 1.8, 7.2 Hz)), 3.83 (dd, J = 3.1, −11.6 Hz), 3.78 (ddd, J = 3.0, 6.0, 8.4 Hz), 3.66 (dd, J = 1.7, 8.5 Hz), 3.63 (dd, J = 6.0, −11.7 Hz). Minor (α) form δ 4.79 (d, J = 3.9 Hz, H-1). Aryl protons (all forms) δ 7.43, 7.20 (both m) 7.36, 7.33 (both broad s). FABMS (glycerol + thioglycerol, matrix) m/z 339 $(M + H)^+$, and, with NaOAc, 361 $(M + Na)^+$. HRFABMS calcd for $C_{13}H_{17}N_2O_5F_3Na$ $(M + Na)^+$ 361.0986, found 361.0990. The product from 2,5-anhydro-D-mannose eluted at 11 min and was concentrated and lyophilized to give **11** (11 μmol, 22%). ^{1}H-NMR (D_2O–DSS, 36 °C), two forms observed (presumed hydrazone isomers), major (>95%) δ 7.32 (d, 1H, J = 6.2 Hz, H-1), 4.45 (dd, 1H, J = 6.5, 6.5 Hz), 4.28 (dd, 1H, J = 6.3, 6.3 Hz), 4.13 (dd, 1H, J = 6.2, 6.2 Hz), 4.01 (ddd, 1H, J = 3.4, 5.7, 6.2 Hz), 3.80 (dd, 1H, J = 3.3, −12.4 Hz), 3.73 (dd, 1H, J = 5.7, −12.5 Hz). Minor form δ 6.74 (d, H-1). Aryl signals (major form) δ 7.45 (dd, 1H, J = 8.0, 8.0 Hz), 7.37 (broad s, 1H), 7.23 (d, 1H, J = 8.2 Hz), 7.21 (d, 1H, J = 7.9 Hz), and also showed barely discernible ($J \sim 1$ Hz) 4-bond couplings. HRFABMS (glycerol + thioglycerol, matrix) calcd for $C_{13}H_{16}N_2O_4F_3$ $(M + H)^+$ 321.1062, found 321.1068.

D-Glucose 2-Pyridylhydrazone (8) and 2,5-Anhydro-D-mannose 2-Pyridylhydrazone (12). For **8**, 50 μmol of D-glucose, 100 μL of water, and 100 μL of freshly distilled 2-hydrazinopyridine (liquid when warm) were mixed in a 200 μL vial and warmed under Ar to 40 °C for 24 h. For **12**, 50 μmol of 2,5-anhydro-D-mannose hydrate was treated identically, except at 23 °C. Samples were transferred in about 1.0 mL of water to a larger tube and lyophilized to remove remaining 2-hydrazinopyridine. Orange aryl impurities remained and were also present in a control sample without the sugars. Samples were taken up in 7.0 mL of water (slow to dissolve), extracted 2× with 5.0 mL of 1-butanol, and the water phases were concentrated. Lyophilization from 3.0 mL of water gave **8** (37.5 μmol, 75%) and **12** (19.5 μmol, 39%). For **8**, ^{1}H-NMR (D_2O–DSS, 36 °C) showed equilibrium of the open-chain and β- and α-glycopyranosylhydrazine derivatives in a ratio of ~30:65:5, respectively. Major (β) form δ 4.15 (d, J = 8.9 Hz, H-1), 3.92 (dd, J = 2.1, −12.4 Hz), 3.73 (dd, J = 5.5, −12.2 Hz), 3.52 (dd, J = 8.9, 8.9 Hz), 3.41 (m), 3.37 (dd, J = 9.0, 9.0 Hz). Open-chain form δ 7.36 (d, J = 5.9 Hz, H-1), 4.47 (dd, J = 6.0, 7.2 Hz), 4.00 (dd, J = 1.8, 7.2 Hz), 3.83 (dd, J = 3.1, −11.6 Hz), 3.79 (ddd, J = 3.1, 6.2, 8.4 Hz), 3.66 (dd, J = 1.9, 8.3 Hz), 3.64 (dd, J = 6.2, −11.7 Hz). Minor (α) δ 4.79 (d, J = 3.9 Hz, H-1). Aryl protons (all forms) δ 8.05, 7.69, 7.02, 6.86 (all m). FABMS (3-NBA, matrix) m/z 272 $(M + H)^+$, and with NaOAc added, 294 $(M + Na)^+$. HRFABMS calcd for $C_{11}H_{18}N_3O_5$ $(M + H)^+$ 272.1247, found 272.1251. For **12**, ^{1}H-NMR (D_2O–DSS, 36 °C) two forms observed (presumed hydrazone isomers), major (~95%) δ 7.39 (d, 1H, J = 6.3 Hz, H-1), 4.46 (dd, 1H, J = 6.5, 6.5 Hz), 4.30 (dd, 1H, J = 6.3, 6.3 Hz), 4.13 (dd, 1H, J = 6.1, 6.1 Hz), 4.01 (ddd, 1H, J = 3.2, 5.6, 6.2 Hz), 3.80 (dd, 1H, J = 3.1, −12.4 Hz), 3.73 (dd, 1H, J = 5.6, −12.4 Hz). Minor form δ 7.43, (d, H-1). Major aryl protons δ 8.07 (apparent d, J = 4.2 Hz), 8.01 (apparent d, J = 5.4 Hz), 7.72 (m), 7.05 (d, J = 8.8 Hz), 6.87 (m). FABMS (3-NBA, matrix) m/z 253 $(M + H)^+$, and with NaOAc added, 276 $(M + Na)^+$. HRFABMS calcd for $C_{11}H_{15}N_3O_4Na$ $(M + Na)^+$ 276.0960, found 276.0960.

