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Synthesis of di- and tri-saccharides with intramolecular NH-glycosidic linkages: molecules with flexible and rigid glycosidic bonds for conformational studies

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ABSTRACT

Attempted dephthalimidation of the trisaccharide 1-*O*-acetyl-3,4-di-*O*-benzyl-2,6-di-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-α-D-mannopyranose (**1**) and its derivatives **2** and **3**, as well as the disaccharide 1-*O*-acetyl-3,4,6-tri-*O*-benzyl-2-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-α-D-mannopyranose (**13**), with hydrazine hydrate in ethanol at 80°C, produced the trisaccharide-6-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3,4-di-*O*-benzyl-β-D-mannopyranose-3′,4′,6′-tri-*O*-acetyl-β-D-glucopyranose 1,2′-*N*:1′,2-*O*-dianhydride (**4**) and 3,4,6-tri-*O*-benzyl-β-D-mannopyranose 3′,4′,6′-tri-*O*-acetyl-β-D-glucopyranose 1,2′-*N*:1′,2-*O*-dianhydride (**14**), respectively, containing an intramolecular NH-glycosidic linkage. The conventional deblocking of compounds **4** and **14** gave the completely deblocked trisaccharide 6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-mannopyranose β-D-glucopyranose 1,2′-*N*:1′,2-*O*-dianhydride (**6**) and the disaccharide β-D-mannopyranose β-D-glucopyrannose 1,2′-*N*:1′,2-*O*-dianhydride (**16**), respectively, containing an intact intramolecular NH-glycosidic bond. The unusual intra NH-glycosyl character makes the linkage rigid, and therefore these compounds should not only be useful for NMR studies but also as substrates or inhibitors of GlcNAc-transferases.

INTRODUCTION

The extent of flexibility about the glycosidic linkages of oligosaccharides is still controversial[1]. It is therefore of particular interest to be able to generate oligosaccharides in which one glycosidic linkage is fixed while another remains potentially flexible. Such model compounds provide ideal test cases for the development of

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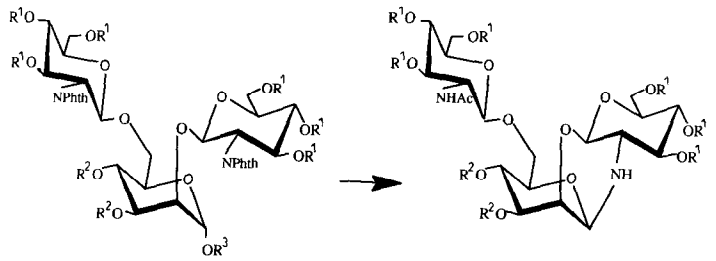
methods for the detection of internal flexibility. For example, it has recently been demonstrated[2,3], for disaccharides, that internal flexibility can be detected from the differential dependence on magnetic field-strength of NOEs (nuclear Overhauser effects). NOE measurements on the title tri- and di-saccharides, having locked glycosidic linkages, should permit direct verification of this proposed detection method. In addition to the foregoing, these oligosaccharides should also be useful in testing theories regarding the molecular basis for the thermodynamic parameters measured for the binding of oligosaccharides to proteins[4,5]. Very few examples of intramolecular glycosylation have been reported in the literature[6–8]. We report herein a synthesis of di- and tri-saccharides with dianhydride bridges involving intramolecular glycosylation.

RESULTS AND DISCUSSION

As part of a program to investigate the conformational properties of N-linked oligosaccharides of glycoproteins[9], we have attempted to synthesize fragments[10,11] of the larger, naturally occurring, complex type of glycans[12–15]. In many of these oligosaccharides, 2-acetamido-2-deoxy-D-glucosamine (GlcNAc) and 2-acetamido-2-deoxy-D-galactosamine (GalNAc) occur. When these sugars are to be β-linked, the phthalimido group is well suited as a blocking group for the amino function since it participates in the glycosylation reaction and hence promotes stereoselectivity[16]. In addition, the phthalimido group provides complete substitution of the primary amine, removing both acidic protons and thus diminishing the nucleophilic character of the nitrogen. However, problems are sometimes encountered in conversion of the phthalimido group into free amine[17]. Conventional deprotection[16] involves hydrazinolysis by hydrazine hydrate. A relatively mild and efficient method[17,18] has been reported where sodium borohydride is used for the reductive opening of the phthalimido group.

While attempting the synthesis of a pentasaccharide[10] with high stereoselectivity, we required synthon **1** (refs 10 and 19) as a glycosylating agent. Investigation of the factors influencing the stereoselectivity of glycosylation of **1** also required its modification[10], for example, by replacing *O*-benzyl groups by *O*-acetyl and phthalimido groups by NHAc groups. For the latter (Scheme 1), compound **1** was hydrazinolyzed with hydrazine hydrate in ethanol at 80°C, which with subsequent acetylation produced **4** in 86% yield. NMR and mass-spectroscopic analysis of **4** revealed an unexpected intramolecular NH_2 glycosylation. As an alternative, the reduction approach[17,18] by sodium borohydride was used. This method is not applicable for compound **2** since the free reducing sugar opens to an aldehyde, which would then be reduced to the corresponding alditol. The method failed to remove the phthalimido group in compound **3**.

