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Rapid assembly of gp120 oligosaccharide moieties via one-pot glycosidation–deprotection sequences

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ABSTRACT

Mannosyl trihaloacetimidate donors equipped with a 2-*O*-Fmoc group can be effectively activated by catalytic $Bi(OTf)_3$ in glycosidations. Despite the expected participating effect of the Fmoc group, the reaction solvent was found to be decisive for obtaining highly selective α-mannosylations. The Fmoc 2-*O*-protecting group can be then simply removed from the obtained di-oligosaccharide in the same vessel where the glycosidation is conducted. The resulting oligosaccharide can thus be directly employed as a glycosyl acceptor for further elongation. The preparation of biologically important linear and branched oligomannoses incorporated into HIV gp120 demonstrates that iteration of this one-pot sequence leads to very straightforward oligosaccharide assembly. As an additional result, a rapid approach has been disclosed for accessing a 3,6-OH mannose building-block to be incorporated in branched structures. This relies on a double reductive opening of a di-*O*-benzylidene mannose intermediate whose regioselectivity appears to be independent of the configuration of the five-membered benzylidene.

1. Introduction

Over the last years the HIV glycoprotein gp120 has attracted wide interest in the search for saccharide epitopes capable of immunogenicity against HIV.[1] It has been shown that gp120 binds the antibody 2G12 and an intense investigation has revealed that substructures of the gp120 oligomannose structure can tightly bind to the antibody. In particular, high affinity was displayed by the tetrasaccharide corresponding to the D1 arm of the oligomannoside and the pentasaccharide moiety incorporating both the D2 and D3 arms.[2] Due to the relevant biological interest associated with such mannose oligomers, numerous synthetic approaches have been described for achieving these sequences.[3–5] In this paper we detail the development of an especially rapid approach for achieving partially protected structures **1** and **2** (Fig. 1),[6] with a particular focus on the optimization of both the glycosidation chemistry and the preparation of the requisite building-blocks.

Indeed, over the few last years the interest of our laboratory has been focused on the development of ever more convenient and practical approaches for oligosaccharide assembly. In this regard we have devoted much effort to the development of easy to handle and moisture stable glycosidation activators of trihaloacetimidate donors[7] such as $Sm(OTf)_3$,[8] $Yb(OTf)_3$,[9] acid washed molecular sieves,[10] and $Bi(OTf)_3$.[11] The latter promoter proved especially efficient in kinetic terms as its reactivity is often comparable with that of standard strong Lewis acid catalysts such as TMSOTf or $BF_3{\cdot}OEt_2$. In addition, we have also been interested in developing efficient strategies for oligosaccharide assembly, and recently we have reported the first examples, also applied to mannose oligomers, of one-pot multiglycosidation schemes exclusively relying on catalytic conditions for glycosyl donor activation.[12] This approach adds another option to the repertoire of sequential glycosidation strategies developed over the last several years,[7,13] and used for the synthesis of numerous biologically significant oligosaccharides, including some moieties from gp120.[2,3b,c]

The search for a convenient strategy to **1** and **2** led us to a synthetically iterable sequence that combines the advantages of both the use of a moisture stable glycosidation catalyst such as $Bi(OTf)_3$, and recourse to one-pot sequential schemes where more than one reaction is performed in the same vessel. In particular, two literature reports describe that a Fmoc (9-fluorenylmethoxycarbonyl)-protecting group can be removed in situ just after the accomplishment of a glycosidation reaction.[14] In this way after a single purification an intermediate oligosaccharide can be directly obtained as a glycosyl acceptor ready for further glycosylation. Unlike most common sequential schemes for oligosaccharide assembly,[13] this iterable scheme entails construction of the oligomer starting from the reducing terminus,[15] and avoids potential problems associated with the opposite elongation sense such as the use of potentially competing glycosyl donors. As detailed below, $Bi(OTf)_3$-promoted couplings followed by the in situ TEA-induced Fmoc removal turned out to be an especially effective sequential one-pot iterable scheme for constructing the target compounds.

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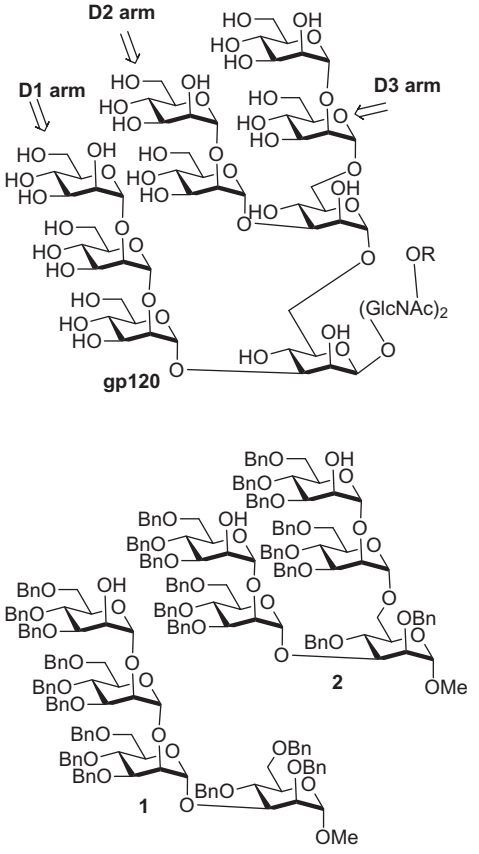


Figure 1. Structure of gp120 and the synthetic targets of the present work.

2. Results and discussion

Synthesis of **1** and **2** entails the adoption of 2-*O*-Fmoc trihaloacetimidate donors where Fmoc is expected to behave initially as a participating group to guarantee α-selectivity and then as a transient group removable by simple addition of excess TEA. To determine the optimized conditions for the glycosidations to occur, trifluoroacetimidate[16] mannosyl donor **3** was prepared as previously described,[6] and then coupled with a range of monosaccharide acceptors (Scheme 1, Table 1).

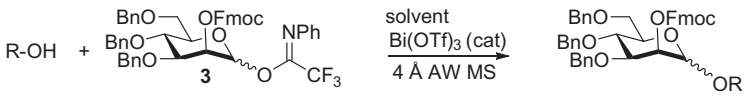
The results indicate that the reaction outcome was strongly dependent on the solvent mixture. Although Fmoc was expected to act as a reliable participating group, in DCE–dioxane solvent mixture, the reaction provided non-negligible amounts of β-mannosides with different acceptors (entries 1–3), and in the case of the thioglycosyl acceptor **4** (entry 1) partial aglycon transfer (with formation of thioethyl 2-*O*-Fmoc mannoside) was also observed as an additional undesired event. To the best of our knowledge, the literature lacks examples of glycosidations reactions where the Fmoc group fails to act as a participating functionality.[17]

Further experimentation was devoted to the screening of alternative solvent mixtures. The use of a 1,2-dimethoxyethane (DME) containing solvent mixture (entry 5), previously found to be highly α-selective with perbenzylated donors,[18] gave disappointing results due to the sluggish activation of the more disarmed 2-*O*-Fmoc donor. This behaviour might be rationalized by considering the ability of 1,2-dimethoxyethane to chelate the Lewis acid. A mixture of dichloromethane–diethyl ether gave **9** in high yield (entry 4) but the stereoselectivity was still poor. More rewarding results were eventually gained by using a toluene–diethyl ether–dioxane solvent mixture (entries 6 and 7), which in previous studies had been applied only to carry out α-selective glycosidations with donors devoid of 2-*O*-participating groups.[9,12] Under these conditions, no β-mannosides could be detected in the reaction mixtures. In entry 7 the result refers to the glycosidation reaction–Fmoc removal sequence conducted by simple addition of TEA on completion of the coupling. As anticipated, the desired base-promoted deprotection occurred uneventfully. The deprotected disaccharide **11** obtained in entry 7, represents the reducing disaccharide terminus of the target sequence **1** and it was obtained in very good 87% overall yield in less than two hours.

