Synthesis of a Methyl Heptaglucoside with Phytoalexin Elicitor Activity Based on Oxidative Coupling of Glucals

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Abstract: Several suitable building blocks for the construction of the phytoalexin elicitor α-methyl-3^2,3^4-di-β-D-glucopyranosylgentiopentaoside (**2**) were readily accessible by oxidative coupling of glucals. Block coupling of trimeric phenylseleno- and ethylthioglucosyl donors **17** and **18** with tetrasaccharide **16** in the presence of the thiophilic promoter *N*-iodosuccinimide and catalytic trifluoromethanesulfonic acid furnished the desired heptaglucan **2** in high overall yield.

Keywords: block synthesis · epoxidations · glycosylations · oligosaccharides · selenoglycosides

Introduction

It is now well documented[1] that the branched 3^2,3^4-di-β-D-glucopyranosylgentiopentaose (**1**) or its methyl congener **2** (Fig. 1) trigger phytoalexin accumulation in soybean. Recently, Verduyn et al. reported that activation of ethyl-1-thioglucosides with the thiophilic promoter *N*-iodosuccinimide (NIS) and catalytic amounts of trifluoromethanesulfonic acid (TfOH) was an efficient and high-yielding approach to the β-linked glucoheptaoside **2**.[2] It was also established that the thioglycoside chemistry could be adopted for a solution-based solid phase synthesis of the methyl heptaglucoside **2** with polyethylene glycol (PEG) as a polymer support.[3]

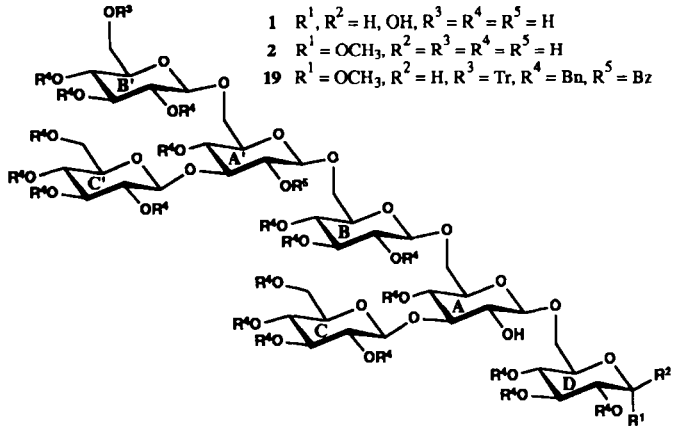

Fig. 1. Structures of naturally occurring heptasaccharide **1**, methyl analogue **2** and its partially protected derivative **19**.

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In a recent paper Danishefsky et al. presented a new and promising approach towards a solid-phase synthesis of oligosaccharides.[4] A characteristic feature of this methodology is the introduction of 1,2-*trans* glycosidic bonds by stereoselective oxidation of an immobilized glycal with 3,3-dimethyldioxirane (DMD) and in situ elongation of the newly generated α-1,2-epoxide with an incoming glycal acceptor under the influence of zinc chloride. The potential of the stereoselective two-step glycosylation was nicely illustrated in a solid support synthesis of several β-linked oligosaccharides.[5] Consequently, the construction of the target heptaglucan **2** by oxidative coupling of glucals with PEG as a polymer support seemed to be an attractive alternative. However, a recent study revealed that zinc chloride mediated condensation of 1,2-anhydro-3,4,6-tri-*O*-benzyl-α-D-glucopyranoside (**6**) with the primary hydroxyl group in glucal **5** was accompanied by the formation of the unwanted α-linked dimer.[6] It is evident that the lack of stereoselectivity does not augur well for our primary objective. On the other hand, reduction in protective group manipulations, together with the feasibility of introducing other functionalities at the anomeric centre by nucleophilic ring opening of a 1,2-epoxide, are additional merits of the glycal approach. These features were a decisive factor in our choice of the glucal approach for the preparation of the phytoalexin elicitor α-methyl-3^2,3^4-di-β-D-glucopyranosylgentiopentaoside (**2**) in solution.

Results and Discussion

The route used towards the synthesis of methyl heptaglucoside **2** is outlined in Scheme 1. It commences with the preparation of the appropriately protected laminaribiosyl derivative **9**, the glucal moiety of which can be used for the further construction of the linear gentiobiosylpentaoside backbone. To this end, commercially available tri-*O*-benzyl-D-glucal **3** was oxidized[7] with DMD[8] to give the corresponding α-1,2-anhydro derivative **6** in excellent yield. Zinc chloride assisted condensation of epoxide donor **6** with the allylic hydroxyl group in the glucal acceptor **8**,