One- and two-dimensional ^1H NMR spectra were acquired for the preliminary assignments of the trisaccharide **4**. Several features of the ^1H NMR spectrum suggested that the dephthalimidation–acetylation reactions had proceeded differ-



	R^1	R^2	R^3
1	Ac	Bn	Ac
2	Ac	Bn	H
3	Ac	Bn	Bz

	R^1	R^2
4	Ac	Bn
5	Ac	Ac
6	H	H

Scheme 1.

ently than expected (Fig. 1). Only seven acetyl resonances, rather than the expected nine, were observed at ~2 ppm. The chemical shift for the H-1 resonance for one of the glucosamine rings appears at lower field than expected (at 5.110 ppm) and the 2D COSY experiment connected it to its corresponding H-2 resonance at 3.277 ppm, which, in turn, correlated with a doublet from an NH resonance at 5.522 ppm, J 7.7 Hz. The latter, and the remaining chemical shifts for H-3 through H-6 are consistent with a typical peracetylated 2-acetamido-2-deoxy-β-D-glucose structure. However the chemical shift for the H-1 and H-3 resonances of the second peracetylated glucosamine appear at 4.317 and 4.930 ppm, respectively. The latter are 0.6–0.8 ppm higher field, compared to the corresponding protons on the other glucosamine ring. Finally the anomeric hydrogen resonance of α-D-mannose (α-Man) acetylated at the anomeric carbon is expected to appear at ~6.1 ppm (personal observations). Instead, the anomeric hydrogen resonance of **4** was identified in the 2D COSY experiment as a singlet at

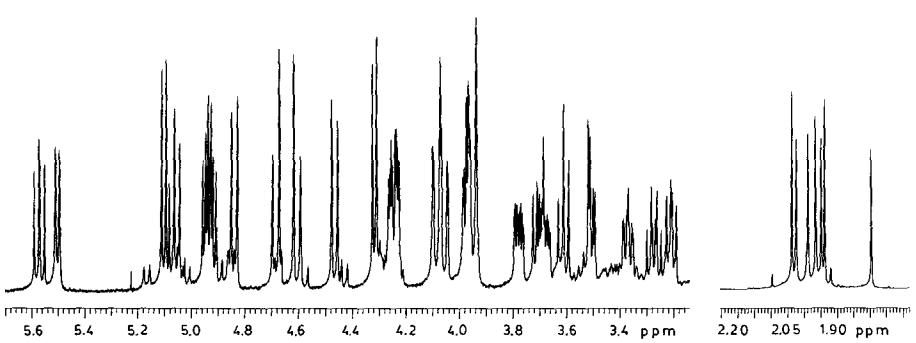


Fig. 1. Expansions of a 500-MHz ^{1}H NMR spectrum of **4** in $CDCl_3$ at 26°C showing the resonances of the sugar ring protons and acetyl groups. The vertical scale of the portion of the spectrum showing the acetyl groups is 1/10th that for the ring protons.

3.940 ppm. The corresponding C-1 shift from an HMQC experiment was 80.67 ppm. The appearance of both of these shifts so far upfield relative to an alkylated or an unsubstituted mannose strongly suggested that the anomeric carbon was perhaps substituted by an amino group.

To account for all of the foregoing observations, the proposed structure of **4** is one where a 3,4-di-*O*-benzylated mannose is substituted at the 6 position with a peracetylated 2-acetamido-2-deoxy-β-D-glucose, as anticipated by the dephthalimidation–acetylation reactions. The latter, however, did not proceed as expected for the conversion of the phthalimido-protected β-D-glucosamine linked at the 2 position of the α-D-mannose. It would appear that, under the conditions of the reaction, the following may have occured: in the initial stage the anomeric acetate is rapidly hydrolyzed to give **2**, the ring opens and the aldehyde forms a Schiff base[20–25] with the intramolecular amine and subsequently closes to form **4**. The stereoselective formation of the β-amine linkage is presumably due to its greater stability[26]. Formation of the α anomer would require either ring-flipping of the mannose residue or the formation of sterically impossible *trans*-1,2-diaxially fused six-membered rings.

In order to prove conclusively the existence of a cyclic structure, a heteronuclear $^{1}H-^{13}C$ correlation experiment (HMQC) was acquired to establish the connectivities between the proton and the carbon resonances, especially those involved in the cyclic portion, namely the C-1–H-1, C-2–H-2 of both glucosamine residues as well as those of the mannose. Both 1D long-range heteronuclear INEPT experiments[27] and 2D HMBC[28] were carried out to show the presence of specific 3-bond $^{13}C-^{1}H$ couplings, which would only be seen if the cyclic structure were present. In the first experiment, each ^{1}H pulse was semiselective ($\gamma B_2/2\pi =$ 20 Hz), affecting only the proton resonance of interest. The appearance of a ^{13}C peak(s) in this experiment confirms that a long-range coupling exists between the irradiated proton and the observed carbon peaks. It is clear that the correct assignment of the ^{13}C spectrum is crucial. In this case, the minimum ^{13}C assignments to prove the cyclic structure are for C-1 and C-2 for both β6 and β2 glucosamines as well as for the mannose residue. The C-1/C-2 pairs are 100.16/56.35, 99.67/53.20 and 80.67/72.89 ppm, respectively.

Hence, two key experiments were carried out to prove the intramolecular cyclic structure; the first to establish the existence of a long-range 3-bond $^{13}C-^{1}H$ coupling from C-1 of the β-D-mannose across the nitrogen bridge to H-2 of the β-2-GlcNH; the second, from C-1 of the β-2-GlcNH across the glycosidic bond to H-2 of the mannose. The delays in the semiselective 1D INEPT were adjusted to optimize the long-range $^{13}C-^{1}H$ couplings, which are normally 4–6 Hz.

As anticipated, irradiation of H-1 of the β-Man resulted in the appearance of peaks corresponding to β-2GlcNH-C-2 (3-bond) as well as peaks corresponding to β-Man-C-2 (2-bond). The inverse experiment consisting of the irradiation of the β-2GlcNH-H-2 resulted in carbon peaks whose shifts corresponded to β-2GlcNH-C-1 and C-3 (2-bond) and β-Man-C-1 (3-bond). These two experiments confirm

that the C-2 of the glucosamine labelled as β-2GlcNH is linked to the mannose-C-1 through NH. Proof that the original (1 → 2)-β-glycosidic linkage between β-2GlcNH and the mannose was still intact comes from the irradiation of the Man-H-2, which produced the expected peak at the β-2-GlcNH-C-1 chemical shift (3-bond). Because the Man-H-2 resonance overlaps with that of Man-H-1 and Man-H-6, all three signals were irradiated in the latter experiment, resulting in additional carbon peaks corresponding to Man-C-2, and Man-C-3, Man-C-4, Man-C-5 and β-6GlcNH-C-1.

