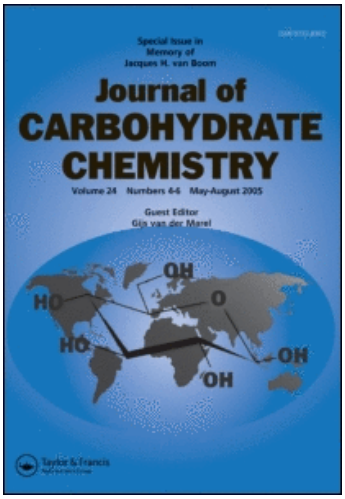

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SYNTHESIS OF A TETRA- AND A HEXASACCHARIDE DONOR CORRESPONDING TO THE NON-REDUCING TERMINI OF MYCOBACTERIAL 3-*O*-METHYLMANNOSE POLYSACCHARIDE (MMP)

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ABSTRACT

The blockwise synthesis of the title compounds, namely the tetra- and the hexasaccharide trichloroacetimidates (**20**) and (**23**), is described. Both acetates and imidates were employed as glycosyl donors in most of the coupling reactions. As nearly all of the synthetic intermediates contain one or more OCH_3 groups, they are easily identified by NMR spectroscopy the methyl signals. The fully functionalized compounds **20** and **23** correspond to the non-reducing terminal fragments of mycobacterial 3-*O*-methylmannose polysaccharide (MMP), and can serve as suitable building blocks for the synthesis of higher-order structures of MMP.

INTRODUCTION

The 3-*O*-methyl-D-mannose-containing polysaccharide (MMP) from the cytoplasm of *Mycobacterium smegmatis*, first described by Gray and Ballou,[1] is composed of 10 to 13 α 1→4-linked 3-*O*-methylmannose units in a linear chain terminated by an unmethylated mannose at the non-reducing end and the reducing end is blocked by an α-methyl aglycon.[2]

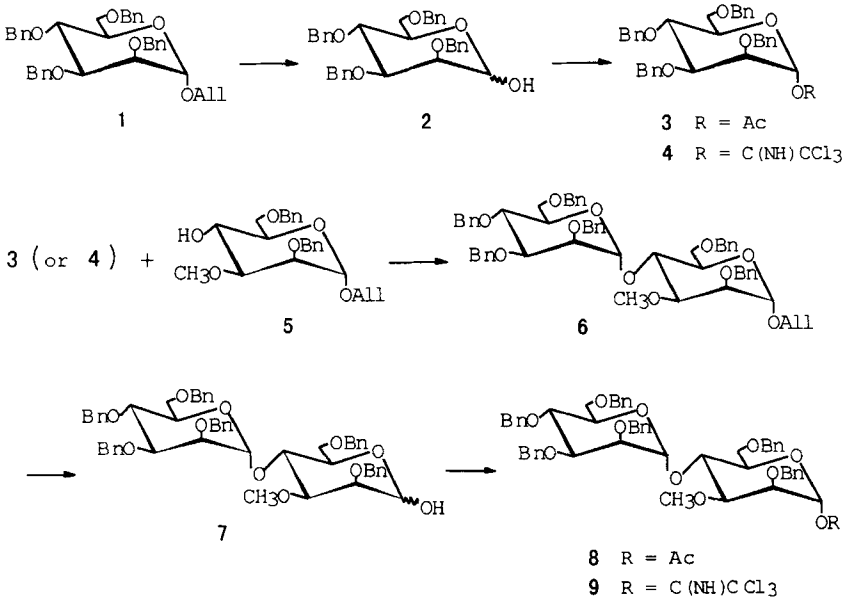
It was reported that MMP has the unusual property of binding long chain fatty acids and acyl coenzyme A derivatives tightly. This polysaccharide can activate the fatty acid

synthetase complex, participate in the regulation of fatty acid synthesis, and alter the fatty acid product distribution,[3,4] apparently as a consequence of the interaction of MMP with the enzyme complex and with the acyl-CoA products.

The important biological functions of MMP had stimulated our efforts towards the total synthesis of MMP. We previously reported the synthesis of a hexasaccharide acceptor corresponding to the reducing end of MMP,[5] and here we report the preparation of the tetra- and the hexasaccharide trichloroacetimidates **20** and **23**. These compounds correspond to the non-reducing termini of MMP, and can suitably function as glycosyl donors for the synthesis of extended fragments of MMP.

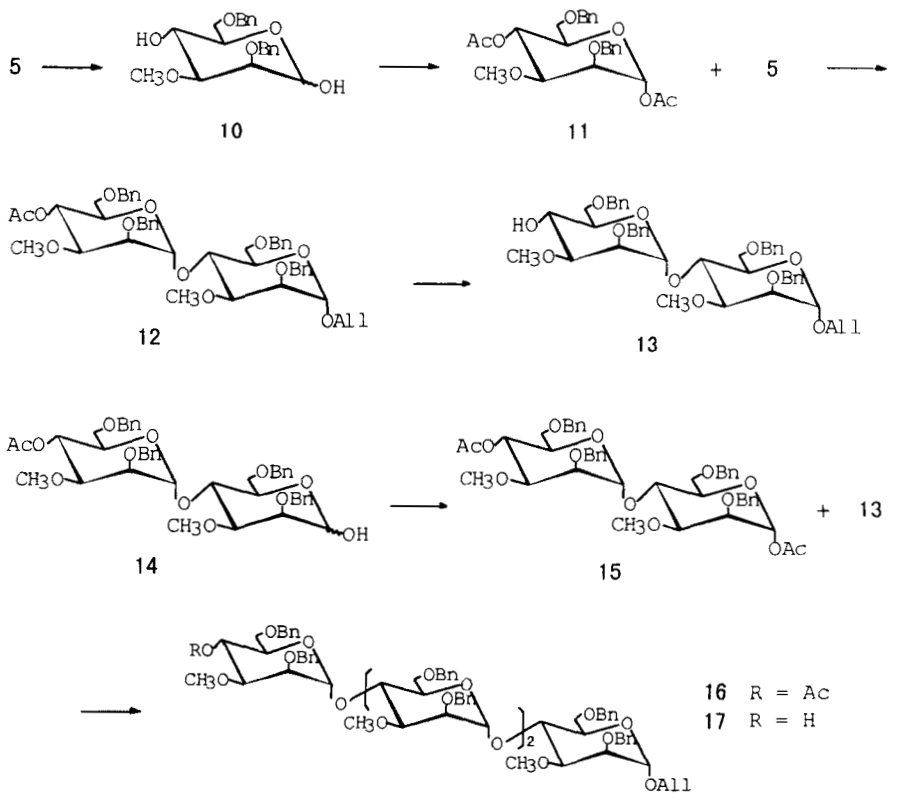
RESULTS AND DISCUSSION

The monosaccharide units.— The monosaccharide glycosyl donors (**3**) and (**4**) were prepared from fully benzylated compound (**1**) in two-steps: I. deallylation of **1** in dry methanol with $PdCl_2$ as catalyst[6,7] generated hemiacetal (**2**) in 87% yield; II. acetylation of **2** with acetic anhydride in pyridine by standard methods furnished acetate **3** in quantitative yield; on the other hand, trichloroacetimidation of **2** with 1,8-diazabicyclo[5.4.0] undec-7-ene (DBU) and CCl_3CN in anhyd CH_2Cl_2[8,9] afforded **4** in 82% yield.