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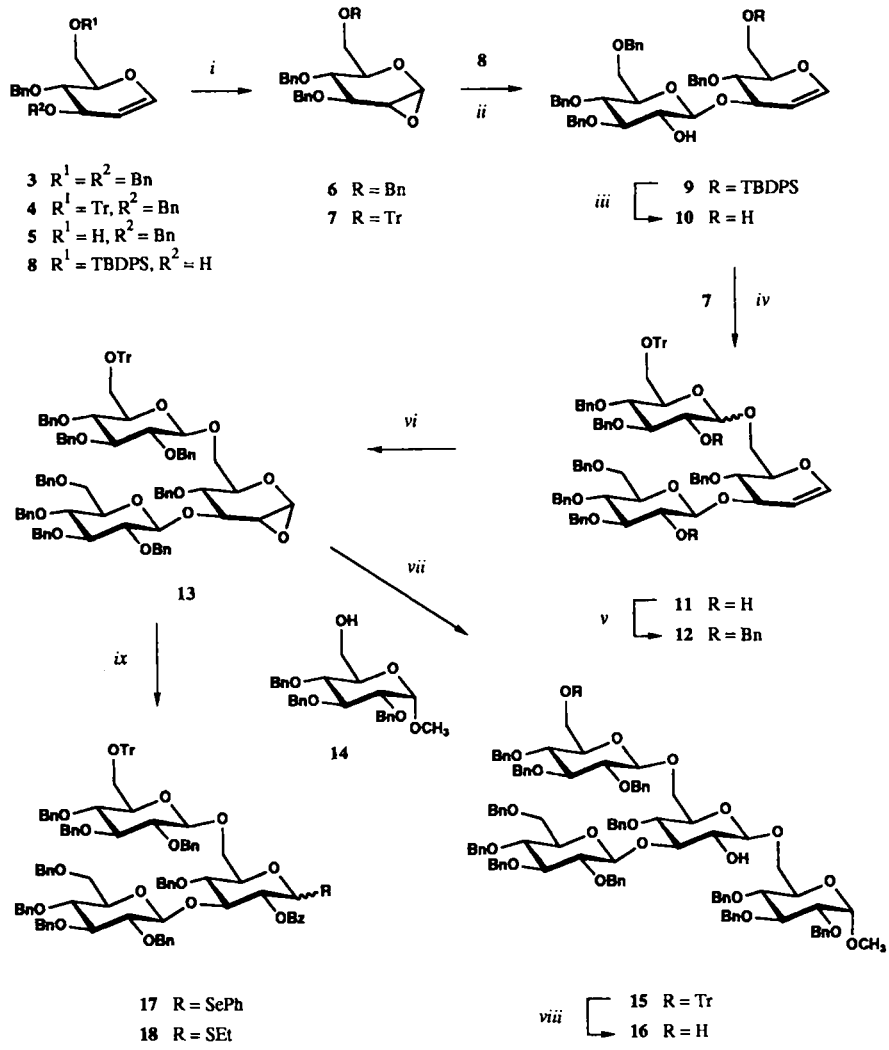

Scheme 1. i) 3,3-Dimethyldioxirane (1.2 equiv), CH_2Cl_2, 0 °C, 5 min, 96%; ii) $ZnCl_2$ (2 equiv), THF, 10 min, 60%; iii) $(nBu)_4NF$, THF, 12 h, 93%; iv) $ZnCl_2$ (2 equiv), THF, 15 min, 65%; v) BnBr, NaH, DMF, 1 h, 87%; vi) 3,3-dimethyldioxirane (1.2 equiv), CH_2Cl_2, 0 °C, 15 min, 92%; vii) $ZnCl_2$ (2 equiv), THF, 15 min, 52%; viii) PhSeH or EtSH (2 equiv), $ZnCl_2$ (2 equiv), THF, 30 min; then BzCl, pyridine, 2 h (**17**: 42% yield; **18**: 69% yield); ix) pyrrole (6 equiv), CF_3CO_2H (2 equiv), CH_2Cl_2, 1 h, 88%.

obtained by regioselective silylation of the known 4-*O*-benzyl-D-glucal **5**[9] with *tert*-butyldiphenylsilyl chloride (TBDPS-Cl), proceeded smoothly and stereoselectively to afford the β-linked dimer **9** in 60% yield (based on consumed acceptor).

Desilylation of **9** with fluoride ion gave dimer **10**, the primary hydroxyl group of which was glycosylated with the α-epoxide **7**, prepared from 6-*O*-trityl-D-glucal[10] by benzylation and sequential epoxidation. Thus, $ZnCl_2$-mediated glycosylation of dimer **10** with **7** furnished trimer **11** as a mixture of anomers ($\alpha:\beta = 1:5$). It is of interest to note that the stereochemical outcome of the condensation endorses the earlier observation that the formation of a $\beta(1 \rightarrow 6)$ glucosidic bond does not proceed stereoselectively.[6] Separation of the individual anomers by chromatography on silica gel afforded the requisite β-linked trimer **11** in 54% yield (based on consumed acceptor). Benzylation of **11** and subsequent epoxidation of **12** led to α-1,2-anhydro derivative **13**, which was condensed with an excess of the terminal building block methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (**14**) in the presence of $ZnCl_2$.[11]

The outcome of the latter $\beta(1 \rightarrow 6)$ glycosylation differs in two respects from the previous one. First of all, an acceptable yield of the β-linked tetramer **15** (52%, based on **12**) could only be obtained by using excess (5 equiv) acceptor **14**. The use of equimolar amounts of **13** and **14**, as in the glycosylation of **10** with **7**, led to an intractable mixture of products. It was also firmly established that the formation of tetramer **15** is a highly stereoselective process, only the β-linked product could be detected in the reaction mixture. It is conceivable that the stereochemistry of the $ZnCl_2$-assisted glycosylation of acceptor **14** with the intrinsically reactive trimeric epoxide **13** may be attributed to steric effects. Steric hindrance may also account for the failure of the condensation of **13** with tetramer **16**, obtained in a near quantitive yield by acidolysis[12] of the trityl group in **15** with trifluoroacetic acid in the presence of pyrrole.

In order to overcome the problem encountered in the latter glycosylation, trimer **13** was transformed[13] into the more conventional 2-*O*-benzoyl phenylseleno[14, 15] and ethylthio[16] glucosides **17** and **18** by Lewis acid catalyzed nucleophilic ring opening of the α-1,2-epoxide function in **13** and subsequent benzoylation of the newly generated 2-hydroxyl group. Thus, reaction of **13** with benzeneselenol in the presence of $ZnCl_2$ gave, after in situ treatment of the resulting phenylselenoglucoside with benzoyl chloride, a 1:3 ratio of 2-*O*-benzoyl phenyl α,β-selenoglucosides **17** in 42% yield. It is worth noting that the nucleophilic ring opening of the α-1,2-epoxide in trimer **13** could not be effected, as in the case of the fully benzylated α-epoxide **6**,[17] with benzeneselenol in the absence of zinc chloride. Similarly, the use of ethanethiol/zinc chloride was required in the first step of the conversion of **13** into the corresponding ethyl 2-*O*-benzoyl-1-thio-α,β-D-glucopyranosides **18** (ratio $\alpha:\beta = 1:3$, overall yield of 69%).

