Optimization of Glycosylation Reactions in a Microreactor

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Glycosylations are notoriously difficult reactions that require extensive optimization regarding the type of anomeric leaving group, solvent, reaction temperature, and reaction time. Described is the use of a silicon-based microreactor to screen reaction conditions and to scale-up synthetic procedures. For the first time, glycosyl phosphates were employed in a microreactor. The optimized reaction conditions were successfully transferred to a batch process.

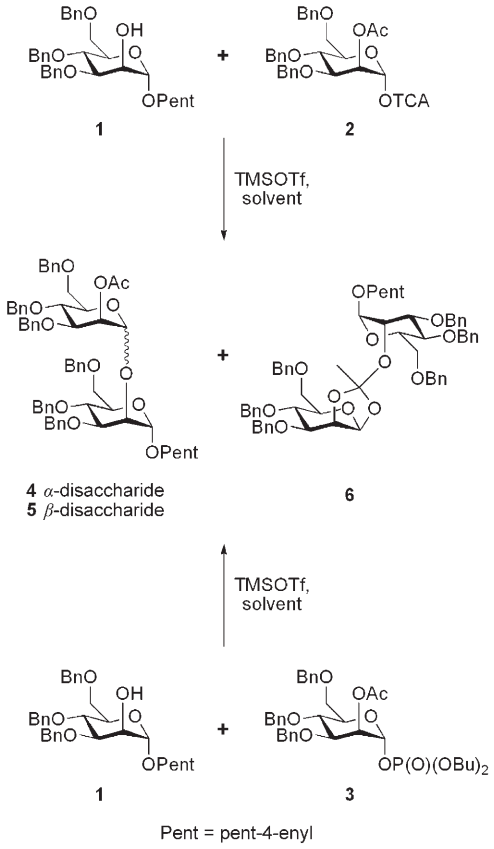
Introduction. – Glycosylations represent a class of challenging synthetic transformations, since the stereochemical outcome depends on various factors such as reaction temperature, solvent, concentration, and the nature of the coupling partners. The building blocks used for the assembly of oligosaccharides require multistep syntheses and are valuable synthetic intermediates. Major challenges for synthetic chemists are the high consumption of material and time required to find ideal reaction conditions. The scale-up of reactions optimized at small scale is an additional hurdle. To overcome these handicaps, continuous-flow technology, in general, and microreactor technology (MRT), in particular, are becoming increasingly interesting for synthetic chemists. Miniaturization of chemical continuous-flow reactions requires small quantities of reagents and allows for high-throughput screening of reaction conditions in a highly controlled manner. The precise control of reaction variables ensures improved safety, an efficient outcome of reactions with temperature-dependent selectivities, and the controlled isolation of unstable intermediates. The scale-up of synthetic procedures can be overcome by scale-out or numbering-up [1–13]. To date, microreactor technology has been investigated mainly by pharmaceutical and fine-chemical companies for production processes [10][14][15], and for proof-of-principle studies on analytical scale [16–21].

Synthetic strategies to install α-(1→2)-linked mannoses are well known [22–29]. We were interested in studying the formation of α-(1→2)-mannose linkages systematically in a silicon-based microreactor as an example for reaction optimization of glycosylation reactions in general.

Results and Discussion. – Three mannose building blocks were compared as glycosylation agents to install α-(1→2)-mannose linkages using continuous-flow microreactors (*Scheme 1*)[1]).

[1]) Synthesis of the building blocks was performed according to the literature [26][30–32].

Scheme 1. *Selective Installation of Mannose α-(1→2)-Linkages Using the Building Blocks* **1**, **2**, *and* **3**



Trichloroacetimidate (TCA) and phosphate building blocks, **2** and **3**, respectively, are well established glycosylating agents that exhibit highly different reactivities, and require different amounts of activator (trimethylsilyl trifluoromethanesulfonate (TMSOTf))[2]. The acetate group at C(2) ensures the selective formation of the α-glycosidic linkage. The synthesis of oligosaccharides in a microreactor using TCA building blocks has already been described [33]; this is the first description of glycosylation reactions in microreactors incorporating glycosyl phosphates.