Deprotection of Pentafluorophenylhydrazones 6, 10, and 14, and Proof of Structure of 19 through Conversion to 24. Deprotection was carried out similarly to that of El Khadem et al. (1972).[9] To **6** (5.0 μmol), **10**, (4.1 μmol), or **14** (5.8 μmol) was added 0.5 mL of a solution prepared by mixing 10 mL of water, 6 mL of ethanol, 0.32 mL of benzaldehyde (Aldrich), and 0.24 mL of acetic acid. Samples were warmed to 50 °C for 5 h, which gave a crystalline product (presumed

to be benzaldehyde pentafluorophenylhydrazone). Solutions were extracted 6× with 0.5 mL diethyl ether, with about 0.3 mL of water added midway to compensate for water lost to the ether phase. The water phase was diluted to 3.0 mL and passed in tandem through 1.0 mL columns of Dowex AG50W, H^+ form, then Dowex AG1, OAc form, washing with an additional 5 × 2.0 mL of water. Eluates were concentrated. Products were from **6**, pure D-glucose **4** (4.4 μmol, 88%), ^{1}H-NMR as described earlier; from **10**, pure **18** (hydrate) (3.7 μmol, 90%), ^{1}H-NMR as described earlier; from **14**, 2,5-anhydro-D-glucose **19**, (5.0 μmol, 86%). ^{1}H-NMR, (D_2O–Ace., 27 °C), decoupling showed two major equilibrating forms (~60:40, attributed to hydrate, and cyclic hemiacetal, respectively. For hydrate, δ 5.14 (d, J = 6.6 Hz, H-1), 4.16 (dd, J = 1.5, 3.7 Hz), 4.08 (dd, J = 1.5, 3.1 Hz), 3.897 (dd, J = 3.7, 6.8 Hz), 3.90 (buried under 3.897, ddd, J = 3.1, 4.3, 5.9 Hz), 3.77 (dd, J = 4.3, −12.2 Hz), 3.72 (dd, J = 5.9, −12.1 Hz). For the hemiacetal, δ 5.05 (J ~ 1.0 Hz, H-1), 4.38 (dd, J = 2.2, <1 Hz), 4.30 (dd, J = 2.0, 6.7 Hz), 4.19 (dd, J = ~1.0, 6.8 Hz), 4.19 (dd, J = 1.9, −11.8 Hz), 4.03 (ddd, J = <1, 1.7, 1.9 Hz), 3.60 (J = 1.7, −11.7 Hz). No data for FABMS. Due to the unusual ^{1}H-NMR data and inability to obtain FABMS data for **19**, it was converted to **23** and then **24**. Reduction of **19** (4.5 μmol), according to the general procedure, gave 2,5-anhydro-D-glucitol **23**. Due to minor impurities in the upfield region of the ^{1}H-NMR spectrum, presumably acquired during workup, HPLC was performed under standard conditions. A peak at 31 min was pure **23** (4.1 μmol, 91%). ^{1}H-NMR (D_2O–Ace., 27 °C) δ 4.19 (dd, 1H, J = 2.6, 4.4 Hz), 4.13 (ddd, 1H, J = 4.4, 4.4, 6.8 Hz), 4.03 (dd, 1H, J = 2.7, 4.3 Hz), 3.85 (ddd, 1H, J = 3.6, 4.4, 5.9 Hz), 3.84 (dd, 1H, J = 4.4, −11.8 Hz), 3.78 (dd, 1H, J = 3.7, −12.0 Hz), 3.74 (dd, 1H, J = 6.7, −11.8 Hz), 3.71 (dd, 1H, J = 5.9, −11.9 Hz). HRFABMS (triethylamine [TEA], matrix) calcd for $C_6H_{11}O_5$ $(M-H)^-$ 163.0607, found 163.0608. Oxidation of 2,5-anhydro-D-mannitol **22** and **23** was carried out similarly to conditions described earlier with **22**,[7] with a different workup. Samples of either **22** (50 μmol) or **23** (3.5 μmol) were dissolved, respectively, in 2.0 mL or 1.0 mL of 0.1 M sodium periodate. After 30 min in the dark at room temperature, samples were diluted with 5.0 mL of water and immediately passed in succession through two columns in tandem: 5.0 mL of Dowex AG1, OAc^- form and then 5.0 mL of Dowex AG50W, H^+ form; followed by 6 × 5 mL washes with water. Eluates were concentrated twice from water to remove residual acetic