Once the best glycosidation solvent was established, elongation of the acceptor disaccharide **11** was attempted through the glycosylation of the sterically encumbered axial 2-OH (Scheme 2). Slight modifications of the optimized protocol gave acceptor trisaccharide **12** in an excellent overall yield (90%), comparable with that of the previous one-pot sequence (Table 1, entry 7). Further coupling of this latter acceptor with donor **3** under analogous conditions gave tetrasaccharide **1** in a rewarding, albeit lower, overall yield (64%). The whole sequence of the three synthetic operations gave the D1 tetrasaccharide **1** in about 50% overall yield for six steps, corresponding to almost 90% average yield for each step, and required only three chromatographic purifications. Comparison of NMR data with those reported[3a] for a differently synthesized tetrasaccharide **1** confirms the identity of the obtained product, with the anomeric linkages all being α-configured.

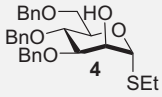
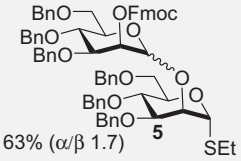
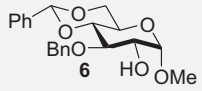
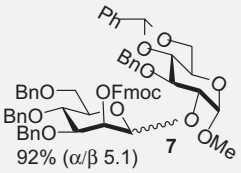
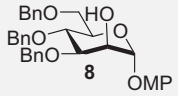
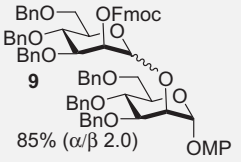
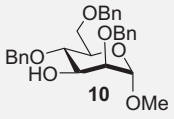
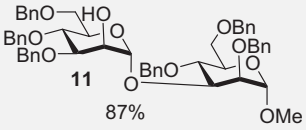
Application of an analogous strategy to the synthesis of the more demanding pentasaccharide **2** requires an efficient access to diol **13**. For this purpose we initially referred to the synthetic scheme described by Ogawa (Scheme 3),[4a] but the initial 3,6-di-O-allylation via stannylidene chemistry was found too sluggish and not satisfying in terms of yield (ca. 30%) due to the prevalent formation of the mono 3-O-allylated derivative. Thus, we turned to an alternative scheme, such as that described by Huang and co-workers on the synthesis of a mannosyl derivative bearing a propylazido aglycon. This approach entails (Scheme 3) regioselective 3,6-di-O-silylation with TBSCl, subsequent 2,4-di-O-benzylation and final removal of the TBS group.[4r] The overall sequence proceeded smoothly but afforded the desired diol in 42% overall yield.

After having tested known three-step procedure, a more convenient access to the target was attempted by resorting to an original two-step approach (Scheme 4). In the first step methyl α-mannopyranoside was submitted to a double benzylidenation by slightly modifying a previously reported procedure described by Liptak and co-workers.[19] Product **17** was obtained after chromatographic purification as an almost equimolar diastereoisomeric mixture (*endo*/*exo* 1.2:1). It is well established by several precedents[4m,20] that the reductive opening of five-membered benzylidenes displays complementary regioselectivity according to the configuration of the starting material. However, we have observed that when the *exo*/*endo* mixture of **17** was directly submitted to reductive opening conditions reported by Hung and co-workers (excess BH_3·THF and 0.3 equiv of copper(II) triflate)[21] the desired 3,6-diol **13** largely

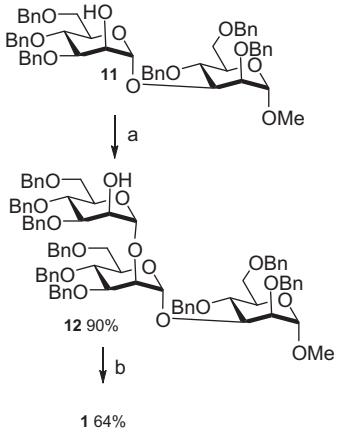


Scheme 1.

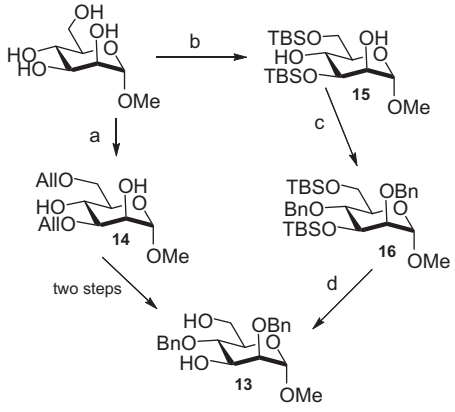
Table 1
Optimization of catalytic α-mannosylations with trifluoroacetimidate donor **3**

Entry	R-OH	Solvent	Products and yield
1		DCE	 63% (α/β 1.7)
2		DCE	 92% (α/β 5.1)
3	 MP: *p*-methoxyphenyl	DCE	 85% (α/β 2.0)
4	**8**	DCM/Et_2O 4:1	**9** 82% (α/β 2.5)
5	**8**	$PhCH_3/DME$ 2:1	Sluggish reaction
6	**8**	$PhCH_3/Et_2O$ 4:1	**9** 85% (α only)
7		$PhCH_3/Et_2O$ 4:1	 87%

General conditions: **3** (1.3–1.6 equiv), acceptor, solvent, $Bi(OTf)_3$ (0.10 equiv) added as a solution in dioxane, −30 °C to rt, 15–180 min. For the final solvent composition see experimental section. In entry 7, 0.08 equiv of $Bi(OTf)_3$ were used and TEA was added to the glycosidation vessel just after completion of the coupling (see Section 4 for details).

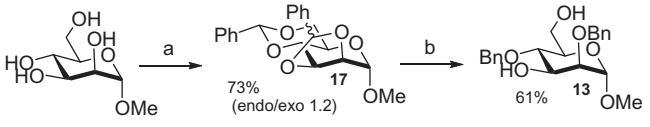


Scheme 2. Synthesis of tetrasaccharide **1** via iterable glycosidations–Fmoc removal sequences. Reagents and conditions (for the final solvent composition see Section 4): (a) **3** (1.7 equiv), $Bi(OTf)_3$ (0.10 equiv) in dioxane, toluene–Et_2O 4:1, −30 to −5 °C, 75 min; then TEA, rt, 1 h; (b) **3** (1.7 equiv), $Bi(OTf)_3$ (0.10 equiv) in dioxane, toluene–Et_2O 4:1, −30 to rt, 150 min; then TEA, rt, 1 h.



