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Synthetic 6-*O*-Methylglucose-Containing Polysaccharides (*s*MGPs): Design and Synthesis

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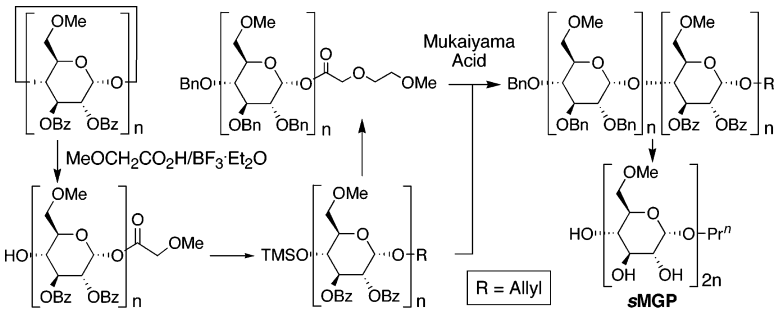

With the hope of mimicking the chemical and biological properties of *natural* 6-*O*-methyl-D-glucose-containing polysaccharides (MGPs), *synthetic* 6-*O*-methyl-D-glucose-containing polysaccharides (*s*MGPs) were designed and synthesized from α-, *β*-, and *γ*-cyclodextrins (CDs). The synthetic route proved to be flexible and general, to furnish a series of *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 precursors to the glycosyl donors and acceptors. The Mukaiyama glycosidation was adopted to couple the glycosyl donors with the glycosyl acceptors. Unlike in the 3-*O*-methyl-D-mannose-containing polysaccharide (*s*MMP) series, the amount of the Mukaiyama acid required in the *s*MGP series increased with an increase of substrate size; that is, 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 (MMPs) and 6-*O*-methyl-D-glucose-containing (lipo)polysaccharides (MG(L)Ps) (Figure 1).[2−4] 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 chemically well-defined and homogeneous materials and (2) synthetic polysaccharides should be structurally

(1) Hsu, M. C.-P.; Lee, J.; Kishi, Y. **2007**, *72*, 1931.

(2) For isolation and structural characterization of MMP, see: (a) Gray, G. R.; Ballou, C. E. *J. Biol. Chem.* **1971**, *246*, 6835. (b) Maitra, S. K.; Ballou, C. E. *J. Biol. Chem.* **1977**, *252*, 2459. (c) Weisman, L. S.; Ballou, C. E. *J. Biol. Chem.* **1984**, *259*, 3457. (d) Weisman, L. S.; Ballou, C. E. *J. Biol. Chem.* **1984**, *259*, 3464. (e) Ilton, M.; Jevans, A. W.; McCarthy, E. D.; Vance, D.; White, H. B., III; Bloch, K. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 87.

(3) For isolation and structural characterization of MGLP, see: (a) Lee, Y. C.; Ballou, C. E. *J. Biol. Chem.* **1964**, *239*, PC3602. (b) Saier, M. H., Jr.; Ballou, C. E. *J. Biol. Chem.* **1968**, *243*, 4332. (c) Smith, W. L.; Ballou, C. E. *J. Biol. Chem.* **1973**, *248*, 7118. (d) Forsberg, L. S.; Dell, A.; Walton, D. J.; Ballou, C. E. *J. Biol. Chem.* **1982**, *257*, 3555. Regarding the structural heterogeneity of MGP, Ballou commented that at least two forms of MGP containing 21 hexoses exist: Kamisango, K.; Dell, A.; Ballou, C. E. *J. Biol. Chem.* **1987**, *262*, 4580. Also see: Tuffal, G.; Albigot, R.; Monsarrat, B.; Ponthus, C.; Picard, C.; Rivière, M.; Puzo, G. *J. Carbohydr. Chem.* **1995**, *14*, 631.

(4) The structure of MG(L)P shown in Figure 1 is the revised structure suggested by Rivière based on the structure of polysaccharide isolated from Mycobacterium bovis BCG. See: Tuffal, G.; Albigot, R.; Rivière, M.; Puzo, G. *Glycobiology* **1998**, *8*, 675.