Reactions were carried out in a silicon-based microreactor (*Fig. 1*). The oxidized silicon and borosilicate interior surface is similar to that of glass flasks. This microreactor can be operated in a wide temperature range and is compatible with a broad range of organic solvents and reagents. The excellent thermal conductivity of silicon ensures precise control of the reaction temperature. An internal volume of 78.3 µl allows for a micro-scaled screening of reaction conditions, as well as the production of several hundreds of milligrams of desired product per day [33][34].

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[2]) Compound **2** requires 0.2–0.4 equiv. TMSOTf, **3** requires stoichiometric amounts of activator.

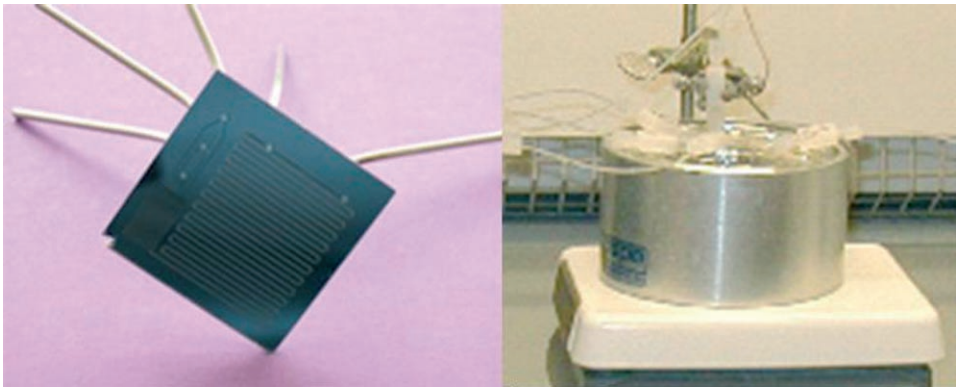

Fig. 1. *The silicon-based microreactor, laboratory setup*

Well-defined solutions of glycosylation agents **2** and **3**, respectively, as well as building block **1** and TMSOTf were introduced at the reactor inlets (*Fig. 2*). After efficient mixing ('mixing loop') and the reaction in the 'reaction loop', Et_3N and an internal reference[3]) for LC/MS analysis were added to quench the reaction.

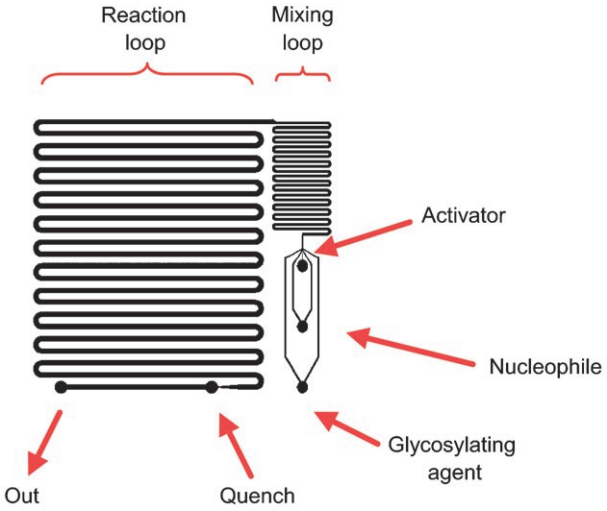

Fig. 2. *Scheme of the microreactor presenting three primary inlets, a secondary port, and the outlet*

Reaction conditions varying in flow rate, solvent, reagent quantities, and temperature were screened (*Table 1*).