It is known that an acetyl group at C-1 of a mannose moiety (e.g., compound **1**) may not survive the mild base hydrazine hydrate; however it was expected that the reacetylation of the dephthalimidated compound would eventually produce the correct synthon. When it was found that the attempt at dephthalimidation of **1** led to the formation of an intramolecular six-membered ring with an NH-glycosidic bond, **3** was chosen as a precursor that might avoid the intraglycosidic reaction. The choice of a benzoyl groups at C-1 of the mannose unit in **3** was made because it is more stable than acetate under mild basic conditions. However, the formation of **4** from **3** in 67% yield after treatment with hydrazine hydrate shows that the benzoyl group at the anomeric carbon atom of mannose was not a good choice. Hydrogenolytic deprotection of the benzyl groups in **4** with hydrogen over palladium-on-charcoal and subsequent acetylation with pyridine–acetic anhydride gave fully acetylated **5** in 65% yield. Verification of the structure of **5** by NMR spectroscopy was simplified, as compared with **4** (see Experimental). In addition, Zemplén deacetylation of **5** produced completely deblocked **6** in 82% yield and high purity, with an intact intramolecular NH-glycosidic bond. This bond was again confirmed unequivocally by an HMBC experiment. Fig. 2 shows the connectivity between proton β-Man-H-1 and carbon β-Man-C-2 (two-bond coupling within the same unit) as well as connectivity through the NH-linkage to carbon β-2GlcNH-C-2.

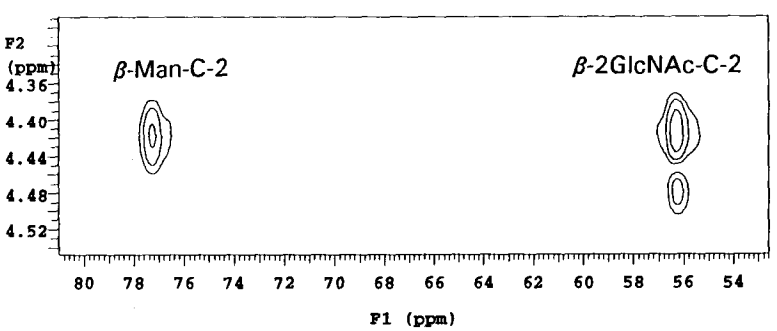
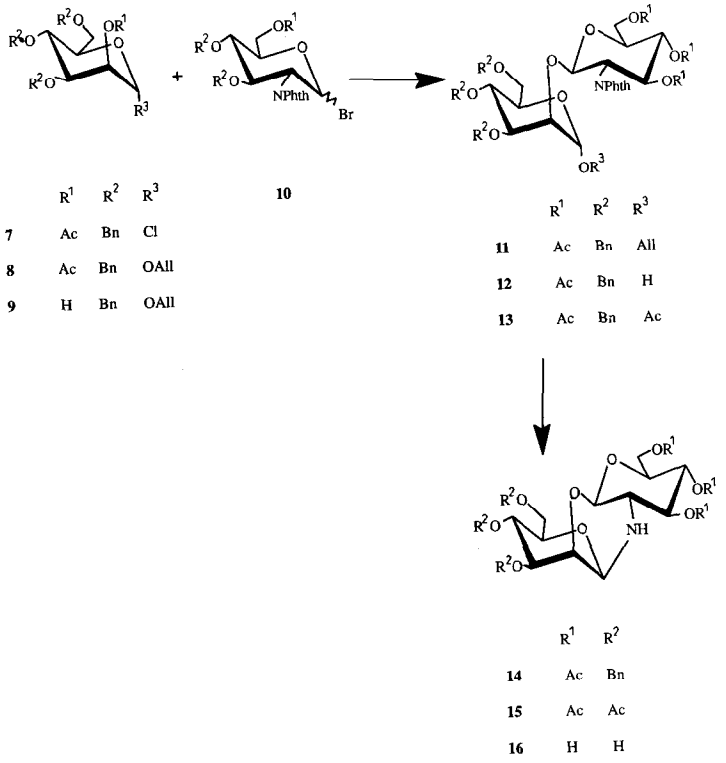


Fig. 2. An expansion of a 500-MHz absolute-value mode 2D HMBC spectrum of **6** in D_2O obtained at 30°C. Correlation peaks show connectivities between the β-Man-H-1 (4.424 ppm) and carbons β-Man-C-2 (77.17 ppm, two-bond coupling within the same unit) and β-GlcNAc-C-2 (56.20 ppm, three-bond coupling across the NH linkage) indicating that the NH-glycosidic linkage stayed intact after deblocking of the protecting groups.



Scheme 2.

The possible utility of the deblocked **6** for conformational studies and for GlcNAc-transferase enzymatic[29] studies encouraged us to explore the synthesis of disaccharide **16** (Scheme 2). The starting compound **9** required for this was prepared first by coupling chloride[30] **7** with allyl alcohol using mercuric cyanide, which gave **8** in 52% yield after chromatographic purification. Compound **8** was then converted into **9** in 95% yield by deacetylation, as described for **6**. The NMR data for **8** and HRMS for **9** given in the experimental section verify the structure. The silver triflate-catalyzed glycosylation of **9** with bromide **10** in acetonitrile gave the protected disaccharide **11** in 54% yield. The deallylation[31,32] of **11** with $PdCl_2$ and subsequent acetylation with pyridine–acetic anhydride produced in 47% yield the disaccharide **13**, which is the precursor for disaccharide **14**. As expected, when compound **13** was subjected to hydrazine hydrate in ethanol as for **1**, deprotection of the phthalimido function proceeded, presumably via the amine, to form the intramolecular NH-glycosidic linkage to the anomeric C-1 of α-D-mannose. Compound **14** thus obtained has the β-D-mannosyl configuration and, in addition, an intramolecular NH-glycosidic linkage to C-2 of the 2-amino-2-deoxy-D-glucose sugar unit. The structure of **14**, and the existence of the intracyclic NH-glycosidic linkage in particular, was confirmed by NMR spectroscopy experiments as per-

formed for trisaccharide **4**. The HRMS data of **14** are in agreement with the described structure. Applying the sequential deblocking procedures described for **4** (dehydrogenation, acetylation, and deacetylation) to **14** produced fully deblocked disaccharide **16** having an intact intramolecular NH-glycosidic linkage. The structure of **16** was again confirmed by NMR and FAB mass spectroscopy as described for the fully deblocked trisaccharide **6**.