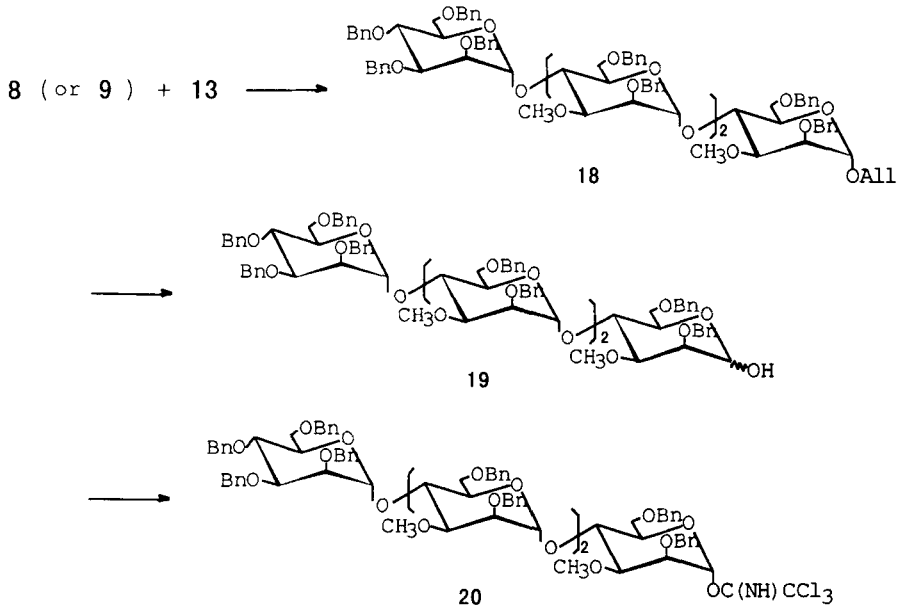
In an analogous fashion to the synthesis of **3**, another monosaccharide donor **11** was prepared from (**5**)[5,10] in 89% overall yield.

Glycosidation reactions.— Coupling of the acetate **3** with the acceptor **5** in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf)[11,12] as Lewis acid catalyst (1.55 mol) and powdered molecular sieve 4A at 0 °C proceeded well to provide protected allyl mannobioside **6** in 86% yield. While employing imidate **4** as glycosyl donor, and triethylsilyltrifluoromethanesulfonate (TESOTf) as catalyst **6** was obtained in an 82% yield. The stereochemistry of the glycosidation was assigned as α-D from the ^{13}C NMR data, which included two signals for C-1_A and C-1_B at δ 91.86 ($^1J_{C,H}$ = 174.1 Hz) and 99.68 ppm ($^1J_{C,H}$ = 171.6 Hz), respectively. Deallylation of **6** wasn't successful with MeOH as the sole solvent as described for monosaccharide **2**, but proceeded smoothly in a mixed solvent of 1:1 (v/v) MeOH-CH_2Cl_2 to generate hemiacetal (**7**) in 87.5% yield. Conversion of **7** to the acetate (**8**) (95%) or the imidate (**9**) (92%), as described for the case of **3** or **4**, afforded the corresponding disaccharide donor.



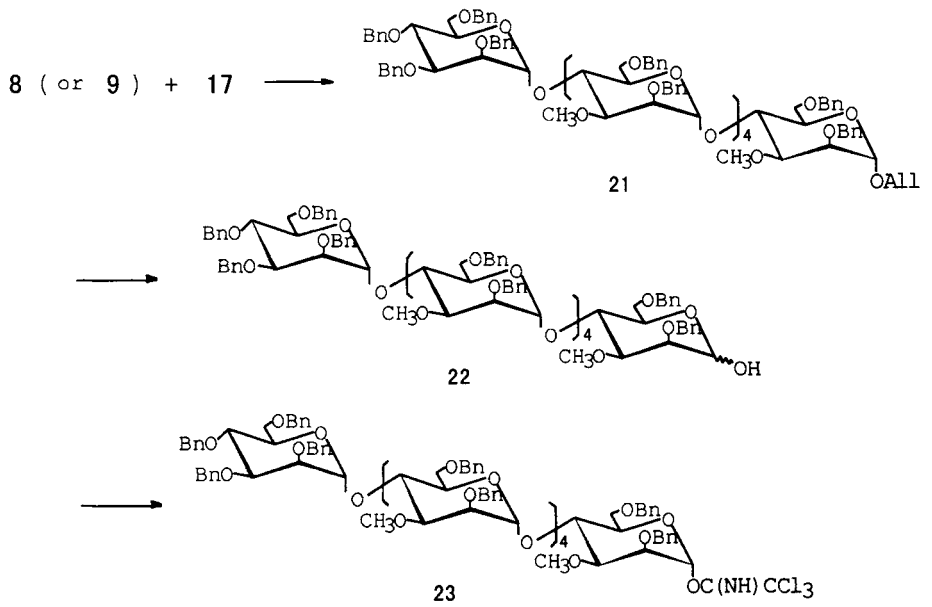
Although the key disaccharide (**12**) can be obtained by Schmidt's method,[5] another way employing the acetate **11** as glycosyl donor was performed and seemed more convenient, especially in large-scale preparation. Condensation of **11** with the alcohol **5** was carried out by using TMSOTf (1.2 mol) as catalyst as described for **6**, to provide allyl mannobioside **12** in 78% yield. Deacetylation of **12** with 0.15 M NaOMe-MeOH in THF produced alcohol **13** as disaccharide acceptor. Acetylation of the hemiacetal (**14**)[5] by standard methods gave diacetate (**15**) as disaccharide donor.