Each glycosylation agent was tested at temperatures from $-78°$ to $+25°$ in $10°$ intervals, and, at each temperature, four different reaction times (0.99, 1.96, 3.94, and 7.88 min) were investigated. A 50-µl aliquot of the reaction mixture was collected for each run and diluted with MeCN prior to further analysis by LC/MS.

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[3]) As internal standard, the UV-active 1-methyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside was used.

Table 1. *Rapidly Screened Reaction Conditions*

Entry	Nucleophile **1** [equiv.]	Building block ([equiv.])	TMSOTf [equiv.]	Solvent
1	1.0	**2** (1.2)	0.2	CH_2Cl_2
2	1.0	**2** (1.3)	0.217	CH_2Cl_2
3	1.0	**2** (1.4)	0.233	CH_2Cl_2
4	1.0	**2** (1.2)	0.2	toluene
5	1.0	**2** (1.3)	0.217	toluene
6	1.0	**2** (1.4)	0.233	toluene
7	1.0	**3** (1.2)	1.2	CH_2Cl_2
8	1.0	**3** (1.3)	1.3	CH_2Cl_2
9	1.0	**3** (1.4)	1.4	CH_2Cl_2
10	1.0	**3** (1.2)	1.2	toluene
11	1.0	**3** (1.3)	1.3	toluene
12	1.0	**3** (1.4)	1.4	toluene

The desired α-(1 $\rightarrow$ 2) linked disaccharide **4** was always the major product, although product distribution was highly dependent on reaction temperature and time. β-Disaccharide **5** was formed in very small quantities, even in the presence of a participating group at C(2). The formation of orthoester **6** was the main side reaction, even at higher temperatures ($> -40^\circ$) when orthoesters are believed to be unstable under acidic conditions. Optimized reaction conditions for each glycosylation agent and solvent were determined (*Table 2* and *Fig. 3*).

Table 2. *Optimal Reaction Conditions Found by Microreactor Screening*

Entry	Building block ([equiv.])	Solvent	T [°C]	Reaction time [min]
1	**2** (1.3)	CH_2Cl_2	-20	3.94
2	**2** (1.3)	toluene	-30	7.88
3	**3** (1.3)	CH_2Cl_2	$+10$	1.96
4	**3** (1.3)	toluene	-10	1.96

Glycosyl trichloroacetimidate **2** was most reactive at low temperatures in both solvent systems (-30° in toluene, -20° in CH_2Cl_2), and at reaction times of 3.94 and 7.88 min, respectively (*Table 2*, *Entries 1* and *2*). In CH_2Cl_2, the conversion of **2** was optimal and most selective at -20°. The formation of side products **5** and **6** was also temperature-dependent, delivering most **5** at high and low temperatures, and most orthoester **6** at -30° (*Fig. 3*,*a*). In toluene, the temperature dependence was striking: below -30°, almost no conversion was observed, whereas at -30° conversion was optimal. Remarkably, only traces of **5** and **6** were formed (*Fig. 3*,*b*).

Glycosyl phosphate **3** reacted faster (1.96 min) and at higher temperatures ($+10^\circ$ in CH_2Cl_2, -10° in toluene; *Table 2*, *Entries 3* and *4*) than the glycosyl trichloroacetimidate **2**. The formation of orthoester **6** was temperature-dependent, and **6** was formed even at $+25^\circ$ under acidic conditions with equimolar use of TMSOTf. β-Disaccharide **5** was formed independent of temperature (*Fig. 3*,*c*). In toluene, the glycosylation again occurred more abrupt at -10° where only traces of **5** and **6** were detected (*Fig. 3*,*d*).

