Published in final edited form as:

J Org Chem. 2007 March 16; 72(6): 1941–1950.

Synthetic 6-*O*-Methylglucose-containing Polysaccharides (sMGP): Design and Synthesis

Malte Meppen, Yonghui Wang, Hwan-Sung Cheon, and Yoshito Kishi*

Department of Chemistry and Chemical Biology, Harvard University, 12 Oxford Street, Cambridge, Massachusetts 02138

Abstract

With the hope of mimicking the chemical and biological properties of *natural* 6-*O*-methyl-D-glucose-containing polysaccharides (MGP), *synthetic* 6-*O*-methyl-D-glucose-containing polysaccharides (sMGP) were designed and synthesized from α -, β -, and γ -cyclodextrins (CDs). The synthetic route proved to be flexible and general, to furnish a series of sMGPs ranging from 6-mer to 20-mer. A practical and scalable method was discovered selectively to cleave the CD derivatives and furnish the linear precursors to the glycosyl donors and acceptors. The Mukaiyama glycosidation was adopted to couple the glycosyl donors with the glycosyl acceptors. Unlike in the sMMP series, an amount of the Mukaiyama acid required in the sMGP series increased with an increase of substrate size; for large oligomers, more than one equivalent of the acid was required.

Introduction

In the preceding paper,^[1] we outlined the background of this research program. Briefly, *Mycobacterium smegmatis* is known to produce two series of polysaccharides, i.e., 3-*O*-methyl-D-mannose-containing polysaccharides (MMP) and 6-*O*-methyl-D-glucose-containing (lipo)polysaccharides MG(L)P (Figure 1).^[2,3,4] Both MMP and MG(L)P affect profoundly the fatty acid biosynthesis catalyzed by fatty acid synthetase I (FAS I) isolated from *Mycobacterium smegmatis*.^[5] We are interested in gaining mechanistic insights for the intriguing biological role(s) of MMP and MG(L)P. However, we felt that naturally occurring MMP and MG(L)P are not necessarily ideal substrates for our study, as they were isolated as complex mixtures of closely related polysaccharides. For this reason, we chose to use synthetic polysaccharides structurally related to natural MMP and MG(L)P for two major reasons: (1) synthetic polysaccharides should be available as the chemically well-defined and homogeneous materials and (2) synthetic polysaccharides should be structurally tunable for the needs of our investigation. Obviously, the most unique structural feature of natural MMP and MG(L)P is the polymeric form of *O*-methylated mannose and glucose. Thus, we decided to incorporate this structural feature in the synthetic polysaccharides and selected the polymers composed of 3-*O*-methyl-D-mannose and 6-*O*-methyl-D-glucose (Figure 2).

For the past one and a half decades, we have witnessed a remarkable development in chemical synthesis of oligosaccharides. A number of glycosyl donors, including halo sugars, pentenyl glycosides, thioglycosides, isopropenyl glycosides, orthoesters, 1-*O*-acyl sugars, 1-*O*-pentenyl sugars, trichloroacetimidates, glycosyl sulfoxides, glycosyl sulfones, glycosyl thiocyanates, glycosyl dialkylphosphites, glycosyl phosphorodithioates, glycosyl tetramethylphosphorodiamidates, glycals and 1,2-anhydrosugars, seleno glycosides, and glycosyl diazirines, are now known to effect the glycosidation in the stereoselective manner

kishi@chemistry.harvard.edu.

in solution- and/or solid-phase syntheses.^[6] Many of these synthetic methods have successfully been applied for the synthesis of a broad range of complex oligosaccharides.^[7] Related to this work, we should specifically quote the work from the Koto laboratory; they reported the synthesis of glucose-containing linear oligosaccharides having $\alpha(1\rightarrow4)$ and $\alpha(1\rightarrow6)$ linkages.^[8]

With a dramatic advancement in chemical synthesis of oligosaccharides, we recognized several promising options to achieve the synthesis of 6-*O*-methyl-D-glucose-containing polysaccharides (*s*MGP). However, we opted to use the synthetic strategy reported in the preceding paper, primarily because it was proved to be effective for the case of synthetic 3-*O*-methyl-D-mannose-containing polysaccharides (*s*MMP).^[1]

Results and Discussion

In the preceding paper, we reported a convergent synthesis of *s*MMP^[1] with use of the modified Mukaiyama glycosidation.^[9] This glycosidation well suited for a highly convergent oligosaccharide synthesis, particularly because of good chemical yields even when using equal-sized donors and acceptors in a molar ratio of *ca.* 1:1. The exceptionally high α/β -selectivity (>50:1 ~ >20:1) observed in the *s*MMP series was due to the result of the selective β -to- α anomerization under the Mukaiyama glycosidation conditions. Unfortunately, this beneficial anomerization was not observed in the *s*MGP series.^[10]

With this knowledge, we reevaluated the synthetic plan for *s*MGP. As the preliminary studies suggested that the Mukaiyama glycosidation^[9] best suit for a convergent synthesis of the *s*MMP/*s*MGP class of polysaccharides, our efforts were focused on identification of the best combination of the glucopyranosyl donor and acceptor. Using the 6-*O*-methylglucopyranosyl donors and acceptors shown in Table 1, the α/β -stereoselectivity and chemical yield were studied, thereby demonstrating: (1) the optimal protecting groups for the donor and the acceptor are benzyl and benzoate groups, respectively, and (2) the newer Mukaiyama ester and *mono*-methyl phthalate^[11] are approximately equal, except that the glycosidation rate with the newer Mukaiyama ester^[9c] was significantly faster than that with *mono*-methyl phthalate.

Having identified the optimal protecting groups for the donor and acceptor, we realized that a modification should be made on the strategy used for the synthesis of *s*MMP (Scheme 1). In the *s*MMP series, **M-a** served as the source of glycosyl donor **M-b** and glycosyl acceptor **M-c**, and the donor and the acceptor were coupled under the modified Mukaiyama protocol, to furnish **M-d**. Importantly, except for n, **M-d** was identical to **M-a** and, therefore, it was not necessary to adjust the protecting groups for the next cycle. In the *s*MGP series, however, the results summarized in Table 1 suggested that the optimal protecting groups for the donor and the acceptor are benzyl and benzoate groups, respectively, to achieve both optimal α/β -stereoselectivity and chemical yield, cf., **G-b** and **G-c**. Therefore, some additional steps would be required to transform the glycosidation product **G-d** to the glycosyl donor and acceptor for the next cycle. cf., **G-d** vs. **G-b** and **G-c**. In order to avoid this potentially cumbersome adjustment of protecting groups after each cycle, we became interested in the possibility of synthesizing the glycosyl donor **G-b** and acceptor **G-c** from α -, β -, and γ -cyclodextrins (CD).

CDs are naturally occurring cyclic oligosaccharides composed of α -(1 $\rightarrow$ 4)-linked D-glucose. α -, β -, and γ -CDs contain 6-, 7-, and 8-units of α -(1 $\rightarrow$ 4)-linked D-glucose, respectively. Assuming that the C6 hydroxyl groups of CDs can be selectively methylated, cf. α -, β -, and γ -CDs $\rightarrow$ **I**, and also that one α -(1 $\rightarrow$ 4) glycosidic bond of CDs can be cleaved preferentially over the α -(1 $\rightarrow$ 4) glycosidic bonds present in the cleaved products,^[11,12] cf., **I** $\rightarrow$ **II**, we anticipated that the 6-, 7-, and 8-mers of **G-b** (n=6, 7, 8) and **G-c** (n=6, 7, 8) should be

synthesized from α -, β -, and γ -CDs (Scheme 2). With this analysis, we began the experimental work on selective functionalization of CDs.

Our first task was to develop a reliable and scalable method for selective *O*-methylation of the C6 hydroxyl groups, which was achieved in four steps in excellent overall yields (Scheme 3). The selective C6 *O*-silylation was reported in the literature.^[11] Under the specified conditions, no scrambling of the silyl or benzoate groups were observed during benzylation, desilylation, and *O*-methylation.^[12,13]

Our second task was to develop a reliable and scalable method to selectively cleave the α -glycosidic bond of CDs. As the cleaved product still contains 6, 7, or 8 glycosidic bonds, the proposed transformation could be problematic. Nonetheless, we hoped that the desired, first cleavage could be faster than the undesired, following cleavages, because of the ring-constraints present in protected CDs. Some examples known in the literature support the proposed step. In particular, the results reported by Kuzuhara were informative; they observed a selective cleavage of one glycosidic bond on peracylated CDs, but observed a complex product formation of permethylated CDs on acetolysis.^[14] These experiments may suggest that the presence of an electron-withdrawing protecting group is the key to achieve the desired selective hydrolysis. Indeed, the controlled acetolysis of **4a~c** yielded the desired products as an approximately 6~7:1 mixture of anomeric α - and β -acetates. The relative rate for the first cleavage was in an order of **4a** (α -CD series) > **4b** (β -CD series) > **4c** (γ -CD series), and importantly the first cleavage rate was significantly faster than the following cleavages even for **4c**. However, the material throughput was best achieved by quenching the acetolysis at approximately 60~70% of completion and recycling the recovered starting materials.