acid, taken up in 1.0 mL of water, and reduced according to the general procedure. Both **22** and **23** gave essentially quantitatively di-2-glyceryl ether **24**, with no evidence for the starting compounds in ^{1}H-NMR spectra (yields 48 and 3.5 μmol from **22** and **23**, respectively, >96% for both). For **24**, ^{1}H-NMR (D_2O–Ace., 27 °C), not first order, δ 3.71 (m), 3.64 (m), in a ratio of 3H:2H. HRFABMS (diethylamine, matrix) calcd for $C_6H_{13}O_5$ $(M-H)^-$ 165.0763, found 165.0764.

4-Acetamido-4-deoxy-2-*O*-β-D-galactopyranosyl-L-xylose (38), and Its Proof of Structure through Conversion to 39, and 40 Plus 41. The disaccharide 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-D-glucose (**36**) (58.1 μmol) was reduced according to the general procedure, which gave 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-D-glucitol (**37**) (57.9 μmol, 99.7%, characterization data in supporting information). Dimethyl sulfoxide (DMSO, Pierce, silylation grade) and 2,3-butanediol (Aldrich, B8,490-4) were vacuum distilled, glacial acetic acid (Aldrich, 99.99%+) was redistilled, and they were stored at −20 °C. The disaccharitol **37** (52.3 μmol) was dissolved in 4.0 mL of 1/1 (v/v) DMSO/acetic acid (prepared beforehand at 0 °C) and the solution taken to −30 °C (methanol bath) in a flask having a 10 mL sidearm and capable of being stoppered. A solution containing 0.5 mmol of lead tetraacetate (Fluka, free of lead oxide) dissolved in 6.0 mL of 1/1 (v/v) DMSO/acetic acid was introduced into the sidearm. The solutions were cooled for 20 min at −30 °C before starting the reaction by tilting the flask. After 40 min, the flask was tilted to introduce the stopping solution (0.5 mL of 2,3-butanediol, 1.0 mL of DMSO, 1.0 mL of acetic acid) into the sidewarm and to allow it to cool, which was added after a total reaction time of 1 h. After another 1 h (−30 °C), 15 mL of water was added and the solution warmed to room temperature and run through a column (5.0 mL) of Dowex AG50W, H^+ form, followed with 3 × 5.0 mL washes with water. The Dowex was prepared beforehand by soaking (4 °C) in a solution containing DMSO/acetic acid/water, 1/1/3, and the column washed with an additional 10 mL of the same solvent mixture and then water (20 mL). The oxidation product was rotary evaporated (35 °C) to remove volatile materials and then placed at <100 mTorr overnight. HPLC was carried out under standard conditions, except 79/21, MeCN/water was used. A major component at 34 min was 4-acetamido-4-deoxy-2-*O*-β-D-galactopyranosyl-L-xylose (**38**) (48.1 μmol, 92%, quantitated after reduction). ^{1}H-NMR (D_2O-Ace., 27 °C) δ 5.39 (d, J = 3.1 Hz), 4.73 (d, J = 7.8 Hz), 4.71 (d, J = 7.6 Hz), 4.52 (d, J = 7.7 Hz), 3.30 (pentet, possibly dd with virtual coupling), 3.91, 3.87, 3.79, 3.74, 3.68, 3.57 (all m, complex), 2.013 (s), 2.010 (s). HRFABMS (TEA, matrix) calcd for $C_{13}H_{22}NO_{10}$ $(M-H)^-$ 352.1244, found 352.1241. Reduction of **38** to 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-D-xylitol **39**, and hydrolysis/*N*-acetylation of **39** to give 2-acetamido-2-deoxy-D-xylitol (**40**) and D-galactose (**41**) are described in the supporting information.