EXPERIMENTAL

General methods.—Optical rotations were measured with a Perkin–Elmer polarimeter (model 140) at 25°C. ^{1}H NMR and ^{13}C NMR spectra were recorded at 300 K in $CDCl_3$ and D_2O with Bruker AM 500 and AM 300 MHz spectrometer with Me_4Si as the internal reference for solutions in $CDCl_3$ and internal acetone for D_2O solutions. Ring-proton assignments were made by first-order analysis of the spectra and were supported by homonuclear decoupling and two-dimensional COSY experiments. The complete assignment of ^{13}C resonances was based on HMQC experiments. Mass spectra were obtained on a VG-Analytical ZAB-SE spectrometer. The samples were ionized by fast-atom bombardment (FAB). The compounds required for analytical purposes were purified by HPLC. All reagents were purchased from Aldrich and solvents from BDH, which were dried by conventional methods.

6-O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3,4-di-O-benzyl-β-D-mannopyranose-3′,4′,6′-tri-O-acetyl-β-D-glucopyranose 1,2′-N : 1′,2-O-dianhydride (**4**).—A solution of compound[19] **1** (150 mg, 0.12 mmol) in EtOH (50 ml, 95%) was stirred for 5 h at 80°C in the presence of hydrazine hydrate (5 mL, 85%). The solution was cooled and concentrated to a syrupy product which, after drying by azeotropic distillation with toluene, was acetylated with 1:1 Ac_2O–pyridine (20 mL) overnight at room temperature. After concentration, the product was purified on silica gel with 1:1 $CHCl_3$–acetone to yield **4** (100 mg, 86%); $[\alpha]_D$ +3° (*c* 0.46, $CHCl_3$); ^{1}H NMR: δ 5.572 (dd, 1 H, $J_{3'',4''}$ 9.4 Hz, H-3′), 5.522 (d, 1 H, $J_{NH'',2''}$ 7.7 Hz, NH″), 5.110 (d, 1 H, $J_{1'',2''}$ 8.2 Hz, H-1″), 5.063 (t, 1 H, $J_{4',5'}$ 9.6 Hz, H-4′), 4.939 (t, 1 H, $J_{4'',5''}$ 9.6 Hz, H-4″), 4.930 (t, 1 H, $J_{3',4'}$ 9.4 Hz, H-3″), 4.842 and 4.473 (AB quartet, 2 H, J_{gem} 11 Hz, Ph-CH_2), 4.686 and 4.60 (AB quartet, 2 H, J_{gem} 12.3 Hz, Ph-CH_2), 4.317 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1′), 4.253 (dd, 1 H, $J_{6'a,6'b}$ 12.3 Hz, H-6′a), 4.243 (dd, 1 H, $J_{6''a,6''b}$ 12.2 Hz, H-6″a), 4.090 (dd, 1 H, $J_{6'b,6'a}$ 12.3 Hz, H-6′b), 4.060 (dd, 1 H, $J_{6''b,6''a}$ 12.2 Hz, H-6″b), 3.974 (dd, 1 H, $J_{6a,6b}$ 11.8 Hz, H-6a), 3.973 (d, 1 H, $J_{2,3}$ 3.5 Hz, H-2), 3.940 (s, 1 H, $J_{1,2} < 1$ Hz, H-1), 3.779 (ddd, 1 H, $J_{5',6'a}$ 4.34 Hz, $J_{5',6'b}$ 2.0 Hz, H-5′), 3.70 (dd, 1 H, $J_{6b,6a}$ 11.8 Hz, H-6b), 3.678 (ddd, 1 H, $J_{5'',6''a}$ 3.1, $J_{5'',6''b}$ 2.3 Hz, H-5″), 3.615 (t, 1 H, $J_{4,5}$ 9.5 Hz, H-4), 3.510 (dd, 1 H, $J_{3,4}$ 9.2 Hz, H-3), 3.373 (ddd, 1 H, $J_{5,6a}$ 1.9, $J_{5,6b}$ 7.4 Hz, H-5), 3.277 (dd, 1 H, $J_{2'',3''}$ 10.2 Hz, H-2″), 3.212 (dd, 1 H, $J_{2',3'}$ 10.5 Hz, H-2′); ^{13}C NMR: δ 100.16 (C-1″), 99.67 (C-1′), 80.67 (C-1), 80.23 (C-3), 76.78 (C-5), 74.38 (C-4), 73.84 (C-5′), 73.69 (C-6), 72.89 (C-2), 71.72 (C-5″), 71.27 (C-3″ and C-4′), 69.01 (C-4″), 68.70

(C-3′), 62.32 (C-6′), 62.26 (C-6″), 56.35 (C-2″), 53.20 (C-2′); HRMS: *m/z* 959.3649 (−1.2 mmu, $C_{46}H_{59}N_2O_{20}$, MH^+). Anal. Calcd. for $C_{46}H_{58}N_2O_{20} \cdot H_2O$: C, 56.5; H, 6.2; N, 2.8. Found: C, 56.4; H, 5.8; N, 2.8.