The next stage of synthesis was to adjust the protecting group at C1 of the reducing end and C4 of the non-reducing end. On treatment with benzylamine, the C1 acetate at the reducing end of **5a~c** was selectively hydrolyzed to yield the desired products. The resultant anomers were allylated with Ag₂O/allyl bromide/DMF, furnishing the α -allylglucosides as the major products (α : β ratio=*ca.* 7:1). We then ran into difficulty in selectively hydrolyzing the acetate at the non-reducing end. Selective hydrolysis of an acetate over a benzoate should not usually present a problem. However, as **6a~c** contained a large number of benzoates (12-, 14-, 16-benzotes for **a**, **b**, and **c**, respectively), it became more challenging to achieve this seemingly simple selective hydrolysis cleanly in an overall sense. Thus far, HBF₄ in MeOH/CH₂Cl₂ was found to give the best result. Even under this condition, it was necessary to quench the reaction at around 50% conversion to avoid the over-hydrolysis (Scheme 4).

An obvious solution to this problem was to replace the C4 acetyl group with a more reactive acyl group such as a trifluoroacetyl or methoxyacetyl group; the reactivity difference between CF₃CO- or MeOCH₂CO- over PhCO- should be more pronounced than the reactivity difference between MeCO- and PhCO-. Thus, we attempted to cleave the CD **4b** under a variety of conditions (Table 2).^[15] After considerable trial-and-error efforts, we finally found that BF₃·Et₂O-promoted cleavage of **4b** in the presence of methoxyacetic acid provides an ultimate solution.

There are several appealing aspects for this cleavage reaction. First, as the reaction progressed, the cleaved product crashed out as a crystalline precipitate, recrystallization of which furnished the desired product (isolated as a 10:1 mixture of the α / β -anomers) in an excellent yield.^[16] Second, this reaction was routinely run in a 5~10 g scale without any technical difficulty. Third and most importantly, the isolated product was shown to have the free hydroxyl group at C4 of the non-reducing end. Thus, unlike in the case of **6b** $\rightarrow$ **7b** (Scheme 4), it was unnecessary to face the problem associated with the selective hydrolysis.

We applied the $\text{BF}_3 \cdot \text{Et}_2\text{O} / \text{MeOCH}_2\text{CO}_2\text{H}$ conditions to the remaining α - and γ -CD substrates. The behavior of γ -CD substrate **4c** was found to be virtually identical to that of β -CD substrate **4b** (Table 3). However, the α -CD substrate **4a** behaved differently; in the α -CD series, the cleaved product did not precipitate out from the reaction mixture. Apparently, this difference was largely due to the solubility of products in the reaction medium. We should note that the precipitation seemed to have two additional beneficial effects, i.e., (1) the precipitation appeared to drive the cleavage reaction to completion and (2) the precipitation appeared to protect the products from further cleavage.

We conducted an optimization work on the cleavage reaction in the α -CD series and found that the reaction rate and selectivity were greatly influenced by solvents. Among several solvents tested, CH_2Cl_2 was found to be the most effective co-solvent for acceleration of the cleavage reaction. Under the optimized conditions, the cleavage reaction proceeded smoothly to near completion and the pure product was isolated in a good yield by recrystallization (Scheme 5).

As pointed out, the linear oligomers **8a~c** have a free hydroxyl group at C4 of the non-reducing end, which facilitated the functional group manipulation required for preparation of the glycosyl acceptors and donors. The glycosyl acceptors **9a~c** were prepared straightforwardly from **8a~c** in three steps: silylation of the C4 hydroxyl group at the non-reducing end, selective removal of the methoxyacetate group at C1 of the reducing end, and allylation of the resultant hemiacetal (Scheme 6). As described in the previous series, allylation of the resultant anomers in DMF furnished α -allylated products with the stereoselectivity of *ca.* 7:1. Interestingly, the stereoselectivity (α : β ratio=*ca.* 7:1) was reversed in allylation in dichloroethane (α : β ratio=*ca.* 1:6). Although we later found that the stereochemistry at the reducing end of the polysaccharides made no noticeable difference in chemical behaviors,^[1] we pursued the homogeneous material to eliminate any concerns possibly related to the stereochemistry. Separation of the mixtures was effectively achieved by Biotage flash chromatography, to afford α -allyl acceptors with purities of >98% in gram quantities.

The glycosyl acceptors **9a~c** thus obtained served as the starting materials for preparation of the glycosyl donors **12a~c**, which was uneventfully accomplished (Scheme 6). For the reason discussed earlier, the newer Mukaiyama ester was chosen for the activator of glycosyl donors.

The glycosidation was first attempted in the β -CD series, i.e., **9b** ($m=7$) + **12b** ($n=7$) $\rightarrow$ **13b** ($m+n=14$) in Table 4. However, we soon realized that, because of poor solubility of the acceptor **9** in Et_2O , the glycosidation conditions optimized for model substrates (Table 1) could not be applied to this case. This difficulty was overcome by using a 1: 2 $\text{Et}_2\text{O} \text{--} \text{CH}_2\text{Cl}_2$ mixture as the solvent, but the α / β -selectivity diminished from 16:1 (Et_2O) down to 6:1 (1:2 $\text{Et}_2\text{O} \text{--} \text{CH}_2\text{Cl}_2$). Fortunately, the lowered selectivity of glycosidation did not present a technical problem, because the desired α -isomer was least polar on silica gel TLC and readily isolable in a pure form by silica gel chromatography.

As mentioned earlier, there was one major difference recognized for the Mukaiyama glycosidation in the *s*MGP series from that in the *s*MMP series. Yet, we recognized another major difference; a catalytic amount (10 mol%) of the Lewis acid $[\text{SnCl}_3\text{ClO}_4]$ was sufficient to achieve a good conversion in the *s*MMP series, whereas a higher loading of the catalysis seemed required to obtain a satisfactory result in the *s*MGP series. We speculated that the lower reactivity of the glucosyl donor in the *s*MGP series than the *s*MMP series accounts for the differences, and conducted an optimization work. In particular, we tested higher loadings of the Mukaiyama acid. As seen from the representative examples listed in Table 4, with an increase in the size of substrates, one or more equivalents of the Mukaiyama acid was required to achieve a good conversion with satisfactory reproducibility. Unlike in the *s*MMP series, the truncation/scrambling^[18] was negligible in the *s*MGP series. In this series, we noticed by-

product formation from the donors,^[19] but found that this by-product formation was significantly suppressed at $-30\text{ }^{\circ}\text{C}$. With these modifications, we were able to achieve the glycosidation with satisfactory reproducibility.

The glycosidation products were subjected to a two-step procedure of deprotection, and the *s*MGP 12-, 14-, and 16-mers were isolated by reverse phase column chromatography on C_{18} -silica gel (Scheme 7). In order to assess the purity of *s*MGP, extensive spectroscopic studies were conducted, thereby demonstrating that no detectable amount of β -anomer was contaminated at the newly introduced anomeric center.^[20]

The successful synthesis of *s*MGP encouraged us to explore the flexibility of the strategy for other closely related polysaccharides. First, in order to address the hydrophobic effect of polysaccharides for the complexation event with fatty acids, we pursued the synthesis of *s*MGP analogs composed of 6-*O*-ethyl-D-glucose **14d**. Gratifyingly, the same synthetic scheme as for the *s*MGP synthesis, except for the ethylation step of the β -CD derivative **3b**, yielded the polysaccharide 14-mer, **14d** (Scheme 8).^[21,22]

Second, in order to test the size-effect of *s*MGPs in the FA biosynthesis catalyzed by FAS I, we wished to have a broad range of *s*MGPs. With use of the reported synthesis, we were able to synthesize *s*MGPs ranging from 6-mer to 20-mer.^[23] The case of *s*MGP 18-mer and 20-mer summarized in Scheme 9 highlights the flexibility and usefulness of the reported synthesis.

