Repetitive Solid Phase Glycosylation on an Alkyl Thiol Polymer Leading to Sugar Oligomers Containing 1,2-*trans*- and 1,2-*cis*-Glycosidic Linkages†

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Repetitive solid phase glycosylation with sugar trichloroacetimidates is performed on an alkyl thiol polymer, demonstrating the selective formation of glycosidic bonds. The α-mannose and α-fucose linkages representing examples of 1,2-*trans*- and 1,2-*cis*-glycosides are synthesized. It is shown that the presented system can be operated in a cyclic manner, thus allowing many consecutive reaction steps to be performed on the solid support. Reaction conditions for solid phase glycosylation and deprotection are described. Analysis of all solid phase reactions is performed by MALDI-TOF mass spectrometry; the yield of solid phase glycosylation is quantitative up to the tetrameric stage judged from the mass spectra obtained from partial product cleavage after each reaction. Cleavage conditions for analysis and preparative isolation by thiophilic Lewis acids and oxidative methods are optimized.

Introduction

The chemical synthesis of oligosaccharides has seen years of dynamic progress, mainly spurred not only by the development of highly reactive sugar donors and advanced protective-group chemistry during the 1980s[1] but also motivated by the eminent role carbohydrate conjugates and oligomers play in different fields of modern biology.[2] Despite these achievements, the synthesis of larger and more complex sugars in solution remains a demanding task.[3] A well-established method for the preparation of sugar oligomers on a solid phase might be superior to the solution techniques with respect to efficiency, applications in combinatorial synthesis,[4] and future automatization. Considerable effort has been spent on the investigation of solid phase glycosylations during the last decades.[5−7] But nevertheless, a generally accepted method has not emerged so far. Perhaps the most limiting difficulty in solid phase synthesis has been the lack of powerful analytical techniques that permit the monitoring of each synthetic step with precision and in reasonable time. Furthermore it is necessary to establish a synthetic protocol that enables repetitive glycosylations and deprotection reactions in high yields. Until now a steep decrease of glycosylation yields was reported for a repeated protocol.[7]

Recently, we introduced a novel strategy for solid phase glycosylation.[8] In this an alkyl thiol linker is coupled to the polymer in a presynthesis step. This procedure yields a generally applicable resin; the first sugar residue can be attached in a standard glycosylation procedure. The linker is easily prepared, resistant to the conditions of glycosylation and deprotection, and rapidly cleavable under variable reaction conditions in high yield. The latter feature permits the monitoring of all solid phase reactions: the supernatant of the cleavage from a small resin sample can be analyzed for the reaction products after only a few minutes either by chromatographic methods or by MALDI-TOF mass spectrometry. Catalytically activated trichloroacetimidates are employed as sugar donors. This concept has so far been employed for the synthesis of anomeric mixtures of glucose (1→6) oligomers up to the pentameric stage. We now wish to report the extension of this method to the stereoselective glycosylation of the less nucleophilic, axial 2-hydroxy group of D-mannose, resulting in the synthesis of α-(1→2)-oligomannosides and of fucosyl-α-(1→2)-mannoside. The target molecules are well-known constituents of N-glycans;[9] they are typical examples of 1,2-*cis* and 1,2-*trans*-glycosidic linkages.

Results and Discussion

For kinetic reasons we chose resins that allow high loadings of 0.1−0.6 mmol/g. Resin functionalization was

† Dedicated to Prof. Peter Welzel on the occasion of his 60th birthday.

