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Synthesis of a selectively protected trisaccharide building block that is part of xylose-containing carbohydrate chains from N-glycoproteins

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ABSTRACT

The synthesis is reported of ethyl 4-*O*-[3-*O*-allyl-4,6-*O*-isopropylidene-2-*O*-(2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl)-β-D-mannopyranosyl]-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**16**), a key intermediate in the synthesis of xylose-containing carbohydrate chains from N-glycoproteins. Condensation of ethyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**5**) with 2,4,6-tri-*O*-acetyl-3-*O*-allyl-α-D-glucopyranosyl bromide, using silver triflate as a promoter, gave the β-linked disaccharide derivative **8** (84%). *O*-Deacetylation of **8** and then isopropylidenation afforded **10**, which was converted via oxidation–reduction into ethyl 4-*O*-(3-*O*-allyl-4,6-*O*-isopropylidene-β-D-mannopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**12**). Silver triflate-promoted condensation of **12** with 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide gave **16** (71%). The Xyl*p* unit in **16** and in de-isopropylidenated **16** (**17**) existed in the $^1C_4$(D) conformation, but that in *O*-deacetylated **17** (**18**) existed in the $^4C_1$(D) conformation.

INTRODUCTION

Xylose-containing N-linked carbohydrate chains occur in glycoproteins of plant and animal origin. Invariably β-D-Xyl*p* is (1 → 2)-linked to β-D-Man*p* of the core structure. If α-L-Fuc*p* is attached to the asparagine-linked D-Glc*p*NAc, it is (1 → 3)-linked in plants and (1 → 6)-linked in animals[1], as in **1** for the proteolytic enzyme bromelain from pineapple stem[2] and in **2** for the α-hemocyanin of *Helix pomatia*[3].

For the study of the conformation[4,5] and biosynthesis[6] of these glycans, the oligosaccharide glycosides β-D-Xyl*p*-(1 → 2)-β-D-Man*p*-OMe, β-D-Xyl*p*-(1 → 2)-

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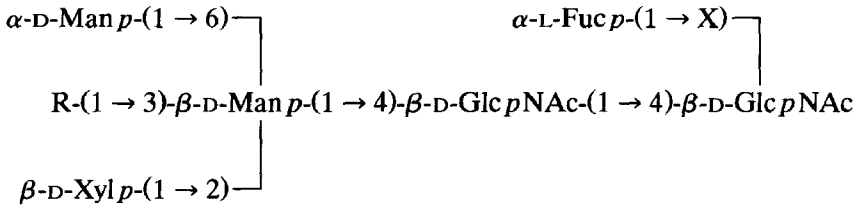
[α-D-Man*p*-(1 → 6)]-β-D-Man*p*-OMe, β-D-Xyl*p*-(1 → 2)-[α-D-Man*p*-(1 → 3)]-β-D-Man*p*-OMe, and β-D-Xyl*p*-(1 → 2)-[α-D-Man*p*-(1 → 3)][α-D-Man*p*-(1 → 6)]-β-D-Man*p*-OMe have been synthesised[7]. We now describe the synthesis of the glycosyl donor ethyl 4-*O*-[3-*O*-allyl-4,6-*O*-isopropylidene-2-*O*-(2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl)-β-D-mannopyranosyl]-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**16**), an essential building block for the preparation of **1** and **2**.



1 R = H, X = 3

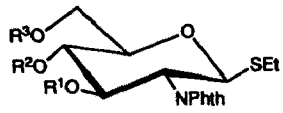
2 R = α-D-Man*p*, X = 6

RESULTS AND DISCUSSION

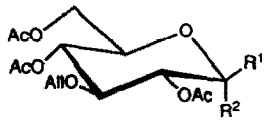
The crucial step in the synthesis of **16** is the creation of the β-D-Man*p* bond. This type of linkage can be synthesised "directly" by using a C-1-activated D-Man*p* donor, having a non-participating group at C-2 with a reactive acceptor[8–10], and "indirectly" by epimerisation of a β-D-Glc*p* unit either by an inter- or intra-molecular S_N2 reaction or via oxidation–reduction[7,11–14]. Of these methods, the oxidation–reduction procedure was chosen because, after reduction, the β-D-Man*p* residue generated has a blocking pattern ideal for the introduction of β-D-Xyl*p* and α-D-Man*p* units.

Ethyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside[15] (**3**) was treated with benzyl bromide in the presence of sodium hydride in boiling tetrahydrofuran to give **4** (90%). Regioselective reductive opening of the 4,6-*O*-benzylidene ring in **4** with the borane–trimethylamine complex and aluminium(III) chloride[16] in tetrahydrofuran yielded the acceptor **5** (84%). Since HO-4 in D-GlcN derivatives has a low reactivity[17], especially in compounds with the $^4C_1$ conformation, forcing conditions are needed to promote the reaction.