The blockwise chain elongation of **15** with the alcohol **13** was performed under similar conditions as described for **6**, to afford tetrasaccharide (**16**) in 58% yield. Four methyl group singlets appeared at 3.28, 3.19, 3.18 and 3.17 ppm in its ^{1}H NMR spectrum, respectively. The assigned structure was also supported by ^{13}C NMR data, in which four signals for C-1 in α-D configuration appeared at δ 99.78 ($^1J_{C,H}$ = 171.2 Hz), 99.69 ($^1J_{C,H}$ = 170.6 Hz), 99.06 ($^1J_{C,H}$ = 169.2 Hz) (C-1_D,1_C,1_B), and 96.80 ppm ($^1J_{C,H}$ = 168.8 Hz, C-1_A). Subsequent deacetylation of **16** provided desired mannotetraosyl glycosyl acceptor (**17**). The ^{1}H NMR spectrum of **17** also showed clearly four methyl singlets at δ 3.30, 3.22, 3.19 and 3.17 ppm, respectively.



The protected non-reducing allyl mannotetraoside (**18**) was obtained in 62% yield by coupling of the disaccharide donor **8** with the acceptor **13**, carried out at 20 °C with TMSOTf (3.2 mol) as catalyst under similar conditions as described for **6**; while employing the imidate **9** as disaccharide donor, 65% yield of **18** was obtained. The structure of **18** was assigned from ^{13}C NMR spectroscopic data, in which four signals for C-1 in the α-D configuration appeared at δ 99.60 ($^{1}J_{C,H}$ = 170.3 Hz), 99.53 ($^{1}J_{C,H}$ = 171.1 Hz), 96.56 ($^{1}J_{C,H}$ = 168.8 Hz) (C-1_D,1_C,1_B), and 91.39 ppm ($^{1}J_{C,H}$ = 174.3 Hz, C-1_A). Deallylation of **18** was carried out in 1:1 MeOH-CH_2Cl_2 with $PdCl_2$, as catalyst as described for **7**, to produce hemiacetal (**19**), followed by treatment with trichloroacetonitrile in the presence of DBU to give the desired tetrasaccharide glycosyl imidate **20**.

The key condensation of the imidate **9** with the tetrasaccharide acceptor **17** employing TESOTf (0.95 mol) as catalyst proceeded at -78 °C without incident, to produce hexasaccharide (**21**) in 56% yield; while using the acetate **8** as glycosyl donor, a relatively low yield (35%) of **21** was obtained. The ^{1}H NMR spectrum of **21** showed five methyl signals at δ 3.20 (6 H, for two methyl groups), 3.18, 3.17 and 3.09 ppm, respectively. Succ-



essive conversion of **21** to the hemiacetal (**22**) (85%) and the imidate **23** (90%), as described for the case of **19** and **20**, afforded the target hexasaccharide glycosyl imidate.

In conclusion, the linear allyl α (1→4)-D-*manno*-tetra- and -hexasaccharide **18** and **21**, which were subsequently converted to the imidates **20** and **23**, were achieved by blockwise strategy employing both acetates and imidates as glycosyl donors. The stereochemistry of all synthetic intermediates were deduced from the synthetic sequences, and also characterized by spectroscopic means, and/or by elemental analyses. Compounds **18**, **20**, **21** and **23** were further characterized by mass spectroscopy. Owing to their hydrophobic nature, the field desorption mass spectroscopy (FDMS) was performed as confirmation of composition. In the case of **23**, the peaks appearing at *m/z* 2304 and *m/z* 2487 corresponded to the ions $[M\text{-}C_2HCl_3NO]^+$ and $[M+Na]^+$ respectively.

EXPERIMENTAL

General methods. Preparative chromatography was performed on silica gel (120-200 mesh) with the solvent systems specified. TLC was performed on silica gel HF, detection being performed either by charring with sulphuric acid in methanol or by UV light. Analytical LC was performed in stainless-steel columns packed with silica gel, with peak detection by a differential refractometer (Perkin-Elmer LC-25 RI Detector). Optical rotations were determined at 25 °C with a Perkin-Elmer model 241 MC automatic polarimeter for solutions in a 1-dm, jacketed cell. ^1H and ^{13}C NMR spectra were recorded with Varian XL-400 and Varian XL-200 spectrometers, with chemical shifts (δ) being given in ppm, downfield from Me_4Si, for solutions in $CDCl_3$. Subscripts A-F refer to the individual sugar residues, with A standing for the reducing-end unit. The field desorption mass spectra (FDMS) were recorded with a Finnigan MAT 90 mass spectrometer.

2,3,4,6-Tetra-*O*-benzyl-D-mannopyranose (2). A mixture of compound **1** (4.8 g, 8.3 mmol) and $PdCl_2$ (240 mg) in dry methanol (50 mL) was stirred vigorously for 6 h at 20 °C and filtered through Celite. The filtrate was concentrated to give **2** (3.9 g, 87%) as an α,β (8:1) mixture which was separated by analytical LC with 3:1 petroleum ether-EtOAc as the eluent. For the α anomer: $[\alpha]_D$ +12.8° (*c* 1.5, $CHCl_3$); Lit.[13] $[\alpha]_D^{20}$ +11° (*c* 0.9, $CHCl_3$); ^1H NMR δ 7.43-7.02 (m, 20 H, 4 Ph), 5.22 (d, 1 H, $J_{1,2}$ = 1.5 Hz, H-1), 4.93-4.40

(m, 8 H, 4 C*H*$_2$Ph), 4.09 (t, 1 H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4), 3.90-3.66 (m, 5 H, H-2,3,5,6,6'), 2.00 (s, 1 H, O*H*).

1-*O*-Acetyl-2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranose (3). Compound **2** (2.5 g, 4.6 mmol) was acetylated with acetic anhydride in pyridine by standard methods to afford **3** in a quantitative yield as a syrup: $[\alpha]_D$ +24.1° (*c* 1.5, $CHCl_3$); Lit.[14] $[\alpha]_D^{27}$ +29.3° (*c* 1.3, $CHCl_3$); ^{1}H NMR δ 7.55-7.04 (m, 20 H, 4 Ph), 6.22 (d, 1 H, $J_{1,2} = 1.9$ Hz, H-1), 4.94-4.44 (m, 8 H, 4 C*H*$_2$Ph), 4.08 (t, 1 H, $J_{3,4} = J_{4,5} = 9.6$ Hz, H-4), 3.88-3.67 (m, 5 H, H-2,3,5,6,6'), 2.00 (s, 3 H, C*H*$_3$CO).