Conclusion

An effective synthetic route to *s*MGPs from CDs was developed. The synthetic route proved to be flexible and general, to furnish a series of *s*MGPs ranging from 6-mer to 20-mer. A practical and scalable method was discovered selectively to cleave the CD derivatives and furnish the linear precursor to the glycosyl donors and acceptors. The Mukaiyama glycosidation was adopted to couple the glycosyl donors with the glycosyl acceptors. Unlike in the *s*MMP series, an amount of the Mukaiyama acid required in the *s*MGP series increased with an increase of substrate size; for large oligomers, more than one equivalent of the acid was required.

*s*MGPs thus obtained provided us, for the first time, with an opportunity to study the chemical and biological properties of *synthetic* MGPs. To our delight, the preliminary experiments demonstrated that *synthetic* and *natural* MGPs exhibit the identical, or at least very similar, properties.^[24] With this encouraging result, we felt it critically important to address the scalability of *s*MGP synthesis to ensure the supply of the materials. Recognizing a difficulty in the scalability of glycosidation, we initiated a study on the second generation of *s*MGP synthesis, resulting in an efficient and scalable synthesis of this class of polysaccharides.^[25]

Experimental Section

Synthesis summarized in Scheme 3

Transformation of 1a to 2a—To a solution of α -CD (**1a**, 10.0 g, 10.3 mmol), dried at $90\text{ }^{\circ}\text{C}$ for 12 h under reduced pressure, in dry pyr (200 mL) was added TBSCl (10.2 g, 67.8 mmol) in dry pyr (100 mL) dropwise at $0\text{ }^{\circ}\text{C}$. The mixture was stirred at RT and the reaction was monitored by TLC every 12 h. Additional TBSCl (up to $0.2 \times 6\text{ eq.}$) was added, if the reaction was incomplete. The excess of pyr was evaporated under reduced pressure and from the residue, white solid was precipitated out by the addition of ice-water (300 mL). The solid was filtered and washed with cold water (500 mL). The solid was taken with CH_2Cl_2 (300 mL) and was washed with 1N HCl, aq. NaHCO_3 , and brine. After drying over Na_2SO_4 and concentration, the product was recrystallized from EtOH (14.1 g, 83%).

With use of the same procedure, β - and γ -CDs (**1b** and **1c**) were converted to the corresponding **2b** and **2c**, respectively. For the spectroscopic data of **2a~c**, see Supporting Information.

Transformation of 2a to 3a—For the α -CD series, the following procedure of benzylation was used. The 6-*O*-TBS- α -CD **2a** (14.0 g, 8.45 mmol) in pyr (400 mL) was cooled to 0 °C to which was added freshly prepared BzOTf (51.3 mL, 310 mmol) slowly. The mixture was warmed gradually to 40 °C and was stirred for 48 h. The progress of reaction was monitored by NMR. The mixture was concentrated down to ca. 300 mL of pyr, to which was added aq. NaHCO₃ (10 mL) at 0 °C and was stirred for 10 min. From the mixture, white solid was precipitated out by the addition of ice-water (30 mL) which was filtered and washed with cold water (30 mL). The solid was taken with CH₂Cl₂ (300 mL) and was washed with 1N HCl, aq. NaHCO₃, and brine. After concentration, the product was recrystallized from EtOH (23.5 g, 95%).

Transformation of 2b,c to 3b,c—For the β - and γ -CDs series, the following procedure of benzylation was used. The 6-*O*-TBS- β -CD **2b** (16 g, 8.3 mmol) in pyr (500 mL) was cooled to 0 °C to which was added BzCl (54 mL, 464 mmol) slowly. The mixture was stirred at 50 °C for 5 days. The progress of reaction was monitored by NMR. The mixture was concentrated by evaporation of pyr (400 mL), to which was added aq. NaHCO₃ (100 mL) at 0 °C and was stirred for 10 min. From the mixture, white solid was precipitated out by the addition of ice-water (300 mL) which was filtered and washed with cold water (300 mL). The solid was taken with CH₂Cl₂ (300 mL) and was washed with 1N HCl, aq. NaHCO₃, and brine. After concentration, the product was recrystallized from EtOH (26 g, 93%).

For the desilylation, the following procedure used for all series. The 6-*O*-TBS-2,3-di-*O*-benzoyl- α -CD thus obtained (1.0 g, 0.34 mmol) was dissolved in CH₃CN-CH₂Cl₂ (20 mL, 3:1) in a plastic reactor equipped with a stirring bar. To this mixture was added 48% aq. HF (2.0 mL, 28 mmol) slowly at RT and was stirred for 2 h. The reaction mixture was diluted with CH₂Cl₂ (25 mL) and was poured into aq. NaHCO₃ (100 mL). The aqueous phase was further extracted with CH₂Cl₂ (15 mL $\times$ 2). The combined organic phase was washed with brine and dried over Na₂SO₄. The product was purified by a flash chromatography (eluent: CH₂Cl₂/MeOH) to give a white solid **3a** (0.63 g, 83%).

For the spectroscopic data for **3a~c** as well as the dibenzoates **2a~c**, see Supporting information.

Transformation of 3a to 4a—To a solution of 2,3-di-*O*-benzoyl- α -CD **3a** (890 mg, 0.40 mmol) in toluene-CH₂Cl₂ (40 mL, 3:1) was added MeI (1.2 mL, 19 mmol), Ag₂O (1.7 g, 7.2 mmol), NaHCO₃ (0.20 g, 2.4 mmol), and crushed 4A molecular sieve (3.3g, dried). The resulting slurry was sonicated for 12 h and stirred for 8 h, after which an additional portion of Ag₂O (0.6 g) and 4A molecular sieve (1 g) was added. Sonication was resumed for a further 8 hours. The slurry was filtered over Celite, rinsing with CH₂Cl₂ and CH₂Cl₂-EtOAc (2:1) successively. The filtrate was reduced *in vacuo* and purified by a flash chromatography on silica gel (eluent: CH₂Cl₂/EtOAc) to give the product as a white solid (790 mg, 85%).

With use of the same procedure, β - and γ -CDs (**3b** and **3c**) were converted to the corresponding **4b** and **4c**, respectively.

Spectroscopic data for 4a—¹H NMR δ 3.56 (s, 3 $\times$ 6 H), 3.85 (d, *J* = 10.0 Hz, 1 $\times$ 6 H), 4.16 (dd, *J* = 11, 4.0 Hz, 1 $\times$ 6 H), 4.19 (dd, *J* = 8.5, 8.5 Hz, 1 $\times$ 6 H), 4.50 (dd, *J* = 9.5, 3.5 Hz, 1 H), 5.04 (dd, *J* = 11.0, 4.0 Hz, 1 $\times$ 6 H), 5.44 (d, *J* = 3.5 Hz, 1 $\times$ 6 H), 6.16 (t, *J* = 8.5 Hz, 1 H), 6.83 (t, *J* = 8.0 Hz, 2 $\times$ 6 H), 6.90 (t, *J* = 7.5 Hz, 2 $\times$ 6 H), 7.11 (t, *J* = 8.0 Hz, 1 $\times$ 6 H), 7.15 (t, *J* = 8.0 Hz, 1 $\times$ 6 H); ¹³C NMR (100 MHz): δ 59.5; 71.4; 71.9; 72.2; 72.4; 78.2;

98.1; 127.7; 127.8; 128.3; 129.7; 130.0; 132.2; 132.6; 164.6; 166.3: **MS(MALDI-TOF)** calculated for ($C_{126}H_{120}O_{42}Na^+$): 2327.71, found 2327.9: $[\alpha]^{25}_D +94.8$ (c 0.26, $CHCl_3$).

Spectroscopic data for 4b— 1H NMR δ 3.55 (s, 3 $\times$ 7 H), 3.81 (d, J = 11.0 Hz, 1 $\times$ 7 H), 4.18 (dd, J = 9.5 Hz, 1 $\times$ 7 H), 4.22 (dd, J = 11.0, 3.5 Hz, 1 $\times$ 7 H), 4.44 (m, 1 $\times$ 7 H), 5.04 (dd, J = 11.0, 3.5 Hz, 1 $\times$ 7 H), 5.50 (d, J = 3.5 Hz, 1 $\times$ 7 H), 5.95 (dd, J = 9.5, 9.5 Hz, 1 $\times$ 7 H), 6.96 (m, 4 $\times$ 7 H), 7.22 (m, 4 $\times$ 7 H), 7.46 (m, 4 $\times$ 7 H); ^{13}C NMR (100 MHz): δ 59.5; 71.2; 71.6; 72.0; 76.3; 97.1; 127.8; 128.0; 128.7; 129.8; 129.95; 130.02; 132.4; 132.6; 164.7; 166.3: **MS(MALDI-TOF)** calculated for ($C_{147}H_{140}O_{49}Na^+$): 2711.84, found 2711.8: $[\alpha]^{25}_D +70.5$ (c 0.33, $CHCl_3$).