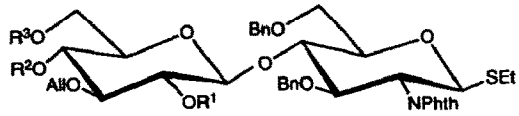
When 2,4,6-tri-*O*-acetyl-3-*O*-allyl-α-D-glucopyranosyl trichloroacetimidate (**7**), obtained from 1,2,4,6-tetra-*O*-acetyl-3-*O*-allyl-β-D-glucopyranoside[18] via treatment with hydrazine acetate[19] (→ **6**, 81%) and trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene[20](→ **7**, 74%), was used as the donor[21], condensation with **5** in dichloromethane using trimethylsilyl triflate as a promoter at −40°C gave only 19% of the desired disaccharide derivative **8**, and the many UV-absorbing products (TLC) indicated degradation of **5**. However, coupling of **5** and 2,4,6-tri-*O*-acetyl-3-*O*-allyl-α-D-glucopyranosyl bromide[18] in dichloromethane–toluene in the presence of silver triflate as a promoter afforded 84% of **8**.



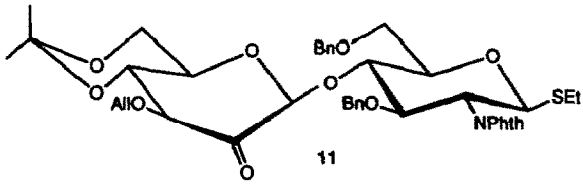
3 $R^1 = H$, $R^2, R^3 = CHPh$
4 $R^1 = Bn$, $R^2, R^3 = CHPh$
5 $R^1 = R^3 = Bn$, $R^2 = H$



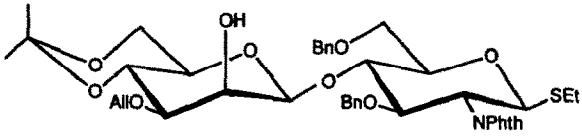
6 $R^1, R^2 = OH, H$
7 $R^1 = H$, $R^2 = OCNHCCl_3$



8 $R^1 = R^2 = R^3 = Ac$
9 $R^1 = R^2 = R^3 = H$
10 $R^1 = H$, $R^2, R^3 = Me_2C$



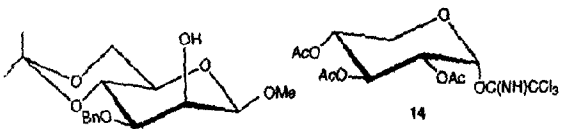
11



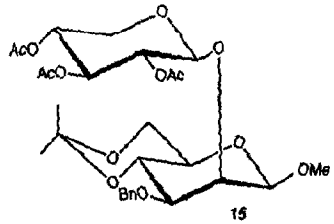
12

Zemplén *O*-deacetylation of **8** (→ **9**, 99%), isopropylidenation with 2,2-dimethoxypropane[22] (→ **10**, 82%), and oxidation of HO-2 using methylsulfoxide–acetic anhydride afforded **11** (88%), without affecting the thioethyl and allyl groups.

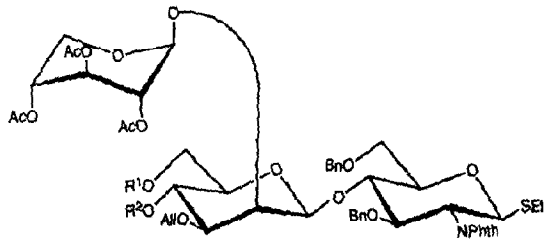
Although borohydride reduction of β-D-*arabino*-hexopyranosyl-2-ulose to β-D-Man*p* derivatives can be achieved in 1:1 dichloromethane–methanol[12,13,23,24] or 12:1 dioxane–water[25], phthalimido groups may be removed[26]. However, when the borohydride reduction of **11** was carried out in dichloromethane, no degradation was observed, and the *manno* (**12**) and *gluco* (**10**) isomers were formed in the ratio ~ 7:3. A similar low stereoselectivity without decomposition occurred in 1:2 dichloromethane–2-propanol [→ **12** (53%) and **10** (38%)]. The isomers **10** and **12** were separated easily by column chromatography, so that **10** could be recycled.



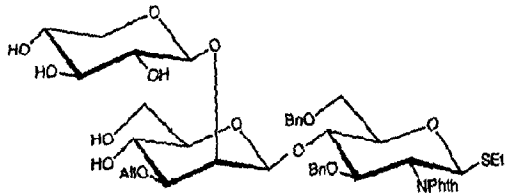
13 14



15



16 $R^1, R^2 = Me_2C$
17 $R^1 = R^2 = H$



18

The xylosylation of methyl 3-*O*-benzyl-4,6-*O*-isopropylidene-β-D-mannopyranoside (**13**) with 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide in toluene–nitromethane in the presence of $Hg(CN)_2$ has been reported[7] but, although the coupling proceeded with a good yield, an α,β-ratio of 1 : 9 was obtained. However, when 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl trichloroacetimidate[27] (**14**) was reacted with **13** in dichloromethane in the presence of trimethylsilyl triflate as a promoter, only the desired β-(1 → 2)-linked disaccharide derivative[7] **15** was formed. Application of the latter method to **12** gave a complex mixture of products, but the coupling of **12** and 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide[28], catalysed by

silver triflate at −40°C in toluene–dichloromethane, yielded only the desired β-linked trisaccharide derivative **16** (70%).