6-O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3,4-di-O-acetyl-β-D-mannopyranose-3′,4′,6′-tri-O-acetyl-β-D-glucopyranose 1,2′-N : 1′,2-O-dianhydride (**5**).—A solution of **4** (100 mg, 0.1 mmol) in 13 : 5 : 1 EtOAc–EtOH–water (19 mL) was hydrogenated over Pd–C (10%, 90 mg) at 45 lb.in.$^{-2}$ for 3 days, and then filtered and concentrated. The syrupy residue was dried in the usual way an acetylated with 1 : 1 pyridine–Ac_2O (8 mL) overnight at room temperature. Column chromatography (2 : 1 $CHCl_3$–acetone) of the product after concentration gave **5** (58 mg, 65%); $[\alpha]_D$ −12.8° (*c* 1.45, $CHCl_3$); ^{1}H NMR: δ 5.736 (NH′), 5.465 (dd, 1 H, $J_{3',4'}$ 9.5 Hz, H-3′), 5.253 (t, 1 H, $J_{4'',5''}$ 10.5 Hz H-4″), 5.120 (t, 1 H, $J_{4,5}$ 9. Hz, H-4), 5.040 (t, 1 H, $J_{4',5'}$ 9.8 Hz, H-4′), 5.010 (dd, 1 H, $J_{3,4}$ 9.9 Hz, H-3), 5.0 (t, 1 H, $J_{3'',4''}$ 10.1 Hz, H-3″), 4.920 (d, 1 H, $J_{1',2'}$ 8.2 Hz, H-1′), 4.410 (d, 1 H, $J_{1'',2''}$ 7.9 Hz, H-1″), 4.310 (dd, 1 H, $J_{6'a,6'b}$ 12.2 Hz, H-6′a), 4.280 (dd, 1 H, $J_{6a,6b}$ 10 Hz, H-6a), 4.220 (s, 1 H, $J_{2,3}$ 3 Hz, H-2), 4.20 (dd, 1 H, $J_{6b,6a}$ 10 Hz, H-6b), 4.140 (dd, 1 H, $J_{6'b,6'a}$ 12.3 Hz, H-6′b), 3.850 (dd, 1 H, $J_{6''a,6''b}$ 11.7 Hz, H-6″a), 3.830 (ddd, 1 H, $J_{5,6a}$ 5.7. $J_{5,6b}$ 3 Hz, H-5), 3.730 (ddd, 1 H, $J_{5',6'a}$ 4.7, $J_{5',6'b}$ 2.35 Hz, H-5′), 3.660 (dd, 1 H, $J_{6''b6''a}$ 11.7 Hz, H-6″b), 3.617 (dd, 1 H, $J_{2',3'}$ 10.4 Hz, H-2′), 3.580 (ddd, 1 H, $J_{5'',6''a}$ 3, $J_{5'',6''b}$ 7.5 Hz, H-5″), 3.290 (dd, 1 H, $J_{2'',3''}$ 10.2 Hz, H-2″), 3.079 (s, 1 H, $J_{1,2}$ < 1 Hz, H-1); HRMS; *m/z* 863.2933 (−1.1 mmu, $C_{36}H_{51}N_2O_{22}$, MH^+), 885.2753 (−0.2 mmu, $C_{36}H_{50}N_2NaO_{22}$, MNa^+).

6-O-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-mannopyranose β-D-glucopyranose 1,2′-N : 1′,2-O-dianhydride (**6**).—Compound **5** (20 mg, 0.23 mmol) in MeOH (5 mL) was deacetylated with NaOMe (33 mg Na in 15 mL MeOH) for 4 h at room temperature. The solution was made neutral with mixed-bed resin (1 mL, BioRad AG501-X8), filtered and evaporated to dryness to give **6** (10 mg, 82%); $[\alpha]_D$ −6° (*c* 0.26, water); ^{1}H NMR: δ 4.603 (d, 1 H, $J_{1'',2''}$ 8.5 Hz, H-1″), 4.467 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1′) 4.424 (s, 1 H, $J_{1,2}$ < 1 Hz, H-1), 4.126 (dd, 1 H, $J_{6a,6b}$ 11.8 Hz, H-6a), 4.074 (d, 1 H, $J_{2,3}$ 3.7 Hz, H-2), 3.940 (dd, 1 H, $J_{6''a,6''b}$ 12.5 Hz, H-6″a), 3.932 (dd, 1 H, $J_{6'a,6'b}$ 12.2 Hz, H-6′a), 3.850 (dd, 1 H, $J_{6a,6b}$ 11.8 Hz, H-6b), 3.758 (dd, 1 H, $J_{6'b,6'a}$ 12.2 Hz, H-6′b), 3.744 (dd, 1 H, $J_{6''b,6''a}$ 12.5 Hz, H-6″b), 3.737 (m, 1 H, $J_{5'',6''a}$ 2.15 Hz, H-5″), 3.711 (dd, 1 H, $J_{2'',3''}$ 8.5 Hz, H-2″), 3.610 (t, 1 H, $J_{4,5}$ 9.5 Hz, H-4), 3.584 (m, 1 H, $J_{5',6'a}$ 1.5 Hz, H-5′), 3.569 (m, 1 H, H-3″), 3.495 (ddd, 1 H, $J_{5,6a}$ 1.9, $J_{5,6b}$ 6.0 Hz, H-5), 3.461 (m, 1 H, H-4′), 3.446 (m, 1 H, H-4″), 3.420 (m, 1 H, H-3′), 2.892 (dd, 1 H, $J_{2',3'}$ 9.7 Hz, H-2′); ^{13}C NMR: δ 103.70 (C-1″), 100.88 (C-1′), 82.01 (C-1), 79.91 (C-5′), 78.27 (C-5), 77.88 (C-4′), 77.17 (C-2), 75.63 (C-3″), 75.61 (C-3′), 74.38 (C-4), 74.04 (C-3), 71.93 (C-4″), 70.00 (C-6), 62.77 (C-5″, C-6″, and C-6′), 57.61 (C-2″), 56.20 (C-2′); pH = 9.85 (*c*, 26 mM, D_2O); HRMS: *m/z* 527.2083 (−0.5 mmu, $C_{20}H_{35}N_2O_{14}$, MH^+).