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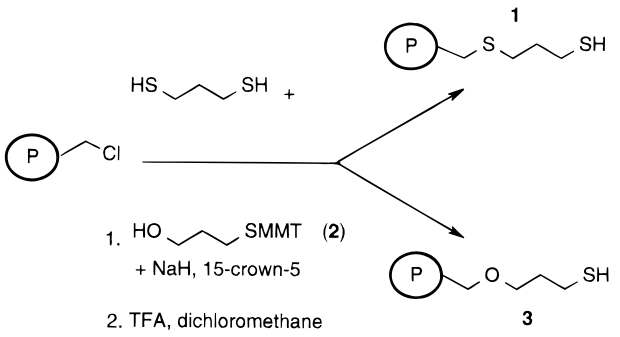
Scheme 1



achieved by S- and O-alkylation with chloromethylated polystyrene/divinylbenzene copolymer,[10] employing 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as base for S-ether formation[11] and sodium hydride in combination with a crown ether (15-crown-5) for O-ether formation (Scheme 1). In the first case, a resin (**1**) with high loading (0.4−0.6 mmol/g) resulted, whereas the O-ether linker **2** yielded only loadings between 0.1 and 0.3 mmol/g (resin **3**). Loadings varied in both cases by changing the excess of reagents and the reaction times. Monomethoxytrityl cleavage from the resin bound linker, generating **3**, can be used as an additional method for the determination of thiol functionalization.

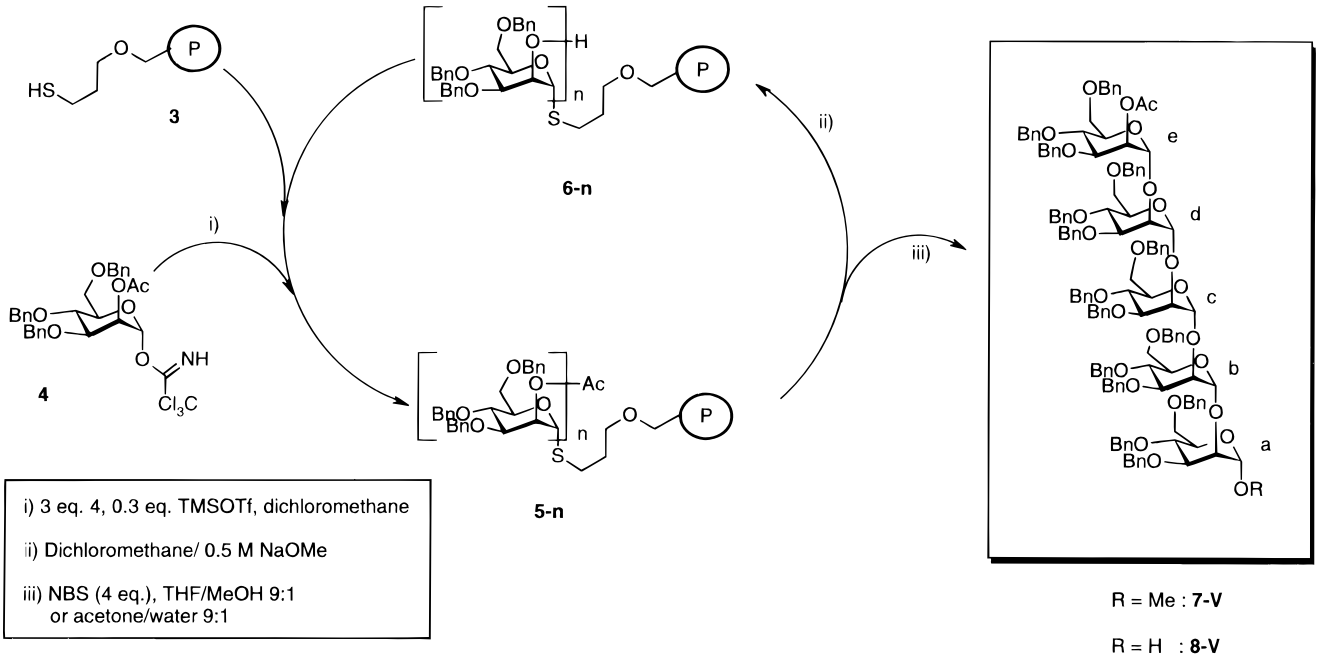
The target oligomannosides were synthesized with the trichloroacetimidate **4** as donor; **4** has already been used in a study on the solution synthesis of oligomannosides.[12,13] Due to neighboring group participation, the 2-*O*-acetyl group will lead to α-product formation; additionally, the *O*-acetyl group provides the required temporary protection for the desired chain extension. The thiol-functionalized resins **1** and **3** were employed in solid phase glycosylation and deprotection steps in a cyclic manner (Scheme 2). For the glycosylations, yielding the fully protected resin bound oligosaccharides **5-*n*** (n = I, II, III, IV, V, VI), trimethylsilyl trifluoromethanesulfonate (TMSOTf) was used as catalyst (0.3 equiv); orthoester formation was not observed under these conditions. The deacetylation steps yielding **6-*n*** were carried out in a 10:1 mixture of CH_2Cl_2 with 0.5 M sodium methoxide in MeOH to avoid resin collapse.