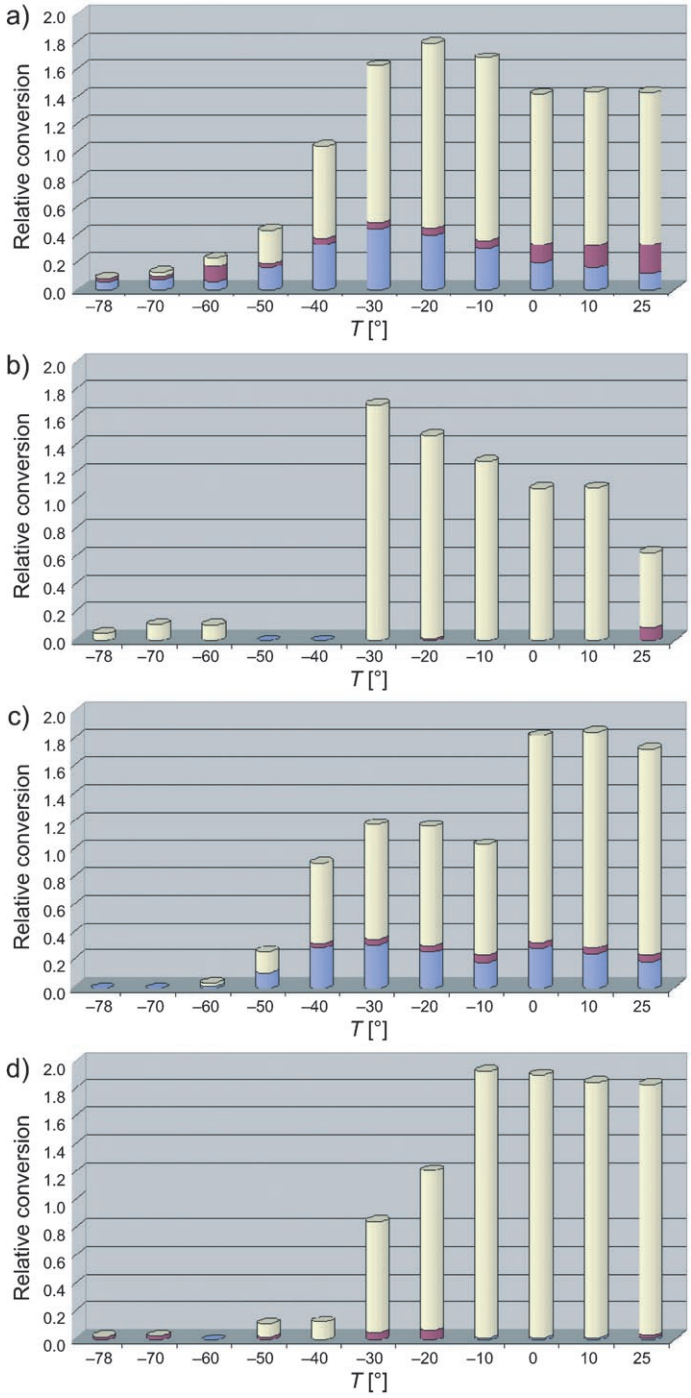
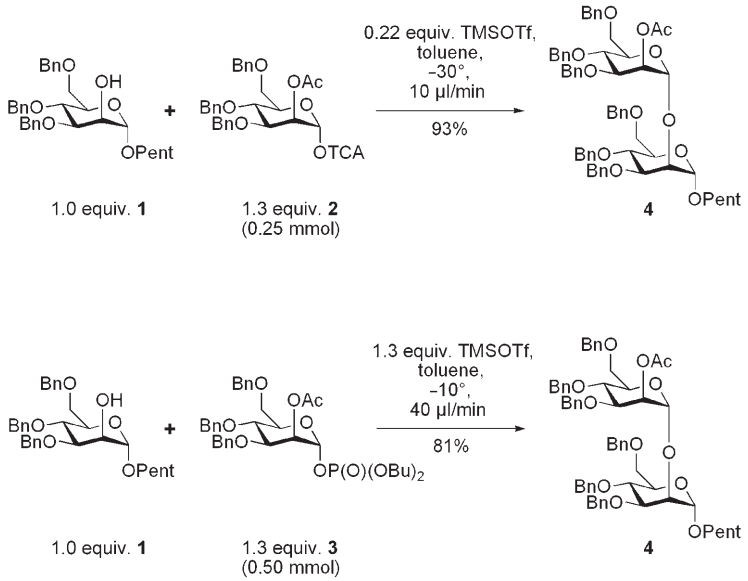