3-Acetamido-3-deoxy-2-*O*-β-D-galactopyranosyl-L-threose (44) and Its Proof of Structure through Conversion to 45, and 46 Plus 41. The disaccharide 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-D-galactose (**42**) (44.3 μmol) was reduced according to the general procedure, which gave 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-D-galactitol (**43**) (44.3 μmol, 99.9%). ^{1}H-NMR, identical to previous report.[21] FABMS (TEA, matrix) m/z 384 $(M-H)^-$. The disaccharitol **43** (39.9 μmol) was oxidized with lead tetraacetate as described for **37**. HPLC of the product was also performed identically, and the major product at 29 min was concentrated to give 3-acetamido-3-deoxy-2-*O*-β-D-galactopyranosyl-L-threose (**44**) (30.7 μmol, 77%, quantitated after reduction). ^{1}H-NMR (D_2O-Ace., 27 °C) δ 5.49 (d, apparent broad s, J ~ 1 Hz), 5.45 (d, J = 4.3 Hz), 4.97 (d, 4.5 Hz, minor), 4.61 (d, J = 7.6 Hz), 4.53 (d, J = 7.5 Hz), 4.48 (ddd), 4.32 (m), 4.23 (dd, apparent broad s, both J ~ 1–1.5 Hz), 3.92 (m), 3.71 (m), 3.57 (dd, J = 7.6, 10.0 Hz), 3.51 (dd, J = 7.7, 10.0 Hz), 2.03 (s, minor), 2.01 (s). HRFABMS (TEA, matrix) calcd for $C_{12}H_{20}NO_9$ $(M-H)^-$ 322.1138, found 322.1123. Reduction of **44** to 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-L-threitol (**45**), and hydrolysis/*N*-acetylation to give 2-acetamido-2-deoxy-L-threitol (**46**) and D-galactose (**41**) is described in the supporting information.

2-*O*-α-D-Glucopyranosyl-glycolaldehyde (49) and Its Proof of Structure through Conversion to 50, and 4 Plus 51. The disaccharide 6-*O*-α-D-glucopyranosyl-D-glucose (**47**) (51.6 μmol) was reduced according to the general procedure, which gave 6-*O*-α-D-glucopyranosyl-D-glucitol (**48**) (51.6 μmol, >99.9%, characterization data in supporting information). The disaccharitol **48** (46.4 μmol) was oxidized with lead tetraacetate as described for **37**. The sample was warmed in water (50 °C) for 2 h prior to HPLC. It was concentrated, and HPLC was performed under standard conditions, except that MeCN/water, 74/26, was used. A major product at 19.5 min, which tailed, was concentrated to give **49** (hydrate, 38 μmol, 82%, quantitated after reduction). ^{1}H-NMR (D_2O–Ace., 36 °C) δ 5.22 (dd, glycolaldehyde hydrate H-1, 1H, J = 4.8, 4.8 Hz), 4.96 (d, α-Glc H-1, 1H, J = 3.8 Hz), 3.86 (dd, 1H, J = 2.0, −11.9 Hz), 3.55 (dd, 1H, J = 3.8, 9.7 Hz), 3.51 (dd, 1H, J = 5.2, 10.6 Hz), 3.41 (dd, 1H, J = 9.5, 9.5 Hz), 3.72 (m, 4H). HRFABMS (TEA, matrix) calcd for $C_8H_{13}O_7$ $(M-H)^-$ 221.0661, found 221.0661. Reduction of **49** to α-D-glucopyranosyl-ethanediol (**50**), and hydrolysis to give D-glucose **4** and ethylene glycol (**51**) is described in the supporting information.