Spectroscopic data for 4c— 1H NMR δ 3.52 (s, 3 $\times$ 8 H), 3.78 (d, J = 10.0 Hz, 1 $\times$ 8 H), 4.10 (m, 1 $\times$ 8 H), 4.27 (dd, J = 9.5, 9.5 Hz, 1 $\times$ 8 H), 4.32 (m, 1 $\times$ 8 H), 5.07 (dd, J = 10.5, 3.5 Hz, 1 $\times$ 8 H), 5.54 (d, J = 3.5 Hz, 1 $\times$ 8 H), 5.90 (dd, J = 8.5, 8.5 Hz, 1 $\times$ 8 H), 6.99 (t, J = 8.0 Hz, 2 $\times$ 8 H), 7.07 (t, J = 8.0 Hz, 2 $\times$ 8 H), 7.17 (t, J = 7.15 Hz, 1 $\times$ 8 H), 7.30 (d, J = 7.5 Hz, 1 $\times$ 8 H), 7.56 (m, 4 $\times$ 8 H); ^{13}C NMR (100 MHz): δ 59.6; 70.9; 71.3; 71.8; 72.1; 74.3; 96.2; 128.0; 128.1; 128.7; 129.7; 129.9; 130.0; 132.6; 132.8; 164.9; 166.1: **MS(MALDI-TOF)** calculated for ($C_{168}H_{160}O_{56}Na^+$): 3095.96, found 3096.1: $[\alpha]^{25}_D +105$ (c 0.26, $CHCl_3$).

Synthesis summarized in Scheme 5

Transformation of 4a to 8a—To a solution of **4a** (5.20 g, 2.24 mmol) in CH_2Cl_2 (210 mL) at RT were added methoxyacetic acid (33 mL) and $BF_3 \cdot OEt_2$ (33 mL) and the mixture was warmed to 40 °C and was stirred for 1 h. The mixture was diluted with CH_2Cl_2 and poured into aq. $NaHCO_3$ (500 mL). The organic phase was separated, washed with brine, dried over Na_2SO_4 , and concentrated. Recrystallization from MeOH (100 mL) afforded the ring-opened product **8a** as a white solid (4.50 g, 82%, $\alpha/\beta=ca.$ 10). 1H NMR (500 MHz) characteristic peaks for α -anomer: seven methoxy- H δ 3.30 (s, 3 H), 3.35 (s, 3 H), 3.37 (s, 3 H), 3.40 (s, 3 H), 3.45 (s, 3 H), 3.47 (s, 3 H), 3.55 (s, 3 H); the reducing end anomeric - H : 6.57 (d, J = 3.4 Hz, 1 H); **MS(MALDI-TOF)** calculated for ($C_{129}H_{126}O_{45}Na^+$): 2417.74, found 2418.0; $[\alpha]^{25}_D +78.1$ (c 0.43, $CHCl_3$).

Transformation of 4b to 8b—Into a 1 L flask containing **4b** (6.00 g, 2.20 mmol) and a stirring bar was added a mixture of methoxyacetic acid (250 mL) and $BF_3 \cdot OEt_2$ (47 mL) at RT. After approximately 2 h of stirring, the starting material dissolved completely, followed by appearance of a white precipitate after about 5 h and was stirred further for 18 h. Then cold water (300 mL) was added to the reaction mixture. The white precipitate was filtered and washed with water (3 $\times$ 100 mL). The solid was taken up with CH_2Cl_2 (200 mL) and washed with aq. $NaHCO_3$ (2 $\times$ 100 mL), and brine (100 mL). After drying over Na_2SO_4 and concentration, recrystallization from EtOAc/hexanes gave **8b** as a white solid (5.44 g, 88%, $\alpha/\beta=ca.$ 3). 1H NMR (500 MHz) characteristic peaks for α -anomer: eight methoxy- H : δ 3.30 (s, 3 H), 3.32 (s, 3 H), 3.35 (s, 3 H), 3.37 (s, 3 H), 3.40 (s, 3 H), 3.45 (s, 3 H), 3.47 (s, 3 H), 3.55 (s, 3 H), the reducing end anomeric - H : 6.57 (d, J = 3.4 Hz, 1 H); **MS(MALDI-TOF)** calculated for ($C_{150}H_{146}O_{52}Na^+$): 2801.87, found 2801.9; $[\alpha]^{25}_D +75.6$ (c 0.31, $CHCl_3$).

Transformation of 4c to 8c—Following the procedure given for **4b** $\rightarrow$ **8b**, **4c** (3.80 g, 12.5 mmol) was converted to **8c** (3.46 g, 88%, $\alpha/\beta=ca.$ 2). 1H NMR (500 MHz) characteristic peaks for α -anomer: δ 3.30 (s, 3 H), 3.32 (s, 3 H), 3.33 (s, 3 H), 3.34 (s, 3 H), 3.35 (s, 3 H), 3.41 (s, 3 H), 3.47 (s, 3 H), 3.49 (s, 3 H), 3.57 (s, 3 H), the reducing end anomeric - H : 6.57 (d, J = 3.4 Hz, 1 H); **MS(MALDI-TOF)** calculated for ($C_{171}H_{166}O_{59}Na^+$): 3185.99, found 3185.9; $[\alpha]^{25}_D +77.2$ (c 0.33, $CHCl_3$).

Synthesis summarized in Scheme 6

Transformation of 8a to 9a—To a solution of **8a** (4.50 g, 1.89 mmol) and Et₃N (1.7 mL, 11.4 mmol) in CH₂Cl₂ (100 mL) at 0 °C was added and TMSOTf (1.05 mL, 5.67 mmol) slowly. The solution was gradually warmed to RT and was stirred for 1 h. Work-up with CH₂Cl₂/aq. NaHCO₃. The crude product was recrystallized from EtOAc/hexanes to give the TMS-ether of **8a** as a white solid (4.40 g, 96%).

To a solution of the TMS-ether of **8a** (4.28 g, 1.73 mmol) in CH₂Cl₂ (60 mL) was added ethanolamine (1.04 mL, 17.3 mmol) at RT and the mixture was stirred for 5 h. The reaction mixture was diluted with CH₂Cl₂ (50 mL), poured into water (100 mL), and partitioned. The organic phase was washed with brine and dried over Na₂SO₄. Flash chromatography (eluent: CH₂Cl₂/EtOAc) yielded the glycoside (3.85 g, 92%, $\alpha/\beta=ca. 2$).

9a (α -anomer enriched)—To a solution of the glycoside thus obtained (2.00 g, 0.842 mmol) in DMF (60 mL) were added Ag₂O (1.56 g, 6.47 mmol), crushed 4A MS (activated, 2.0 g), allyl bromide (0.73 mL, 8.42 mmol), and NaHCO₃ (0.42 g, 5.05 mmole). The dark suspension was stirred for 18 h. The slurry was filtered over Celite, rinsing with CH₂Cl₂ and CH₂Cl₂-EtOAc (2:1) successively. The filtrate was reduced *in vacuo* and purified by flash chromatography on silica gel (eluent: CH₂Cl₂/EtOAc) to give the product **9a** as a white solid (2.03 g, 92%, $\alpha/\beta=ca. 7$). The anomeric mixture (1.5 g) was loaded on a Biotage® column (40+M, $\phi = 4.0$ cm), and was eluted by EtOAc/CHCl₃ with a linear gradient from 4% to 20% (1.2 L), to furnish the α -anomer (1.0 g, $\alpha/\beta=ca. 100:1$) and the β -enriched anomeric mixture (0.34 g, $\alpha/\beta=ca. 1:2$). Spectroscopic data for **9a** ($\alpha/\beta=ca. 100:1$): ¹H NMR (500 MHz) characteristic peaks for α -anomer: TMS-*H*: δ -0.02 (s, 9 H); six methoxy-*H*: 3.30 (s, 3 H), 3.33 (s, 3 H), 3.35 (s, 3 H), 3.44 (s, 3 $\times$ 2 H), 3.56 (s, 3 H); the reducing end anomeric -*H*: 5.26 (d, $J = 3.4$ Hz, 1 H); **MS (MALDI-TOF)** calculated for (C₁₃₂H₁₃₄O₄₃SiNa⁺): 2457.80, found 2457.9; $[\alpha]^{25}_D +68.8$ (c 0.28, CHCl₃).