Fig. 3. *Product distribution for the optimized reaction conditions found by LC/MS analysis. a*) Building block **2** in CH_2Cl_2 (*Entry 1*, *Table 2*). *b*) Building block **2** in toluene (*Entry 2*, *Table 2*). *c*) Building block **3** in CH_2Cl_2 (*Entry 3*, *Table 2*). *d*) Building block **3** in toluene (*Entry 4*, *Table 2*). Yellow: α-disaccharide **4**, *red: β*-disaccharide **5**, *blue:* orthoester **6**.

α-Disaccharide **4** was formed faster, more selectively, and with greater conversion in toluene than in CH_2Cl_2. Glycosyl phosphate **3** furnished disaccharide **4** in higher yield and in shorter reaction times than glycosyl trichloroacetimidate **2**. The solvent-dependent formation of orthoester **6** may be explained by better stabilization of the cationic orthoester intermediate in more polar solvents.

The optimized reaction parameters for the α-selective mannosylation of **1** were applied to a larger-scale continuous-flow synthesis of disaccharide **4**: trichloroacetimidate **2** (0.25 mmol) and glycosyl phosphate **3** (0.50 mmol) yielded disaccharide **4** after column chromatography in 93 and 81% yield, respectively (*Scheme 2*).

Scheme 2. *Large-Scale Synthesis of α-Disaccharide* **4** *by Scale-Out*



Having established the applicability of the silicon-based microreactor for the synthesis of α-disaccharide **4** even at a larger scale, the optimized reaction parameters were transferred back to batch processes. The optimized reaction conditions afforded disaccharide **4** in 98% yield from **2** (0.50 mmol) and in 91% yield from **3** (0.5 mmol).

Conclusions. – The temperature, reaction time, and solvent dependencies of glycosylation reactions render them difficult to optimize using traditional experimental methods. Microreactors were utilized to compare different glycosylation reagents under a host of conditions. The best reaction conditions were readily 'scaled-out' to produce gram quantities of product. Importantly, the reaction conditions determined by microreactors were readily transferred back to the batch mode and gave identical results.

Experimental Part

General. All commercial materials were used without further purifications, unless otherwise noted. CH_2Cl_2, THF, and toluene were purified by a *J. C. Meyer Solvent Dispensing System* (two packed columns of neutral alumina, or, in the case of toluene, one packed column of alumina, followed by one packed column of *Q5* reactant, *i.e.*, a copper oxide oxygen scavenger). Et_3N was freshly distilled over CaH_2 under N_2 before use. All reactions were performed in oven-dried glassware under an inert atmosphere (N_2 or Ar) unless noted otherwise. Solvents for chromatography and workup procedures were distilled from commercially available technical-grade solvents. Building blocks **1**–**3** were synthesized according to the literature [26][30–32].