2-*O*-α-D-Glucopyranosyl-L-threose (54) and 2-*O*-α-D-Glucopyranosyl-D-arabinose (55) and Proof of Their Structures through Conversion to 56 and 4 Plus 57, and 58 and 4 Plus 59, Respectively. The disaccharide 3-*O*-α-D-glucopyranosyl-D-glucose (**52**) (97.6 μmol) was reduced according to the general procedure, which gave 3-*O*-α-D-glucopyranosyl-D-glucitol **53** (96.5 μmol, 99%, characterization

(21) Kamerling, J. P.; Vliegenthart, J. F. G., *Biol. Magn. Reson.* **1992**, *10*, 1.

data in supporting information). The disaccharitol **53** (43.9 μmol) was oxidized with lead tetraacetate as described for **37**. The product was concentrated, and HPLC was performed under standard conditions, except MeCN/water, 74/26, was used. A product eluting at 21 min was pure 2-*O*-α-D-glucopyranosyl-L-threose (**54**) (36.8 μmol, 84%, quantitated after reduction). ^{1}H-NMR (D_2O–Ace., 27 °C) showed cyclic forms and possible hydrate; δ 5.52 (d, J = 4.1 Hz), 5.39 (d, J = 1.4 Hz), 5.30 (d, J = 3.9 Hz, minor), 5.27 (d, J = 8.5 Hz), 5.20 (d, J = 5.2 Hz, minor), 5.19 (d, J = 3.4 Hz), 5.09 (d, J = 3.7 Hz), 5.06 (d, J = 3.9 Hz), 4.49 (ddd), 4.42 (ddd), 4.12 (dd, J = 4.3, 4.3 Hz), 4.09 (dd, both $J \sim 1.7-2.3$ Hz), 3.96 (dd, J = 4.2, 10.0 Hz), 3.88 (dd, J = 1.8, −12.0 Hz), 4.21, 3.73, 3.57, 3.44 (all m). HRFABMS (TEA, matrix) calcd for $C_{10}H_{17}O_9$ $(M - H)^-$ 281.0873, found 281.0870. A product eluting at 25 min showed (^{1}H-NMR) one major component and a minor one. The major component was isolated by rechromatography (twice) as above, except MeCN/water 77/23 was used, which gave at 32.5 min 2-*O*-α-D-glucopyranosyl-D-arabinose **55** (3.8 μmol, 9%, quantitated after reduction). ^{1}H-NMR (D_2O-Ace., 27 °C) showed cyclic forms; δ 5.42 (d, J = 3.7 Hz), 5.41 (d, J = 2.4 Hz), 5.38 (d, J = 4.5 Hz), 5.25 (d, J = 3.8 Hz), 5.15 (d, J = 3.8 Hz), 5.13 (d, J = 4.2 Hz), 5.12 (d, J = 4.1 Hz), 4.25 (dd, minor, J = 7.6, 7.6 Hz), 3.42–4.17 (complex series of overlapping signals, see supporting information). HRFABMS (TEA, matrix) calcd for $C_{11}H_{19}O_{10}$ $(M - H)^-$ 311.0978, found 311.0982. Reduction of **54** and **55** to give, respectively, 2-*O*-α-D-glucopyranosyl-L-threitol (**56**) and 2-*O*-α-D-glucopyranosyl-D-arabinitol (**58**), and hydrolysis of **56** and **58** to give D-glucose and, respectively, L-threitol **57** and D-arabinitol **59**, is described in the supporting information.