At this stage, the intended acceptor **16** containing a free primary OH group was regioselectively glycosylated under the influence of NIS and catalytic amounts of TfOH[15] with the respective phenylseleno- and ethylthioglucosyl donors **17** and **18**. Workup and purification of the individual reaction mixtures by silica gel chromatography gave heptamer **19** in 49% and 58% yield (based on acceptor **16**) starting from **17** and **18**, respectively. Zemplén deacylation of **19** and subsequent hydrogenolysis with palladium hydroxide on charcoal afforded, after purification by chromatography on Sephadex LH-20, homogeneous methyl heptaglucoside **2** in 72% yield. The spectroscopic and analytical data of **2** were in full accord with those of the same oligomer that we prepared earlier[2, 3] by a thioglycoside approach.

Conclusion

The results presented in this paper show that the efficacy of glycals as glycosyl donors decreases beyond the dimeric stage, thus limiting the general application of glycals in the construc-

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tion of oligosaccharides by a block condensation procedure. However, this drawback can be overcome by the introduction[13] of an appropriate function at the anomeric centre of the glycal moiety in the donor molecule and subsequent protection of the newly generated 2-OH by a participating acyl or nonparticipating ether group (cf. conversion of trimeric glycal **12** into the respective phenylseleno- or ethylthioglucosyl donors **17** or **18**). In conclusion, the attractive features inherent in the glycal approach promise to be of great value for the future construction of complex oligosaccharides.

Experimental Procedure

Materials and methods: ^{1}H and ^{13}C NMR spectra were recorded with a Jeol JNM-FX-200 (200 and 50.1 MHz, respectively) or a Bruker WM-300 spectrometer (300 and 75.1 MHz, respectively). Chemical shifts are given relative to tetramethylsilane as internal standard. Optical rotations were determined at 20 °C by means of a PROPOL polarimeter. Dichloromethane, pyridine and toluene were refluxed with CaH_2 for 3 h, distilled and stored over molecular sieves (4 Å). Triethylamine (TEA) was heated under reflux with CaH_2 for 3 h and then distilled. 1,2-Dichloroethane (Biosolvent, HPLC grade), *N*,*N*-dimethylformamide (DMF, Baker, p.a.) and tetrahydrofuran (THF, Biosolvent, HPLC-grade) were stored over molecular sieves (4 Å). Zinc chloride (Merck, p.a.) was dissolved in THF (1.0 M solution) and stored over molecular sieves (3 Å). Pyrrole (Janssen) was freshly distilled before use. Benzoyl chloride (Merck), benzyl bromide (Merck), *tert*-butyldimethylsilyl chloride (TBDMS-Cl, Janssen), *tert*-butyldiphenylsilyl chloride (TBDPS-Cl, Janssen), imidazole (Merck), *N*-iodosuccinimide (NIS, Aldrich), palladium hydroxide on charcoal (20% $Pd(OH)_2$/C, Aldrich), sodium hydride (Janssen, 60% dispersion in mineral oil), trifluoroacetic acid (Aldrich), trifluoromethanesulfonic acid (TfOH, Fluka) and trityl chloride (Tr-Cl, Janssen) were used as received. Column chromatography was performed on Merck Kieselgel 60 (230–400 mesh). Gel permeation chromatography was accomplished on Sephadex LH-20 (Pharmacia). TLC analysis was performed on DC-fertigfolien (Schleicher & Schüll F1500, LS254) with detection by UV absorption (254 nm), where applicable, and charring with 20% H_2SO_4 in MeOH or ammonium molybdate (25 g L^{-1}) and ceric ammonium sulfate (10 g L^{-1}) in 10% aq. H_2SO_4. Reactions were run at ambient temperature, unless otherwise stated. Prior to the NIS/TfOH mediated coupling reactions, powdered molecular sieves (4 Å) were added to the reaction mixture and stirred for 30 min at 20 °C.

1,5-Anhydro-3,4-di-*O*-benzyl-6-*O*-trityl-2-deoxy-D-*arabino*-hex-1-enopyranoside (4): To a stirred solution of 6-*O*-trityl-D-glucal[10] (3.88 g, 10.0 mmol) in DMF (50 mL) was added NaH (0.600 g, 25.0 mmol) and benzyl bromide (2.98 mL, 25.0 mmol). After the reaction mixture had been stirred for 2 h, MeOH (5 mL) was added, and the solvent was evaporated under reduced pressure. The residue was redissolved in EtOAc (150 mL), washed with water (2 × 50 mL), dried ($MgSO_4$) and concentrated in vacuo. Purification by silica gel chromatography (0–20% EtOAc/hexanes) furnished glucal **4** as a colourless oil (5.06 g, 89%). $[\alpha]_D = 3.1$ ($c = 1$, $CHCl_3$). ^{1}H NMR ($CDCl_3$): $\delta = 7.50-7.01$ (25H, m, H_{arom}), 6.49 (1H, d, H_1, $J_{1,2} = 6.2$ Hz), 4.87 (1H, m, H_2), 4.61 (2H, AB, CH_2 Bn), 4.57 (2H, AB, CH_2 Bn), 4.16 (1H, m, H_3), 3.99 (2H, m, H_4/H_5), 3.46 (10H, AB, $H_6/H_{6'}$). ^{13}C NMR ($CDCl_3$): $\delta = 144.5$ (C_1), 143.6 (C_{quat} Tr), 138.1, 137.9 (2 × C_{quat} Bn), 128.4–126.6 (C_{arom}), 99.6 (C_2), 86.2 (C(Ph)$_3$), 76.6, 75.8, 74.4 ($C_3/C_4/C_5$), 73.4, 71.7 (2 × CH_2 Bn), 61.9 (C_6).