Anal. TLC was performed on *E. Merck* silica gel *60 F_{254}* plates (0.25 mm). Compounds were visualized by UV (λ 254 nm) and/or by dipping the plates in a cerium sulfate–ammonium molybdate (CAM) solution or phosphomolybdic acid (PMA) solution, followed by heating. Liquid column chromatography was performed using forced flow of the indicated solvent on *Fluka* silica gel (230–400 mesh). Optical rotations $[\alpha]_D^{rt}$ were measured on a *Perkin-Elmer 241* polarimeter (10 cm, 1-ml cell). The solvents and concentrations (in g/100 ml) are indicated. IR Spectra were measured in $CHCl_3$ on a *Perkin-Elmer 782* spectrophotometer. ^{1}H-NMR Spectra were obtained with a *Varian Mercury XL 300* spectrometer (300 MHz) and are reported in δ relative to $CHCl_3$ (7.16 ppm) as an internal reference. Coupling constants (J) are reported in Hz. ^{13}C-NMR spectra were recorded with a *Varian Mercury XL 300* spectrometer (75 MHz) and are reported in δ relative to $CDCl_3$ (77.23 ppm) as an internal reference. LC/MS Spectra were obtained with a *Agilent 1100 LC MSD* high-performance liquid chromatograph with a *Waters Symmetry® C18* column (3.9 × 150 nm, 5 μm), and a gradient of MeCN/i-PrOH and H_2O/i-PrOH as the mobile phase (flow rate 1 ml · min^{-1}). The spectra were detected at 208 and 210 nm. Prep. HPLC was performed with a *Waters* HPLC apparatus with a *Waters SunFire™ Prep C8* column (10 × 150 mm, 5 μm), and a gradient of MeCN/i-PrOH and H_2O/i-PrOH as the mobile phase (flow rate 6.60 ml · min^{-1}). The spectra were detected at a wavelength of 208 and 210 nm. High-resolution mass spectra (HR-MS) analyses were performed by the MS service at the Laboratory for Organic Chemistry at ETH-Zürich. MALDI-MS were obtained on an *IonSpec Ultra* instrument, 2,5-dihydroxybenzoic acid (DHB) served as the matrix.

General Procedure for Screening of Reaction Conditions. Before introducing the reagents, the microreactor was rinsed with 20–50 reactor volumes of each anh. THF and the anh. solvent used in the reaction. Reagent solns. were prepared by azeotroping the building blocks separately with toluene and drying overnight under high vacuum. The reagents were diluted with anh. solvent to the desired concentrations (25 mM for **1**). The gas-tight syringes were flushed with anh. solvents before loading with the reagents. Inlet 1 was used for the glycosylating agent (2.5-ml syringe), inlet 2 for the nucleophile (2.5-ml syringe), inlet 3 for the activator (0.5-ml syringe), and inlet 4 for anh. Et_3N (1.0-ml syringe) in order to quench the reaction, also containing 1-methyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside as UV-active compound for quantification by LC/MS. After loading, the syringes were connected to the device, and the microreactor was set to the desired temp. by either an ice bath or an acetone/dry-ice bath. Two reactor volumes (160 μl) were delivered at the desired flow rate to flush the device, followed by the collection of 50 μl of reaction mixture diluted with 800 μl of MeCN for analysis. While maintaining the temp., four flow rates were screened (80, 40, 20, and 10 μl · min^{-1}), and a temp. range of $-78°$ to $+25°$ in 10° intervals was investigated. The optimized reaction conditions were applied for large-scale synthesis using continuous-flow.

General Procedure for Large-Scale Synthesis Using the Silicon-Based Microreactor. Microreactor and gastight syringes were prepared according to the screening procedure. The building blocks were co-evaporated with toluene and dried under high vacuum overnight. Both reactants were dissolved in toluene to give solns. with defined concentrations (25-mM solns. for **1**). Trimethylsilyl trifluoromethanesulfonate (TMSOTf) was dissolved in toluene to the corresponding defined soln., Et_3N was diluted with toluene. After flushing the microreactor and the syringes, the gas-tight glass syringes were filled with the corresponding reagent solns. and connected to the microreactor system. The reaction was carried out at the optimal reaction temp. and reaction time until consumption of the stock solns. After concentration, the reaction mixture was purified by flash silica-gel column chromatography (FC; hexanes/AcOEt 4 : 1).