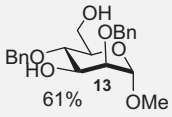
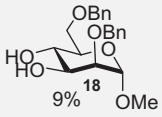
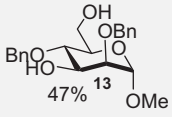
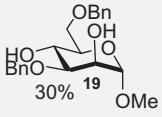
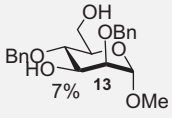
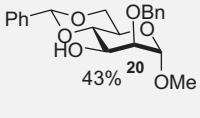
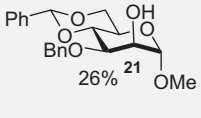
Scheme 3. Attempted three-step routes to diol **13**. Reagents and conditions: (a) Bu_2SnO (2.2 equiv), MeOH reflux, 3 h; solvent removal then toluene, TBAI (2 equiv), AllBr (20 equiv), 70 °C, 72 h; (b) TBSCl, DMF, imidazole, 66% yield; (c) NaH, BnBr, DMF; (d) excess Pyr·HF, 64% yield over two steps.

prevailed (Scheme 4 and Table 2, entry 1). Minor amounts (ca. 10%) of the unexpected 3,4-diol **18** were also isolated and characterized in its acetylated form (Table 2, entry 1). Other experiments showed that the outcome of the benzylidene reductive opening was critically dependent on the experimental procedure. As shown in Table 2, the reaction was much more sluggish and much less regioselective when it was conducted in the presence of molecular sieves (entries 2 and 3). Preliminary coevaporation of copper(II) triflate in toluene also strongly influenced the final composition of the reaction mixture (compare entries 2 and 3).



Scheme 4. A two-step route to diol **13**. Reagents and conditions: (a) $PhCH(OMe)_2$, DMF, CSA, 70 °C, 250 mbar; (b) BH_3·THF, $Cu(OTf)_2$, 0 °C to rt.

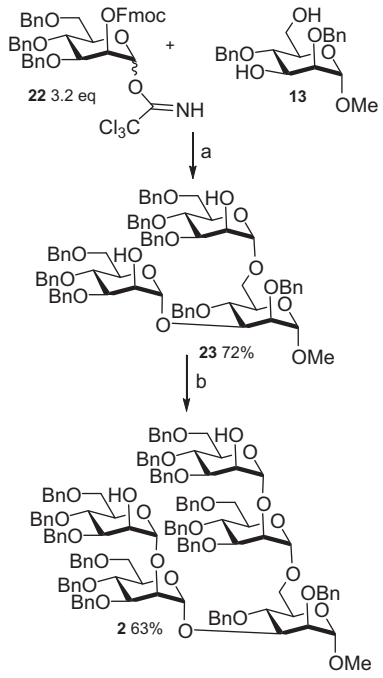
Table 2
Optimization of double reductive opening of **17**

Entry	Conditions	Time (h)	Products and yields		
1	A	1.7	 61% **13**		 9% **18**
2	B	48	 47% **13**		 30% **19**
3	C	48	 7% **13**	 43% **20**	 26% **21**

General conditions: **17**, BH_3·THF (1 M in THF, 10 equiv), then $Cu(OTf)_2$ (sol in THF, 0.3 equiv), 0 °C to rt. Condition A: neither use of molecular sieves, nor coevaporation in toluene of $Cu(OTf)_2$ prior to its use. Condition B: use of 4 Å molecular sieves and coevaporation in toluene of $Cu(OTf)_2$ prior to its use. Condition C: use of 4 Å molecular sieves and no coevaporation in toluene of $Cu(OTf)_2$ prior to its use.

Having established a straightforward access to the reducing terminus precursor of **2**, its direct double glycosidation was attempted. Simultaneous attachment of two mannose residues to acceptor diol **13** is not a trivial task (Scheme 5), and in some cases it is preferable to employ longer synthetic procedures in which the mannose residues are attached to the branching unit at different stages.[22] After a preliminary screening, trichloroacetimidate **22**[23] (Scheme 5) proved more efficient than the trifluoro counterpart (**3**) at providing trisaccharide **23**.

Indeed, by using the less reactive trifluoroacetimidate donor **3** higher amounts of intermediate dimannosides were detected, whereas with trichloroacetimidate donor **22** the sequence of bis-glycosylation and bis-deprotection afforded trisaccharide **23** under very mild conditions, in short times, and in a very high overall yield (72% for four synthetic steps, Scheme 5). This latter compound was then exposed to trichloroacetimidate **22** under $Bi(OTf)_3$ activation to afford pentasaccharide **2** in 63% overall yield. Assembly of **2** was performed in only two synthetic operations with the overall yield of its eight synthetic steps (ca. 45%) corresponding once again to a ca. 90% average yield for each step.



Scheme 5. Synthesis of pentasaccharide **2** via iterable glycosidations–Fmoc removal sequences. Reagents and conditions (for the final solvent composition see Section 4): (a) $Bi(OTf)_3$ (0.10 equiv.) in dioxane, toluene–Et_2O 4:1, −30 to 10 °C, 45 min; then TEA, rt, 1 h; (b) **22** (4.3 equiv), $Bi(OTf)_3$ (0.20 equiv) in dioxane, toluene–Et_2O 4:1, −30 to 5 °C, 1 h; then TEA, rt, 1 h.

3. Conclusions

In conclusion, in this paper we have shown that 2-*O*-Fmoc mannosyl trihaloacetimidate donors can be effectively activated by catalytic $Bi(OTf)_3$ in couplings with saccharide acceptors. In the same reaction vessel the Fmoc 2-*O*-protecting group can be then simply removed from the obtained di- or oligosaccharide by addition of excess TEA. Despite the expected participating effect of the Fmoc group, we found that the nature of the solvent is decisive for obtaining highly selective α-mannosylations. Iteration of this glycosidation–deprotection one-pot sequence has been applied to biologically useful mannose oligosaccharides related to HIV gp120 by resorting to a smaller number of synthetic operations than in other reported procedures towards similar oligosaccharide sequences. As an additional result, we have disclosed a rapid approach for accessing a mannose building-block to be incorporated into branched structures that relies on a regioselective double reductive opening of a di-*O*-benzylidene mannose intermediate.

The described synthetic strategy features a combination of simultaneous advantages, rather unusual in other schemes, such as the reduced experimental work, the attainment of high yields, and the exclusive use of a catalytic and moisture stable glycosidation promoter. This iterable scheme is expected to be of broad applicability, thus adding a further useful complement to the recent strategical advances in oligosaccharide synthesis.

4. Experimental

4.1. General methods

^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ (internal standard, for ^1H: $CHCl_3$ at δ 7.26; for ^{13}C: $CDCl_3$ at δ 77.0). ^1H NMR assignments were based on homo-decoupling experiments. MALDI-MS spectra were recorded in the positive mode: compounds were dissolved in CH_3CN at a concentration of 0.1 mg/mL and 1 μL of these solutions were mixed with 1 μL of a 20 mg/mL

solution of 2,5-dihydroxybenzoic acid in 7:3 CH_3CN–H_2O or, in the case of trifluoroacetimidate derivatives, with a 10 mg/mL solution of trihyroxyacetophenone in 1:1 MeOH–H_2O. Analytical thin layer chromatography (TLC) was performed on aluminium plates precoated with Silica Gel 60 F_{254} as the adsorbent. The plates were developed with 5% H_2SO_4 ethanolic solution and then heating to 130 °C. Column chromatography was performed on silica gel (63–200 mesh). $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. Glycosidations were performed with commercial anhydrous solvents. Bismuth(III) triflate was coevaporated three times in toluene and dried under vacuum for 30–45 min and then dissolved in dioxane in the presence of freshly activated 4 Å MS.

4.2. General procedure of glycosidation with donor 3

A mixture of donor **3** (0.13–0.16 mmol) and an acceptor (0.10 mmol) was coevaporated three times with anhydrous toluene (3 × 2 mL), and then dried in vacuo for 30 min. After adding 4 Å AW 300 MS, the mixture was dissolved under argon with 4:1 toluene–Et_2O (4.1 mL), cooled to −30 °C, and stirred for 15 min. A solution of $Bi(OTf)_3$ in dioxane (14.5 mg–mL, 10 μmol, 0.45 mL) was then added, and the temperature was allowed to slowly raise to room temperature. On completion of the glycosidation (TLC analysis, 15–180 min for entries in Table 1), a few drops of pyridine were added and the reaction mixture was filtered on a short plug of silica gel repeatedly washed with CH_2Cl_2–MeOH–CH_3CN 85:10:5. The filtrate was concentrated, and the residue purified by silica-gel flash-chromatography (eluent: *n*-hexane–EtOAc or toluene–EtOAc).