Allyl 2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranoside (**8**).—Allyl alcohol (6 mL, 88 mmol) was added to a mixture of $Hg(CN)_2$ (1.3 g, 5.14 mmol) and Drierite (1.3 g) in dry benzene (6 mL). After stirring the mixture for 1 h at room

temperature, a solution of freshly prepared 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl chloride[30] (1 g, 2 mmol) in benzene (6 mL) was added. This was further stirred at room temperature for 24 h and filtered through Celite. Washing of the filtrate with aq $NaHCO_3$ and water, followed by drying and evaporation left a syrupy product which was purified by chromatography using 5:1 hexane–EtOAc as an eluant to provide **8** (0.56 g, 52%); $[\alpha]_D$ +34.5° (*c* 3.37, $CHCl_3$); (lit.[33] $[\alpha]_D$ +30.5° (*c* 0.5, $CHCl_3$); ^1H NMR: δ 5.874 (m, 1 H, O–C–CH=C), 5.390 (dd, 1 H, $J_{2,3}$ 3.3 Hz, H-2), 5.256 (m, 1 H, O–C–C=CH *trans*), 5.178 (m, 1 H, O–C–C=CH *cis*), 4.887 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 4.885 and 4.478 (AB quartet, 2 H, J_{gem} 10.7 Hz, Ph-CH_2), 4.702 and 4.534 (AB quartet, 2 H, J_{gem} 11.1 Hz, Ph-CH_2), 4.678 and 4.511 (AB quartet, 2 H, J_{gem} 12.1 Hz, Ph-CH_2), 4.159 (m, 1 H, O–CHa–C=C), 4.009 (dd, 1 H, $J_{3,4}$ 9.3 Hz, H-3), 3.978 (m, 1 H, O–CHb–C=C), 3.888 (t, 1 H, $J_{4,5}$ 9.5 Hz, H-4), 3.821 (ddd, 1 H, $J_{5,6a}$ 3.9, $J_{5,6b}$ 1.4 Hz, H-5), 3.799 (dd, 1 H, $J_{6a,6b}$ 10.5 Hz, H-6a), 3.70 (dd, 1 H, $J_{6b,6a}$ 10.55 Hz, H-6b); HRMS: *m/z* 533.2210 (−0.0 mmu, $C_{28}H_{37}O_8S$, MH + thioglycerol − BnOH). It has been observed previously (unpublished results) whenever an allyl group is present, the thioglycerol adduct-ion becomes relatively more abundant. Anal. Calcd. for $C_{32}H_{36}O_7$: C, 72.2; H, 6.8. Found: C, 71.8; H, 6.9.

Allyl 3,4,6-tri-O-benzyl-α-D-mannopyranoside[33] (**9**).—Compound **9** (297 mg) was obtained from **8** (340 mg, 0.64 mmol) in 95% yield by the procedure described for the preparation of **6**; $[\alpha]_D$ +60.2° (*c* 1.23, $CHCl_3$); (lit.[33] $[\alpha]_D$ +56° (*c*, 1.5, $CHCl_3$); ^1H NMR: δ 5.879 (m, 1 H, O–C–CH=C), 5.257 (m, 1 H, O–C–C=CH *trans*), 5.174 (m, 1 H, O–C–C=CH *cis*), 4.946 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 4.822 and 4.508 (AB quartet, 2 H, J_{gem} 10.9 Hz, Ph-CH_2), 4.707 and 4.675 (AB quartet, 2 H, J_{gem} 12.15 Hz, Ph-CH_2), 4.65 and 4.532 (AB quartet, 2 H, J_{gem} 12.3 Hz, Ph-CH_2), 4.176 (m, 1 H, O–CHa–C=C), 4.055 (d, 1 H, $J_{2,3}$ 3.8 Hz, H-2), 3.989 (m, 1 H, O–CHb–C=C), 3.908 (dd, 1 H, $J_{3,4}$ 9.1 Hz, H-3), 3.853 (t, 1 H, $J_{4,5}$ 9.3 Hz, H-4), 3.797 (ddd, 1 H, $J_{5,6a}$ 4.4, $J_{5,6b}$ 1.8 Hz, H-5), 3.755 (dd, 1 H, $J_{6a,6b}$ 10.6 Hz, H-6a), 3.699 (dd, 1 H, $J_{6b,6a}$ 10.6 Hz, H-6b); HRMS: *m/z* 491.2400 (−3.4 mmu, $C_{30}H_{35}O_6$, MH^+).

Allyl 3,4,6-tri-O-benzyl-2-O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-α-D-mannopyranoside (**11**).—To a suspension of granular 4 Å molecular sieves (14 g) and powdered 4 Å molecular sieves (9 g) in MeCN (44 mL) was added a solution of **9** (0.50 g, 1 mmol) in MeCN (20 mL). The mixture was cooled to −30°C and silver triflate (1.5 g, 6 mmol) and 2,4,6-collidine (0.8 mL, 6 mmol) were added sequentially. After 5 min of stirring at this temperature, bromide **10** (2.8 g, 5.6 mmol) in MeCN (35 mL) was added dropwise. The mixture was stirred for a further 20 h at room temperature, filtered through Celite and concentrated. The residual solid was chromatographed over a column of silica gel (2:1 hexane–EtOAc) to give **11** (0.5 g, 54%); $[\alpha]_D$ −3.9° (*c* 5.13, $CHCl_3$); ^1H NMR: δ 5.819 (dd, 1 H, $J_{3',4'}$ 11.4 Hz, H-3'), 5.791 (m, 1 H, O–C–CH=C), 5.515 (d, 1 H, $J_{1',2'}$ 8.6 Hz, H-1'), 5.193 (dd, 1 H, $J_{4',5'}$ 12 Hz, H-4'), 5.150 (m, 1 H, O–C–C=CH *trans*), 5.124 (m, 1 H, O–C–C=CH *cis*), 4.804 and 4.395 (AB quartet, 2 H, J_{gem} 10.9 Hz,