***O*-2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl trichloroacetimidate (4).** To a mixture of compound **2** (500 mg, 0.92 mmol) and Cl_3CCN (0.25 mL) in anhyd CH_2Cl_2 (10 mL) at -10 °C was added 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (20 μL). The mixture was stirred at 0 °C under argon for 1 h. The solvent was removed by evaporation and the residue was purified by column chromatography with 3:1 petroleum ether-EtOAc as eluent to yield **4** (516 mg, 82%): $[\alpha]_D$ +29.7° (*c* 0.7, $CHCl_3$); Lit.[15] $[\alpha]_D$ +39° (*c* 0.9, $CHCl_3$); ^{1}H NMR δ 8.54 (s, 1 H, OC(N*H*)CCl_3), 7.48-7.14 (m, 20 H, 4 Ph), 6.39 (d, 1 H, $J_{1,2} = 1.6$ Hz, H-1), 4.94-4.49 (m, 8 H, 4 C*H*$_2$Ph), 4.18 (t, 1 H, $J_{3,4} = J_{4,5} = 9.3$ Hz, H-4), 4.12-3.69 (m, 5 H, H-2,3,5,6,6').

Allyl *O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl)-(1→4)-2,6-di-*O*-benzyl-3-*O*-methyl-α-D-mannopyranoside (6). [*A*]. A mixture of the acetate **3** (1.5 g, 2.58 mmol) and the alcohol **5** (1.14 g, 2.75 mmol) in anhyd CH_2Cl_2 (35 mL) was stirred with powdered molecular sieve 4A (6 g) under argon for 30 min at room temperature. The solution was cooled to -20 °C and TMSOTf (0.75 mL) was added dropwise. The mixture was stirred for 4 h at 0 °C, neutralized with Et_3N (4.0 mL), filtered through Celite and the filtrate was concentrated. The residue was chromatographed on a column of silica gel in 3:1 petroleum ether-EtOAc to yield syrupy **6** (2.08 g, 86%);

[*B*]. A mixture of the imidate **4** (900 mg, 1.31 mmol) and the acceptor **5** (540 mg, 1.30 mmol) in anhyd CH_2Cl_2 (20 mL) was stirred with powdered molecular sieve 4A (5 g) under argon for 30 min at room temperature. The solution was cooled to -78 °C and TESOTf (55 μL, 0.22 mmol) was added dropwise. The mixture was stirred for 20 min, then neutralized with Et_3N (4 mL). Processing and chromatography on silica gel in 3:1 petroleum ether-EtOAc yielded **6** (1.0 g, 82%);

$[\alpha]_D$ +23.4° (*c* 1.4, $CHCl_3$); ^1H NMR δ 7.48-7.15 (m, 30 H, 6 Ph), 6.00-5.87 (m, 1 H, CH_2=C*H*-CH_2), 5.35 (d, 1 H, $J_{1,2}$ = 1.6 Hz, H-1_B), 5.34-5.20 (m, 2 H, CH_2=CH-CH_2), 4.97 (d, 1 H, $J_{1,2}$ = 1.4 Hz, H-1_A), 4.95-4.46 (m, 12 H, 6 CH_2Ph), 3.48 (dd, 1 H, $J_{2,3}$ = 3.4 Hz, $J_{3,4}$ = 9.7 Hz, H-3_A), 3.19 (s, 3 H, OCH_3); ^{13}C NMR δ 138.5-137.6 (aromatic C-1), 133.6 (CH_2=*C*H-CH_2), 128.1-127.0 (aromatic C), 117.3 (*C*H_2=CH-CH_2), 99.68 ($^1J_{C,H}$ = 171.6 Hz, C-1_B), 91.86 ($^1J_{C,H}$ = 174.1 Hz, C-1_A), 81.61, 79.70 (C-3_B,3_A), 56.41 (O*C*H_3). Anal. Calcd for $C_{58}H_{64}O_{11}$: C, 74.34; H, 6.88. Found: C, 74.12; H, 6.86.

***O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl)-(1→4)-2,6-di-*O*-benzyl-3-*O*-methyl-D-mannopyranose (7).** A mixture of **6** (1.5 g, 1.60 mmol) and $PdCl_2$ (150 mg) in CH_3OH–CH_2Cl_2 (30 mL, 1:1) was stirred vigorously for 4 h at room temperature and filtered through Celite. The filtrate was concentrated to give **7** (1.25 g, 87.5%) as an α,β (6:1) mixture which was separated by analytical LC with 2:1 petroleum ether-EtOAc as the eluent; For the α anomer: $[\alpha]_D$ +16.5° (*c* 1.0, $CHCl_3$); ^1H NMR δ 7.69-7.03 (m, 30 H, 6 Ph), 5.35-5.22 (m, 2 H, H-1_B,1_A), 4.98-4.34 (m, 12 H, 6 CH_2Ph), 3.11 (s, 3 H, OCH_3), 1.65 (bs, 1 H, O*H*); ^{13}C NMR δ 138.5-137.7 (aromatic C-1), 128.1-127.2 (aromatic C), 99.89 (C-1_B), 91.87 (C-1_A), 81.11, 79.72 (C-3_B,3_A), 56.41 (O*C*H_3).

Anal. Calcd for $C_{55}H_{60}O_{11}$: C, 73.64; H, 6.74. Found: C, 73.56; H, 6.70.

***O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl)-(1→4)-1-*O*-acetyl-2,6-di-*O*-benzyl-3-*O*-methyl-α-D-mannopyranose (8).** Compound **7** (300 mg, 0.34 mmol) was acetylated by standard methods and compound **8** was obtained in 95% yield (295 mg): $[\alpha]_D$ +21.3° (*c* 0.9, $CHCl_3$); ^1H NMR δ 7.57-7.00 (m, 30 H, 6 Ph), 6.23 (d, 1 H, $J_{1,2}$ = 2.0 Hz, H-1_A), 5.30 (d, 1 H, $J_{1,2}$ = 1.5 Hz, H-1_B), 4.93-4.37 (m, 12 H, 6 CH_2Ph), 3.10 (s, 3 H, OCH_3), 2.10 (s, 3 H, CH_3CO); ^{13}C NMR δ 168.9 (CH_3*C*O), 138.7-137.6 (aromatic C-1), 128.3-127.3 (aromatic C), 99.82 (C-1_B), 91.57 (C-1_A), 81.03, 79.78 (C-3_B,3_A), 56.62 (O*C*H_3), 21.09 (*C*H_3CO).

Anal. Calcd for $C_{56}H_{62}O_{11}$: C, 73.82; H, 6.86. Found: C, 73.64; H, 6.82.