In the ^{13}C NMR spectrum of **16**, the resonance of C-1 of the β-D-Xyl*p* unit appeared at relatively high field (δ 98.66), whereas the ^{1}H NMR spectrum revealed relatively small coupling constants for the Xyl*p* ring ($J_{1,2}$ 3.0, $J_{2,3}$ 5.0, $J_{3,4}$ 4.9 Hz). Based on the findings for ring protons in *gauche–gauche* arrangements and the chemical shift for the H-5 resonance[29], it is concluded that the Xyl*p* unit in **16** has the $^{1}C_4$ conformation. This behaviour is not uncommon for protected β-Xyl*p* derivatives[30–32] and it has been suggested[30,31] that the anomeric effect counters the destabilising effect of the three axially oriented *O*-acetyl groups. Removal of the isopropylidene group from **16** (→ **17**, 97%) did not change the conformation of the Xyl*p* unit (^{13}C, C-1 δ 98.92; ^{1}H, $J_{1,2}$ 3.5, $J_{2,3}$ 5.5, $J_{3,4}$ 5.5 Hz). However, after saponification of **17** (→ **18**, 95%), the NMR parameters completely changed (^{13}C, C-1 δ 103.32; ^{1}H, $J_{1,2}$ 6.7 Hz). The usual $^{4}C_1$(D) chair conformation was observed for **15**.

EXPERIMENTAL

General methods.—The ^{1}H (200 and 360 MHz) and ^{13}C (APT, 50 MHz) NMR spectra were recorded at 25°C with a Bruker WP 200 SY, WP 200, or HX 360 spectrometer. Chemical shifts (δ) are given in ppm relative to the signal for internal Me_4Si ($CDCl_3$) for ^{1}H and for ^{13}C (indirectly to $CDCl_3$, δ 76.9). Column chromatography was performed on Kieselgel 60 (Merck, < 230 mesh) and fractions were monitored by TLC on Kieselgel 60 F_{254} (Merck) by detection with UV light and then charring with H_2SO_4. Optical rotations were measured for solutions in $CHCl_3$ at 20°C with a Perkin–Elmer 241 polarimeter, using a 10-cm 1-mL cell. Melting points (uncorrected) were determined on a Kofler apparatus. Solvents were evaporated under reduced pressure at 40°C (bath). All solvents were distilled from the appropriate drying agents.

Ethyl 3-O-benzyl-4,6-O-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-gluco-pyranoside (**4**).—A solution of **3**[15] (6.5 g, 14.7 mmol) and benzyl bromide (2.7 mL, 22.7 mmol) in tetrahydrofuran (75 mL) was added dropwise to NaH (700 mg, 29.17 mmol), and the mixture was boiled under reflux. After 1 h, TLC (95:5 CH_2Cl_2–acetone) indicated the reaction to be complete. The mixture was cooled, diluted with EtOAc (300 mL), filtered through Celite, and washed with water (3 × 30 mL), and the combined washings were extracted with EtOAc (50 mL). The combined organic phases were washed with water (50 mL), dried ($MgSO_4$), filtered, and concentrated. Column chromatography (95:5 CH_2Cl_2–acetone) of the residue gave **4**, isolated as a syrup (7.1 g, 90%); $[\alpha]_D$ +97° (*c* 1); R_f 0.60. NMR data ($CDCl_3$): ^{1}H, δ 7.87–6.84 (m, 14 H, 2 Ph and Phth), 5.635 (s, 1 H, PhC*H*), 5.348 (d, 1 H, $J_{1,2}$ 10.6 Hz, H-1), 4.799 and 4.516 (2 d, each 1 H, PhCH_2O), 2.657 (m, 2 H, CH_3CH_2S), 1.169 (t, 3 H, CH_3CH_2S); ^{13}C, δ 137.6, 137.1, 133.7, 131.4, 128.8–127.2, 125.9, and 123.1 (2 C) ($C_6H_5CH_2O$, C_6H_5CH, and Phth), 101.1

(PhCH), 82.8, 81.6, 75.2, and 70.2 (C-1,3,4,5), 74.0 and 68.5 (C-6 and $PhCH_2O$), 54.5 (C-2), 23.8 (CH_3CH_2S), 14.7 (CH_3CH_2S). Anal. Calcd for $C_{30}H_{29}NO_6S$: C, 67.78; H, 5.50. Found: C, 67.76; H, 5.37.

Ethyl 3,6-di-O-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glycopyranoside (**5**).—A mixture of the borane–trimethylamine complex (5.7 g, 78.1 mmol), powdered 4A molecular sieves (5 g), **4** (2.6 g, 4.9 mmol), and tetrahydrofuran (50 mL) was stirred for 1 h. Aluminium(III) chloride (10.5 g, 78.7 mmol) was added and the mixture was stirred for 5 h in the dark. TLC (9:1 CH_2Cl_2–EtOAc) then showed the conversion of **4** (R_f 0.80) into **5** (R_f 0.46). The mixture was diluted with CH_2Cl_2 (250 mL), filtered through Celite, washed with cold 0.5 M H_2SO_4, water, aq 5% $NaHCO_3$, and water, dried ($MgSO_4$), filtered, and concentrated. Column chromatography (9:1 CH_2Cl_2–EtOAc) of the residue gave **5** (2.2 g, 84%), which crystallised on storage and had mp 110°C (from EtOH); $[\alpha]_D$ +43° (*c* 1.5); R_f 0.46. NMR data ($CDCl_3$): ^1H, δ 7.83–6.94 (m, 14 H, 2 Ph and Phth), 5.275 (d, 1 H, $J_{1,2}$ 10.0 Hz, H-1), 4.750 and 4.543 (2 d, each 1 H, $PhCH_2O$), 4.639 and 4.583 (2 d, each 1 H, $PhCH_2O$), 2.949 (d, 1 H, $J_{HO,4}$ 2.5 Hz, HO-4), 2.623 (m, 2 H, CH_3CH_2S), 1.160 (t, 3 H, CH_3CH_2S); ^{13}C, δ 168.0 and 167.4 (*C*O Phth), 138.0, 137.4, 133.7, 131.5, 128.4–127.3, 123.4, and 123.2 (2 $C_6H_5CH_2O$ and Phth), 81.0, 79.4, 77.4, and 74.5 (C-1,3,4,5), 74.3, 73.7, and 70.8 (C-6 and 2 $PhCH_2O$), 54.3 (C-2), 23.9 (CH_3CH_2S), 14.8 (CH_3CH_2S). Anal. Calcd for $C_{30}H_{31}NO_6S$: C, 67.52; H, 5.86. Found: C, 68.04; H, 5.78.