4.3. Ethyl 3,4,6-tri-*O*-benzyl-2-*O*-fluorenylmethoxycarbonyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-1-thio-α-D-mannopyranoside 5α

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91–7.00 (Ar), 5.45 (s, 1H, H-1), 5.40 (br d, 1H, H-2′), 5.21 (s, 1H, H-1′), 5.00–4.41 (m, 6 × AB, 12H, 6 × –C*H*$_2$Ph), 4.40–4.23 (m, 3H, –C*H*$_2$O and H-9 Fmoc), 4.20–4.01 (m, 4H, H-2, H-5, H-3′ and H-5′), 4.00–3.78 (m, 6H), 3.72 (d, $J_{6a,6b}$ = 11.1 Hz, 1H, H-6b), 2.64–2.49 (m, 2H, –SC*H*$_2$CH$_3$), 1.24 (t, 3H, J = 7.2 Hz, –SCH$_2$C*H*$_3$). ^{13}C NMR (50 MHz, $CDCl_3$) δ 154.7 (–O*C*O$_2$–), 143.7, 143.4, 141.3, 141.2 (Fmoc aromatic C), 138.6 (×2), 138.5, 138.4, 138.2, 138.1 (aromatic C), 128.3–120.0 (aromatic CH), 99.4 (C-1′), 80.2 (C-1), 78.2, 75.9, 75.6, 75.2, 75.1, 75.0, 74.6, 73.4, 73.3, 72.8, 72.3 (×3), 72.0, 70.3, 69.3 (×2), 46.7 (Fmoc –*C*HCH$_2$), 25.5 (–SC*H*$_2$CH$_3$), 15.0 (–SCH$_2$C*H*$_3$). MALDI-TOF MS: calcd for [M+Na]$^+$ 1171.47. Found 1171.4. Anal. Calcd for $C_{71}H_{72}O_{12}S$: C, 74.19; H, 6.31. Found: C, 74.45; H, 6.25.

4.4. Ethyl 3,4,6-tri-*O*-benzyl-2-*O*-fluorenylmethoxycarbonyl-β-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-1-thio-α-D-mannopyranoside 5β

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80–6.90 (Ar), 5.57 (d, $J_{1,2}$ = 2.7 Hz, 1H, H-1), 5.43 (d, $J_{2,3}$ = 1.5 Hz, 1H, H-2′), 5.07–4.77 (m, 4H, –C*H*$_2$Ph and H-1′), 4.76–4.43 (m, 4 × AB, 8H, 4 × –C*H*$_2$Ph), 4.42–4.19 (m, 4H), 4.18–4.05 (m, 2H), 3.94 (t, J = 9.4 Hz, 1H), 3.93–3.58 (m, 7H), 3.57–3.48 (m, 1H, H-5′), 2.77–2.43 (m, 2H, –SC*H*$_2$CH$_3$), 1.26 (t, 3H, J = 7.5 Hz, –SCH$_2$C*H*$_3$). ^{13}C NMR (50 MHz, $CDCl_3$) δ 155.1 (–O*C*O$_2$–), 143.9, 143.4, 141.2, 141.1 (Fmoc aromatic C), 138.6, 138.5, 138.4, 138.3 (×2), 137.6 (aromatic C), 128.4–119.8 (aromatic CH), 95.8 (C-1′), 81.1 (C-1), 80.1, 78.2, 75.9, 75.2, 75.1, 74.5, 74.4, 73.6, 73.4, 73.1, 72.3, 71.8, 71.4, 70.6, 70.3, 69.6, 69.4, 46.8 (Fmoc –*C*HCH$_2$), 25.5 (–SC*H*$_2$CH$_3$), 15.0 (–SCH$_2$C*H*$_3$). MALDI-TOF MS: calcd for [M+Na]$^+$ 1171.47. Found 1171.7. Anal. Calcd for $C_{71}H_{72}O_{12}S$: C, 74.19; H, 6.31. Found: C, 74.40; H, 6.20.

4.5. Methyl 3,4,6-tri-*O*-benzyl-2-*O*-fluorenylmethoxycarbonyl-α-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside 7α

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87–7.03 (m, Ar), 5.58 (s, 1H, benzylidene –CHPh), 5.37 (br d, 1H, H-2′), 5.17 (s, 1H, H-1′), 4.96 (d, $J_{1,2}$ = 3.2, 1H, H-1), 5.00–4.38 (m, 4 × AB, 8H, 4 × –C*H*$_2$Ph), 4.50–4.45 (m, 1H, Fmoc –CHCH$_2$O–), 4.40 (d, J = 12.0 Hz,1H, Fmoc –CHCH$_a$*H*$_b$O–), 4.35–4.28 (m, 3H, H-6a, and Fmoc –CHCH$_2$O–), 4.25–4.20 (m, 1H, H-5′), 4.14 (dd, $J_{2,3}$ = 2.8 Hz, $J_{3,4}$ = 9.6 Hz, 1H, H-3′), 4.07 (t, $J_{3,4}$ = 9.6 Hz, 1H, H-4′), 4.00–3.95 (m, 2H, H-2 and H-3), 3.88 (td, $J_{5,6a}$ = 4.6 Hz, $J_{5,6b}$ = 9.8 Hz, 1H, H-5), 3.76 (t, $J_{5,6b}$ = $J_{6a,6b}$ = 10.0 Hz,1H, H-6b), 3.73 (dd, $J_{5,6b}$ = 4.0 Hz, $J_{6a,6b}$ = 10.2 Hz, 1H, H-6′a), 3.67–3.57 (m, 2H, H-4 and H-6′b), 3.49 (s, 3H, –OMe). ^{13}C NMR (50 MHz, $CDCl_3$) δ 154.8 (–O*C*O$_2$–), 143.5, 143.1, 141.25, 141.20 (Fmoc aromatic C), 138.6, 138.3, 137.9, 137.8, 137.3 (aromatic C), 129.0–119.9 (aromatic CH), 101.2 (benzylidene –CHPh), 97.0, 94.3 (anomeric CH), 82.1, 79.1, 78.0, 75.6, 75.2, 74.1, 73.6, 73.0, 72.7, 71.8, 71.2, 70.2, 68.9, 68.5, 62.2, 55.4 (–OMe), 46.5 (Fmoc –*C*HCH$_2$). MALDI-TOF MS: calcd for [M+Na]$^+$ 1049.41. Found 1049.3. Anal. Calcd for $C_{63}H_{62}O_{13}$: C, 73.67; H, 6.08. Found: C, 73.45; H, 6.15.