Several conditions were tested for cleavage of the products. The cleavage procedure had to be optimized to meet two objectives: to enable analysis and to allow preparative isolation. For the first demand, a resin sample of 1−2 mg was treated with 50 μL of the cleavage solution. After 5−15 min, depending on the cleavage reagent, the reaction products **7-*n*** or **8-*n*** were analyzed directly from the supernatant either by chromatographic methods like TLC and HPLC or by MALDI-TOF mass spectrometry. For the analytical cleavage, yielding **7-*n***, silver ions without base buffering were suitable. More rapid analysis was achieved with [(dimethylmethyl)thio]-sulfonium salts (the triflate DMTST or the more convenient and commercially available tetrafluoroborate DMTSB). However, in the case of larger oligomers, cleavage with DMTST can lead to partial degradation of the products. For analytical cleavage, the presence of a buffer or succinimide in the solution had to be avoided as the products became undetectable by mass spectrometry in this case. With these methods there is a tool available to analyze solid phase reactions in detail.

Crucial for the successful repetition of the synthetic cycle (glycosylation followed by a deprotection step) was the optimization of the reaction and washing protocol. All solid phase reactions were conducted in glass tubes closed with Teflon stoppers that carried a glass filter inlet. During reactions, the reaction vessels were placed horizontally and kept under vigorous shaking. Removal of the deacetylation reagent was accomplished most effectively by THF containing traces of acetic acid and 15-crown-5 as complexing agent.

All glycosylation reactions were carried out in nearly quantitative yield as judged by the disappearance of the preceding oligomer in the mass spectrum. By employing the described analytic procedures it was possible to accomplish complete glycosylation before proceeding with the next synthetic cycle. Best results were obtained with

Scheme 2

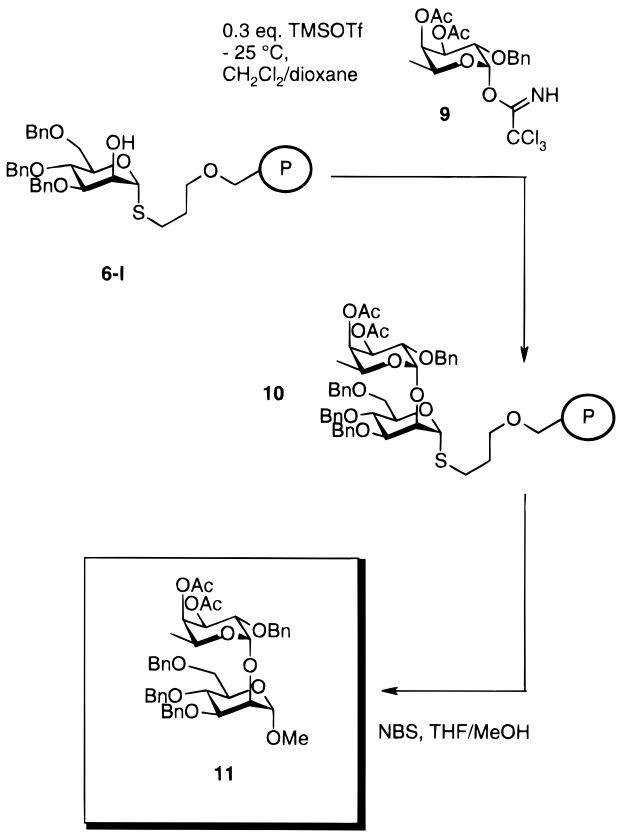


polymer **3** functionalized with 0.15−0.3 mmol/g; with this resin complete reaction was realized up to the tetrameric stage.