2,4,6-Tri-O-acetyl-3-O-allyl-α-D-glucopyranosyl trichloroacetimidate (**7**).—A solution of 1,2,4,6-tetra-*O*-acetyl-3-*O*-allyl-β-D-glucopyranoside[18] (3.88 g, 9.99 mmol) in *N,N*-dimethylformamide (10 mL) was stirred with hydrazine acetate (1.1 g, 11.9 mmol) at 50°C for 15 min, cooled, diluted with EtOAc (250 mL), and washed with aq 5% NaCl (3 × 30 mL), and the combined washings were extracted with EtOAc (30 mL). The combined organic phases were washed with water (30 mL), dried ($MgSO_4$), filtered, and concentrated. Column chromatography (9:1 CH_2Cl_2–acetone) of the residue gave **6**, isolated as a syrup (2.8 g, 81%); $[\alpha]_D$ +47° (*c* 0.5); R_f 0.40 (9:1 CH_2Cl_2–acetone).

To a solution of **6** (1.04 g, 3.00 mmol) in dry CH_2Cl_2 (10 mL) and trichloroacetonitrile (3.0 mL, 29.9 mmol) was added 1,8-diazabicyclo[5.4.0]undec-7-ene (0.56 mL, 3.74 mmol). The mixture was stirred for 20 min, when the reaction was complete (TLC), then concentrated. Column chromatography [3:2 light petroleum (bp 40–60°C)–EtOAc] of the residue gave **7** (1.09 g, 74%), which crystallised on storage and had mp 54°C (from ether); $[\alpha]_D$ +64° (*c* 0.5); R_f 0.58. NMR data ($CDCl_3$): ^1H, δ 8.63 (s, 1 H, NH), 6.48 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 5.78 (m, 1 H, $H_2C{=}CHCH_2O$), 5.24-5.06 (m, 3 H, $H_2C{=}CHCH_2O$ and H-4), 5.00 (dd, 1 H, $J_{2,3}$ 10.0 Hz, H-2), 2.06, 2.03, and 2.02 (3 s, each 3 H, 3 Ac). Anal. Calcd for $C_{17}H_{22}Cl_3NO_9$: C, 41.61; H, 4.52. Found: C, 42.03; H, 4.43.

Ethyl 3,6-di-O-benzyl-2-deoxy-2-phthalimido-4-O-(2,4,6-tri-O-acetyl-3-O-allyl-β-D-glucopyranosyl)-1-thio-β-D-glucopyranoside (**8**).—A solution of **5** (1.15 g, 2.16 mmol) and 2,4,6-tri-*O*-acetyl-3-*O*-allyl-α-D-glucopyranosyl bromide[18] (2.6 g, 6.4

mmol) in CH_2Cl_2 (20 mL) and toluene (20 mL) containing powdered 4A molecular sieves (7.5 g) was stirred for 30 min under Ar. A solution of silver triflate (2.2 g, 8.6 mmol) in toluene (40 mL) was added dropwise in the dark during 2 h at −45°C and stirring was continued for 2 h at −40°C. TLC [3:2 light petroleum (bp 40–60°C)–EtOAc] then showed the absence of **5** (R_f 0.54) and the formation of **8** (R_f 0.47). Pyridine (5 mL) was added, and the mixture was diluted with CH_2Cl_2 (400 mL), filtered through Celite, washed with aq 10% sodium thiosulfate (3 × 50 mL) and water (3 × 50 mL), dried ($MgSO_4$), filtered, and concentrated. Column chromatography [3:2 light petroleum (bp 40–60°C)–EtOAc] of the residue gave **8**, isolated as a syrup (1.56 g, 84%); $[\alpha]_D$ +24° (*c* 0.5); R_f 0.47. NMR data ($CDCl_3$): ^{1}H, δ 7.80–6.75 (m, 14 H, 2 Ph and Phth), 5.767 (m, 1 H, H_2C=C*H*CH_2O), 5.207 and 5.136 (2 m, 2 H, *H*$_2$C=CHC*H*$_2$O), 5.197 (d, 1 H, $J_{1,2}$ 9.8 Hz, H-1), 4.804 and 4.526 (2 d, each 1 H, PhC*H*$_2$O), 4.786 and 4.410 (2 d, each 1 H, PhC*H*$_2$O), 4.587 (d, 1 H, $J_{1',2'}$ 8.1 Hz, H-1'), 2.617 (m, 2 H, CH_3CH_2S), 2.067, 2.056, and 1.963 (3 s, each 3 H, 3 Ac), 1.162 (t, 3 H, CH_3CH_2S); ^{13}C, δ 170.5–166.8 (*C*OCH$_3$ and *C*O Phth), 138.3, 137.8, 134.0, 133.5, 131.4, 128.3–127.6, 126.8, and 123.1 ($C_6H_5CH_2O$, Phth, and H_2C=*C*HCH_2O), 116.5 (H_2C=CHCH_2O), 100.1 (C-1'), 80.9, 79.8, 78.8, 78.0, 77.4, 72.6, 71.5, and 69.3 (C-1,3,4,5,2',3',4',5'), 74.5, 73.4, 72.5, 67.6, and 61.7 (C-6,6', 2 PhCH_2O, and H_2C=CHCH_2O), 54.5 (C-2), 23.7 (CH_3CH_2S), 23.7, 20.7, and 20.4 (3 CO*C*H$_3$), 14.7 (CH_3CH_2S). Anal. Calcd for $C_{45}H_{51}NO_{14}S$: C, 62.71; H, 5.96. Found: C, 62.33, H, 6.07.