(5) For reviews on MMP and MGLP/MGP, see: (a) Bloch, K. *Adv. Enzymol.* **1977**, *45*, 1. (b) Ballou, C. E. *Acc. Chem. Res.* **1968**, *1*, 366. (c) Ballou, C. E. *Pure Appl. Chem.* **1981**, *53*, 107.

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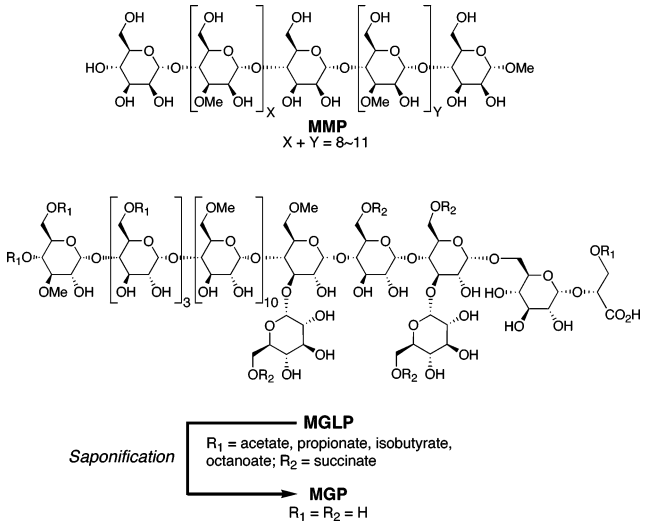


FIGURE 1. Structures of mycobacterial polysaccharides 3-*O*-methyl-D-mannose-containing polysaccharides (MMPs) and 6-*O*-methyl-D-glucose-containing lipopolysaccharides (MGLPs)/6-*O*-methyl-D-glucose-containing polysaccharides (MGPs).

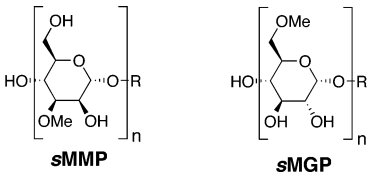


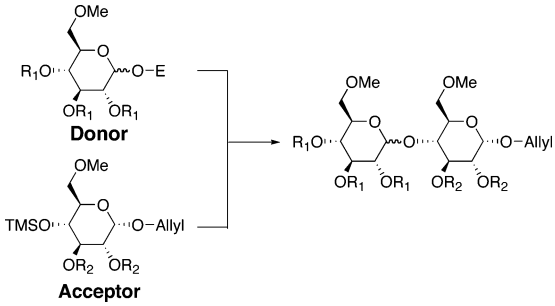
FIGURE 2. Structures of synthetic analogues of mycobacterial polysaccharides, *s*MMP and *s*MGP.

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*-pentenoyl 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 a stereoselective manner 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, as they reported the synthesis of glucose-containing linear oligosaccharides having α(1→4) and α(1→6) 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*MGPs). 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*MMPs).[1]

TABLE 1. Screening of Protection Groups of Glycosyl Donors and Acceptors[a]



entry	R_1	R_2	−E	reaction time (h)	yield (%)[b]	α/β ratio[c]
1	Bz	Bz	−CO−C_6H_4−CO_2Me-(*o*)	37	7 (β)	1:4
2	Bn	Bz	−CO−C_6H_4−CO_2Me-(*o*)	39	86 (α)	13:1
3	Bn	Bz	−CO−$CH_2O(CH_2)_2$OMe	4	81 (α)	16:1
4	Bn	Bn	−CO−$CH_2O(CH_2)_2$OMe	12	76 (α)	6:1

[a] Glycosidation conditions: 10 mol % of $AgClO_4$−$SnCl_4$, Et_2O, 0 °C. [b] Isolated yield. [c] Data based on ^{1}H NMR analysis.

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

(6) For examples, see the reviews cited in refs 9 and 10 in the preceding paper.