Ph-CH_2), 4.756 and 4.519 (AB quartet, 2 H, J_{gem} 11.4 Hz, Ph-CH_2), 4.634 (d, 1 H, $J_{1,2}$ 2.1 Hz, H-1), 4.497 (dd, 1 H, $J_{2',3'}$ 10.8 Hz, H-2'), 4.306 (dd, 1 H, $J_{6'a,6'b}$ 12.2 Hz, H-6'a), 4.218 (dd, 1 H, $J_{6'b,6'a}$ 12.2 Hz, H-6'b), 4.147 (dd, 1 H, $J_{2,3}$ 3.3 Hz, H-2), 4.121 and 4.090 (AB quartet, 2 H, J_{gem} 12.1 Hz, Ph-CH_2), 4.010 (m, 1 H, O–CHa–C=C), 3.879 (ddd, 1 H, $J_{5',6'a}$ 12.2, $J_{5',6'b}$ 2.5 Hz, H-5'), 3.857 (dd, 1 H, $J_{3,4}$ 6.6 Hz, H-3), 3.796 (m, 1 H, O–CHb–C=C), 3.583 (dd, 1 H, $J_{4,5}$ 10.8 Hz, H-4), 3.575 (dd, 1 H, $J_{6a,6b}$ 10 Hz, H-6a), 3.439 (dd, 1 H, $J_{6b,6a}$ 10 Hz, H-6b), 3.11 (ddd, 1 H, $J_{5,6a}$ 1.8, $J_{5,6b}$ 4 Hz, H-5); HRMS: *m/z* 930.3286 (−2.6 mmu, $C_{50}H_{53}NNaO_{15}$, MNa^+). Anal. Calcd. for $C_{50}H_{53}NO_{15} \cdot H_2O$: C, 64.8; H, 5.9; N, 1.5. Found: C, 64.2; H, 6.1; N, 1.4.

1-O-Acetyl-3,4,6-tri-O-benzyl-2-O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-α-D-mannopyranose (**13**).–A mixture of **11** (600 mg, 0.66 mmol) and $PdCl_2$ (200 mg) in 20:1 AcOH–H_2O (5 mL) containing NaOAc (100 mg) was stirred for 24 h at room temperature. The suspension was filtered and concentrated. The resulting residue was treated with 1:1 pyridine–Ac_2O (20 mL) overnight at room temperature, concentrated and purified on a column of silica gel by eluting with 1:1 hexane–EtOAc to give **13** (280 mg, 47%); $[\alpha]_D$ +11.5° (*c* 2.8, $CHCl_3$); ^{1}H NMR: δ 5.882 (d, 1 H, $J_{1,2}$ 2 Hz, H-1), 5.823 (dd, 1 H, $J_{3',4'}$ 9.3 Hz, H-3'), 5.61 (d, 1 H, $J_{1',2'}$ 8.5 Hz, H-1'), 5.211 (t, 1 H, $J_{4',5'}$ 9.8 Hz, H-4'), 4.788 and 4.398 (AB quartet, 2 H, J_{gem} 10.8 Hz, Ph-CH_2), 4.750 and 4.550 (AB quartet, 2 H, J_{gem} 11.5 Hz, Ph-CH_2), 4.488 (d, 1 H, $J_{2',3'}$ 10.7 Hz, H-2'), 4.30 (dd, 1 H, $J_{6'a,6'b}$ 12.2 Hz, H-6'a), 4.23 (dd, 1 H, $J_{6'b,6'a}$ 12.3 Hz, H-6'b), 4.130 and 4.060 (AB quartet, 2 H, J_{gem} 12 Hz, Ph-CH_2), 4.120 (dd, 1 H, $J_{2,3}$ 3.4 Hz, H-2), 3.920 (ddd, 1 H, $J_{5',6'a}$ 4.82, $J_{5',6'b}$ 2.39 Hz, H-5'), 3.780 (dd, 1 H, $J_{3,4}$ 8.3 Hz, H-3), 3.660 (m, 1 H, $J_{5,6b}$ 5 Hz, H-5), 3.650 (d, 1 H, $J_{4,3}$ 8.3 Hz, H-4), 3.388 (d, 1 H, $J_{6a,6b}$ 10.9 Hz, H-6a), 3.110 (dd, 1 H, $J_{6b,6a}$ 10.9 Hz, H-6b); HRMS: *m/z* 927.3566 (+3.7 mmu, $C_{49}H_{55}N_2O_{16}$, MNH_4^+). The acetyl group is cleaved off easily during the analysis, as a result, an ion at *m/z* 885.3453 (+0.7 mmu, $C_{47}H_{53}N_2O_{15}$, $MNH_4^+ - CH_3CO$) was detected with higher abundance during the high-resolution analysis.

3,4,6-Tri-O-benzyl-β-D-mannopyranose 3',4',6'-tri-O-acetyl-β-D-glucopyranose 1,2'-N:1',2-O-dianhydride (**14**).—Compound **13** (50 mg, 0.055 mmol) was treated with hydrazine hydrate (18 mL, 85%) in EtOH (200 mL, 95%) under the conditions described for the preparation of **4**. After conventional work-up, the residual syrup was acetylated with 1:1 pyridine–Ac_2O (8 mL) overnight at room temperature. The resulting mixture was purified on silica gel column with 1:2 hexane–EtOAc as eluant to give **14** (28 mg, 70%); $[\alpha]_D$ −55.2°C (*c* 0.93; $CHCl_3$); ^{1}H NMR: δ 5.079 (t, 1 H, $J_{3',4'}$ 9.5 Hz, H-3'), 5.008 (dd, 1 H $J_{4',5'}$ 9.4 Hz, H-4'), 4.880 and 4.482 (AB quartet, 2 H, J_{gem} 10.9 Hz, Ph-CH_2), 4.750 and 4.683 (AB quartet, 2 H, J_{gem} 12.4 Hz, Ph-CH_2), 4.598 and 4.563 (AB quartet, 2 H, J_{gem} 12.3 Hz, Ph-CH_2), 4.378 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1'), 4.290 (dd, 1 H, $J_{6'a,6'b}$ 12.3 Hz, H-6'a), 4.162 (dd, 1 H, $J_{6'b,6'a}$ 12.3 Hz, H-6'b), 4.108 (s, 1 H, $J_{12} < 1$ Hz, H-1), 4.022 (d, 1 H, $J_{2,3}$ 3.4 Hz, H-2), 3.852 (ddd, 1 H, $J_{5',6'a}$ 4.9 Hz, $J_{5',6'b}$ 2.1 Hz, H-5'), 3.736 (dd, 1 H, $J_{6a,6b}$ 10.5 Hz, H-6a), 3.626 (dd, 1 H, $J_{6b,6a}$ 10.5 Hz, H-6b), 3.589 (dd, 1 H, $J_{3,4}$