*Ethyl 4-O-(3-O-allyl-4,6-O-isopropylidene-β-*D*-glucopyranosyl)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-1-thio-β-*D*-glucopyranoside* (**10**).—A solution of **8** (2.0 g, 2.3 mmol) and NaOMe (250 mg, 4.63 mmol) in MeOH (50 mL) was stirred overnight. TLC (9:1 CH_2Cl_2–MeOH) then showed a complete *O*-deacetylation (→ **9**, R_f 0.58). The solution was neutralised with Amberlite IR-120 (H^+) resin, filtered, and concentrated, and CH_2Cl_2 (2 × 10 mL) was evaporated from the residue to afford amorphous **9** (1.7 g, 99%).

To a solution of **9** (1.50 g, 2.04 mmol) in 2,2-dimethoxypropane (15 mL, 122 mmol) was added *p*-toluenesulfonic acid monohydrate (150 mg, 0.79 mmol). After 30 min, TLC (95:5 CH_2Cl_2–acetone) indicated the reaction to be complete, and solid $NaHCO_3$ was added. The mixture was diluted with CH_2Cl_2 (250 mL), washed with water, dried ($MgSO_4$), filtered, and concentrated. Column chromatography (95:5 CH_2Cl_2–acetone) of the residue gave **10**, isolated as a white glass (1.30 g, 82%); $[\alpha]_D$ +50° (*c* 0.4); R_f 0.42. NMR data ($CDCl_3$): ^{1}H, δ 7.80–6.84 (m, 14 H, 2 Ph and Phth), 5.935 (m, 1 H, H_2C=C*H*CH_2O), 5.296 and 5.183 (2 m, 2 H, *H*$_2$C=CHCH_2O), 5.199 (d, 1 H, $J_{1,2}$ 10.4 Hz, H-1), 4.765 and 4.402 (2 d, each 1 H, PhC*H*$_2$O), 4.724 and 4.592 (2 d, each 1 H, PhC*H*$_2$O), 4.604 (d, 1 H, $J_{1',2'}$ 7.6 Hz, H-1'), 2.631 (m, 2 H, CH_3CH_2S), 1.416 and 1.369 (2 s, each 3 H, CMe_2), 1.169 (t, 3 H, CH_3CH_2S); ^{13}C, δ 138.2, 137.7, 135.0, 133.6, 131.4, 128.2–126.9, and 123.1–123.0 ($C_6H_5CH_2O$, Phth, and H_2C=*C*HCH_2O), 116.7 (H_2C=CHCH_2O), 103.1 (C-1'), 98.9 (Me$_2$*C*), 80.8, 80.5, 78.9, 78.6, 78.4, 74.6, 73.8, and 66.9 (C-1,3,4,5,2',3',4',5'), 74.5, 73.2, 73.0, 68.1, and 61.9 (C-6,6', 2 PhCH_2O, and H_2C=CHCH_2O), 54.5

(C-2), 28.9 and 18.9 [2 (CH_3)$_2$C], 23.4 (CH_3CH_2S), 14.7 (CH_3CH_2S). Anal. Calcd for $C_{42}H_{49}NO_{11}S$: C, 65.02; H, 6.37. Found: C, 64.61; H, 6.58.

*Ethyl 4-O-(3-O-allyl-4,6-O-isopropylidene-β-*D*-arabino-hexopyranosyl-2-ulose)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-1-thio-β-*D*-glucopyranoside* (**11**).—A solution of **10** (1.10 g, 1.42 mmol) in 1:2 acetic anhydride–Me_2SO (45 mL) was stirred for 16 h at room temperature, then concentrated, and toluene (3 × 50 mL) was evaporated from the residue. Column chromatography (95:5 CH_2Cl_2–acetone) then gave **11**, isolated as a white glass (955 mg, 87%); $[\alpha]_D$ +52° (*c* 1); R_f 0.42–0.62 (tailing, 3:1 toluene–acetone). NMR data ($CDCl_3$): ^{1}H, δ 7.80–6.80 (m, 14 H, 2 Ph and Phth), 5.920 (m, 1 H, H_2C=CHCH$_2$O), 5.230 (d, 1 H, $J_{1,2}$ 10.5 Hz, H-1), 4.940 (s, 1 H, H-1′), 2.637 (m, 2 H, CH_3CH_2S), 1.545 and 1.415 (2 s, each 3 H, CMe_2), 1.170 (t, 3 H, CH_3CH_2S); ^{13}C, δ 187.3 (C-2′ as carbonyl), 102.6 and 101.7 (C-1′ as carbonyl and *gem*-diol), 93.9 (C-2′ as *gem*-diol), 99.2 and 99.0 (Me_2C as carbonyl and *gem*-diol), 54.6 (C-2), 23.6 (CH_3CH_2S), 28.7 and 18.7 [2 (CH_3)$_2$C], 14.7 (CH_3CH_2S). Anal. Calcd for $C_{42}H_{47}NO_{11}S \cdot H_2O$: C, 63.70; H, 6.24. Found: C, 63.95; H, 6.18.