4.6. Methyl 3,4,6-tri-*O*-benzyl-2-*O*-fluorenylmethoxycarbonyl-β-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside 7β

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87–7.06 (Ar), 5.59 (s, 1H, benzylidene H), 5.35 (d, 1H, $J_{2,3}$ = 2.8 Hz, H-2′), 5.01–4.37 (4 × –C*H*$_2$Ph, 8H), 4.94 (d, 1H, $J_{1,2}$ = 3.9 Hz, H-1), 4.78 (s, 1H, H-1′), 4.50–4.35 (m, 2H, Fmoc –CHC*H*$_2$O–), 4.36–4.28 (m, 2H, H-6a and Fmoc –C*H*CH$_2$O–), 4.08 (t, $J_{2,3}$ = $J_{3,4}$ = 9.6 Hz 1H, H-3), 3.91–3.71 (m, 6H), 3.64 (t, $J_{4,5}$ = 9.6 Hz, 1H, H-4), 3.49 (dd, 1H, $J_{3,4}$ = 9.2 Hz, H-3′), 3.47–3.43 (m, 1H, H-5′), 3.30 (s, 3H, –OMe). ^{13}C NMR (50 MHz, $CDCl_3$) δ 155.0 (–O*C*O$_2$–), 143.6, 143.5, 141.2, 141.1 (Fmoc aromatic C), 138.8, 138.2, 138.0, 137.5, 137.3 (aromatic C), 128.9–119.9 (aromatic CH), 101.3 (benzylidene CH), 100.2, 100.1 (anomeric CH), 82.4, 80.5, 79.5, 78.5, 75.5, 75.3, 74.1, 73.5, 72.1, 71.5, 70.2, 69.3, 69.1, 67.0, 62.3, 55.3 (–OMe), 46.7 (Fmoc –*C*HCH$_2$). [M+Na]$^+$ 1049.41. Found 1049.3. Anal. Calcd for $C_{63}H_{62}O_{13}$: C, 73.67; H, 6.08. Found: C, 73.50; H, 6.15.

4.7. *p*-Methoxyphenyl 3,4,6-tri-*O*-benzyl-2-*O*-fluorenylmethoxycarbonyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside 9α

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90–7.12 (Ar), 6.97 (d, J = 9.1 Hz, 2H, Ar-H$_{PMP}$), 6.74 (d, J = 9.1 Hz, 2H, Ar-H$_{PMP}$), 5.59 (d, $J_{1,2}$ = 2.0 Hz, 1H, H-1), 5.41 (dd, $J_{2,3}$ = 3.0 Hz, H-2′), 5.25 (d, $J_{1,2}$ = 1.6 Hz,1H, H-1′), 4.97–4.43 (m, 6 × AB, 12H, 6 × –C*H*$_2$Ph), 4.32 (d, J = 8.0 Hz, 1H, Fmoc –CHC*H*$_a$CH$_a$O–), 4.30 (d, J = 7.9 Hz, 1H, Fmoc –CHCH$_a$C*H*$_b$O–), 4.25 (t, 1H, Fmoc –C*H*CH$_a$CH$_b$O–), 4.21 (t, $J_{2,3}$ = 2.5 Hz, 1H, H-2), 4.14 (dd, 1H, $J_{2,3}$ = 3.0 Hz, $J_{3,4}$ = 8.8 Hz, H-3), 4.11–4.02 (m, 2H, H-5′ and H-3′), 3.97 (t, 1H, J = 9.3 Hz), 3.94–3.86 (m, 2H), 3.84–3.72 (m, 3H), 3.74 (s, 3H, –OMe), 3.69 (dd, $J_{5,6}$ = 1.6 Hz, $J_{6a,6b}$ = 11.2 Hz, 1H, H-6). ^{13}C NMR (50 MHz, $CDCl_3$) δ 155.0 (*p*-methoxyphenyl aromatic C), 154.7 (–O*C*O$_2$), 150.1 (*p*-methoxyphenyl aromatic C), 143.6, 143.3, 141.3, 141.2 (Fmoc aromatic C), 138.5, 138.4, 138.3(3), 138.3(0), 138.2, 138.0 (aromatic C), 128.4–120.0 (aromatic CH), 117.9, 114.6 (*p*-methoxyphenyl aromatic CH), 99.3, 97.7 (C-1′ and C-1), 79.5, 78.2, 75.24, 75.20, 75.0, 74.6, 74.5, 73.3, 73.2, 72.7, 72.5, 72.4, 72.2, 72.0, 70.2, 69.2 (×2), 55.6 (–OMe), 46.7 (Fmoc –*C*HCH$_2$). [M+Na]$^+$ 1233.50. Found 1233.6. Anal. Calcd for $C_{76}H_{74}O_{14}$: C, 75.35; H, 6.16. Found: C, 75.20; H, 6.05.

4.8. *p*-Methoxyphenyl 3,4,6-tri-*O*-benzyl-2-*O*-fluorenylmethoxycarbonyl-β-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside 9β

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94–6.94 (Ar), 6.77 (d, J = 9.1 Hz, 2H, Ar-H_{PMP}), 5.57 (d, $J_{2,3}$ = 3.0 Hz, 1H, H-2′), 5.54 (d, $J_{1,2}$ = 2.4 Hz, 1H, H-1), 4.84 (s, 1H, H-1′), 4.52 (t, $J_{2,3}$ = 2.4 Hz, 1H, H-2), 4.94–4.46 (m, 12H, 6x –CH_2Ph), 4.34 (d, J = 8.0 Hz, 2H, Fmoc –CHCH_2O–), 4.24 (t, 1H, Fmoc –CHCH$_2$O–), 4.17–4.07 (m, 2H, H-3 and H-4′), 3.96–3.88 (m, 2H, H-4 and H-5), 3.85–3.70 (m, 3H, H-6a′, H-6b′, H-3′), 3.74 (s, 3H, –OMe), 3.66 (dd, $J_{5,6b}$ = 5.1 Hz, $J_{6a,6b}$ = 10.9 Hz, 1H, H-6b), 3.62 (dd, $J_{5,6a}$ = 2.3 Hz, 1H, H-6a), 3.58–3.61 (m, $J_{5,6a}$ = 1.7 Hz, $J_{5,6b}$ = 4.8 Hz, $J_{4,5}$ = 9.7 Hz, 1H, H-5′). ^{13}C NMR (50 MHz, $CDCl_3$) δ 155.0 (*p*-methoxyphenyl aromatic C and –OCO_2–), 150.5 (*p*-methoxyphenyl aromatic C), 143.8, 143.4, 141.2, 141.1 (Fmoc aromatic C), 138.8, 138.4 (×2), 138.2 (×2), 137.6 (aromatic C), 128.4–119.8 (aromatic CH), 118.0, 114.6 (*p*-methoxyphenyl aromatic CH), 96.8, 96.7 (anomeric CH), 80.1, 78.5, 75.8, 75.3, 74.9, 74.5, 74.2, 73.6, 73.1, 72.4, 72.2, 72.1, 71.4, 71.0, 70.3, 69.6, 69.3, 55.6 (–OMe), 46.7 (Fmoc –CHCH$_2$). $[M+Na]^+$ 1233.50. Found 1233.5. Anal. Calcd for $C_{76}H_{74}O_{14}$: C, 75.35; H, 6.16. Found: C, 75.15; H, 6.10.