1,2-Anhydro-3,4-di-*O*-benzyl-6-*O*-trityl-α-D-glucopyranoside (7): To a cooled (0 °C) and stirred solution of glucal **4** (1.42 g, 2.50 mmol) in CH_2Cl_2 (10 mL) was added dropwise a solution of 3,3-dimethyldioxirane [8] in acetone (0.075 M, 40.0 mL, 3.00 mmol). After the reaction mixture had been stirred for 5 min, it was concentrated under reduced pressure. The epoxide **7** was obtained as a white solid (1.37 g, 94%). $[\alpha]_D = 4.9$ ($c = 1$, $CHCl_3$). ^{1}H NMR ($CDCl_3$): $\delta = 7.51-6.85$ (25H, m, H_{arom}), 5.09 (1H, d, H_1, $J_{1,2} = 2.4$ Hz), 4.73 (2H, AB, CH_2 Bn), 4.51 (2H, AB, CH_2 Bn), 3.93 (1H, d, H_3, $J_{3,4} = 7.5$ Hz), 3.81 (1H, dd, H_4, $J_{4,5} = 7.7$ Hz), 3.72 (1H, m, H_5), 3.27 (1H, dd, H_6, $J_{5,6} = 3.0$ Hz, $J_{6,6'} = 11.3$ Hz), 3.22 (1H, dd, $H_{6'}$, $J_{5,6'} = 2.5$ Hz), 3.12 (1H, d, H_3). ^{13}C NMR ($CDCl_3$): $\delta = 143.6$ (C_{quat} Tr), 137.8, 137.2 (2 × C_{quat} Bn), 128.5–126.7 (C_{arom}), 86.1 (C(Ph)$_3$), 78.8 (C_3), 77.2 (C_1), 74.2, 74.2 (2 × CH_2 Bn), 74.1 (C_4), 69.3 (C_5), 61.5 (C_6), 52.3 (C_2). $C_{39}H_{36}O_5$ (584.7): calcd. C 80.11, H 6.21; found C 79.99, H 6.26.

1,5-Anhydro-4-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-D-*arabino*-hex-1-enopyranoside (8): TBDPS-Cl (1.37 mL, 5.25 mmol) was added to a stirred solution of 4-*O*-benzyl-D-glucal [9] (1.18 g, 5.00 mmol) in pyridine (50 mL). After the reaction mixture had been stirred for 15 h, MeOH (3 mL) was added, and the solvent was evaporated in vacuo. The resulting oil was redissolved in EtOAc (100 mL), washed with aq. $NaHCO_3$ (1 M, 2 × 30 mL), dried ($MgSO_4$) and concentrated under reduced pressure. Pyridine was removed by coevaporation with toluene (2 × 50 mL) and the residue was purified by silica gel chromatography (10–25% EtOAc/hexanes) to yield glucal **8** as a white solid (1.87 g, 79%). $[\alpha]_D = 11.2$ ($c = 1$, $CHCl_3$). ^{1}H NMR ($CDCl_3$): $\delta = 7.73-7.25$ (15H, m, H_{arom}), 6.39 (1H, dd, H_1, $J_{1,2} = 6.2$ Hz, $J_{1,3} = 1.5$ Hz), 4.83 (2H, AB, CH_2 Bn), 4.72 (1H, dd, H_2, $J_{2,3} = 2.2$ Hz), 4.36 (1H, m, H_3), 4.01 (2H, m, H_4/H_5), 3.84 (2H, m, $H_6/H_{6'}$), 2.00 (1H, d, OH, $J_{3,OH} = 5.9$ Hz), 1.08, 1.07, 1.06 (3 × 3H, 3 × s, 3 × CH_3 *t*Bu). ^{13}C NMR ($CDCl_3$): $\delta = 144.5$ (C_1), 138.6 (C_{quat} Bn), 135.8–129.7 (C_{arom} TBDPS), 133.4, 133.0 (2 × C_{quat} TBDPS), 128.5–127.6 (C_{arom} Bn), 102.5 (C_2), 77.7, 77.1 (C_4/C_5), 73.9 (CH_2 Bn), 69.1 (C_3), 62.5 (C_6), 26.8 (CH_3 *t*Bu), 19.3 (C_{quat} *t*Bu). $C_{29}H_{34}O_4Si$ (474.7): calcd. C 73.38, H 7.22; found C 73.12, H 7.29.