*Ethyl 4-O-(3-O-allyl-4,6-O-isopropylidene-β-*D*-mannopyranosyl)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-1-thio-β-*D*-glucopyranoside* (**12**).—To a solution of **11** (1.03 g, 1.33 mmol) in CH_2Cl_2 (5 mL) was added a solution of $NaBH_4$ (200 mg, 5.29 mmol) in 2-propanol (10 mL) at 0°C. After 10 min, TLC (3:1 toluene–acetone) showed the absence of **9**, and the presence of the *gluco*- (**10**, R_f 0.62) and *manno*- (**12**, R_f 0.42) derivatives in the ratio ~ 3:7. The mixture was diluted with CH_2Cl_2 (200 mL), washed with water, dried ($MgSO_4$), filtered, and concentrated. Column chromatography (3:1 toluene–acetone) of the residue gave **12**, isolated as a white glass (547 mg, 53%); $[\alpha]_D$ +41° (*c* 0.2); and **10** (392 mg, 38%), partially contaminated with **12**, which was recycled. NMR data ($CDCl_3$) for **12**: ^{1}H, δ 7.80–6.85 (m, 14 H, 2 Ph and Phth), 5.892 (m, 1 H, H_2C=CHCH$_2$O), 5.231 (d, 1 H, $J_{1,2}$ 10.5 Hz, H-1), 5.280 and 5.184 (2 m, 2 H, H_2C=CHCH$_2$O), 4.824 and 4.449 (2 d, each 1 H, PhCH_2O), 4.737 and 4.527 (2 d, each 1 H, PhCH_2O), 4.661 (s, 1 H, H-1′), 2.975 (m, 1 H, H-5′), 2.642 (m, 2 H, CH_3CH_2S), 1.432 and 1.379 (2 s, each 3 H, CMe_2), 1.175 (t, 3 H, CH_3CH_2S); ^{13}C, δ 138.2, 137.6, 134.6, 133.6, 131.4, 128.3–126.9, and 123.3 ($C_6H_5CH_2O$), Phth, and H_2C=CHCH$_2$O), 116.9 (H_2C=CHCH$_2$O), 100.6 (C-1′), 99.3 (Me_2C), 80.9, 78.6, 78.4 (2 C), 77.1, 70.4, 69.3, 67.6 (C-1,3,4,5,2′,3′,4′,5′), 74.5, 73.4, 71.0, 68.4, and 61.8 (C-6,6′, 2 PhCH_2O, and H_2C=CHCH_2O), 54.5 (C-2), 29.0 and 19.0 [(CH_3)$_2$C], 23.5 (CH_3CH_2S), 14.7 (CH_3CH_2S). Anal. Calcd for $C_{42}H_{49}NO_{11}S$: C, 65.02; H, 6.37. Found: C, 64.50; H, 6.60.

*Methyl 3-O-benzyl-4,6-O-isopropylidene-2-O-(2,3,4-tri-O-acetyl-β-*D*-xylopyranosyl)-β-*D*-mannopyranoside*[7] (**15**).—A solution of methyl 3-*O*-benzyl-4,6-*O*-isopropylidene-β-D-mannopyranoside[7] (**13**; 80 mg, 0.25 mmol) and 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl trichloroacetimidate[27] (**14**; 126 mg, 0.30 mmol) in CH_2Cl_2 (4 mL) containing 4A molecular sieves (1 g) was stirred for 30 min under Ar. A solution of trimethylsilyl triflate (55 μL, 0.30 mmol) in CH_2Cl_2 (2 mL) was added dropwise at −40°C. TLC (85:10:5 CH_2Cl_2–EtOAc–acetone) showed that **15** (R_f 0.56) was

formed within 10 min. Pyridine (1 mL) was added, the mixture was filtered through Celite and concentrated, and toluene (3 × 5 mL) was evaporated from the residue. Column chromatography (85:10:5 CH_2Cl_2–EtOAc–acetone) then gave **15**, isolated as a syrup (102 mg, 70%), $[\alpha]_D$ −90° (*c* 1); R_f 0.56. For the ^1H NMR data, see ref. 7.