Transformation of 8b,c to 9b,c—Using the same procedure, **8b** and **c** were converted to **9b** and **c**, respectively. The α -enriched **9b** and **9c** ($\alpha/\beta=ca. 100:1$) were obtained by using the same chromatographic method.

Spectroscopic data for 9b (α -anomer enriched)—TMS-*H*: δ 0.00 (s, 9 H); seven methoxy-*H*: 3.30 (s, 3 $\times$ 3 H), 3.31 (s, 3 H), 3.34 (s, 3 H), 3.44 (s, 3 $\times$ 2 H), 3.56 (s, 3 H); the reducing end anomeric -*H*: 5.26 (d, $J = 3.4$ Hz, 1 H); **MS (MALDI-TOF)** calculated for (C₁₅₃H₁₅₄O₅₀SiNa⁺): 2841.92, found: 2881.9; $[\alpha]^{25}_D +76.4$ (c 0.38, CHCl₃).

Spectroscopic data for 9c (α -anomer enriched)—¹H NMR characteristic peaks for α -anomeric-OH product: 4-OTMS-*H*: δ -0.02 (s, 9 H); eight methoxy-*H*: 3.29 (s, 3 H), 3.30 (s, 3 $\times$ 3 H), 3.34 (s, 3 H), 3.44 (s, 3 $\times$ 2 H), 3.56 (s, 3 H); the reducing end anomeric -*H*: 5.25 (d, $J = 3.4$ Hz, 1 H); (d, $J = 8.0$ Hz, 1 H); **MS (MALDI-TOF)** calculated for (C₁₅₃H₁₅₄O₅₀SiNa⁺): 3226.04, found: 3226.0; $[\alpha]^{25}_D +78.0$ (c 0.45, CHCl₃).

Transformation of 9a~c to 10a~c—The following procedure was applied for all series. To **9a** (1.65 g, 0.677 mmol) was added mixture of 1N HCl/acetone (40 mL, 1:9) and the mixture was stirred at RT for 1h. Then the mixture was reduced *in vacuo* and the residue was taken with CH₂Cl₂ (50 mL) and washed with NaHCO₃ and brine successively. After drying over Na₂SO₄, solvents of the organic phase were exchanged with CH₂Cl₂-CH₃OH (30-15 mL).

To the resultant residue was added NaOMe (400 mg, 7.40 mmol). The mixture was stirred at RT for 22 h and then evaporated to dryness.

To the resultant residue were added THF (40 mL), DMF (10 mL), NaH (95%, 602 mg, 24.0 mmol), and TBAI (250 mg, 0.677 mmol). The heterogeneous mixture was stirred at RT for 30 min before benzyl bromide (3.13 mL, 26.4 mmol) was added. The mixture was stirred at RT for 21 h. After quenched by 5 mL of methanol at 0 °C, the mixture was taken with CH₂Cl₂ (100 mL) and rinsed with 1 N HCl, aq. NaHCO₃, and brine. Flash chromatography on silica gel (eluent: benzene/EtOAc) afforded **10a** (1.20 g, 77%). ¹H NMR (500 MHz) characteristic peaks for α-anomer: six methoxy-*H*: δ 3.29 (s, 3 H), 3.31 (s, 3 H), 3.32 (s, 3 H), 3.33 (s, 3 H), 3.37 (s, 3 H), 3.42 (s, 3 H); allyl-CH₂: 5.22 (dd, *J* = 11.0, 1.5 Hz, 1 H), 5.36 dd, *J* = 17.0, 1.5 Hz, 1 H); six α-anomeric-*H*: 5.60 (d, *J* = 4.0 Hz, 1 H), 5.63 (d, *J* = 3.5 Hz, 1 H), 5.68 (m, 4 H); **MS (MALDI-TOF)** calculated for (C₁₃₆H₁₆₀O₃₄Na⁺): 2308.05, found: 2307.9; [α]²⁵_D +72.0 (*c* 0.23, CHCl₃).

Spectroscopic data for 10b—¹H NMR (500 MHz) characteristic peaks for α-anomer: seven methoxy-*H*: δ 3.29 (s, 3 H), 3.30 (s, 3 H), 3.32 (s, 3 H), 3.33 (s, 3 H), 3.37 (s, 3 H), 3.39 (s, 3 H), 3.42 (s, 3 H); allyl-CH₂: 5.22 (dd, *J* = 11.0, 1.5 Hz, 1 H), 5.36 (dd, *J* = 17.0, 1.5 Hz, 1 H); seven α-anomeric-*H*: 5.60 (d, *J* = 4.0 Hz, 1 H), 5.63 (d, *J* = 3.5 Hz, 1 H), 5.68 (m, 5 H); **MS (MALDI-TOF)** calculated for (C₁₅₇H₁₈₀O₃₆Na⁺): 2664.21, found: 2664.3; [α]²⁵_D +75.3 (*c* 0.15, CHCl₃).

Spectroscopic data for 10c—¹H NMR (500 MHz) characteristic peaks for α-anomer: eight methoxy-*H*: δ 3.29 (s, 3 H), 3.30 (s, 3 H), 3.316 (s, 3 H), 3.322 (s, 3H) 3.36 (s, 3 H), 3.37 (s, 3 H), 3.39 (s, 3 H), 3.42 (s, 3 H); allyl-CH₂: 5.21 (dd, *J* = 11.0, 1.5 Hz, 1 H), 5.36 (dd, *J* = 17.0, 1.5 Hz, 1 H); eight α-anomeric-*H*: 5.59 (d, *J* = 3.5 Hz, 1 H), 5.64 (d, *J* = 3.5 Hz, 1 H), 5.67 (m, 6 H); **MS (MALDI-TOF)** calculated for (C₁₇₈H₂₀₄O₄₁Na⁺): 3020.38, found: 3020.1; [α]²⁵_D +68.3 (*c* 0.43, CHCl₃).

Transformation of 10a–c to 11a–c—The following procedure was applied for all series. To a solution of **10a** (0.80 g, 0.35 mmol) in EtOH-toluene (6–3 mL) were added (Ph₃P)₃RhCl (16 mg, 0.018 mmol) and DABCO (5.9 mg, 0.053 mmol). The mixture was heated at 80 °C for 20 h. After the removal of solvents, the residue was stirred with 1N HCl-acetone (1:9, 20 mL) at 80 °C for 6 h. The solvents were removed *in vacuo* and the residue was taken up with CH₂Cl₂ and was washed with aq. NaHCO₃ and brine. Flash chromatography on silica gel (eluent: CH₂Cl₂/EtOAc) afforded **11a** (0.65 g, 82%, α/β=*ca.* 2:1). ¹H NMR (500 MHz) characteristic peaks for α-anomer: the reducing end C1 OH: δ 2.94 (d, *J* = 2.5 Hz, 1 H); six methoxy-*H*: 3.29 (s, 3 H), 3.30 (s, 3 H), 3.32 (s, 3 H), 3.33 (s, 3 H), 3.37 (s, 3 H), 3.38 (s, 3 H); six α-anomeric-*H*: 5.23 (d, *J* = 3.0 Hz, 1 H), 5.59 (d, *J* = 3.5 Hz, 1 H), 5.62 (d, *J* = 3.5 Hz, 1 H), 5.67 (m, 3 H); **MS (MALDI-TOF)** calculated for (C₁₃₃H₁₅₂O₃₁Na⁺): 2268.02, found: 2268.4; [α]²⁵_D +57.5 (*c* 0.40, CHCl₃).

Spectroscopic data for 11b (α/β=*ca.* 2:1)—¹H NMR (500 MHz) characteristic peaks for α-anomer: the reducing end C1 OH: δ 2.94 (d, *J* = 2.5 Hz, 1 H); seven methoxy-*H*: 3.29 (s, 3 H), 3.30 (s, 3 H), 3.32 (s, 3 H), 3.33 (s, 3 H), 3.37 (s, 3 H), 3.39 (s, 3 H), 3.42 (s, 3 H); seven α-anomeric-*H*: 5.24 (m, 1 H), 5.60 (d, *J* = 3.5 Hz, 1 H), 5.64 (m, 5 H); **MS (MALDI-TOF)** calculated for (C₁₅₄H₁₇₆O₃₆Na⁺): 2624.18, found: 2624.2; [α]²⁵_D +79.1 (*c* 0.18, CHCl₃).