For preparative isolation of the products, a protocol is required that enables complete reaction in a reasonable reaction time without destroying the oligomeric products e.g. by glycosidic cleavage. This could be achieved in acetone/water (9:1) by oxidative cleavage with NBS buffered with 1,6-di-*tert*-butylpyridine (DTBP) as sterically hindered base, yielding 75% of product **8-II** after two synthesis cycles; this is an excellent overall yield for a four-step procedure. Yields were lower when using other oxidants like *m*-chloroperbenzoic acid (MCPBA) and iodonium ion reagents or Lewis acids like silver ions and DMTST. The cleavage with NBS generating sugar adehyde was high-yielding but it was not suited for NMR studies in order to determine the stereoselectivity of the solid phase glycosylation. For this purpose, cleavage was conducted with NBS in the presence of DTBP in THF/MeOH leading to the methyl glycoside products **7-*n***. The trimeric, tetrameric, pentameric, and hexameric oligomannosides (**7-III**, **7-IV**, **7-V**, and **7-VI**) were synthesized, isolated, and identified by mass specrometry. To determine the stereoselectivity of the solid phase glycosylation the cleavage products were isolated by reversed phase chromatography. This method separates only according to the oligomer size. NMR spectra of the isolated tri- and tetrasaccharide methyl glycosides showed only the per-α-product. Trisaccharide **7-III** and tetrasaccharide **7-IV** were synthesized and isolated after HPLC-purification with an overall yield of 54% (**7-III**) and 34% (**7-IV**), respectively.

It is a general experience of carbohydrate synthesis that stereoselective preparation of 1,2-*cis*-glycosides is more demanding than that of 1,2-*trans*-glycosides. Whereas formation of the 1,2-*trans*-glycosides is strongly favored by neighboring group participation (generation of intermediate acetoxonium ions), the formation of 1,2-*cis*-glycosides relies often on the anomeric effect. The anomeric effect can be exploited most effectively with diethyl ether as solvent.[1c] Unfortunately, acyclic ethers are useless in this case because they do not swell polystyrene resins. We found a solution to this problem in the use of 1,4-dioxane as cosolvent (Scheme 3). Donor **9**[14] gave at −25 °C with the monosaccharide resin **6-I** the polymer-bound disaccharide **10** which was cleaved to methyl glycoside **11** under the conditions described above. Disaccharide **11** was isolated in a total yield of 54%, and no β-anomeric product was detected.

Scheme 3



Conclusions

In summary, we have demonstrated the repetitive solid phase glycosylation of the axial 2-hydroxy group of mannose which is to our knowledge the least nucleophilic acceptor so far employed in solid phase glycosylations. All solid phase glycosylations gave nearly quantitative yields. Product cleavage and isolation were conducted in acceptable yields and furnished sugar oligomers in a stereoselective manner.

Experimental Section

Flash chromatography is performed over silica 40 (Baker), solvents are redistilled, and petroleum ether is used in the boiling range of 35−65 °C. Preparative HPLC is conducted over Eurospher 100 Si, 7 μm.

Resin Functionalizations: Divinylbenzene−polystyrene Copolymer Functionalized with Propane-1,3-dithiol (1) Chloromethylated 1% divinylbenzene−polystyrene copolymer (mesh 200−400) (1 g, 1 mmol) of Rapp Polymere GmbH, Tübingen, Germany, is swollen in toluene (10 mL). Propane-1,3-dithiol (1 mL, 10 mmol) is added and the solution mixed through gentle shaking. After 15 min 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU) (0.45 mL, 3 mmol) is added dropwise and with occasional shaking the reaction mixture is kept at room temperature for 24 h. The resin is filtered over porous glass and washed several times switching between CH_2Cl_2 and DMF. Afterward it is dried *in vacuo* for 12 h at 90 °C. The degree of functionalization is determined by elemental analysis with 0.5 mmol/g. When the same reaction was carried out for only 2 h the degree of functionalization dropped to 0.3 mmol/g. IR: SH stretch at 2565 cm^{-1}.