2-*O*-[6-*O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-galactopyranosyl]-D-erythrose (62) and Its Proof of Structure through Conversion to 64, and 41 Plus 65 Plus 66. The trisaccharide 4-*O*-[6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-galactopyranosyl]-D-glucose (**60**) (33.4 μmol) was reduced according to the general procedure, giving 4-*O*-[6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-galactopyranosyl]-D-glucitol (**61**) (33.3 μmol, 99.7%, characterization data in supporting information). The trisaccharitol **61** (10.0 μmol) was dissolved in 1.0 mL of water, and with 3.2 mL of acetic acid and 12.3 mL of acetone, was taken to −73 °C in a flask having a 10 mL sidearm and capable of being stoppered. Lead tetraacetate (0.5 mmol) was added, and the solution was stirred at −73 °C in a methanol bath. A stopping solution of 0.48 mL of water, 1.55 mL of acetic acid, 5.95 mL of acetone, and 0.28 mL of ethylene glycol was added to the sidearm after about 1.5 h and allowed to cool prior to mixing with the reaction solution after a total time period of 2 h. After an additional 1 h at −73 °C, the solution was removed from the bath and 20 mL of water added. It was concentrated to ~5 mL and run through a column of Dowex AG50W, H^+ form, followed with 3 × 5 mL washes with water. Eluate was concentrated to ~3 mL and chromatographed on a well-washed Sephadex G-10 column (1.5 × 95 cm), collecting 3.0 mL fractions. Product was concentrated, and HPLC was performed under standard conditions except MeCN/water, 77/23, was used. A major peak at 37 min was **62** (9.3 μmol, 93%, quantitated after reduction). ^{1}H-NMR (D_2O-Ace., 36 °C) showed cyclic forms and possible hydrate formation at the reducing erythrose, δ 5.44 (d, J = 3.0 Hz), 5.31 (d, J = 4.6 Hz), 5.18 (d, J = 1.9 Hz), 4.21 (dd, J = 4.9, 9.9 Hz), 4.16 (dd, J = 3.2, 4.6 Hz), 4.08 (dd, J = 4.8, 10.1 Hz), 3.45–4.03 (complex series of overlapping signals, see supporting information), 2.06 (s), 2.04 (s). HRFABMS (TEA, matrix) calcd for $C_{18}H_{30}NO_{14}$ $(M - H)^-$ 484.1667, found 484.1670. Reduction of **62** to give 2-*O*-[6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-galactopyranosyl]-D-erythritol (**64**) and hydrolysis/*N*-acetylation of **64** to give D-galactose (**41**), 2-acetamido-2-deoxy-D-glucose (**65**), and erythritol (**66**) is described in the supporting information.