Spectroscopic data for 11c (α/β=*ca.* 2:1)—¹H NMR (500 MHz) characteristic peaks for α-anomer: the reducing end C1 OH: δ 2.94 (d, *J* = 2.5 Hz, 1 H); eight methoxy-*H*: 3.29 (s, 3 H), 3.30 (s, 3 H), 3.32 (s, 3 H), 3.33 (s, 3 H), 3.37 (s, 3 H), 3.39 (s, 3 H), 3.42 (s, 3 H); eight α-anomeric-*H*: 5.23 (t, *J* = 3.5 Hz, 1 H), 5.60 (d, *J* = 3.5 Hz, 1 H), 5.63 (d, *J* = 3.5 Hz, 1 H), 5.67 (m, 5 H); **MS (MALDI-TOF)** calculated for (C₁₇₅H₂₀₀O₄₁Na⁺): 2980.35, found: 2980.2; [α]²⁵_D +79.3 (*c* 0.19, CHCl₃).

Transformation of 11a–c to 12a–c—The following procedure was applied for all series. To a solution of **11a** (0.50 g, 0.22 mmol), 2-(2-methoxyethoxy)acetic acid (38.4 mL, 0.33 mmol), and DMAP (13.6 mg, 0.11 mmol) in CH₂Cl₂ (15 mL) at 0 °C was added EDCI (85 mg, 0.446 mmol) in portions. The cooling bath was removed after 10 min and the mixture was stirred at RT for 2 h. The mixture was diluted with CH₂Cl₂ (20 mL) and washed with aq. NaHCO₃ and brine. Flash chromatography on silica gel (eluent: CH₂Cl₂/EtOAc) afforded **12a** as a colorless solid (0.50 g, 96%, $\alpha/\beta=ca. 2$). ¹H NMR (500 MHz) characteristic peaks for α -anomer: seven methoxy-*H*: δ 3.30 (s, 3 H), 3.31 (s, 3 H), 3.32 (s, 3 H), 3.34 (s, 3 H), 3.38 (s, 3 $\times$ 2H), 3.41 (s, 3 H); six α -anomeric-*H*: 5.61 (d, *J* = 3.5 Hz, 1 H), 5.63 (d, *J* = 3.5 Hz, 1 H), 5.66 (d, *J* = 3.5 Hz, 1 H), 5.68 (d, *J* = 3.5 Hz, 1 H), 5.74 (d, *J* = 3.5 Hz, 1 H), 6.46 (d, *J* = 3.5 Hz, 1 H); **MS (MALDI-TOF)** calculated for (C₁₃₈H₁₆₀O₃₄Na⁺): 2384.07, found: 2384.2; $[\alpha]^{25}_D +80.1$ (*c* 0.23, CHCl₃).

Spectroscopic data for 12b ($\alpha/\beta=ca. 2:1$)—¹H NMR (500 MHz) characteristic peaks for α -anomer: eight methoxy-*H*: δ 3.29 (s, 3 H), 3.30 (s, 3 H), 3.31 (s, 3 H), 3.32 (s, 3 H), 3.36 (s, 3 H), 3.377 (s, 3 H), 3.38 (s, 3 $\times$ 2H), 3.41 (s, 3 H); seven α -anomeric-*H*: 5.59 (d, *J* = 3.5 Hz, 1 H), 5.63 (m, 4 H), 5.74 (d, *J* = 3.5 Hz, 1 H), 6.45 (d, *J* = 3.5 Hz, 1 H); **MS (MALDI-TOF)** calculated for (C₁₅₉H₁₈₄O₃₉Na⁺): 2740.23, found: 2740.0; $[\alpha]^{25}_D +81.2$ (*c* 0.26, CHCl₃).

Spectroscopic data for 12c ($\alpha/\beta=ca. 2:1$)—¹H NMR (500 MHz) characteristic peaks for α -anomer: nine methoxy-*H*: δ 3.29 (s, 3 H), 3.30 (s, 3 H), 3.318 (s, 3 H), 3.322 (s, 3 H), 3.36 (s, 3 H), 3.37 (s, 3 H), 3.38 (s, 3 $\times$ 2H), 3.41 (s, 3 H); eight α -anomeric-*H*: 5.59 (d, *J* = 3.5 Hz, 1 H), 5.64 (d, *J* = 3.5 Hz, 1 H), 5.66 (m, 4 H), 5.75 (d, *J* = 3.5 Hz, 1 H), 6.45 (d, *J* = 3.5 Hz, 1 H); **MS (MALDI-TOF)** calculated for (C₁₈₀H₂₀₈O₄₄Na⁺): 3096.39, found: 3095.8; $[\alpha]^{25}_D +83.2$ (*c* 0.25, CHCl₃).

Glycosidations summarized in Scheme 7

General note on glycosidation reactions—*Precautions*: For handling of anhydrous AgClO₄, see: Perrin, D. D.; Armarego, W. L. F. *Purification of Laboratory Chemicals*, 3rd ed.; Pergamon, New York, NY, 1988 and Encyclopedia of Reagents for Organic Synthesis, Paquette, L. A. Ed, John Wiley & Son, New York, NY, 1995. All solvents were freshly distilled prior to use (CH₂Cl₂ from CaH₂; Et₂O from LiAlH₄) and transferred *via* cannula into the solvent flasks. All flasks were flame dried just before use. Syringes, septa, needles, AgClO₄ (placed in a pear-shaped flask equipped with a stirrer bar, septum and needle), aluminum foil, stoppers, and plastic bags were dried over P₂O₅/Drierite in a desiccator *in vacuo* overnight. The starting materials were also dried over P₂O₅ under reduced pressure overnight. After all the reagents, solvents, and the desiccator have been placed in a glove bag, the atmosphere is exchanged three times with nitrogen (introduced through Drierite plug).

Glycosidation to form 13a—To AgClO₄ (52 mg, 0.25 mmol) in a 10 mL pear-shaped flask equipped with a stirring bar was added Et₂O (5 mL). This suspension was stirred for twenty minutes and the flask was wrapped with aluminum foil to avoid light. To the AgClO₄ suspension was added SnCl₄ (30 mL, 0.25 mmol) was slowly and the mixture was stirred for an additional twenty minutes. The suspension was then left to stand for 5 minutes to allow AgCl-precipitate to settle down. To the reaction flask, which was equipped with a stirring bar and sealed with a septum, was added the supernatant of the Mukaiyama acid solution (0.36 mL, 0.018 mmol). The starting materials (α -anomer enriched **9a** (45 mg, 0.018 mmol) and **12a** (44 mg, 0.018 mmol)) were dissolved in CH₂Cl₂ (0.36 mL) and the solution was transferred into a 1 mL plastic syringe whose needle exit was fixed on the septum of the catalyst solution and they were carefully placed in two plastic bags to isolate them from outer atmosphere. They were cooled down to –30 °C in a cryobath. After allowing for thermal equilibration, the starting

material solution was added slowly over ten minutes and the solution was stirred for 24 h. The reaction mixture was quenched with aq. NaHCO_3 and extracted with CH_2Cl_2 (2 ml), dried over Na_2SO_4 and reduced *in vacuo*. The crude mixture was stirred in 1N HCl/acetone (10 ml, 1:9) for 1 h at RT to desilylate the unreacted **9a** (20 mg, 43% recovered). The product (α/β selectivity of glycosidation=*ca.* 5.5:1 by ^1H NMR spectrum) was purified by two successive flash chromatography on silica gel. The first chromatography (hexanes/THF) was used to remove the by-product and the second chromatography ($\text{CH}_2\text{Cl}_2/\text{EtOAc}$) was used to isolate the desired α -glycosidation product (isolated α -isomer: 43 mg; 48%. ^1H NMR (500 MHz) characteristic peaks for the product with α -allyl terminal: twelve methoxy-*H*: δ 3.284 (s, 3 H), 3.297 (s, 3 H), 3.300 (s, 3 H), 3.311 (s, 3 H), 3.318 (s, 3 H), 3.328 (s, 3 H), 3.346 (s, 3 $\times$ 2 H), 3.358 (s, 3 H), 3.402 (s, 3 H), 3.477 (s, 3 H), 3.64 (s, 3 H); **MS (MALDI-TOF)** calculated for ($\text{C}_{262}\text{H}_{276}\text{O}_{73}\text{Na}^+$): 4612.78, found: 4612.3; $[\alpha]^{25}_{\text{D}} +72.9$ (*c* 0.48, CHCl_3).