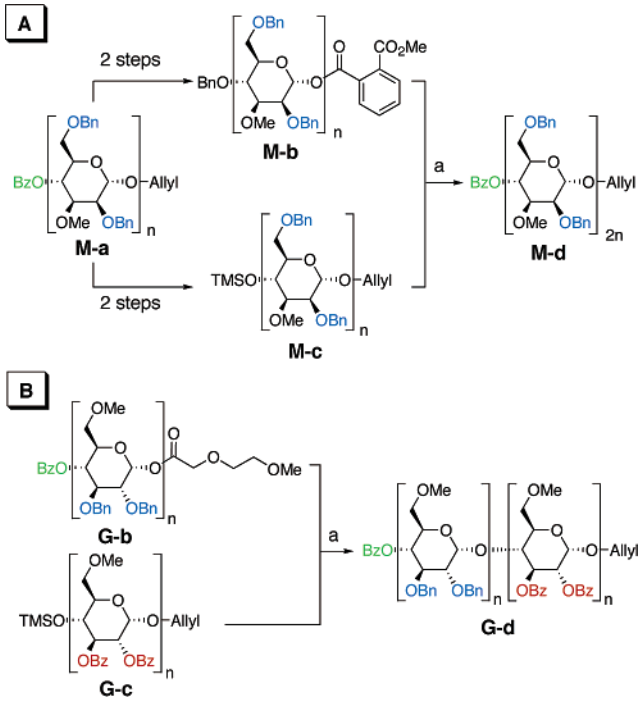
(7) For examples, see the syntheses cited in ref 11 in the preceding paper.

(8) Koto, S.; Haigoh, H.; Shichi, S.; Hirooka, M.; Nakamura, T.; Maru, C.; Fijita, M.; Goto, A.; Sato, T.; Okada, M.; Zen, S.; Yago, K.; Tomonaga, F. *Bull. Chem. Soc. Jpn.* **1995**, *68*, 2331.

(9) (a) Mukaiyama, T.; Takashima, T.; Katsurada, M.; Aizawa, H. *Chem. Lett.* **1991**, 533. (b) Mukaiyama, T.; Katsurada, M.; Takashima, T. *Chem. Lett*. **1991**, 985. (c) Mukaiyama, T.; Matsubara, K.; Sasaki, T.; Mukaiyama, T. *Chem. Lett.* **1993**, 1373.

(10) Wang, Y.; Cheon, H.-S.; Lee, J.; Kishi, Y., in preparation.

SCHEME 1. Divergent Synthetic Strategies of *s*MMP (Panel A) and *s*MGP (Panel B)

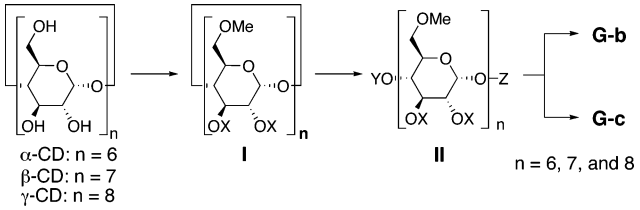


[a] Mukaiyama glycosidation: 10 mol % of $AgClO_4$−$SnCl_4$, Et_2O, 0 °C.

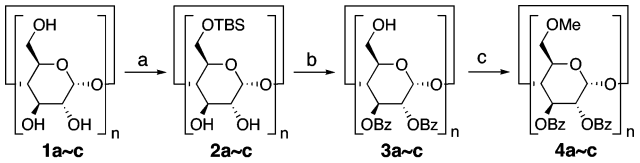
M-b and glycosyl acceptor **M-c**, and the donor and the acceptor were coupled under the modified Mukaiyama protocol, to furnish **M-d**. Importantly, except for *n*, **M-d** was identical to **M-a** and, therefore, it was not necessary to adjust the protecting groups for the next cycle. In the *s*MGP 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**). 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 (CDs).

CDs are naturally occurring cyclic oligosaccharides composed of α-(1→4)-linked D-glucose. α-, *β*-, and *γ*-CDs contain 6-, 7-, and 8-units of α-(1→4)-linked D-glucose, respectively. Assuming that the C6 hydroxyl groups of CDs can be selectively methylated (cf. α-, *β*-, and *γ*-CDs → **I**) and also that one α-(1→4) glycosidic bond of CDs can be cleaved preferentially over the α-(1→4) glycosidic bonds present in the cleaved products[11,12] (cf. **I** → **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.