3-*O*-(3,4,6-Tri-*O*-benzyl-β-D-glucopyranosyl)-1,5-anhydro-4-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-D-*arabino*-hex-1-enopyranoside (9): $ZnCl_2$ (1.0 M solution in THF, 4.00 mL) was added dropwise, under a continuous stream of dry nitrogen, to a solution of 1,2-anhydro-3,4,6-tri-*O*-benzyl-α-D-glucopyranoside [7] (**3**, 0.865 g, 2.00 mmol) and glucal **8** (1.42 g, 3.00 mmol) in THF (10 mL). After 10 min of stirring, the reaction mixture was diluted with EtOAc (100 mL), washed with water (25 mL) and aq. $NaHCO_3$ (1 M, 2 × 25 mL), dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by Sephadex LH-20 column chromatography (eluent: CH_2Cl_2/MeOH, 2/1 v/v); this resulted in the isolation of dimer **9** as a white solid (0.924 g, 60%) and unreacted acceptor **8** (0.616 g, 1.3 mmol). $[\alpha]_D = -9.8$ ($c = 1$, $CHCl_3$). ^{1}H NMR ($CDCl_3$): $\delta = 7.71-7.15$ (30H, m, H_{arom}), 6.41 (1H, d, H_1, $J_{1,2} = 6.2$ Hz), 4.97 (1H, d, $H_{1'}$, $J_{1',2'} = 11.3$ Hz) 4.96–4.78 (4H, m, 2 × CH_2 Bn), 4.83 (1H, d, H_2), 4.68–4.41 (4H, m, 2 × CH_2 Bn), 4.39 (1H, d, H_3, $J_{3,4} = 7.2$ Hz), 4.03–3.87 (3H, m, $H_4/H_5/H_{2'}$), 3.68 (2H, AB, H_{6A}/H_{6B}), 3.65–3.54 (3H, m, H_3/$H_{4'}/H_{5'}$), 3.48 (2H, AB, $H_{6'A}/H_{6'B}$), 2.30 (1H, bs, OH). 1.06 (9H, s, CH_3 *t*Bu). ^{13}C NMR ($CDCl_3$): $\delta = 145.6$ (C_1), 138.9, 138.8, 138.3, 138.3 (4 × C_{quat} Bn), 135.9–129.9 (C_{arom} TBDPS), 133.6, 133.4 (2 × C_{quat} TBDPS), 128.5–127.8 (C_{arom} Bn), 100.7 ($J_{C,H} = 156.8$ Hz, $C_{1'}$), 98.9 (C_2), 84.7 ($C_{3'}$), 77.9, 77.6, 75.4, 74.9, 74.2, 73.8 ($C_3/C_4/C_5/C_{2'}/C_{4'}/C_{5'}$), 75.2, 75.1, 73.6, 73.4 (4 × CH_2 Bn), 69.1 ($C_{6'}$), 62.5 (C_6), 27.1 (CH_3 *t*Bu), 19.5 (C_{quat} *t*Bu). $C_{56}H_{62}O_9Si$ (907.2): calcd. C 74.14, H 6.89; found C 73.88, H 6.99.

3-*O*-(3,4,6-Tri-*O*-benzyl-β-D-glucopyranosyl)-1,5-anhydro-4-*O*-benzyl-2-deoxy-D-*arabino*-hex-1-enopyranoside (10): A solution of dimer **9** (0.924 g, 1.02 mmol) in THF (10 mL) was treated with (nBu)$_4$NF (1.0 M solution in THF, 1.53 mL) and stirred overnight. Ethyl acetate (50 mL) was added and the reaction mixture was washed with aq. $NaHCO_3$ (1 M, 2 × 10 mL), dried ($MgSO_4$) and concentrated in vacuo. The resulting oil was purified by silica gel chromatography (10–40% EtOAc/hexanes) to yield dimer **10** as a white solid (0.633 g, 93%). $[\alpha]_D = -11.1$ ($c = 1$, $CHCl_3$). ^{1}H NMR ($CDCl_3$): $\delta = 7.49-7.10$ (20H, m, H_{arom}), 6.43 (1H, dd, H_1, $J_{1,2} = 6.2$ Hz, $J_{1,3} = 0.8$ Hz, 4.91–4.78 (4H, m, 2 × CH_2 Bn), 4.90 (1H, d, $H_{1'}$, $J_{1',2'} = 11.3$ Hz), 4.88 (1H, dd, H_2, $J_{2,3} = 1.0$ Hz), 4.71 (2H, AB, CH_2 Bn), 4.49 (2H, AB, CH_2 Bn), 4.32 (1H, dt, H_3, $J_{3,4} = 5.1$ Hz), 4.12 (1H, ddd, H_5, $J_{5,6A} = J_{5,6B} = 6.1$ Hz), 3.97 (1H, dd, H_4, $J_{4,5} = 5.3$ Hz), 3.89 (1H, m, $H_{2'}$), 3.77 (2H, m, H_{6A}/H_{6B}), 3.62 (1H, dd, $H_{4'}$, $J_{4',5'} = 10.6$ Hz), 3.56 (1H, ddd, $H_{5'}$, $J_{5',6'} = 1.2$ Hz), 3.49 (2H, AB; d, $H_{6'A}/H_{6'B}$, $J_{6'A,6'B} = 8.3$ Hz), 3.36 (1H, ddd, $H_{3'}$, $J_{3',4'} = 4.5$ Hz, $J_{3',5'} = 2.1$ Hz), 2.57 (2H, bs, 2 × OH). ^{13}C NMR ($CDCl_3$): $\delta = 145.5$ (C_1), 138.7, 138.3, 138.1, 138.1 (4 × C_{quat} Bn), 128.9–127.7 (C_{arom} Bn), 101.6 ($C_{1'}$), 98.6 (C_2), 84.5 ($C_{3'}$), 77.4, 76.6, 75.0, 74.6, 73.5, 72.9 ($C_3/C_4/C_5/C_{2'}/C_{4'}/C_{5'}$), 75.2, 75.1, 73.6, 72.6 (4 × CH_2 Bn), 68.8 ($C_{6'}$), 60.6 (C_6). $C_{40}H_{44}O_9$ (668.8): calcd. C 71.84, H 6.63; found C 71.81, H 6.65.