Ethyl 4-O-[3-O-allyl-4,6-O-isopropylidene-2-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-β-D-mannopyranosyl]-3,6-di-O-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**16**).—A solution of **12** (300 mg, 0.39 mmol) and 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide[28] (264 mg, 0.78 mmol) in CH_2Cl_2 (3 mL) and toluene (3 mL) containing powdered 4A molecular sieves (900 mg) was stirred for 30 min under Ar. A solution of silver triflate (400 mg, 1.56 mmol) in toluene (4 mL) was added dropwise in the dark at −45°C during 40 min, and stirring was continued for 20 min at −45°C. TLC [1:1 light petroleum (bp 40–60°C)–EtOAc] then showed the absence of **12** (R_f 0.29) and the formation of **16** (R_f 0.48). Pyridine was added, and the mixture was diluted with CH_2Cl_2 (100 mL), filtered through Celite, washed with aq 10% sodium thiosulfate (3 × 10 mL) and water (3 × 10 mL), dried ($MgSO_4$), filtered, and concentrated. Column chromatography [1:1 light petroleum (bp 40–60°C)–EtOAc] of the residue gave **16**, isolated as a white glass (283 mg, 70%); $[\alpha]_D$ −25° (*c* 0.5); R_f 0.48. NMR data ($CDCl_3$): ^1H, δ 7.83–6.78 (m, 14 H, 2 Ph and Phth), 5.89 (m, 1 H, H_2C=C*H*CH_2O), 5.30 and 5.16 (2 m, 2 H, *H*$_2$C=CHCH_2O), 5.25 (d, 1 H, $J_{1,2}$ 10.0 Hz, H-1), 5.14 (d, 1 H, $J_{1'',2''}$ 3.0 Hz, H-1''), 5.02 (t, 1 H, $J_{3'',4''}$ 4.9 Hz, H-3''), 4.98 (dd, 1 H, $J_{2'',3''}$ 5.0 Hz, H-2''), 4.85 (m, 1 H, $J_{4'',5''ax/eq}$ 3.3 and 3.7 Hz, H-4''), 4.85 and 4.35 (2 d, each 1 H, PhC*H*$_2$O), 4.73 and 4.51 (2 d, each 1 H, PhC*H*$_2$O), 4.56 (s, 1 H, H-1'), 4.55 and 3.51 (2 dd, each 1 H, $J_{5''ax,5''eq}$ −12.9 Hz, H-5''*ax*, 5''*eq*), 4.34 (dd, 1 H, $J_{3,4}$ 8.0 Hz, H-3), 4.24 (t, 1 H, $J_{2,3}$ 10.0 Hz, H-2), 4.03 (d, 1 H, $J_{2',3'}$ 3.0 Hz, H-2'), 3.99 (dd, 1 H, $J_{4,5}$ 9.9 Hz, H-4), 3.84 (t, 1 H, $J_{4',5'}$ 9.5 Hz, H-4'), 3.75 (1 H, $J_{6a,6b}$ −11.5 Hz, H-6a), 3.71 (1 H, $J_{6'a,6'b}$ −10.6 Hz, H-6'a), 3.60 (m, 1 H, $J_{5,6a} = J_{5,6b} \sim 2.5$ Hz, H-5), 3.39 (t, 1 H, H-6'b), 3.17 (dd, 1 H, $J_{3',4'}$ 9.5 Hz, H-3'), 2.95 (m, 1 H, $J_{5',6'a}$ 5.1, $J_{5',6'b}$ 10.3 Hz, H-5'), 2.65 (m, 2 H, CH_3CH_2S), 2.13, 2.10, and 2.00 (3 s, each 3 H, 3 Ac), 1.40 and 1.37 (2 s, each 3 H, CMe_2), 1.18 (t, 3 H, CH_3CH_2S); ^{13}C, δ 169.6, 168.8, and 168.7 ($COCH_3$), 138.6, 137.6, 134.9, 133.5, 131.4, 128.5–126.6, and 123.1 ($C_6H_5CH_2O$, Phth, and H_2C=*C*HCH_2O), 116.1 (H_2C=CHCH_2O), 102.1 (C-1'), 99.2 (Me_2C), 98.4 (C-1''), 80.9, 80.3, 78.7, 78.2, 77.1, 74.0, 70.4, 68.5, 68.2, 68.1, and 67.8 (C-1,3,4,5,2',3',4',5',2'',3'',4''), 74.5, 73.4, 70.8, 68.8, 61.8, and 59.5 (C-6,6',5'', 2 PhCH_2O, and H_2C=CH*C*H_2O), 54.6 (C-2), 28.9 and 18.9 [(CH_3)$_2$C], 23.4 (CH_3CH_2S), 20.7 (2 C) and 20.4 (3 CO*C*H_3), 14.7 (*C*H_3CH_2S). Anal. Calcd for $C_{53}H_{63}NO_{18}S$: C, 61.56; H, 6.14. Found: C, 61.23; H, 6.09.

Ethyl 4-O-[3-O-allyl-2-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-β-D-mannopyranosyl]-3,6-di-O-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**17**). —A solution of **16** (200 mg, 0.19 mmol) in 6:4 acetic acid–water (4 mL) was kept for 20 min at 60°C, then concentrated, and toluene (5 × 5 mL) was evaporated from the residue to afford **17**, as a white glass (186 mg, 97%); $[\alpha]_D$ −30° (*c* 0.3);