***O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl)-(1→4)-2,6-di-*O*-benzyl-3-*O*-methyl-α-D-mannopyranosyl trichloroacetimidate (9).** To a mixture of compound **7** (600 mg, 0.67 mmol) and Cl_3CCN (0.2 mL) in anhyd CH_2Cl_2 (16 mL) at -10 °C was added 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (40 μL). The mixture was stirred at 0 °C under argon for 1 h. Processing and chromatography on silica gel in 2:1 petroleum ether-EtOAc

yielded **9** (650 mg, 92%): $[\alpha]_D$ +28.3° (*c* 1.0, $CHCl_3$); ^1H NMR δ 8.60 (s, 1 H, OC(N*H*)CCl_3), 7.60-7.12 (m, 30 H, 6 Ph), 6.40 (d, 1 H, $J_{1,2}$ = 2.0 Hz, H-1_A), 5.30 (d, 1 H, $J_{1,2}$ = 1.5 Hz, H-1_B), 4.94-4.38 (m, 12 H, 6 C*H*$_2$Ph), 3.08 (s, 3 H, OC*H*$_3$); ^{13}C NMR δ 160.2 (*C*=NH), 138.5-137.6 (aromatic C-1), 128.2-127.1 (aromatic C), 99.78 (C-1_B), 91.02 (C-1_A), 81.33, 79.47 (C-3_B,3_A), 56.62 (O*C*H$_3$).

FDMS: *m/z* 879 $[M-C_2HCl_3NO]^+$, 1040 $[M+H]^+$.

2,6-Di-*O*-benzyl-3-*O*-methyl-D-mannopyranose (10). Deallylation of **5** (5.4 g, 13.0 mmol) in dry methanol (60 mL) with $PdCl_2$ (400 mg) as catalyst was performed as described for **2**, to give **10** as a syrup (4.33 g, 89%) consisting of α and β anomers in a ratio of 6:1 which was separated by analytical LC with 1:1 petroleum ether-EtOAc as the eluent; For the α anomer: $[\alpha]_D$ -18.8° (*c* 3.3, $CHCl_3$); ^1H NMR δ 7.49-7.10 (m, 10 H, 2 Ph), 5.31 (d, 1 H, $J_{1,2}$ = 1.5 Hz, H-1), 4.73 and 4.65 (ABq, 2 H, J = 12.2 Hz, C*H*$_2$Ph), 4.59 (S, 2 H, C*H*$_2$Ph), 3.35 (s, 3 H, OC*H*$_3$); ^{13}C NMR δ 138.0, 137.8 (aromatic C-1), 128.5–127.7 (aromatic C), 92.61 (C-1), 80.68 (C-3), 73.43, 72.49 (2 *C*H$_2$Ph), 72.70 (C-2), 71.22 (C-5), 70.50 (C-6), 67.53 (C-4), 57.02 (O*C*H$_3$).

Anal.Calcd for $C_{21}H_{26}O_6 \cdot 1/3H_2O$: C, 66.30; H, 7.06. Found: C, 66.33; H, 7.04.

1,4-Di-*O*-acetyl-2,6-di-*O*-benzyl-3-*O*-methyl-α-D-mannopyranose (11). Compound **10** (4.0 g, 10.7 mmol) was acetylated by standard methods and **11** was obtained in a quantitative yield as a syrup: $[\alpha]_D$ +19.7° (*c* 3.6, $CHCl_3$); ^1H NMR δ 7.45–7.20 (m, 10 H, 2 Ph), 6.21 (d, 1 H, $J_{1,2}$ = 2.1 Hz, H-1), 5.35 (t, 1 H, $J_{3,4}$ = $J_{4,5}$ = 9.8 Hz, H-4), 4.75, 4.53 (2 s, 4 H, 2 C*H*$_2$Ph), 3.92 (ddd, 1 H, H-5), 3.80 (dd, 1 H, $J_{1,2}$ = 2.1 Hz, $J_{2,3}$ = 3.4 Hz, H-2), 3.60-3.51 (m, 3 H, H-3,6 and 6'), 3.31 (s, 3 H, OC*H*$_3$), 2.11, 1.98 (2 s, 6 H, 2 C*H*$_3$CO); ^{13}C NMR δ 169.7, 168.8 (2 CH_3CO), 137.7, 137.5 (aromatic C-1), 128.3-127.5 (aromatic C), 91.63 (C-1), 78.34 (C-3), 73.51, 72.54 (2 *C*H$_2$Ph), 72.59 (C-2), 72.17 (C-5), 69.66 (C-6), 68.38 (C-4), 57.70 (O*C*H$_3$), 21.02, 20.86 (2 *C*H$_3$CO).

Anal. Calcd for $C_{25}H_{30}O_8$: C, 65.49; H, 6.59. Found: C, 65.74; H, 6.66.

Allyl *O*-(4-*O*-Acetyl-2,6-di-*O*-benzyl-3-*O*-methyl-α-D-mannopyranosyl)-(1→ 4)-2,6-di-*O*-benzyl-3-*O*-methyl-α-D-mannopyranoside (12). Coupling of the acetate **11** (3.5 g, 7.64 mmol) with the alcohol **5** (3.32 g, 8.02 mmol) was performed as described for **6** (method [*A*]), to yield **12** as a syrup (4.83 g, 78%): $[\alpha]_D$ +28.5° (*c* 2.0, $CHCl_3$); Lit. [5] $[\alpha]_D$ +28.7° (*c* 2.8, $CHCl_3$).

Allyl *O*-(2,6-Di-*O*-benzyl-3-*O*-methyl-α-D-mannopyranosyl)-(1→4)-2,6-di-*O*-benzyl-3-*O*-methyl-α-D-mannopyranoside (13). A solution of compound **12** (2.0 g, 2.46 mmol) in THF (20 mL) and 0.15 M NaOMe-MeOH (24 mL) was stirred for 2 h at room temperature. Neutralization with Amberlite H-120 and chromatography on a column of silica gel in 2:1 petroleum ether-EtOAc afforded **13** as an amorphous solid (1.76 g, 93%): $[\alpha]_D$ +12.7° (*c* 1.4, $CHCl_3$); ^1H NMR δ 7.56-7.21 (m, 20 H, 4 Ph), 6.00-5.86 (m, 1 H, CH_2=C*H*-CH_2), 5.38 (d, 1 H, $J_{1,2}$ = 1.6 Hz, H-1_B), 5.35-5.19 (m, 2 H, CH_2=CH-CH_2), 4.98 (d, 1 H, $J_{1,2}$ =1.6 Hz, H-1_A), 4.82-4.48 (m, 8 H, 4 CH_2Ph), 3.53 (dd, 1 H, $J_{2,3}$ = 3.4 Hz, $J_{3,4}$ = 9.4 Hz, H-3_A), 3.42 (dd, 1 H, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 9.4 Hz, H-3_B), 3.35, 3.25 (2 s, 6 H, OCH_3-3_A,3_B), 2.63 (bs, 1 H, O*H*); ^{13}C NMR δ 138.6-138.1 (aromatic C-1), 133.7 (CH_2=*C*H-CH_2), 128.3-127.2 (aromatic C), 117.6 (*C*H_2=CH-CH_2), 99.76 (C-1_B), 96.66 (C-1_A), 81.76, 80.55 (C-3_B,3_A), 56.95, 56.62 (OCH_3-3_B,3_A).