4.9. Methyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-α-D-mannopyranoside 11

A mixture of donor **3** (147 mg, 0.174 mmol) and acceptor **10** (49 mg, 0.105 mmol) was coevaporated three times with anhydrous toluene (3 × 2 mL), and then dried in vacuo for 30 min. After adding 4 Å AW 300 MS, the mixture was dissolved under argon with 4:1 toluene–Et_2O (3.3 mL), cooled to −30 °C, and stirred for 15 min. A solution of $Bi(OTf)_3$ in dioxane (14.5 mg/mL, 0.38 mL, 8.4 μmol) was then added, and the temperature was allowed to slowly raise. After 50 min (temperature at −20 °C), having realized the completion of the glycosidation step (TLC analysis, eluent: *n*-hexane–EtOAc 7:3), TEA (0.8 mL) was added and the reaction vessel was immediately warmed to rt. After one hour the reaction mixture was filtered on a short plug of silica gel repeatedly washed with CH_2Cl_2–MeOH–CH_3CN 85:10:5. The filtrate was concentrated, and the residue purified by silica-gel flash-chromatography (eluent: *n*-hexane–EtOAc from 3:1 to 2:1) to yield disaccharide **9** as an oil (84 mg, 87% overall yield); $[\alpha]_D^{28}$ +29.1 (*c* 1.0 in $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.40−7.10 (Ar), 5.22 (d, $J_{1,2}$ = 1.2 Hz, 1H, 1′-H), 4.72 (d, $J_{1,2}$ = 1.6 Hz, 1H, 1-H), 4.85–4.46 (12H, 6x-CH_2Ph), 4.13 (dd, J = 3.2 and 10.6 Hz, 1H), 4.02–3.60 (11H), 3.29 (s, 3H, –OCH_3), 2.31 (d, J = 2.4 Hz, 1H, OH-2). ^{13}C NMR (50 MHz; $CDCl_3$): δ 138.6, 138.3 (×3), 138.1, 137.9 (aromatic C); 128.5–127.5 (aromatic CH); 101.2 and 98.2 (anomeric CH), 80.0, 78.4, 75.2, 75.0, 74.9, 74.4, 72.1, 72.0, 71.9, 71.7, 69.2, 69.0, 68.6, 54.8. MALDI-TOF MS: calcd for $[M+Na]^+$ 919.40. Found 919.27. Anal. Calcd for $C_{55}H_{60}O_{11}$: C, 73.64; H, 6.74. Found: C, 73.50; H, 6.65.

4.10. Methyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-α-D-mannopyranoside 12

A mixture of donor **3** (74 mg, 88 μmol) and acceptor **11** (45 mg, 49 μmol) was coevaporated three times with anhydrous toluene (3 × 1 mL), and then dried in vacuo for 30 min. After adding 4 Å AW 300 MS, the mixture was dissolved under argon with 4:1 toluene–Et_2O (3.3 mL), cooled to −30 °C, and stirred for 15 min. A solution of $Bi(OTf)_3$ in dioxane 14.5 mg/mL, 0.22 mL, 4.9 μmol) was then added, and the temperature was allowed to raise up to −5 °C over 75 min. Having realized the completion of the glycosidation step (TLC analysis, eluent *n*-hexane–EtOAc 7:3), TEA (0.83 mL) was added and the reaction vessel was immediately warmed to rt. After one hour the reaction mixture was filtered through a short plug of silica gel repeatedly washed with CH_2Cl_2–MeOH–CH_3CN 85:10:5. The filtrate was concentrated, and the residue purified by silica-gel flash-chromatography (eluent: *n*-hexane–EtOAc from 3:1 to 2:1) to yield trisaccharide **12** as an oil (59 mg, 90% overall yield); $[\alpha]_D^{28}$ +34.2 (*c* 1.3 in $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.45–7.10 (Ar), 5.31 (d, $J_{1,2}$ = 1.6 Hz, 1H, 1″-H), 5.13 (d, $J_{1,2}$ = 1.6 Hz, 1H, 1′-H), 4.85 (d, $J_{1,2}$ = 1.6 Hz, 1H, 1-H), 4.92–4.41 (18H, 9 x-CH_2Ph), 4.18–4.12 (2H), 4.10–3.80 (9H), 3.80–3.65 (6H), 3.55 (br d, J = 10.0 Hz, 1H,), 3.33 (s, 3H, –OCH_3), 2.48 (br s, 1H, 2.OH). ^{13}C NMR (100 MHz; $CDCl_3$): δ 138.8, 138.6, 138.55, 138.48 (×2), 138.41 (×2), 138.2, 138.1 (aromatic C); 128.5–127.3 (aromatic CH); 101.0 (×2), 98.2 (anomeric CH), 79.9, 79.5, 77.7, 75.4, 75.0, 74.9, 74.7, 74.3, 73.4, 73.3, 73.2, 72.7, 72.2, 72.1, 72.0, 71.8, 69.7, 69.3, 68.6, 68.5 54.8. MALDI-TOF MS: calcd for $[M+Na]^+$ 1351.60. Found 1351.48. Anal. Calcd for $C_{82}H_{88}O_{16}$: C, 74.18; H, 6.67. Found: C, 73.95; H, 6.71.

4.11. Methyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,4, 6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-α-D-manno- pyranoside 1

A mixture of donor **3** (11 mg, 13 μmol) and acceptor **12** (10 mg, 7.5 μmol) was coevaporated three times with anhydrous toluene (3 × 1 mL), and then dried in vacuo for 30 min. After adding 4 Å AW 300 MS, the mixture was dissolved under argon with 4:1 toluene–Et_2O (0.51 mL), cooled to −30 °C, and stirred for 15 min. A solution of $Bi(OTf)_3$ in dioxane (14.3 mg/mL, 34 μL, 0. 75 μmol) was then added, and the temperature was allowed to raise up to rt over 2.5 h. Having realized the completion of the glycosidation step (TLC analysis, eluent: *n*-hexane–EtOAc 7:3), TEA (0.13 mL) was added. After one hour from the addition, the reaction mixture was filtered on a short plug of silica gel repeatedly washed with CH_2Cl_2–MeOH–CH_3CN 85:10:5. The filtrate was concentrated, and the residue purified by silica-gel flash-chromatography (eluent: toluene–EtOAc from 10:0 to 85:15) to yield tetrasaccharide **1** as an oil (8 mg, 64% overall yield); $[\alpha]_D^{28}$ +28.5 (*c* 0.8 in $CHCl_3$); ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.40–7.05 Ar), 5.25 (br s, 1H, 1‴-H), 5.18 (br s, 1H, 1″-H), 5.14 (br s, 1H, 1′-H), 4.70 (d, $J_{1,2}$ = 1.5 Hz, 1H, 1-H), 4.85–4.22 (24H, 12 x-CH_2Ph), 4.12–4.08 (2H), 4.00 (1H, J = 1.5 Hz), 3.98–3.60 (18H), 3.55 (dd, J = 3.0 and 11.5 Hz, 1H), 3.47 (br d, J = 11.5, 1H), 3.43 (br d, J = 10.0 Hz, 1H), 3.26 (s, 3H, –OCH_3), 2.39 (br s, 1H, 2-OH). ^{13}C NMR (100 MHz; $CDCl_3$): δ 138.8−138.1 (aromatic C); 128.5–127.2 (aromatic CH); 101.0, 100.9, 100.8 and 98.2 (anomeric CH), 80.0, 79.6, 79.4, 79.2, 79.0, 77.6, 77.2, 75.6, 75.1, 74.9, 74.7, 74.2, 73.4, 73.3, 73.0, 72.6, 72.5, 72.4, 72.1, 72.0, 71.9, 71.7, 71.6, 69.7, 69.3, 69.0, 68.7, 68.5, 54.7. MALDI-TOF MS: calcd for $[M+Na]^+$ 1784.79. Found 1784.45. Calc. for $C_{109}H_{116}O_{21}$: C, 74.30; H, 6.64. Found: C, 73.95; H, 6.50.