R_f 0.62 (7:3 CH_2Cl_2–acetone). NMR data ($CDCl_3$): ^1H, δ 7.82–6.83 (m, 14 H, 2 Ph and Phth), 5.91 (m, 1 H, H_2C=CHCH_2O), 5.29 (d, 1 H, $J_{1,2}$ 10.2 Hz, H-1), 5.28 and 5.19 (2 m, 2 H, H_2C=CHCH_2O), 5.06 (dd, 1 H, $J_{3'',4''}$ 5.5 Hz, H-3″), 5.02 (d, 1 H, $J_{1'',2''}$ 3.5 Hz, H-1″), 4.94 (dd, 1 H, $J_{2'',3''}$ 5.5 Hz, H-2″), 4.91 and 4.43 (2 d, each 1 H, PhCH_2O), 4.84 (m, 1 H, $J_{4'',5''ax/eq}$ 4.5 and 4.6 Hz, H-4″), 4.72 and 4.53 (2 d, each 1 H, PhCH_2O), 4.60 (s, 1 H, H-1′), 4.43 and 3.49 (2 dd, each 1 H, $J_{5''ax,5''eq}$ −12.6 Hz, H-5″*ax*,5″*eq*), 4.38 (t, 1 H, $J_{3,4}$ 8.4 Hz, H-3), 4.24 (t, 1 H, $J_{2,3}$ 10.0 Hz, H-2), 4.13 and 3.88 (2 m, 2 H, CH_2=CHCH_2O), 4.02 (d, 1 H, $J_{2',3'}$ 3.0 Hz, H-2′), 3.99 (t, 1 H, H-4), 3.50 (dd, 1 H, $J_{6'a,5'}$ 2.8, $J_{6a',6b'}$ −11.6 Hz, H-6′a), 3.18 (m, 1 H, $J_{5',4'}$ 9.4, $J_{5',6'b}$ 6.0 Hz, H-5′), 3.07 (dd, 1 H, $J_{3',4'}$ 9.4 Hz, H-3′), 2.65 (m, 2 H, $CH_3C$$H_2$S), 2.11, 2.04, and 1.96 (3 s, each 3 H, 3 Ac), 1.18 (t, 3 H, C$H_3$$CH_2$S); ^{13}C, δ 169.7, 169.3, and 169.0 (3 *C*OCH$_3$), 138.3, 137.8, 134.5, 133.6, 131.6, 128.4–127.0, and 123.2 ($C_6H_5CH_2O$, Phth, and H_2C=*C*HCH_2O), 117.1 (H_2C=CHCH_2O), 101.2 (C-1′), 98.9 (C-1″), 81.0, 80.4, 80.1, 78.8, 78.4, 76.1, 72.4, 69.3, 68.7, 68.1, and 66.4 (C-1,3,4,5,2′,3′,4′,5′,2″,3″,4″), 74.7, 73.5, 70.0, 69.4, 62.3, and 60.1 (C-6,6′,5″, 2 PhCH_2O, and H_2C=CHCH_2O), 54.9 (C-2), 23.6 ($CH_3C$$H_2$S), 20.5 (2 C) and 20.4 (CO*C*H$_3$), 14.8 (CH_3CH_2S). Anal. Calcd for $C_{50}H_{59}NO_{18}S \cdot H_2O$: C, 59.34; H, 6.07. Found: C, 59.68; H, 5.98.

*Ethyl 4-O-(3-O-allyl-2-O-β-*D*-xylopyranosyl-β-*D*-mannopyranosyl)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-1-thio-β-*D*-glucopyranoside* (**18**).—A solution of **17** (60 mg, 60 μmol) in methanolic M NaOMe (4 mL) was kept for 16 h, then neutralised with Dowex 50W (H^+) resin, filtered, and concentrated to give **18**, as a white glass (50 mg, 95%); $[\alpha]_D$ −26° (*c* 0.2); R_f 0.62 (85:15 CH_2Cl_2–MeOH). NMR data ($CDCl_3$): ^1H, δ 7.83–6.72 (m, 14 H, 2 Ph and Phth), 5.86 (m, 1 H, H_2C=CHCH_2O), 5.21 (d, 1 H, $J_{1,2}$ 10.2 Hz, H-1), 4.77 and 4.49 (2 d, each 1 H, PhCH_2O), 4.58 (s, 1 H, H-1′), 4.49 (d, 1 H, $J_{1'',2''}$ 6.7 Hz, H-1″), 4.30 (t, 1 H, $J_{3,4}$ 8.4 Hz, H-3), 4.17 (t, 1 H, $J_{2,3}$ 10.0 Hz, H-2), 2.61 (m, 2 H, $CH_3C$$H_2$S), 1.15 (C$H_3$$CH_2$S); ^{13}C, δ 138.5, 137.9, 134.4, 133.7, 131.6, 128.5–127.0, and 123.4 ($C_6H_5CH_2O$, Phth, and H_2C=*C*HCH_2O), 117.8 (H_2C=CHCH_2O), 103.3 (C-1″), 102.2 (C-1′), 81.0, 80.5, 80.1, 78.8 (2 C), 78.6, 76.5, 75.0, 73.0, 72.6, and 69.3 (C-1,3,4,5,2′,3′,4′,5′,2″,3″,4″), 74.8, 73.6 (2 C), 70.3, 69.2, and 61.0 (C-6,6′,5″, 2 PhCH_2O, and H_2C=CHCH_2O), 54.9 (C-2), 23.7 ($CH_3C$$H_2$S), 14.9 ($CH_3CH_2S$). Anal. Calcd for $C_{44}H_{53}NO_{15}S \cdot H_2O$: C, 59.65; H, 6.26. Found: C, 59.98; H, 6.05.

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