3-*O*-(3,4,6-Tri-*O*-benzyl-β-D-glucopyranosyl)-6-*O*-(3,4-di-*O*-benzyl-6-*O*-trityl-α/β-D-glucopyranosyl)-1,5-anhydro-4-*O*-benzyl-2-deoxy-D-*arabino*-hex-1-enopyranoside (11): Under a continous stream of dry nitrogen, $ZnCl_2$ (1.0 M solution in THF, 1.90 mL) was added dropwise to a stirred solution of epoxide **7** (0.554 g, 0.948 mmol) and dimer **10** (0.633 g, 0.948 mmol) in THF (5.0 mL). After 15 min of stirring, EtOAc (50 mL) was added, and the reaction mixture was washed with water (10 mL) and aq. $NaHCO_3$ (1 M, 2 × 15 mL), dried ($MgSO_4$) and concentrated under reduced pressure. Purification of the residue by silica gel chromatography (40% EtOAc/hexanes) furnished trimer **11** as a colourless oil (α anomer: 0.091 g, 11%; β anomer: 0.452 g, 54%), as well as unreacted dimer **10** (0.187 g, 0.280 mmol). ^{13}C NMR ($CDCl_3$); β anomer: $\delta = 144.9$ (C_{1A}), 143.6 (C_{quat} Tr), 138.4, 138.4, 138.1, 137.9, 137.9, 137.6 (6 × C_{quat} Bn), 128.5–126.2 (C_{arom}), 103.4 ($J_{C,H} = 158.3$ Hz, C_{1B}), 100.3 ($J_{C,H} = 155.3$ Hz, C_{1C}), 98.3 (C_{2A}), 86.1 (C(Ph)$_3$), 84.6, 84.4 (C_{3B}/C_{3C}), 77.2, 77.2, 76.1, 74.9, 74.3, 74.3, 74.1, 73.6, 73.3 ($C_{2B-C}/C_{3A}/C_{4A-C}/C_{5A-C}$), 75.0, 74.7, 74.7, 73.1, 73.1, 72.4 (6 × CH_2 Bn), 68.6, 67.7 (C_{6A}/C_{6C}), 62.2 (C_{6B}); α anomer: $\delta = 100.6$ (C_{1C}), 98.7 (C_{1B}), 98.3 (C_{2A}), 84.5 (C_{3C}), 83.3 (C_{3B}).

3-*O*-(2,3,4,6-Tetra-*O*-benzyl-β-D-glucopyranosyl)-6-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-trityl-β-D-glucopyranosyl)-1,5-anhydro-4-*O*-benzyl-2-deoxy-D-*arabino*-hex-1-enopyranoside (12): To a stirred solution of trimer **11** (452 mg, 0.361 mmol) in DMF (5 mL) was added NaH (26.0 mg, 1.08 mmol) and benzyl bromide (129 μL, 1.08 mmol). After the reaction mixture had been stirred for 1 h, MeOH (1 mL) was added, and the solvent was evaporated under reduced pressure. The residue was redissolved in EtOAc (50 mL), washed with water (2 × 10 mL), dried ($MgSO_4$) and concentrated in vacuo. Purification by silica gel chromatography (10–30% EtOAc/hexanes) resulted in the isolation of crude **12**, which was subjected to Sephadex LH-20 column chromatography (eluent: CH_2Cl_2/MeOH, 2/1 v/v) to yield trimer **12** as a white solid (450 mg, 87%). $[\alpha]_D = -3.8$ ($c = 1$, $CHCl_3$). ^{13}C NMR ($CDCl_3$): $\delta = 144.6$ (C_{1A}), 143.7 (C_{quat} Tr), 138.5, 138.4, 138.3, 138.1, 138.0, 137.9, 137.9, 137.7

($8 \times C_{quat}$ Bn), 128.6–126.8 (C_{arom}), 104.1 (C_{1B}), 101.4 (C_{1C}), 98.9 (C_{2A}), 86.1 ($C(Ph)_3$), 84.5, 84.4 (C_{3B}/C_{3C}), 82.1, 82.1, 77.6, 77.6, 76.3, 74.3, 74.1, 74.1, 72.9 ($C_{2B-C}/C_{3A}/C_{4A-C}/C_{5A-C}$), 75.7, 75.4, 74.8, 74.8, 74.7, 73.3, 73.3, 72.4 ($8 \times CH_2$ Bn), 68.8, 68.2 (C_{6A}/C_{6C}), 62.1 (C_{6B}). $C_{93}H_{92}O_{14}$ (1433.8): calcd. C 77.91, H 6.47; found C 77.98, H 6.47.

Methyl 6-*O*-(3-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-6-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-trityl-β-D-glucopyranosyl)-4-*O*-benzyl-β-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (15): A solution of 3,3-dimethyldioxirane in acetone (0.080 M, 1.63 mL) was added dropwise to a cooled (0 °C) and stirred solution of glucal **12** (155 mg, 0.109 mmol) in CH_2Cl_2 (1.0 mL). After the reaction mixture had been stirred for 10 min, it was concentrated in vacuo. Epoxide **13** was obtained as a white solid (145 mg, 92%). To a stirred solution of epoxide **13** (145 mg, 0.100 mmol) and methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside [11] (**14**, 232 mg, 0.500 mmol) in THF (2.0 mL), under a continuous stream of dry nitrogen, was added $ZnCl_2$ (1.0 M solution in THF, 200 μL). After stirring for 15 min, EtOAc (25 mL) was added and the reaction mixture was washed with water (5 mL) and aq. $NaHCO_3$ (1 M, 2 × 5 mL), dried ($MgSO_4$) and concentrated under reduced pressure. Purification of the residue by silica gel chromatography (20–40% EtOAc/hexanes) furnished crude **15**, which was further purified by Sephadex LH-20 column chromatography (eluent: CH_2Cl_2/MeOH, 2/1 v/v) to yield tetramer **15** as a white solid (99.5 mg, 52%). $[\alpha]_D = 27.2$ ($c = 1$, $CHCl_3$). ^{13}C NMR ($CDCl_3$): $\delta = 143.9$ (C_{quat} Tr), 138.9–137.2 (C_{quat} Bn), 128.7–126.8 (C_{arom}), 104.1 ($J_{C,H} = 160.0$ Hz), 103.9 ($J_{C,H} = 159.7$ Hz; C_{1A}/C_{1B}), 102.1 ($J_{C,H} = 161.2$ Hz, C_{1C}), 97.9 ($J_{C,H} = 170.0$ Hz; C_{1D}), 86.8 (C_{3A}), 86.2 ($C(Ph)_3$), 85.1, 84.8, 84.4, 82.5, 82.2, 81.9, 79.8, 79.0, 77.9, 77.7, 77.4, 77.2, 74.5, 74.5, 69.4 ($C_{2A-D}/C_{3B-D}/C_{4A-D}/C_{5A-D}$), 75.8–73.0 ($CH_2$ Bn), 68.7, 68.2, 67.9 ($C_{6A}/C_{6C}/C_{6D}$), 62.3 (C_{6B}), 55.1 (OCH_3). $C_{121}H_{124}O_{24}$ (1914.3): calcd. C 75.92, H 6.53; found C 75.69, H 6.68.