4.12. Methyl 3,6-di-*O*-*tert*-butyldimethylsilyl-α-D-mannopyranoside 15

Compound **15** was prepared and elaborated to diol **13** by reproducing the procedures described in Ref. 4r. Compound **15**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.69 (d, $J_{1,2}$ = 0.9 Hz, 1H, H-1), 3.83 (d, 2H, H-6a and H-6b), 3.80 (dd, 1H, H-3), 3.72 (br d, $J_{2,3}$ = 3.6 Hz, 1H, H-2), 3.66 (td, $J_{3,4}$ = 9.1 Hz, 1H, H-4), 3.52 (dt, $J_{5,6a}$ = 4.5 Hz, $J_{5,6b}$ = 5.2 Hz, 1H, H-5), 3.33 (s, 3H, –OMe), 2.78 (d, $J_{4,OH}$ = 2.1 Hz, 1H, 4-O*H*), 2.59

(br s, 1H, 2-O*H*), 0.88 (s, 9H, –C(CH_3)$_3$), 0.87 (s, 9H, –C(CH_3)$_3$), 0.12 (s, 3H, –Si(CH_3)$_2$), 0.10 (s, 3H, –Si(CH_3)$_2$), 0.06 (s, 6H, –Si(CH_3)$_2$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 100.1 (anomeric CH), 73.0, 71.0, 70.6, 70.3, 64.8, 54.6 (–OMe), 25.8 and 25.7 (2 × –C(CH_3)$_3$), 18.2, 18.0 (2 × –*C*(CH$_3$)$_3$), −5.3, −4.6 (2 × –Si(CH_3)$_2$), −4.3 (2 × –Si(*C*H$_3$)$_2$).

4.13. Methyl 2,4-di-*O*-benzyl-α-D-mannopyranoside 13

Compound **17**[19,20a] (*endo*/*exo* 1.2:1, 55 mg, 0.15 mmol) was dissolved at 0 °C under argon with 1 M BH_3 in THF (1.5 mL, 1.5 mmol). To the resulting solution was added at 0 °C a solution of copper(II) triflate in THF (0. 069 M prepared dissolving the salt under argon in the presence of 4 Å MS, 0.65 mL, 0.045 mmol). After 30 min, the ice-bath was removed and the reaction vessel was allowed to warm to rt. After a few minutes a black precipitate appeared. After 1 h and 40 min from the start, the reaction was quenched with TEA (25 μL) and MeOH (0.2 mL). The mixture was concentrated in vacuo and the residue was purified by silica-gel flash-chromatography (eluent: *n*-hexane–EtOAc from 32:18 to 55:45) to yield diol **13** as a colourless oil (34 mg, 61% yield). Acetylation of a sample confirmed the presence of free hydroxyls at *O*-3 and *O*-6 as indicated by ^{1}H NMR (200 MHz; $CDCl_3$): signals at δ 5.16 (dd, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 9.0 Hz, 1H, 3-H), 4.30 (dd, $J_{5,6a}$ = 3.0 Hz, $J_{6a,6b}$ = 12.4 Hz, 1H, 6a-H), 4.22 (dd, $J_{5,6b}$ = 4.2 Hz, 1H, 6b-H). Compound **13**: $[\alpha]_D^{28}$ +21.5 (*c* 1.1 in $CHCl_3$), lit.:[19] $[\alpha]_D$ +23.5 (*c* 0.8 in $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.40−7.25 (Ar), 4.75 (d, $J_{1,2}$ = 1.6 Hz, 1H, anomeric proton), 4.92–4.59 (4H, 2 × –CH_2Ph), 3.99 (dd, $J_{2,3}$ = 3.2 Hz, $J_{3,4}$ = 9.6 Hz, 1H, 3-H), 3.86 (dd, $J_{5,6a}$ = 3.2 Hz, $J_{6a,6b}$ = 11.6 Hz, 1H, 6a-H), 3.77 (dd, $J_{5,6b}$ = 4.4 Hz, 1H, 6b-H), 3.73 (dd, 1H, 2-H), 3.68 (t, $J_{4,5}$ = 9.6 Hz, 1H, 4-H), 3.60–3.55 (m, 1H, 5-H), 3.32 (s, 3H, –OCH_3). ^{13}C NMR (50 MHz; $CDCl_3$) δ 138.3, 137.6 (aromatic C); 128.6–127.8 (aromatic CH); 98.1 (C-1), 78.3, 74.8, 73.1, 71.7, 71.1, 62.3, 54.8 (–OCH_3). MALDI-TOF MS: calcd for $[M+Na]^+$ 397.17. Found 397.25. Anal. Calcd for $C_{21}H_{26}O_6$: C, 67.36; H, 7.00. Found: C, 67.05; H, 7.10.

4.14. Other reductive opening procedures (conditions B and C of Table 2)

The reductive openings were essentially accomplished as illustrated above for preparation of **13** (condition A of Table 2). Only differences were the addition of molecular sieves to the reaction vessel and coevaporation of $Cu(OTf)_2$ with toluene prior to be dissolved in THF, according to the indications in Table 2 (conditions B and C). Listed below are the NMR data of all products obtained from the reductive openings. In several cases acetylation was exploited for structural confirmation.

4.15. Methyl 3,4-di-*O*-acetyl-2,6-di-*O*-benzyl-α-D-mannopyranoside (acetylated 18)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42–7.29 (m, 10H, ArH), 5.39 (t, $J_{3,4}$ = $J_{4,5}$ = 9.9 Hz, 1H, H-4), 5.21 (dd, 1H, $J_{2,3}$ = 3.4 Hz, H-3), 4.75 (d, $J_{1,2}$ = 1.7 Hz, 1H, H-1), 4.66–4.54 (2 × AB, 4H, 2 × –CH_2Ph), 3.88 (ddd, 1H, $J_{5,6a}$ = 2.3 Hz, $J_{5,6b}$ = 3.5 Hz, H-5), 3.82 (dd, 1H, H-2), 3.61 (dd, 1H, H-6a), 3.56 (dd, 1H, $J_{6a,6b}$ 10.9 Hz, H-6b), 3.38 (s, 3H, –OMe), 1.98 and 1.91 (2 × s, 6H, 2 × –COCH_3).

4.16. Methyl 3,4-di-*O*-benzyl-α-D-mannopyranoside 19

^{1}H NMR (200 MHz, $CDCl_3$) δ 7.49–7.28 (Ar), 4.94–4.60 (m, 5H, 2 × –CH_2Ph and H-1), 4.06–4.00 (br d, 1H, H-2), 3.94–3.72 (m, 4H, H-3, H-4, H-6a and H-6b), 3.70–3.60 (m, 1H, H-5), 3.36 (s, 3H, –OMe). ^{13}C NMR (50 MHz, $CDCl_3$) δ 138.3, 137.8 (aromatic C), 128.5–127.8 (aromatic CH), 100.2 (anomeric CH), 80.0, 75.1, 74.1, 72.1, 71.3, 68.3, 62.1, 54.9 (–OMe).

4.17. Methyl 2,6-di-*O*-acetyl-3,4-di-*O*-benzyl-α-D-mannopyranoside (acetylated 19)

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43–7.28 (m, 10H, ArH), 5.37 (dd, 1H, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.3 Hz, H-2), 4.92–4.50 (m, 5H, 2 × –CH_2Ph and H-1), 4.38–4.32 (d, 2H, H-6a and H-6b), 4.00 (dd, $J_{4,5}$ = 9.9 Hz, 1H, H-3), 3.84 (dt, 1H, $J_{5,6a}$ = 2.7 Hz, $J_{5,6b}$ = 3.6 Hz, H-5), 3.74 (t, 1H, H-4), 3.36 (s, 3H, –OMe), 2.16 and 2.08 (2 × s, 6H, 2 × –COCH_3).