*Pent-4-enyl (2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl)-(1 → 2)-3,4,6-tri-*O*-benzyl-α-*D*-mannopyranoside* (**4**). General procedure with building blocks **3** (343 mg, 0.5 mmol, 1.3 equiv., 32.5-mM soln.), **1** (200 mg, 0.39 mmol, 25-mM soln.), TMSOTf (90.6 µl, 0.5 mmol, 1.3 equiv., 163-mM soln.), and Et_3N (3 ml) in toluene at $-10°$ and 1.96 min reaction time gave **4** in 80%. Characterization data were consistent with those in [24].

*Pent-4-enyl (2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl)-(1 → 2)-3,4,6-tri-*O*-benzyl-α-*D*-mannopyranoside* (**4**). General procedure with building blocks **2** (160 mg, 0.25 mmol, 1.3 equiv., 32.5-mM soln.), **1** (100 mg, 0.19 mmol, 25-mM soln.), TMSOTf (7.6 µl, 0.04 mmol, 0.22 equiv., 30-mM soln.), and Et_3N (3 ml) in toluene at $-30°$ and 7.88 min reaction time gave **4** in 93%. Characterization data were consistent with those in [24].

Batch Process Large-Scale synthesis of Disaccharide **4** *Using the Improved Reaction Conditions Found by Microreactor Screening.* Building blocks **1** (200 mg, 0.39 mmol) and **3** (343 mg, 0.5 mmol, 1.3 equiv.) were azeotroped with toluene and dried under high vacuum overnight. The reagents were dissolved in toluene to afford the corresponding concentrated solns. (25 mM for **1**, 32.5 mM for **3**). The solns. were mixed at $-10°$ and under vigorous stirring a soln. of TMSOTf (90.6 µl, 0.5 mmol, 1.3 equiv., 163-mM soln.) in toluene was quickly added. The reaction was quenched after 1.96 min by addition of Et_3N (2 ml). FC (silica gel; hexanes/AcOEt 4 : 1) yielded **4** in 91%. Characterization data were consistent with those in [24].

Building blocks **1** (200 mg, 0.39 mmol) and **2** (319 mg, 0.5 mmol, 1.3 equiv.) were azeotroped with toluene and dried on high vacuum overnight. The reagents were dissolved in toluene to afford the corresponding concentrated solns. (25 mM for **1**, 32.5 mM for **2**). The solns. were mixed at $-30°$ and under vigorous stirring a soln. of TMSOTf (15.2 µl, 0.08 mmol, 0.22 equiv., 30-mM soln.) in toluene was quickly added. The reaction was quenched after 7.88 min by addition of Et_3N (2 ml). FC (silica gel; hexanes/AcOEt 4 : 1) yielded **4** in 98%. Characterization data were consistent with those in [24].

Synthesis of **5** *and* **6** *in the Microreactor. Pent-4-enyl (2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-*D*-mannopyranosyl)-(1 → 2)-3,4,6-tri-*O*-benzyl-α-*D*-mannopyranoside* (**5**). General procedure with building blocks **2** (166 mg, 260 mmol, 1.3 equiv., 32.5-mM soln.), **1** (104 mg, 200 mmol, 25-mM soln.), TMSOTf (9.8 µl, 43.4 mmol, 0.22 equiv., 27-mM soln.), and Et_3N (2 ml) in CH_2Cl_2 at $-40°$ and 7.88 min reaction time gave **5** in 4%. R_f (hexanes/AcOEt 2 : 1) 0.65. $[\alpha]_D^{20} = -4.12$ ($c = 0.34$, $CHCl_3$). IR ($CHCl_3$) 3682, 3515, 3007, 29609, 1710, 1602, 1416, 1363, 1248, 1090, 1010, 812, 600. ^{1}H-NMR ($CDCl_3$): 7.41 – 7.08 (*m*, 30 H); 5.74 (*ddt*, $J = 16.5, 11.4, 6.6$, 1 H); 5.61 (*dd*, $J = 3.6, 2.1$, 1 H); 5.23 (*d*, $J = 1.8$, 1 H); 5.01 – 4.86 (*m*, 2 H); 4.86 – 4.80 (*m*, 2 H); 4.77 – 4.65 (*m*, 7 H); 4.49 – 4.37 (*m*, 4 H); 4.34 (br. *s*, 1 H); 4.21 (*d*, $J = 2.4$, 1 H); 4.14 – 4.06 (*m*, 1 H); 3.99 – 3.50 (*m*, 8 H); 3.46 – 3.34 (*m*, 2 H); 2.10 (*s*, 3 H); 2.14 – 2.02 (*m*, 2 H); 1.69 – 1.56 (*m*, 2 H). ^{13}C-NMR ($CDCl_3$): 170.2; 138.8; 138.3; 138.0; 138.0; 137.8; 128.2; 128.2; 128.1; 128.1; 128.0; 127.7; 127.6; 127.4; 127.1; 127.0; 114.6; 99.8; 98.4; 82.4; 78.4; 77.1; 75.6; 75.1; 74.6; 74.1; 73.4; 71.9; 71.6; 70.9; 69.3; 69.1; 68.7; 30.1; 28.6. HR-MALDI-MS: 1015.4622 ($[M + Na]^+$, $C_{61}H_{68}NaO_{12}^+$; calc. 1015.4603).