Methyl 6-*O*-(3-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-6-*O*-(2,3,4-tri-*O*-benzyl-β-D-glucopyranosyl)-4-*O*-benzyl-β-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (16): A stirred solution of tetramer **15** (99.5 mg, 0.052 mmol) in CH_2Cl_2 (5.0 mL) was treated with pyrrole (21.6 μL, 0.312 mmol) and trifluoroacetic acid (8.0 μL, 0.104 mmol). After the mixture had been stirred for 1 h, aq. $NaHCO_3$ (1 M, 15 mL) was slowly added. The reaction mixture was extracted with CH_2Cl_2 (30 mL), dried ($MgSO_4$) and concentrated in vacuo. Purification by silica gel chromatography (40% EtOAc/hexanes) resulted in the isolation of the crude tetrasaccharide, which was subjected to Sephadex LH-20 column chromatography (eluent: CH_2Cl_2/MeOH, 2/1 v/v) to yield acceptor **16** as a white solid (76.4 mg, 88%). $[\alpha]_D = 7.0$ ($c = 1$, $CHCl_3$). ^{13}C NMR ($CDCl_3$): $\delta = 138.9–137.2$ (C_{quat} Bn), 128.4–127.4 (C_{arom}), 104.2, 103.7 (C_{1A}/C_{1B}), 102.0 (C_{1C}), 98.0 (C_{1D}), 86.8 (C_{3A}), 85.1, 84.5, 82.5, 81.9, 81.9, 81.8, 79.1, 77.9, 77.4, 77.4, 77.2, 75.8, 75.2, 75.0, 69.5 ($C_{2A-D}/C_{3B-D}/C_{4A-D}/C_{5A-D}$), 75.6–73.1 ($CH_2$ Bn), 68.7, 68.7, 67.9 ($C_{6A}/C_{6C}/C_{6D}$), 61.8 (C_{6B}), 55.0 (OCH_3).

Phenyl 3-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-6-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-trityl-β-D-glucopyranosyl)-2-*O*-benzoyl-4-*O*-benzyl-1-seleno-α/β-D-glucopyranoside (17): Epoxide **13** was prepared as described for the synthesis of tetramer **15**. To a stirred solution of trimer **13** (140 mg, 0.096 mmol) in THF (2.0 mL) was, under a continuous stream of dry nitrogen, subsequently added phenylselenol (PhSeH, 20.3 μL, 0.193 mmol) and $ZnCl_2$ (1.0 M solution in THF, 193 μL). After the mixture had been stirred for 30 min, pyridine (2 mL) was added, and the reaction mixture was treated with benzoyl chloride (BzCl, 44.8 μL, 0.386 mmol). After 2 h, excess benzoyl chloride was removed by addition of water (1 mL) and the solvent was evaporated under reduced pressure. The residue was redissolved in EtOAc (25 mL) and washed with aq. $NaHCO_3$ (1 M, 2 × 5 mL), dried ($MgSO_4$) and concentrated in vacuo. Pyridine was removed by coevaporation with toluene (3 × 10 mL). Purification of the resulting oil by silica gel chromatography (10–25% EtOAc/hexanes) yielded donor **17** as a colourless oil (69.3 mg, 42%, $\alpha:\beta = 1:3$). ^{13}C NMR ($CDCl_3$); β anomer: $\delta = 165.2$ (C=O Bz), 143.8 (C_{quat} Tr), 138.6–137.8 (C_{quat} Bn), 133.6 (C_{arom} PhSe), 133.4–126.5 (C_{arom}), 129.4 (C_{quat} Bz), 103.8, 103.6 (C_{1B}/C_{1C}), 86.3 ($C(Ph)_3$), 82.2 (C_{1A}), 84.6, 82.7, 80.9, 78.6, 77.8, 77.5, 76.1, 75.9, 75.2, 74.6, 73.9, 72.7 ($C_{2A-C}/C_{3A-C}/C_{4A-C}/C_{5A-C}$), 75.8–73.4 ($CH_2$ Bn), 69.1, 67.9 (C_{6A}/C_{6C}), 62.3 (C_{6B}); α anomer: $\delta = 165.0$ (C=O Bz), 133.9 (C_{arom} PhSe), 103.8, 103.1 (C_{1B}/C_{1C}), 81.9 (C_{1A}).