4.18. Methyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside 20

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.53–7.28 (Ar), 5.54 (s, 1H, benzylidene PhCH), 4.72 (s, 1H, H-1), 4.71 (d, 1H, J_{gem} = 11.5 Hz, –CH_aH$_b$Ph), 4.67 (d, 1H, –CH$_a$$H_b$Ph), 4.23 (dd, 1H, $J_{5,6a}$ = 4.2 Hz, $J_{6a,6b}$ = 9.7 Hz, H-6a), 4.05 (dd, 1H, $J_{2,3}$ = 3.7 Hz, $J_{3,4}$ = 9.7 Hz, H-3), 3.88 (t, 1H, H-4), 3.84–3.70 (m, 3H, H-2, H-5 and H-6b), 3.33 (s, 3H, –OMe), 2.43 (br s, 1H, 3-O*H*).

4.19. Methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside 21

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.53–7.28 (Ar), 5.61 (s, 1H, benzylidene PhCH), 4.84 (d, 1H, J_{gem} = 12.0 Hz, –CH_aH$_b$Ph), 4.76 (d, $J_{1,2}$ = 1.0 Hz, 1H, H-1), 4.70 (d, 1H, J_{gem} = 12.0 Hz, –CH$_a$$H_b$Ph), 4.27 (dd, 1H, $J_{5,6a}$ = 4.2 Hz, $J_{6a,6b}$ = 9.7 Hz, H-6a), 4.09 (t, 1H, H-4), 4.04 (dd, 1H, H-2), 3.90 (dd, 1H, $J_{2,3}$ = 3.7 Hz, $J_{3,4}$ = 9.7 Hz, H-3), 3.88–3.77 (m, 2H, H-5 and H-6b), 3.37 (s, 3H, –OMe). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.7, 170.2 (2 × –*C*OCH$_3$), 138.1, 137.8 (aromatic C), 128.4–127.8 (aromatic CH), 98.8 (anomeric CH), 78.1, 75.1, 74.1, 71.7, 69.6, 68.5, 63.4, 54.9 (–OMe), 21.0, 20.8 (2 × –CO*C*H$_3$).

4.20. Methyl (3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→3))-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6))-2,4-di-*O*-benzyl-α-D-mannopyranoside 23

A mixture of donor **22** (78 mg, 95 μmol) and diol **13** (12 mg, 29 μmol) was coevaporated three times with anhydrous toluene (3 × 2 mL), and then dried in vacuo for 30 min. After adding 4 Å AW 300 MS, the mixture was dissolved under argon with 4:1 toluene–Et_2O (2.8 mL), cooled to −30 °C, and stirred for 15 min. A solution of $Bi(OTf)_3$ in dioxane (14.5 mg/mL, 135 μL, 0.29 μmol) was then added, and the temperature was allowed to raise up to −10 °C over 45 min. Having realized the completion of the glycosidation step (TLC analysis, eluent: petroleum ether–EtOAc 7:3), TEA (0.70 mL) was added and the reaction vessel was immediately warmed to rt. After one hour the reaction mixture was filtered through a short plug of silica gel repeatedly washed with CH_2Cl_2–MeOH–CH_3CN 85:10:5. The filtrate was concentrated, and the residue purified by silica-gel flash-chromatography (eluent: *n*-hexane–acetone–CH_2Cl_2 from 3:1:0.5 to 2:1:0.5) to yield trisaccharide **23** as an oil (26 mg, 72% overall yield). $[\alpha]_D^{26}$ +25.9 (*c* 0.8 in $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$: δ 7.40−7.10 Ar), 5.23 (d, $J_{1,2}$ = 1.2 Hz, 1H, 1′^{3}-H), 5.09 (d, $J_{1,2}$ = 1.2 Hz, 1H, 1′^{6}-H), 4.66 (d, $J_{1,2}$ = 1.6 Hz, 1H, 1-H), 4.87–4.46 (16H, 8 × –CH_2Ph), 4.15–4.10 (2H), 4.05–3.95 (2H), 3.95–3.80 (8H), 3.80–3.60 (6H), 3.25 (s, 3H, –OCH_3). ^{13}C NMR (100 MHz; $CDCl_3$): δ 138.5, 138.4, 138.3 (×2), 138.2, 138.1, 137.9 (×2) (aromatic C); 128.6–127.5 (aromatic CH); 101.4, 98.7, and 98.2 (anomeric CH), 80.1, 79.5, 78.9, 77.7, 75.0, 74.9, 74.8, 74.4, 74.2, 73.6, 73.3, 72.3, 72.0, 71.9, 71.4, 71.1,

69.4, 68.8, 68.7, 68.0, 66.1, 54.7. MALDI-TOF MS: calcd for $[M+Na]^+$ 1261.55. Found 1261.15. Anal. Calcd for $C_{75}H_{82}O_{16}$: C, 72.68; H, 6.67. Found: C, 72.35; H, 6.50.

4.21. Methyl (3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,4, 6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→3))-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyr- anosyl-(1→6))-2,4-di-*O*-benzyl-α-D-mannopyranoside 2

A mixture of donor **22** (40 mg, 49 μmol) and diol **23** (14 mg, 11 μmol) was coevaporated three times with anhydrous toluene (3 × 1 mL), and then dried in vacuo for 30 min. After adding 4 Å AW 300 MS, the mixture was dissolved under argon with 4:1 toluene–Et_2O (2.1 mL), cooled to −30 °C, and stirred for 15 min. A solution of $Bi(OTf)_3$ in dioxane (14.5 mg/mL, 100 μL, 2.2 μmol) was then added, and the temperature was allowed to raise up to +5 °C over one hour. Having realized the completion of the glycosidation step (TLC analysis, eluent petroleum ether–EtOAc 7:3), TEA (0.35 mL) was added and the reaction mixture immediately warmed to rt. The reaction mixture was then filtered through a short plug of silica gel repeatedly washed with CH_2Cl_2–MeOH–CH_3CN. The filtrate was concentrated, and the residue purified by silica-gel flash-chromatography (eluent: *n*-hexane–acetone–CH_2Cl_2 3:1:0.5) to yield pentasaccharide **2** as an oil (15 mg, 63% overall yield); $[\alpha]_D^{28}$ +34.7 (*c* 0.7 in $CHCl_3$); ^1H NMR (400 MHz; $CDCl_3$: δ 7.40–7.10 Ar), 5.25, 5.11 and 5.05 (3 × d, $J_{1,2}$ = 1.6 Hz, 3H, 2 × 1″-H and 1′^{3}-H), 4.97 (d, $J_{1,2}$ = 1.6 Hz, 1H, 1′^{6}-H), 4.64 (d, $J_{1,2}$ = 1.6 Hz, 1H, 1-H), 4.98–4.30 (28H, 14 × $-CH_2Ph$), 4.15–4.05 (4H), 4.05–3.95 (3H), 3.95–3.75 (13H), 3.75–3.63 (6H), 3.60–3.40 (4H), 3.16 (s, 3H, $-OCH_3$). ^{13}C NMR (100 MHz; $CDCl_3$: δ 138.7–138.0 (aromatic C); 128.6–127.0 (aromatic CH); 101.1 (×3), 99.1, and 97.9 (anomeric CH), 80.3, 80.0, 79.9, 79.6, 78.9, 77.8, 75.3, 75.0, 74.9, 74.8, 74.7, 74.6, 74.5, 74.3, 74.2, 73.4, 73.3, 72.7, 72.1, 72.0, 71.8, 71.6, 71.5, 71.0, 69.7, 69.1, 68.9, 68.6, 68.5, 66.5, 54.6 ppm. MALDI-TOF MS: calcd for $[M+Na]^+$ 2126.94. Found 2126.11. Anal. Calcd for $C_{129}H_{138}O_{26}$: C, 73.62; H, 6.61. Found: C, 73.35; H, 6.70.

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