9.3 Hz, H-3), 3.484 (ddd, 1 H, $J_{5',6a}$ 1.6, $J_{5,6b}$ 6.9 Hz, H-5), 3.329 (dd, 1 H, $J_{2',3'}$ 10.3 Hz, H-2'); ^{13}C NMR: δ 99.66 (C-1'), 80.35 (C-1 and C-3), 76.39 (C-5'), 74.38 (C-4), 74.1 (C-4'), 73.61 (C-5), 73.03 (C-2), 69.75 (C-6), 68.91 (C-3'), 62.26 (C-6'), 52.87 (C-2'); HRMS; *m/z* 720.3036 (+1.6 mmu, $C_{39}H_{46}NO_{12}$, MH^+). Anal. Calcd. for $C_{39}H_{45}NO_{12} \cdot H_2O$: C, 63.5; H, 6.4; N, 1.9. Found: C, 63.8; H, 6.6; N, 1.9.

3,4,6-Tri-O-acetyl-β-D-mannopyranose 3',4',6'-tri-O-acetyl-β-D-glucopyranose 1,2'-N:1',2-O-dianhydride (**15**).—Compound **15** (31 mg) was obtained from **14** (70 mg, 0.09 mmole) in 55% yield in a similar way to that described for the preparation of **5**; $[\alpha]_D$ −21.7° (*c* 1.88, $CHCl_3$); ^1H NMR: δ 5.360 (t, 1 H, $J_{3',4'}$ 9.9 Hz, H-3'), 5.081 (t, 1 H, $J_{4,5}$ 9.7 Hz, H-4), 5.028 (dd, 1 H, $J_{3,4}$ 9.8 Hz, H-3), 5.020 (t, 1 H, $J_{4',5'}$ 9.76 Hz, H-4'), 4.437 (d, 1 H, $J_{1',2'}$ 8 Hz, H-1'), 4.319 (s, 1 H, $J_{1,2} < 1$ Hz, H-1), 4.285 (dd, 1 H, $J_{6'a,6'b}$ 12.4 Hz, H-6a), 4.220 (d, 1 H, $J_{2,3}$ 3.4 Hz, H-2), 4.19 (d, 1 H, $J_{6a,6b}$ 12.2 Hz, H-6a), 4.175 (dd, 1 H, $J_{6'b,6'a}$ 12.4 Hz, H-6'b), 4.167 (d, 1 H, $J_{6b,6a}$ 12.2 Hz, H-6b), 3.86 (ddd, 1 H, $J_{5',6'a}$ 4.8, $J_{5',6'b}$ 2 Hz, H-5'), 3.63 (ddd, 1 H, $J_{5,6a}$ 4.6, $J_{5,6b}$ 3.3 Hz, H-5), 3.31 (dd, 1 H, $J_{2',3'}$ 9.9 Hz, H-2'); HRMS: *m/z* 576.1913 (−1.5 mmu, $C_{24}H_{34}NO_{15}$, MH^+).

β-D-Mannopyranose β-D-glucopyranose 1,2'-N:1',2-O-dianhydride (**16**).—Compound **16** (8 mg) was obtained from **15** (31 mg, 0.05 mmol) in 46% yield in a similar way to that described for the preparation of **6**; $[\alpha]_D$ +10.9° (*c* 0.27, H_2O); ^1H NMR: δ 4.496 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1'), 4.459 (s, 1 H, $J_{1,2} < 1$ Hz, H-1), 4.101 (d, 1 H, $J_{2,3}$ 3.5 Hz, H-2), 3.924 (dd, 1 H $J_{6'a,6'b}$ 12.3 Hz, H-6'a), 3.949 (dd, 1 H, $J_{6a,6b}$ 12.1 Hz, H-6a), 3.808 (dd, 1 H, $J_{3,4}$ 9.8 Hz, H-3), 3.773 (dd, 1 H, $J_{6'b,6'a}$ 12.3 Hz, H-6'b) 3.645 (t, 1 H, $J_{4,5}$ 9.9 Hz, H-4), 3.602 (ddd, 1 H, $J_{5',6'a}$ 2.7, $J_{5',6'b}$ 6.3 Hz, H-5'), 3.450 (t, 1 H, $J_{3',4'}$ 9.5 Hz, H-3'), 3.462 (d, 1 H, $J_{4',5'}$ 9.4 Hz, H-4'), 3.414 (ddd, 1 H, $J_{5,6a}$ 2.6, $J_{5,6b}$ 6.5 Hz, H-5), 2.938 (t, 1 H, $J_{2',3'}$ 10.3 Hz, H-2'); ^{13}C NMR: δ 101.26 (C-1'), 82.1 (C-1), 80.03 (C-5'), 79.51 (C-5), 77.44 (C-2), 75.89 (C-4'), 74.01 (C-3), 72.26 (C-3'), 68.63 (C-4), 63.19 (C-6' and C-6), 56.20 (C-2'); pH 10.1, *c* 18 mM, D_2O; HRMS: *m/z* 324.1288 (−0.6 mmu, $C_{12}H_{22}NO_9$ MH^+).

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