Glycosidation to form 13b—With **9b** (α -anomer enriched, 52 mg, 0.0184 mmol), **12b** (50 mg, 0.0184 mmol), and the Mukaiyama acid (0.0184 mmol), the glycosidation was conducted under the essentially same conditions: α/β selectivity=*ca.* 5:1; α -isomer isolated: 48 mg, 50%. ^1H NMR (500 MHz) characteristic peaks for the product with α -allyl terminal: fourteen methoxy-*H*: δ 3.289 (s, 3 H), 3.301 (s, 3 H), 3.306 (s, 3 $\times$ 2 H), 3.316 (s, 3 H), 3.321 (s, 3 H), 3.327 (s, 3 H), 3.358 (s, 3 $\times$ 3H), 3.364 (s, 3 H), 3.408 (s, 3 H), 3.483 (s, 3 H), 3.647 (s, 3 H); **MS (MALDI-TOF)** calculated for ($\text{C}_{304}\text{H}_{320}\text{O}_{85}\text{Na}^+$): 5353.06, found: 5353.1; $[\alpha]^{25}_{\text{D}} +76.3$ (*c* 0.66, CHCl_3).

Glycosidation to form 13c—With **9c** (α -anomer enriched, 32 mg, 0.010 mmol), **12c** (31 mg, 0.010 mmol), and the Mukaiyama acid (0.010 mmol), the glycosidation was conducted under the essentially same conditions: α/β selectivity=*ca.* 5:1; α -isomer isolated: 28 mg, 46%. ^1H NMR (500 MHz) characteristic peaks for the product with α -allyl terminal: sixteen methoxy-*H*: δ 3.288 (s, 3 H), 3.300–3.325 (7s, 3 $\times$ 7H), 3.360 (s, 3 $\times$ 5 H), 3.407 (s, 3 H), 3.481 (s, 3 H), 3.646 (s, 3 H); **MS (MALDI-TOF)** calculated for ($\text{C}_{346}\text{H}_{364}\text{O}_{97}\text{Na}^+$): 6093.34, found: 6093.6; $[\alpha]^{25}_{\text{D}} +83.0$ (*c* 0.32, CHCl_3).

Glycosidations with β -anomer enriched acceptors **9a~c** were also carried out under the essentially same conditions. For details, see Supporting Information.

Deprotection summarized in Scheme 7

α -14a—To a solution of **13a** (α -allyl anomer, 20 mg, 4.3 mmol) in THF-MeOH (3 mL, 2:1) was added 10% $\text{Pd}(\text{OH})_2$ on carbon (10 mg). The mixture was equipped with a H_2 balloon and was stirred for 24 h. The reaction mixture was passed through a sintered-glass filter with thorough rinsing with CH_2Cl_2 -MeOH (30 mL, 2:1). The filtrate was concentrated with an evaporator and the residue was taken up with CH_2Cl_2 (2.0 mL). To the mixture was added 0.1 M NaOMe in MeOH (1.0 mL, 0.1 mmol). The mixture was stirred at RT for 12 h and neutralized by adding 1N HCl (0.1 mL, 0.1 mmol). After evaporation of solvents, the residue was subjected to reverse phase C_{18} -column chromatography (eluent: $\text{H}_2\text{O}/\text{MeOH}$: 3/1 to 1/1) to furnish *s*MGP 12-mer **14a** (8.1 mg, 86%). The product was further purified by HPLC on a reverse phase C_{18} -column with an RI detector (eluent: $\text{MeOH}/\text{H}_2\text{O}$). ^1H NMR (D_2O , 500MHz): δ 0.90 (t, $J = 7.5$ Hz, 3 H), 1.61 (m, 2 H), 3.38 (s, 3 $\times$ 11 H), 3.39 (s, 3 H), 3.43 (t, $J = 9.5$ Hz, 1 H), 3.44–3.95 (m, 73 H), 4.88 (d, $J = 4.0$ Hz, 1 H), 5.37 (d, $J = 4.0$ Hz, 1 H), 5.42 (m, 10 H); **MS (MALDI-TOF)** calculated for ($\text{C}_{87}\text{H}_{152}\text{O}_{61}\text{Na}^+$): 2195.87, found: 2196.0; $[\alpha]^{25}_{\text{D}} +213$ (*c* 0.28, H_2O).

α -14b—The deprotection was performed under the same conditions, where **13b** (α -anomer enriched, 20 mg, 7.5 mmol) gave **14b** (10.2 mg (68%)). ^1H NMR (D_2O , 500MHz): δ 0.89 (t, $J = 7.5$ Hz, 3 H), 1.60 (m, 2 H), 3.37 (s, 3 $\times$ 13 H), 3.38 (s, 3 H), 3.42 (t, $J = 9.5$ Hz, 1 H),

3.54–3.94 (m, 85 H), 4.89 (d, $J = 4.0$ Hz, 1 H), 5.36 (d, $J = 4.0$ Hz, 1 H), 5.42 (m, 12 H); **MS** (MALDI-TOF) calculated for ($C_{101}H_{176}O_{71}Na^+$): 2548.01, found: 2548.2; $[\alpha]^{25}_D +203$ (c 0.29, H_2O).

α -14c—The deprotection was performed under the same conditions, where **13c** (α -anomer enriched, 62 mg, 10 mmol) gave **14c** (19.5 mg (68%)). **1H NMR** (D_2O , 500MHz): δ 0.89 (t, $J = 7.5$ Hz, 3 H), 1.61 (m, 2 H), 3.37 (s, 3×15 H), 3.38 (s, 3 H), 3.43 (t, $J = 9.5$ Hz, 1 H), 3.54–3.94 (m, 97 H), 4.89 (d, $J = 4.0$ Hz, 1 H), 5.36 (d, $J = 4.0$ Hz, 1 H), 5.42 (m, 14 H); **MS** (MALDI-TOF) calculated for ($C_{115}H_{200}O_{81}Na^+$): 2900.14, found: 2900.0; $[\alpha]^{25}_D +205$ (c 0.28, H_2O).

Using the same protection procedure, β -enriched sMGPs 12-, 14-, and 16-mers were prepared. For details, see Supporting Information.

For details for the synthesis summarized in Schemes 8 and 9, see Supporting Information.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

Financial support from the National Institutes of Health (NS 12108) and Eisai Research Institute is gratefully acknowledged.

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12. 4a was previously prepared by methylation ($\text{BF}_3/\text{CH}_2\text{N}_2$) of 3a: see ref. 11a.
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16. The products were isolated as a mixture of α/β -anomers at the reducing ends with ca. 10:1 ratios favoring α -anomers. The homogeneity of products was assessed by ^1H NMR and MS (MALDI-TOF), and was further confirmed by transforming them to the known acceptors 9a~c.
17. Following the same sequences as shown in Schemes 6 and 7, β -anomer enriched 9a~c were converted to the β -anomer enriched sMGP 14a~c. For details, see Supporting Information.
18. The profile of Mukaiyama glycosidation in the glucose series was different from that in the mannose series. However, considering the total number of glycosidic bonds present in the product as well as the starting materials, we were concerned with the possibility that the "truncated/scrambled" products might be contaminated in the products in the gluco series as well. To address this issue, the glycosidation was purposely run for a prolonged time at 0 °C, and the product was subjected to the mass spectrometry and size-exclusion chromatography, thereby showing that the product mixture thus obtained was indeed contaminated by a small amount of "truncated" oligomers. Interestingly, these oligomers were formed by cleavage of glycosidic bonds exclusively at the hexoses bearing benzyl protection groups, suggesting that electron-withdrawing groups on C2 hydroxyl groups destabilize carbocation-formation and suppress the truncation. When the reaction was carried out at -30 °C, the process forming the "truncated" oligomers was completely suppressed.
19. Based on the ^1H NMR analysis, the major by-product appeared to be a benzyl glycoside of the donors.
20. An extensive study on the ^1H NMR spectrum was carried out; the absence of the doublet peak ($J = 8.0$ Hz) at 4.48 ppm demonstrated no contamination with the β -anomer at the newly introduced anomeric center. Similarly, the absence of the doublet ($J = 8.0$ Hz) at 4.45 ppm demonstrated no contamination with the β -propyl anomer at the reducing end.
21. For details, see Supporting Information.
22. Structure assignment in the sEMG 14-mer series was made, based on the ^1H NMR analysis in comparison with the corresponding sMGP 14-mer series
23. With use of the reported synthetic route, sMGP 6-, 7-, 8-, 10-, 12-, 14-, 16-, 18-, and 20-mers were synthesized. For details, see Supporting Information.
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Figure 1. Structures of mycobacterial polysaccharides 3-*O*-methyl-D-mannose-containing polysaccharides (MMP) and 6-*O*-methyl-D-glucose-containing lipopolysaccharides (MGLP)/6-*O*-methyl-D-glucose-containing polysaccharide (MGP).

Figure 2.
Structures of synthetic analogs of mycobacterial polysaccharides, *sMMP* and *sMGP*.