Anal. Calcd for $C_{45}H_{54}O_{11}$: C, 70.11; H, 7.06. Found: C, 70.05; H, 7.26.

***O*-(4-*O*-Acetyl-2,6-di-*O*-benzyl-3-*O*-methyl-α-D-mannopyranosyl)-(1→4)-1-*O*-acetyl-2,6-di-*O*-benzyl-3-*O*-methyl-α-D-mannopyranose (15).** Compound **14** (2.5 g, 3.24 mmol) was acetylated by standard methods and compound **15** was obtained in a quantitative yield as an amorphous solid: $[\alpha]_D$ +22° (*c* 0.7, $CHCl_3$); ^1H NMR δ 7.48-7.20 (m, 20 H, 4 Ph), 6.25 (d, 1 H, $J_{1,2}$ = 1.9 Hz, H-1_A), 5.33 (d, 1 H, $J_{1,2}$ = 1.3 Hz, H-1_B), 5.30 (t, 1 H, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-4_B), 4.81-4.42 (m, 8 H, 4 CH_2Ph), 4.04 (t, 1 H, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-4_A), 3.34, 3.15 (2 s, 6 H, OCH_3-3_A,3_B), 2.11, 1.98 (2 s, 6 H, 2 CH_3CO); ^{13}C NMR δ 169.8, 168.9 (2 CH_3*C*O), 138.3-137.5 (aromatic C-1), 128.3-127.3 (aromatic C), 99.76 (C-1_A), 91.48 (C-1_B), 80.96, 78.62 (C-3_B,3_A), 57.54, 56.52 (O*C*H_3-3_B,3_A), 21.03, 20.90 (2 *C*H_3CO).

Anal. Calcd for $C_{46}H_{54}O_{13}$: C, 67.80; H, 6.68. Found: C, 67.68; H, 6.65.

Allyl *O*-(4-*O*-Acetyl-2,6-di-*O*-benzyl-3-*O*-methyl-α-D-mannopyranosyl)-[(1→4)-*O*-(2,6-di-*O*-benzyl-3-*O*-methyl-α-D-mannopyranosyl)]$_2$-(1→4)-2,6-di-*O*-benzyl-3-*O*-methyl-α-D-mannopyranoside (16). A mixture of the diacetate **15** (1.5 g, 1.84 mmol) and the alcohol **13** (1.44 g, 1.88 mmol) in anhyd CH_2Cl_2 (20 mL) was stirred with finely powdered molecular sieve 4A (4 g) under argon for 30 min at room temperature. The solution was cooled to -10 °C and TMSOTf (1.05 mL) was added dropwise. The mixture was stirred for 4 h at 20 °C. Processing and chromatography on silica gel in 2:1 petroleum ether-EtOAc yielded **16** as an amorphous solid (1.63 g, 58%): $[\alpha]_D$ +26.7° (*c* 5.0, $CHCl_3$);

^{1}H NMR δ 7.29-7.00 (m, 40 H, 8 Ph), 5.84-5.72 (m, 1 H, CH_2=C*H*-CH_2), 5.29-5.05 (m, 6 H, H-4_D, H-1_D,1_C,1_B, CH_2=CH-CH_2), 4.98 (d, 1 H, $J_{1,2}$ = 1.6 Hz, H-1_A), 4.83-4.27 (m, 16 H, 8 CH_2Ph), 3.28, 3.19, 3.18, 3.17 (4 s, 12 H, OCH_3-3_D,3_C,3_B,3_A), 1.89 (s, 3 H, CH_3CO); ^{13}C NMR δ 169.8 (CH_3*C*O), 138.7-138.0 (aromatic C-1), 133.8 (CH_2=*C*H-CH_2), 128.3-127.1 (aromatic C), 117.4 (*C*H_2=CH-CH_2), 99.78 ($^1J_{C\text{-}1,H\text{-}1}$ = 171.2 Hz), 99.69 ($^1J_{C\text{-}1,H\text{-}1}$ = 170.6 Hz), 99.06 ($^1J_{C\text{-}1,H\text{-}1}$ = 169.2 Hz) (C-1_D,1_C,1_B), 96.80 ($^1J_{C\text{-}1,H\text{-}1}$ = 168.8 Hz, C-1_A), 81.64, 81.35, 81.29 (C-3_D,3_C,3_B), 78.74 (C-3_A), 57.62, 56.68, 56.44, 56.42 (O*C*H_3-3_D,3_C,3_B,3_A).

Anal. Calcd for $C_{89}H_{104}O_{22}$: C, 70.06; H, 6.87. Found: C, 69.72; H, 6.90.

Allyl *O*-(2,6-Di-*O*-benzyl-3-*O*-methyl-α-D-mannopyranosyl)-[(1→4)-*O*-(2,6-di-*O*-benzyl-3-*O*-methyl-α-D-mannopyranosyl)]$_2$-(1→4)-2,6-di-*O*-benzyl-3-*O*-methyl-α-D-mannopyranoside (17). A solution of **16** (500 mg, 0.33 mmol) in THF (8 mL) and 0.10 M NaOMe-MeOH (12 mL) was stirred for 2 h at room temperature. Processing and chromatography on silica gel in 1:1 petroleum ether-EtOAc gave **17** (445 mg, 90%) as an amorphous solid: $[\alpha]_D$ +20.1° (*c* 0.8, $CHCl_3$); ^{1}H NMR δ 7.40-7.14 (m, 40 H, 8 Ph), 5.93-5.81 (m, 1 H, CH_2=C*H*-CH_2), 5.37 (d, 1 H, $J_{1,2}$ = 1.6 Hz, H-1_D), 5.31-5.15 (m, 4 H, H-1_C,1_B, CH_2=CH-CH_2), 4.92 (d, 1 H, $J_{1,2}$ = 1.7 Hz, H-1_A), 4.77-4.37 (m, 16 H, 8 CH_2Ph), 3.30, 3.22, 3.19, 3.17 (4 s, 12 H, OCH_3-3_D,3_C,3_B,3_A); ^{13}C NMR δ 138.9-138.2 (aromatic C-1), 133.8 (CH_2=*C*H-CH_2), 128.5-127.3 (aromatic C), 117.7 (*C*H_2=CH-CH_2), 99.97, 99.89, 99.79 (C-1_D,1_C,1_B), 96.91 (C-1_A), 81.94, 81.80, 81.56 (C-3_D,3_C,3_B), 80.28 (C-3_A), 56.86, 56.78 (O*C*H_3-3_D,3_C), 56.58 (O*C*H_3-3_B,3_A).