***S*-(Monomethoxytrityl)-1-mercaptopropan-3-ol (2).** (Monomethoxytriphenyl)methyl chloride (6.17 g, 20 mmol) is dissolved in pyridine (40 mL). At 10 °C methyl 3-mercaptopropionate (2.16 g, 18 mmol) is added and the mixture stirred at room temperature for 14 h. Then the reaction mixture is diluted with diethyl ether (150 mL) and washed with water (20 mL). After treatment with magnesium sulfate the organic layer is concentrated *in vacuo* and coevaporated several times with toluene. The raw product obtained by this procedure is dissolved in THF (40 mL). At 0 °C lithium aluminum hydride (820 mg, 21.6 mmol) is added in small portions. After 2 h the reaction is complete (TLC, toluene/EtOAc 5:1). For quenching, the solution is diluted with EtOAc (10 mL), after 15 min with diethyl ether (100 mL), and then with water (80 mL). After stirring for another 60 min the suspension is filtered over

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Kieselguhr silica and washed several times with diethyl ether. After desiccation the organic phase is concentrated and compound **2** (5.6 g, 86%) is obtained after double crystallization from (petroleum ether/EtOAc) or short column separation. ^{1}H-NMR (250 MHz, $CDCl_3$): δ = 1.25 (bs, 1 H), 1.64 (tt, ^3J = 6.6 Hz, 2 H), 2.28 (t, ^3J = 7.2 Hz, 2 H), 3.58 (dt, J = 5.7 Hz, J = 6.6 Hz, 2 H), 3.79 (s, 3 H), 6.7−7.4 (m, 14 H). Anal. Calcd for $C_{23}H_{24}O_2S$ (M = 364.51): C, 75.79; H, 6.64. Found: C, 75.70; H, 6.66.

Divinylbenzene−polystyrene Copolymer Functionalized with 1-Mercaptopropan-3-ol (3). Chloromethylated 1% divinylbenzene−polystyrene copolymer (1 g) is swollen in THF (10 mL). *S*-(Monomethoxytrityl)-1-thiopropan-3-ol (**2**) (760 mg, 3.84 mmol), 15-crown-5 (0.76 mL, 3.84 mmol), and sodium hydride (92 mg, 3.84 mmol) are added and mixed through gentle shaking. The reaction is heated for 24 h at 60 °C. The resin is filtered over porous glass and washed several times with switching between CH_2Cl_2 and DMF. Then it is washed with a 5% solution of trifluoroacetic acid in CH_2Cl_2 until the solution is colorless. Afterward it is again washed with DMF and CH_2Cl_2 and dried *in vacuo* for 12 h at 90 °C. The degree of functionalization is determined by elemental analysis with 0.3 mmol/g. When the same reaction was carried out with only 2 equiv of alcohol **2**, a functionalization of 0.15 mmol/g resulted. In addition the OD of the trityl cleavage solution is measured and employed to calculate the degree of functionalization with the specific absorption of ϵ = 20 850 M^{-1} cm^{-1} at 478 nm. IR: SH stretch at 2565 cm^{-1}.

Protocol for Solid Phase Glycosylations: Glycosylated, Fully Protected Resin (5-*n*). Resin **1**, **3** (200 mg), or, alternatively, **6-*n*** is placed under dry nitrogen in a glass tube (8 × 80 mm) and sealed tightly with Teflon stoppers. Sugar donor **4** (3 equiv) is dissolved in CH_2Cl_2 (2 mL). This solution is injected in the reaction vessel, which is shaken horizontally for 15 min. Then a freshly prepared 0.5 M solution of TMSOTf in CH_2Cl_2 (0.3 equivs) is added and the stoppered glass tube is shaken horizontally for 1 h. Afterwards it is washed by switching between CH_2Cl_2 and THF several times and dried *in vacuo*. Completion of the glycosylation is monitored with MALDI-TOF mass spectrometry.