3-*O*-α-D-Glucopyranosyl-D-*arabino*-2-hexosulose Bis(phenylhydrazone) (67) and 3-*O*-β-D-Galactopyranosyl-D-*erythro*-2-pentosulose Bis(Phenylhydrazone) (70) and Their Proof of Structure through Conversion to the Phenylosotriazoles 68 and 71. Preparation of both types of derivatives was performed similarly to previous protocols applied to other mono- and disaccharides.[10] To 3-*O*-β-D-galactopyranosyl-D-arabinose (**69**) (Sigma, 330 μmol), phenylhydrazine hydrochloride (1.1 mmol), and NaOAc (1.0 mmol) was added 2.0 mL of water, and the sample was heated under N_2 to 70 °C for 6 h. The yellow precipitate was filtered, washed with 5 mL of water, and taken up in 5.0 mL of hot methanol. Crystals appeared overnight at −20 °C, whereupon 5.0 mL of water was added to enhance further crystallization. The product was filtered and washed with 10 mL of methanol/water, 1/1 (0 °C), before drying, which gave **70** (133 μmol, 40.3%). ^{1}H-NMR (DMSO-d_6, 2% D_2O, TMS, 24 °C, after equilibration for 2 days) showed one major open-chain form only, δ 7.73 (s, 1H), 7.34 (dd, 2H, J = 7.5, 8.5 Hz), 7.31 (dd, 2H, J = 7.6, 8.5 Hz), 7.14 (d, 2H, J = 8.3 Hz), 7.01 (d, 2H, J = 8.4 Hz), 6.87 (dd, 1H, J = 7.4 Hz, 7.4 Hz), 6.86 (dd, 1H, J = 7.4, 7.4 Hz); downfield (aryl) protons showed barely discernable ($J \sim 1$ Hz) 4-bond couplings, 4.29 (d, 1H, J = 7.1 Hz), 4.21 (d, 1H, J = 6.8 Hz), 3.79 (ddd, 1H, J = 4.4, 6.0, 6.2 Hz), 3.63 (m, 2H), 3.47, 3.29 (both m, 3.40–3.42 obscured by HOD peak). FABMS (3-NBA, matrix) m/z 491 $(M + H)^+$, and with NaOAc added, 513 $(M + Na)^+$. HRFABMS calcd for $C_{23}H_{31}N_4O_8$ $(M + H)^+$ 491.2142, found 491.2144. To **52** (100 μmol), 360 μmol phenylhydrazine hydrochloride, and 330 μmol of NaOAc was added 0.67 mL of water, and the sample was heated under N_2 at 70 °C for 14 h. Upon cooling a yellow precipitate was observed which was filtered and washed with 5.0 mL of water. Product was dissolved in a minimum (0.4 mL) of ethanol at 65 °C and then filtered hot, which upon cooling gave yellow crystals. They were washed with 2.0 mL of ice-cold ethanol, giving **67** (41.6 μmol, 41.6%). ^{1}H-NMR (DMSO-d_6, 2% D_2O, TMS, 36 °C, after equilibration for 2 days) showed one major open-chain form only, 7.83 (s, 1H), 7.34 (dd, 2H, J = 7.5, 8.5 Hz), 7.30 (dd, 2H, J = 7.5, 8.5 Hz), 7.14 (d, 2H, J = 8.5 Hz), 7.01 (d, 2H, J = 8.6 Hz), 6.87 (dd, 1H, J = 7.5, 7.5 Hz), 6.86 (dd, 1H, J = 7.5, 7.5 Hz); downfield (aryl protons) showed barely discernible ($J \sim 1$ Hz) 4-bond couplings, 4.88 (d, 1H, J = 3.8 Hz), 4.47 (d, 1H, J = 3.4 Hz), 3.63 (m, 3H), 3.54 (dd, 1H, J = 3.5, 7.9 Hz) 3.41–3.52 (complex m, 4H) 3.36 (m, 1H, partially obscured by HOD peak), 3.21 (m, 2H). HRFABMS (3-NBA, with NaOAc added), calcd for $C_{24}H_{32}N_4O_9Na$ $(M + Na)^+$ 543.2067, found 543.2068. Due to the unusual properties of bis(phenylhydrazones),[10] **67** and **70** were converted to their respective phenylosotriazole derivatives 3-*O*-α-D-glucopyranosyl-D-*arabino*-hexose phenylosotriazole (**68**) and 3-*O*-β-D-galactopyranosyl-D-*erythro*-pentose phenylosotriazole (**71**) which gave unambiguous characterization data (reported in supporting information).

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Supporting Information Available: ^{1}H-NMR spectra (500 MHz) for compounds **6–8**, **10–12**, **14**, **15**, **18–24**, **29–32**, **37–40**, **43–46**, **48–50**, **53–59**, **61–64**, **66–68**, **70**, and **71**. ^{1}H-NMR data (chemical shifts and coupling constants) for **4**, **18**, **21**, **22**, **30–32**, **35**, **37**, **39**, **40**, **41**, **43**, **45**, **46**, **48**, **50**, **51**, **53**, **56–59**, **61**, **63–66**, **68**, **71**. ESMS data for **18**. FABMS data for **37**, **48**, **53**, **61**. HRFABMS data for **30**, **31**, **32**, **39**, **40**, **45**, **46**, **50**, **56**, **58**, **64**, **68**, **71**. Experimental detail for preparation and/or isolation of **30–32**, **39–41**, **45**, **46**, **50**, **51**, **56–59**, **64–66**, **68**, **71** (55 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

Note Added in Proof: The hydrazino derivative (2,3,5,6-tetrafluorophenyl)hydrazine (Aldrich) reacted similarly to (pentafluorophenyl)hydrazine with **1**, giving the (2,3,5,6-tetrafluorophenyl)hydrazone derivatives of **4**, **18**, and **19**.

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