Anal. Calcd for $C_{87}H_{102}O_{21}$: C, 70.43; H, 6.93. Found: C, 70.12; H, 6.89.

Allyl *O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl)-[(1→4)-*O*-(2,6-di-*O*-benzyl-3-*O*-methyl-α-D-mannopyranosyl)]$_2$-(1→4)-2,6-di-*O*-benzyl-3-*O*-methyl-α-D-mannopyranoside (18). [*A*]. Coupling of the acetate **8** (450 mg, 0.49 mmol) with the disaccharide acceptor **13** (380 mg, 0.49 mmol) was performed by using TMSOTf (0.35 mL) as catalyst as described for **16**, to yield **18** as a syrup (0.5 g, 62%);

[*B*]. Coupling of the imidate **9** (200 mg, 0.19 mmol) with **13** (140 mg, 0.18 mmol) was performed by using a catalytic amount of TESOTf (15 μL, 60.7 μmol) as described for **6** (method [*B*]), to yield **18** (190 mg, 65%);

$[\alpha]_D$ +20.7° (*c* 0.8, $CHCl_3$); ^{1}H NMR δ 7.57-6.97 (m, 50 H, 10 Ph), 5.93-5.82 (m, 1 H, CH_2=C*H*-CH_2), 5.38-5.14 (m, 5 H, H-1_D,1_C,1_B, CH_2=CH-CH_2), 4.93 (d, 1 H, $J_{1,2}$ = 1.7

Hz, H-1_A), 4.91-4.35 (m, 20 H, 10 CH_2Ph), 3.23, 3.22, 3.19 (3 s, 9 H, OCH_3-3_C,3_B,3_A); ^{13}C NMR δ 138.7-137.4 (aromatic C-1), 133.6 (CH_2=CH-CH_2), 128.3-127.1 (aromatic C), 117.3 (CH_2=CH-CH_2), 99.60 ($^1J_{C,H}$ = 170.3 Hz), 99.53 ($^1J_{C,H}$ = 171.1 Hz), 96.56 ($^1J_{C,H}$ = 168.8 Hz) (C-1_D, 1_C,1_B), 91.39 ($^1J_{C,H}$ = 174.3 Hz, C-1_A), 81.46, 81.20, 80.90 (C-3_D,3_C,3_B), 79.71 (C-3_A), 56.40, 56.21, 56.09 (OCH_3-3_C,3_B,3_A).

Anal. Calcd for $C_{100}H_{112}O_{21}$: C, 72.79; H, 6.84. Found: C, 72.68; H, 6.79. FDMS: *m/z* 1649 $[M]^+$, 1672 $[M+Na]^+$.

***O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl)-[(1→4)-*O*-(2,6-di-*O*-benzyl-3-*O*-methyl-α-D-mannopyranosyl)]$_2$-(1→4)-2,6-di-*O*-benzyl-3-*O*-methyl-D-mannopyranose (19).** Deallylation of **18** (150 mg, 92.4 μmol) in CH_3OH-CH_2Cl_2 (5.0 mL, 1:1, v/v) with $PdCl_2$ (20 mg) as catalyst was performed as described for **7**, to give **19** as a syrup (125 mg, 85.4%), consisting of α and β anomers in a ratio of 7:1, which was separated by analytical LC with 2:1 petroleum ether-EtOAc as the eluent; For the α anomer: $[\alpha]_D$ +10.4° (*c* 0.7, $CHCl_3$); ^{1}H NMR δ 7.82-6.78 (m, 50 H, 10 Ph), 5.43-5.20 (m, 4 H, H-1_D,1_C,1_B,1_A), 4.95-4.31 (m, 20 H, 10 CH_2Ph), 3.19, 3.17, 3.15 (3 s, 9 H, OCH_3-3_C,3_B,3_A); ^{13}C NMR δ 139.3-138.8 (aromatic C-1), 128.7-127.8 (aromatic C), 100.4, 100.3, 100.2 (C-1_D,1_C,1_B), 92.86 (C-1_A), 82.02, 81.94, 81.84 (C-3_D,3_C,3_B), 80.73 (C-3_A), 57.11, 56.94, 56.82 (OCH_3-3_C,3_B,3_A).

Anal. Calcd for $C_{97}H_{108}O_{21}$: C, 72.37; H, 6.76. Found: C, 72.22; H, 6.72.

***O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl)-[(1→4)-*O*-(2,6-di-*O*-benzyl-3-*O*-methyl-α-D-mannopyranosyl)]$_2$-(1→4)-2,6-di-*O*-benzyl-3-*O*-methyl-α-D-mannopyranosyl trichloroacetimidate (20).** To a mixture of compound **19** (100 mg, 63 μmol) and Cl_3CCN (20 μL) in anhyd CH_2Cl_2 (2 mL) at -10 °C was added 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (10 μL). The mixture was stirred at 0 °C under argon for 1 h. Processing and chromatography on silica gel in 2:1 petroleum ether-EtOAc yielded **20** (100 mg, 91.8%): $[\alpha]_D$ +17.6° (*c* 1.3, $CHCl_3$); ^{1}H NMR δ 8.61 (s, 1 H, OC(N*H*)CCl_3), 7.49-7.09 (m, 50 H, 10 Ph), 6.41 (d, 1 H, $J_{1,2}$ = 2.0 Hz, H-1_A), 5.38-5.20 (m, 3 H, H-1_D,1_C,1_B), 4.95-4.35 (m, 20 H, 10 CH_2Ph), 3.20, 3.17, 3.15 (3 s, 9 H, OCH_3-3_C,3_B,3_A); ^{13}C NMR δ 160.5 (*C*=NH), 138.8-137.8 (aromatic C-1), 128.3-127.3 (aromatic C), 99.88, 99.75, 96.17 (C-1_D,1_C,1_B), 91.19(C-1_A), 81.55 (C-3_D), 81.40 (C-3_C,3_B), 80.23 (C-3_A), 57.39, 56.79, 56.46 (OCH_3-3_C,3_B,3_A).