Protocol for Solid Phase Deacetylation: Glycosylated, 2-O-Deacetylated Resin (6-*n*). Glycosylated resin (**5-*n***) (0.02 mmol) is placed in the reaction vessel and suspended in CH_2Cl_2 (2.5 mL), and 0.5 M sodium methoxide in MeOH is added (0.25 mL). The glass tube is shaken for 2 h in the horizontal position. Then the resin is filtered off and washed in a batchwise procedure first with 0.05 M solution of 15-crown-5 in THF/acetic acid 20:1, followed by several alternating washing steps with THF and CH_2Cl_2. The resin is dried first with a stream of dry nitrogen and then *in vacuo*. Analysis is performed as described.

Protocol for MALDI-TOF Mass Spectrometry from a Small Resin Sample. A small resin sample (1−2 mg of resin **5-*n*** or **6-*n***) is placed in an Eppendorf cup. A freshly prepared solution (50 μL) of dry silver triflate (4 mg/mL) in CH_2Cl_2/MeOH 10:1 is added. The suspension is mixed by gentle swirling or preferably ultrasound. After 10 min 10 μL of the supernatant is mixed with the same volume of a matrix solution (10 mg/mL of 2,5-dihydroxybenzoic acid in THF). A small portion of this solution is placed on the laser target and directly measured in the positive ion mode.

Protocol of Oligosaccharide Cleavage from Glycosylated Polymer 5-*n*: 1-*O*-Methyl-α-(1→2)-D-mannopyranosyl oligosaccharide (7-*n*). Glycosylated resin **5-*n*** (0.01 mmol) is placed in the solid phase synthesis vessel and swollen in THF/MeOH 10:1 (2.5 mL). After 15 min NBS (9 mg, 4 equiv) and DTBP is added. The glass tube is shaken for 90 min in the horizontal position. Then it is filtered off and the resin is washed repeatedly with THF and CH_2Cl_2. Triethylsilane (10 μl) is added for reduction of unreacted NBS, and the collected filtrates are concentrated *in vacuo*. After coevaporation with toluene the oily residue is subjected to flash chromatography (toluene/EtOAc 6:1). Further purification is carried out following two alternative procedures: Either the sugar product is purified by RP-18 flash chromatography with an acetone/water gradient (see Table 1) or it is subjected to normal phase HPLC employing hexane/EtOAc 3:1 (see the second column of Table 1). Trisaccharide **7-III** is isolated in 52% yield (7 mg); tetrasaccharide **7-IV** is isolated 34% yield (6 mg).

Table 1. Chromatographic Data of Compounds 7-III, 7-IV, 7-V, 7-VI

compd	normal phase HPLC:[a] retention time (min)	RP flash chromatography: acetone/water
7-III	11.2	8:1
7-IV	12.0	10:1
7-V	12.9	12:1
7-VI	14.4	14:1

[a] Hexane/EtOAc 3:1, 10 mL/min.

Analytical data of compound **7-III**: ^{1}H-NMR (600 MHz, $CDCl_3$): δ = 2.10 (s, 3 H), 3.20 (s, 3 H), 3.48−3.65 (m, 3 H), 3.62 (m, 1 H), 3.65−3.75 (m, 3 H), 3.70−3.85 (m, 3 H), 3.78 (dd, 1 H), 3.82 (dd, 1 H), 3.87 (dd, 1 H), 3.91 (dd, J = 8.9 Hz, 1 H), 3.97 (d, J < 1 Hz, 1 H), 3.98 (dd, 1 H), 4.08 (d, J < 1 Hz, 1 H), 4.25−4.80 (m, 18 H), 4.78 (d, J < 1 Hz, 1 H), 5.02 (d, J < 1 Hz, 1 H), 5.17 (d, J < 1 Hz, 1 H), 5.51 (dd, J < 1 Hz, 1 H), 7.12−7.32 (m, 45 H). Calcd: M($C_{84}H_{90}O_{17}$) = 1371.63 *m/z*; $(M + Na)^+$ = 1394.63 *m/z*. Found: MALDI-MS (DHB, THF) = 1395 *m/z*.