Ethyl 3-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-6-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-trityl-β-D-glucopyranosyl)-2-*O*-benzoyl-4-*O*-benzyl-1-thio-α/β-D-glucopyranoside (18): Epoxide **13** was prepared as described for the synthesis of tetramer **15**. To a stirred solution of trimer **13** (145 mg, 0.100 mmol) in THF (2.0 mL) was added, under a continuous stream of dry nitrogen, ethanethiol (14.8 μL, 0.200 mmol) and $ZnCl_2$ (1.0 M solution in THF, 200 μL). After the mixture had been stirred for 30 min, pyridine (2 mL) was added. The reaction mixture was then treated with benzoyl chloride (46.4 μL, 0.400 mmol). After 2 h, excess benzoyl chloride was removed by addition of water (1 mL), and the solvent was evaporated under reduced pressure. The residue was redissolved in EtOAc (25 mL) and washed with aq. $NaHCO_3$ (1 M, 2 × 5 mL), dried ($MgSO_4$) and concentrated in vacuo. Pyridine was removed by coevaporation with toluene (3 × 10 mL). Purification of the resulting oil by silica gel chromatography (5–20% EtOAc/hexanes) furnished donor **18** as a colourless oil (111 mg, 69%, $\alpha:\beta = 1:3$). ^{13}C NMR ($CDCl_3$); β anomer: $\delta = 165.2$ (C=O Bz), 143.8 (C_{quat} Tr), 138.6–137.7 (C_{quat} Bn), 134.5–126.8 (C_{arom}), 129.4 (C_{quat} Bz), 103.7, 103.5 (C_{1B}/C_{1C}), 86.3 ($C(Ph)_3$), 84.6 (C_{1A}), 84.6, 82.6, 82.5, 82.2, 82.0, 81.6, 78.1, 77.8, 77.8, 75.2, 74.2, 73.0 ($C_{2A-C}/C_{3A-C}/C_{4A-C}/C_{5A-C}$), 75.8–73.3 ($CH_2$ Bn), 69.1, 67.3 (C_{6A}/C_{6C}), 62.4 (C_{6B}), 23.3 (CH_2 SEt), 14.6 (CH_3 SEt); α anomer: $\delta = 165.0$ (C=O Bz), 103.5, 103.2 (C_{1B}/C_{1C}), 84.4 (C_{1A}), 24.1 (CH_2 SEt), 14.5 (CH_3 SEt).

Methyl 6-*O*-(3-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-6-*O*-(6-*O*-(3-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-6-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-trityl-β-D-glucopyranosyl)-2-*O*-benzoyl-4-*O*-benzyl-β-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-β-D-glucopyranosyl)-4-*O*-benzyl-β-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (19): To a stirred and cooled (0 °C) mixture of the appropriate donor (**17** or **18**, 0.050 mmol), acceptor **16**, (70 mg, 0.042 mmol), powdered molecular sieves (4 Å) and 1,2-dichloroethane (1.0 mL) was added dropwise, under a continuous stream of dry nitrogen, a freshly prepared solution of NIS (12.4 mg, 0.055 mmol) and TfOH (0.5 μL, 5.5 μmol) in THF (1.0 mL). After 30 min of stirring at 0 °C, triethylamine (0.25 mL) was added and the molecular sieves were removed by filtration. The filtrate was diluted with CH_2Cl_2 (25 mL) and washed with aq. $Na_2S_2O_3$ (1 M, 5 mL) and aq. $NaHCO_3$ (1 M, 2 × 5 mL), dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by silica gel chromatography (10–30% EtOAc/hexanes), and heptamer **19** was isolated as a colourless oil (with donor **17**: yield = 66.4 mg, 49%; with donor **18**: yield = 78.6 mg, 58%). $[\alpha]_D = 36$ ($c = 1$, $CHCl_3$). ^{13}C NMR ($CDCl_3$): $\delta = 165.7$ (C=O Bz), 143.9 (C_{quat} Tr), 138.6–137.3 (C_{quat} Bn), 135.9–126.9 (C_{arom}), 104.2 (double int.; $J_{C,H} = 158.6$ Hz), 103.7 (double int.; $J_{C,H} = 159.8$ Hz; $C_{1A}/C_{1B}/C_{1A'}/C_{1B'}$), 102.4 (double int.; $J_{C,H} = 161.1$ Hz, $C_{1C}/C_{1C'}$), 98.0 ($J_{C,H} = 170.0$ Hz; C_{1D}), 86.4 ($C(Ph)_3$), 85.8, 85.1 ($C_{3A}/C_{3A'}$), 85.1–69.2 ($7 \times C_2$, $5 \times C_3$, $7 \times C_4$, $7 \times C_5$), 75.9–73.1 (CH_2 Bn), 69.3, 69.2, 69.2, 69.1, 69.1, 68.6 ($C_{6A}/C_{6A'}/C_{6B}/C_{6C}/C_{6C'}/C_{6D}$), 62.6 ($C_{6B'}$), 55.0 ($OCH_3$). MS (plasma desorption): $m/2z = 1605–1625$ (calcd. 1612.9).

Methyl 6-*O*-(6-*O*-(6-*O*-(3,6-di-*O*-(β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-3-*O*-(β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl-α-D-glucopyranoside (2): NaOMe (0.01 M solution in MeOH, 0.5 mL) was added to a stirred solution of heptamer **19** (100 mg, 0.031 mmol) in MeOH/CH_2Cl_2 (2/1 v/v, 2 mL). After the mixture had been stirred for 6 h, it was carefully neutralized with Dowex 50XW4 resin (H^+ form, 100–200 mesh) and evaporated in vacuo. The residue was redissolved in *tert*-butanol/water (5/1 v/v, 5 mL), treated with $Pd(OH)_2$ on charcoal (20% Pd, 100 mg) and hydrogenated at elevated pressure ($P(H_2) = 40$ psi). After 12 h, the metal catalyst was removed by filtration and the filtrate was evaporated under reduced pressure. Purification by Sephadex LH-20 column chromatography (eluent: H_2O/MeOH, 1/1 v/v) furnished the completely deprotected heptasaccharide **2** as a white solid (26 mg, 72%). The optical rotation, mass spectrum, ^1H and ^{13}C NMR spectra of compound **2** agreed completely with those reported [2].

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