SCHEME 2. Synthetic Plan for the Glycosyl Donor G-b and the Glycosyl Acceptor G-c from α-, *β*-, and *γ*-CDs



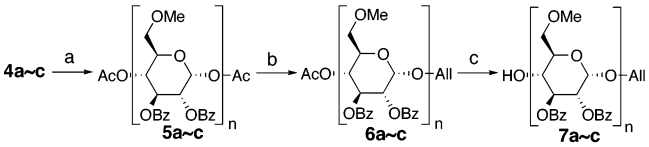
SCHEME 3 [a]



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

[a] 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) aqueous HF, CH_2Cl_2−CH_3CN, rt (**a** 83%; **b** 80%; **c** 82%); (c) Ag_2O, MeI, MS (4 Å), toluene, rt (**a** 85%; **b** 83%; **c** 81%).

SCHEME 4 [a]



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

[a] 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 Å), 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%).

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 was observed during benzoylation, desilylation, and O-methylation.[12,13]

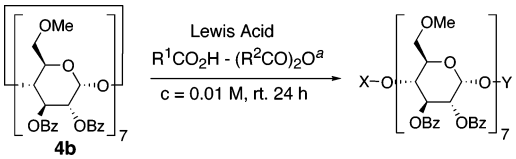
Our second task was to develop a reliable and scalable method to selectively cleave the α-glycosidic bond of CDs. As the cleaved product still contains six, seven, or eight glycosidic bonds, the proposed transformation could be problematic. Nonetheless, we hoped that the desired, first cleavage could 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, as 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

(11) (a) Fugedi, P. *Carbohydr. Res.* **1989**, *192*, 366. (b) Pregel, M. J.; Buncel, E. *Can. J. Chem.* **1991**, *69*, 130. (c) Takeo, K.; Uemura, K.; Mitoh, M. *J. Carbohydr. Chem.* **1998**, *7*, 293.

(12) **4a** was previously prepared by methylation (BF_3/CH_2N_2) of **3a**: see ref 11a.

(13) **3a** was previously prepared by controlled hydrolysis (^{i}PrOK/^{i}PrOH−benzene) of perbenzoylated α-CD. See: (a) Boger, J.; Corcoran, R. J.; Lehn, J. M. *Helv. Chim. Acta* **1978**, *61*, 2190. (b) Uccello-Barretta, G.; Cuzzola, A.; Balzana, F.; Menicagli, R.; Iuliano, A.; Salvadori, P. *J. Org. Chem.* **1997**, *62*, 827.

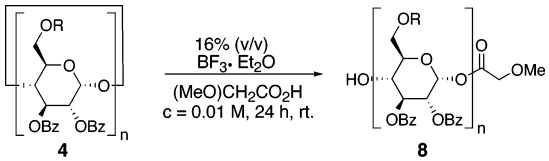
TABLE 2. Screening of the Selective Cleavage of β-CD Derivative 4b



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	$BF_3{\cdot}Et_2O$ (2%)	$(MeO)CH_2$	$(MeO)CH_2$	H	(MeO)Ac	ca. 20%
6	$BF_3{\cdot}Et_2O$ (2%)	$(MeO)CH_2$	none[d]	H	(MeO)Ac	ca. 25%
7	$BF_3{\cdot}Et_2O$ (4%)	$(MeO)CH_2$	none[d]	H	(MeO)Ac	ca. 30%
8	$BF_3{\cdot}Et_2O$ (12%)	$(MeO)CH_2$	none[d]	H	(MeO)Ac	ca. 90%
9	$BF_3{\cdot}Et_2O$ (16%)	$(MeO)CH_2$	none[d]	H	(MeO)Ac	ca. 97%

[a] The ratio of R^1CO_2H to $(R^2CO)_2O$ was 1:1 where the acid anhydride was used. [b] Conversion was estimated from ^1H NMR of the crude reaction mixture. [c] No desired product was found. [d] The acid anhydride was omitted.