Analytical data of compound **7-IV**. ^{1}H-NMR (600 MHz, $CDCl_3$): δ = 2.09 (s, 3 H), 3.40 (s, 3 H), 4.81 (d, J < 1 Hz, 1 H), 5.06 (d, J < 1 Hz, 1 H), 5.18 (d, J < 1 Hz, 1 H), 5.25 (d, J < 1 Hz, 1 H), 5.54 (dd, J < 1 Hz, 1 H), 7.12−7.32 (m, 60 H). Calcd: M($C_{111}H_{118}O_{22}$) = 1804.1 *m/z*; $(M + Na)^+$ = 1827.1 *m/z*. Found: MALDI-MS (DHB, THF) = 1827 *m/z*.

Analytical data of compound **7-V**. Calcd: M($C_{138}H_{146}O_{27}$) = 2236.62 *m/z*; $(M + Na)^+$ = 2259.62 *m/z*. Found: MALDI-MS (DHB, THF) = 2260 *m/z*.

Analytical data of compound **7-VI**. Calcd: M($C_{165}H_{174}O_{32}$) = 2669.13 *m/z*; $(M + Na)^+$ = 2692.13 *m/z*. Found: MALDI-MS (DHB, THF) = 2692 *m/z*.

3,4-Di-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl 1-*O*-Methyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (11). Glycosylated and 2-O-deacetylated resin **6-I** (0.02 mmol) is placed in a 10 mL round bottom flask and suspended in CH_2Cl_2 and dioxane (0.5 mL each). Donor **9** (0.06 mmol) is added and the suspension is stirred slowly at −25 °C. After 10 min a 0.5 M solution of TMSOTf in CH_2Cl_2 (0.3 equivs) is added and the stirring is continued for 1 h. The solution is warmed to room temperature, and then it is washed and dried as described for compound **6-*n***. Cleavage with NBS is conducted as described for **7-*n***, and the product (8.5 mg, 54%) is isolated by flash chromatography (toluene/EtOAc 6:1) followed by HPLC (hexane/EtOAc 3:1). ^{1}H-NMR (600 MHz, $CDCl_3$): δ = 0.85 (d, J = 6.5 Hz, 3 H), 1.98, 2.08 (2 s, 6 H), 3.45 (s, 3 H), 3.4−3.5 (m, 1 H), 3.52 (dd, J = 2.4 Hz, J = 9.4 Hz, 1 H), 3.84 (dd, J = 3.6 Hz, J = 10.7 Hz, 1 H), 3.86 (dd, J = 9.4 Hz, 1 H), 4.27 (dd, J < 1 Hz, J = 2.4 Hz, 1 H), 4.4−4.9 (m, 8 H), 4.54 (m, 1 H), 4.59 (d, J < 1 Hz, J = 2.4 Hz, 1 H), 5.23 (dd, J = 3.2 Hz, J < 1Hz, 1 H), 5.38 (dd, J = 10.7 Hz, J = 3.2 Hz, 1 H), 5.64 (d, J = 3.6 Hz, 1 H). Calcd: M($C_{45}H_{52}O_{12}$) = 784.9 *m/z*; $(M + Na)^+$ = 807.9 *m/z*. Found: MALDI-TOF (DHB/THF) = 808 *m/z*.

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Supporting Information Available: ^{1}H-NMR peak and coupling assignments derived from 2D-NMR-experiments are given for compounds **7-III**, **7-IV**, and **11** (3 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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