*3,4,6-Tri-*O*-benzyl-1,2-[3,4,6-tri-*O*-benzyl-1-*O*-(pent-4-enyl)-α-*D*-mannopyranoside-2-yl]ethylidene-α-*D*-mannopyranoside* (**6**). General procedure with building blocks **2** (166 mg, 260 mmol, 1.3 equiv., 32.5-mM soln.), **1** (104 mg, 200 mmol, 25-mM soln.), TMSOTf (9.8 µl, 43.4 mmol, 0.22 equiv., 27-mM soln.), and Et_3N (2 ml) in CH_2Cl_2 at $-40°$ and 7.88 min reaction time gave **6** in 10%. R_f (hexanes/AcOEt 2 : 1) 0.70. $[\alpha]_D^{20} = +30.10$ ($c = 0.21$, $CHCl_3$). IR ($CHCl_3$): 3690, 3600, 3066, 3040.1, 2924, 2856, 1602, 1496, 1453, 1363, 1261, 1099, 1012, 896, 818. ^{1}H-NMR ($CDCl_3$): 7.45 – 7.20 (*m*, 30 H); 5.76 (*ddt*, $J = 17.1$, 10.2, 6.6, 1 H); 5.40 (*d*, $J = 2.6$, 1 H); 5.05 – 4.71 (*m*, 6 H); 4.70 – 4.42 (*m*, 10 H); 4.09 (br. *s*, 1 H); 3.93 – 3.57 (*m*, 10 H); 3.45 (*dt*, $J = 9.0, 3.2$, 1 H); 3.34 (*dt*, $J = 9.6, 6.6$, 1 H); 2.05 (*dt*, $J = 7.3, 7.3$, 2 H); 1.81 (*s*, 3 H); 1.63 (*tt*, $J = 7.4, 7.4$, 2 H). ^{13}C-NMR ($CDCl_3$): 138.4; 138.4; 138.4; 138.1; 138.0; 137.9; 137.5; 128.3; 128.3; 128.2; 128.2; 127.9; 127.9; 127.8; 127.8; 127.7; 127.7; 127.6; 127.5; 127.5; 127.4; 127.3; 124.5; 114.8; 99.3; 97.5; 78.4; 77.1; 76.7; 76.2; 75.5; 74.5; 74.3; 74.0; 73.2; 73.2; 73.2; 71.7; 71.6; 71.4; 69.4; 69.3; 69.0; 68.9; 66.9; 30.2; 29.6; 28.7; 25.2. HR-MALDI-MS: 1015.4592 ($[M + Na]^+$, $C_{61}H_{68}NaO_{12}^+$; calc. 1015.4603).

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