FDMS: *m/z* 1592 $[M-C_2HCl_3NO]^+$, 1752 $[M]^+$.

Allyl *O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl)-[(1→4)-*O*-(2,6-di-*O*-benzyl-3-*O*-methyl-α-D-mannopyranosyl)]$_4$-(1→4)-2,6-di-*O*-benzyl-3-*O*-methyl-α-D-mannopyranoside (21). [*A*]. Coupling of the acetate **8** (65 mg, 71 μmol) with the alcohol **17** (80 mg, 54 μmol) was performed by using TMSOTf (0.1 mL) as catalyst as described for **16**, to yield **21** (45 mg, 35%);

[*B*]. Coupling of the imidate **9** (400 mg, 0.38 mmol) with the tetrasaccharide acceptor **17** (280 mg, 0.19 mmol) was performed by using a catalytic amount of TESOTf (45 μL, 182 μmol) as described for **18** (method [*B*]), to yield **21** as a syrup (251 mg, 56%);

$[\alpha]_D$ +18.1° (*c* 0.6, $CHCl_3$); ^{1}H NMR δ 7.56-7.03 (m, 70 H, 14 Ph), 5.97-5.84 (m, 1 H, CH_2=C*H*-CH_2), 5.37 (d, 1 H, $J_{1,2}$ = 1.6 Hz, H-1_F), 5.35-5.16 (m, 6 H, H-1_E,1_D,1_C,1_B, CH_2=CH-CH_2), 4.94 (d, 1 H, $J_{1,2}$ = 1.5 Hz, H-1_A), 4.90-4.33 (m, 28 H, 14 CH_2Ph), 3.20 (s, 6 H, 2 OCH_3), 3.18, 3.17, 3.09 (3 s, 9 H, 3 OCH_3); ^{13}C NMR δ 138.7-138.0 (aromatic C-1), 133.8 (CH_2=CH-CH_2), 128.4-127.4 (aromatic C), 117.6 (CH_2=CH-CH_2), 99.74, 99.67, 99.62, 99.59, 96.80 (C-1_F,1_E,1_D,1_C,1_B), 96.76 (C-1_A), 81.94, 81.80, 81.74, 81.69, 81.56 (C-3_F,3_E,3_D,3_C,3_B), 80.28 (C-3_A), 56.68, 56.66, 56.42, 56.39, 56.38 (OCH$_3$-3_E,3_D,3_C,3_B,3_A).

Anal. Calcd for $C_{142}H_{160}O_{31}$: C, 72.18; H, 6.83. Found: C, 72.08; H, 6.78. FDMS: *m/z* 2362 $[M+H]^+$.

O-(2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl)-[(1→4)-*O*-(2,6-di-*O*-benzyl-3-*O*-methyl-α-D-mannopyranosyl)]$_4$-(1→4)-2,6-di-*O*-benzyl-3-*O*-methyl-D-mannopyranose (22). Deallylation of **21** (180 mg, 76 μmol) in CH_3OH-CH_2Cl_2 (6 mL, 1:1) with $PdCl_2$ (30 mg) as catalyst was performed as described for **7**, to give **22** as a syrup (150 mg, 85%), consisting of α and β anomers in a ratio of 8:1, which was separated by analytical LC with 2:1 petroleum ether-EtOAc as the eluent; For the α anomer: $[\alpha]_D$ +11.2° (*c* 0.8, $CHCl_3$); ^{1}H NMR δ 7.83-6.84 (m, 70 H, 14 Ph), 5.44-5.21 (m, 6 H, H-1_F,1_E,1_D,1_C,1_B,1_A), 4.94-4.30 (m, 28 H, 14 CH_2Ph), 3.18, 3.17, 3.16, 3.15, 3.10 (5 s, 15 H, 5 OCH_3); ^{13}C NMR δ 139.2-138.3 (aromatic C-1), 128.8-127.5 (aromatic C), 100.54, 100.45, 99.97, 99.89, 96.90 (C-1_F,1_E,1_D,1_C,1_B), 96.86 (C-1_A), 82.44, 82.35, 82.23, 82.10, 81.29 (C-3_F,3_E,3_D,3_C,3_B), 80.38 (C-3_A), 57.38, 57.16, 57.12, 57.09, 57.02 (OCH$_3$-3_E,3_D,3_C,3_B,3_A).

Anal. Calcd for $C_{139}H_{156}O_{31}$: C, 71.88; H, 6.77. Found: C, 71.66; H, 6.72.

O-(2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl)-[(1→4)-*O*-(2,6-di-*O*-benzyl-3-*O*-methyl-α-D-mannopyranosyl)]$_4$-(1→4)-2,6-di-*O*-benzyl-3-*O*-methyl-α-D-mannopyr-

anosyl trichloroacetimidate (23). To a mixture of compound **22** (120 mg, 51.7 μmol) and Cl_3CCN (20 μL) in anhyd CH_2Cl_2 (3 mL) at -10 °C was added 1,8-diazabicyclo [5.4.0]undec-7-ene (DBU) (12 μL). The mixture was stirred at 0 °C under argon for 2 h. Processing and chromatography on silica gel in 2:1 petroleum ether-EtOAc yielded **23** as an amorphous solid (115 mg, 90%): $[\alpha]_D$ +16.4° (*c* 0.7, $CHCl_3$); ^{1}H NMR δ 8.60 (s, 1 H, OC(N*H*)CCl_3), 7.48-7.06 (m, 70 H, 14 Ph), 6.42 (d, 1 H, $J_{1,2}$ = 1.9 Hz, H-1_A), 5.39-5.21 (m, 5 H, H-1_F,1_E,1_D,1_C,1_B), 4.94-4.34 (m, 28 H, 14 CH_2Ph), 3.19, 3.17, 3.16, 3.15, 3.11 (5 s, 15 H, 5 OCH_3); ^{13}C NMR δ 160.7 (*C*=NH), 138.9-137.3 (aromatic C-1), 128.4-127.1 (aromatic C), 99.96, 99.89, 99.84, 99.78, 96.66 (C-1_F,1_E,1_D,1_C,1_B), 96.54 (C-1_A), 82.05, 81.90, 81.84, 81.76, 81.58 (C-3_F,3_E,3_D,3_C,3_B), 80.78 (C-3_A), 57.70, 57.56, 57.41, 57.37, 57.30 (O*C*H_3-3_E,3_D,3_C,3_B,3_A).

FDMS: *m/z* 2304 $[M-C_2HCl_3NO]^+$, 2487 $[M+Na]^+$.

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