Scheme 1.

Divergent synthetic strategies of *s*MMP (Panel **A**) and *s*MGP (Panel **B**). (a) Mukaiyama glycosidation: 10 mol% AgClO₄-SnCl₄, Et₂O, 0 °C.

Scheme 2.

Synthetic plan for the glycosyl donor **G-b** and the glycosyl acceptor **G-c** from α -, β -, and γ -CDs.

a: α -CD series, $n = 6$; **b:** β -CD series, $n = 7$; **c:** γ -CD series, $n = 8$

Scheme 3.

Reagents and conditions: (a) TBSCl, pyr, rt (**a**: 83%; **b**: 83%; **c**: 78%); (b) 1. BzOTf, pyr, rt (**a**: 95%) or BzCl, pyr, 50 °C (**b**: 93%; **c**: 90%); 2. aq. HF, CH₂Cl₂-CH₃CN, rt (**a**: 83%; **b**: 80%; **c**: 82%); (c) Ag₂O, MeI, MS (4Å), toluene, rt (**a**: 85%; **b**: 83%; **c**: 81%).

Scheme 4.

Reagents and conditions: (a) 2% H_2SO_4 in Ac_2O , rt (**a**: 71%; **b**: 66%; **c**: 55%); (b) 1. BnNH_2 , THF, rt (**a**: 96%; **b**: 93%; **c**: 92%); 2. Ag_2O , MS ($4\text{\AA}$), allyl bromide, DMF, rt (**a**: 90%; **b**: 97%; **c**: 93%); (c) HBF_4 , CH_2Cl_2 -MeOH (3:1), 40 °C (**a**: 54%, **b**: 44%; **c**: 52%).

a: α -CD series, $n = 6$; **b:** β -CD series, $n = 7$; **c:** γ -CD series, $n = 8$

Scheme 5.

Reagents and conditions: (a) α -CD **4a**: $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (12% v/v), $\text{MeOCH}_2\text{CO}_2\text{H} \cdot \text{CH}_2\text{Cl}_2$ (12% v/v; $[\text{C}] = 0.01 \text{ M}$), 40°C , 1 h, 82% yield (recrystallization). β -CD **4b** and γ -CD **4c**: see Table 3.

Scheme 6.

Reagents and conditions: (a) 1. TMSOTf, Et_3N , CH_2Cl_2 (**a**: 96%; **b**: 93%; **c**: 95%); 2. ethanolamine, CH_2Cl_2 (**a**: 92%; **b**: 86%; **c**: 86%); 3. Allyl bromide, Ag_2O , MS(4Å), DMF, rt (**a**: 92%; **b**: 86%; **c**: 91%); (b) (i) H_3O^+ ; (ii) NaOMe, MeOH- CH_2Cl_2 (1:1), rt; (iii) NaH, BnBr, DMF, rt (**a**: 77%; **b**: 87%; **c**: 71%). (c) 1. $(\text{Ph}_3\text{P})_3\text{RhCl}$, Dabco, EtOH-toluene- H_2O (6:3:1), 90 °C; 2. acetone-1N HCl (9:1), 90 °C (**a**: 82%; **b**: 76%; **c**: 77%); (d) EDCI, DMAP, 2-(2-methoxyethoxy)acetic acid, CH_2Cl_2 , 0 °C (**a**: 96 %; **b**: 96%; **c**: 91%).

Scheme 7.

Reagents and conditions: (a) 1. 100 mol% $[\text{SnCl}_3\text{ClO}_4]$, CH_2Cl_2 - Et_2O (2:1), -30°C (**a**: 49%; **b**: 50%; **c**: 51%); (b) (i) H_2 , $\text{Pd}(\text{OH})_2$ on C, THF, rt; (ii) NaOMe , CH_2Cl_2 - MeOH (3:1), rt (**a**: 86%, **b**: 66%; **c**: 68%).

Scheme 8.

Reagents and conditions: (a) iodoethane, Ag₂O, MS (4Å), toluene, 75%; (b) Follow the steps in Table 3; (c) Follow the step a in Scheme 6 (77% overall yield); (d) Follow the steps b, c and d in Scheme 6 (69% overall yield); (e) (1). 200 mol% [SnCl₃ClO₄], CH₂Cl₂-Et₂O (2:1), -30 °C, 50%; (2) Follow the step b in Scheme 7 (54% overall yield). For the details, see Supporting Information.

Scheme 9.

Reagents and conditions: (a) 1. (i) NaOMe, MeOH-CH₂Cl₂ (1:1), rt.; (ii) NaH, BnBr, DMF, rt. (**a**: 70%, **b**: 72%); 2. (i) Follow the steps c and d in Scheme 6 (**a**: 57%, **b**: 57%). (b) 300 mol % [SnCl₃ClO₄], CH₂Cl₂-Et₂O (2:1), -30 °C (**a**: 44%, **b**: 43%). (c) Follow the step b in Scheme 7 (**14e**: 58%, **14f**: 64%).

Table 1

Screening of protection groups of glycosyl donor and acceptor. Glycosidation conditions: 10 mol% AgClO₄-SnCl₄, Et₂O, 0 °C.

entry	R ₁	R ₂	-E	Reaction time (h)	Yield (%) ^a	α/β ratio ^b
1	Bz	Bz	-CO-C ₆ H ₄ -CO ₂ Me-(o)	37	7(β)	1:4
2	Bn	Bz	-CO-C ₆ H ₄ -CO ₂ Me-(o)	39	86(α)	13:1
3	Bn	Bn	-CO-CH ₂ O(CH ₂) ₂ OMe	4	81(α)	16:1
4	Bn	Bn	-CO-CH ₂ O(CH ₂) ₂ OMe	12	76(α)	6:1

^a Isolated yield.

^b Data based on ¹H NMR analysis.

Table 2

Screening of the selective cleavage of β -CD derivative **4b**.

entry	Lewis acid (v/v%)	R ¹	R ²	X	Y	conversion ^b
1	none	CH ₃	CF ₃	Ac	Ac	ca. 50%
2	none	CF ₃	CF ₃	-	-	n.a. ^c
3	none	(MeO)CH ₂	CF ₃	-	-	n.a. ^c
4	HBF ₄ (2%)	(MeO)CH ₂	(MeO)CH ₂	H	(MeO)Ac	< 5 %
5	BF ₃ ·Et ₂ O (2%)	(MeO)CH ₂	(MeO)CH ₂	H	(MeO)Ac	ca. 20%
6	BF ₃ ·Et ₂ O (2%)	(MeO)CH ₂	none ^d	H	(MeO)Ac	ca. 25%
7	BF ₃ ·Et ₂ O (4%)	(MeO)CH ₂	none ^d	H	(MeO)Ac	ca. 30%
8	BF ₃ ·Et ₂ O (12%)	(MeO)CH ₂	none ^d	H	(MeO)Ac	ca. 90%
9	BF ₃ ·Et ₂ O (16%)	(MeO)CH ₂	none ^d	H	(MeO)Ac	ca. 97%

^aThe ratio of R1CO₂H to (R2CO)₂O was 1:1 where the acid anhydride was used.

^bConversion was estimated from ¹H NMR of the crude reaction mixture.

^cNo desired product was found.

^dThe acid anhydride was omitted.

Table 3
Substrate-scope of the selective cleavage reaction of CD derivatives.

entry	n	R	conversion	crystalline precipitation	isolated yield
1	6	Me	ca. 85% ^a	No	61%
2	7	Me	ca. 97% ^b	Yes	86%
3	7	Et	ca. 97% ^b	Yes	88%
4	8	Me	ca. 97% ^b	Yes	88%

^aConversion was based on recovered SM; the reaction mixture contains unidentified side-products.

^bConversion was estimated from ¹H NMR of the crude reaction mixture.

Table 4
Selected examples for screening the catalyst loading for the glycosidation.

12	9	9:12	Eq. of L.A.	Temp.	Time	Yield^d	α/β^b
m = 6	n = 6						
6	6	1.0	0.2	0 °C	6h	60%	6/1
7	7	1.0	1.0	-30 °C	24h	60%	6/1
7	7	1.0	0.2	0 °C	6h	38%	5/1
8	8	1.0	1.0	-30 °C	24h	59%	5/1
8	8	1.0	1.0	-30 °C	24h	54%	5/1
14	6	2.0	2.0	-30 °C	24h	61%	5/1
14	6	2.0	2.0	-30 °C	24h	41%	6/1
	6	2.0	3.0	-30 °C	24h	51%	5/1

^a Combined yield of α - and β -anomers.

^b Stereoselectivity estimated by ¹H NMR.