TABLE 3. Substrate Scope of the Selective Cleavage Reaction of CD Derivatives



entry	*n*	R	conversion	crystalline precipitation	purification	isolated yield (%)
1	6	Me	ca. 85%[a]	no	chromatography	61
2	7	Me	ca. 97%[b]	yes	recrystallization	86
3	7	Et	ca. 97%[b]	yes	recrystallization	88
4	8	Me	ca. 97%[b]	yes	recrystallization	88

[a] Conversion was based on recovered starting material. The reaction mixture contains unidentified side products. [b] Conversion was estimated from ^1H NMR of the crude reaction mixture.

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 the 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 at C4 of the nonreducing 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 Ag_2O/allyl bromide/DMF, furnishing the α-allylglucosides as the major products (α/β ratio = ca. 7:1). We then ran into difficulty when selectively hydrolyzing the acetate at the nonreducing 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, and 16 benzoates for **a**, **b**, and **c**, respectively), it became more challenging to achieve this seemingly simple selective hydrolysis cleanly in an overall sense. Thus far, HBF_4 in MeOH/CH_2Cl_2 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 overhydrolysis (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 CF_3CO- or $MeOCH_2CO-$ and PhCO− should be more pronounced than the reactivity difference between MeCO− and PhCO−.[15] Thus, we attempted to cleave the CD **4b** under a variety of conditions (Table 2). After considerable trial-and-error efforts, we finally found that $BF_3{\cdot}Et_2O$-promoted cleavage of **4b** in the presence of methoxyacetic acid provides an ultimate solution.

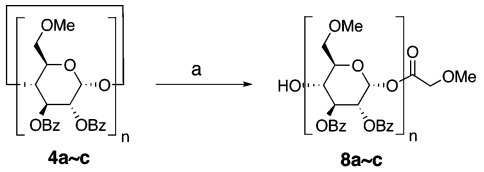
There are several appealing aspects for this cleavage reaction. First, as the reaction progressed, the cleaved product crashed out as a crystalline precipitate, recrystallization of which furnished the desired product (isolated as a 10:1 mixture of the α/β-anomers) in an excellent yield.[16] Second, 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

(14) For cleavage of peracylated cyclodextrins, see: (a) Sakari, N.; Wang, L.-X.; Kuzuhara, H. *J. Chem. Soc., Chem. Commun.* **1991**, 289. (b) Sakari, N.; Wang, L.-X.; Kuzuhara, H. *J. Chem. Soc., Perkin Trans. 1* **1995**, 437. (c) Sakari, N.; Matsui, K.; Kuzuhara, H. *Carbohydr. Res.* **1995**, *266*, 263. (d) Sakairi, N.; Kuzuhara, H. *Carbohydr. Res.* **1996**, *280*, 139.

(15) Green, T. W.; Wuts, P. G. *Protective Groups in Organic Synthesis*, 3rd ed.; John Wiley & Sons: New York, 1999; pp 160−166 and references cited therein.

(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**.

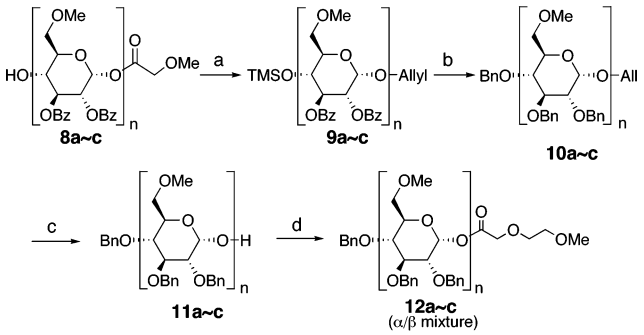
SCHEME 5 [a]



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

[a] Reagents and conditions: (a) α-CD **4a**: BF_3·Et_2O (12% v/v), $MeOCH_2CO_2H-CH_2Cl_2$ (12% v/v; [*C*] = 0.01 M, 40 °C, 1 h, 82% yield (recrystallization)). *β*-CD **4b** and *γ*-CD **4c**: see Table 3.

SCHEME 6 [a]



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

[a] 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 (4 Å), 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) $(Ph_3P)_3RhCl$, Dabco, EtOH/toluene/H_2O (6:3:1), 90 °C; (2) acetone−1 N 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%).

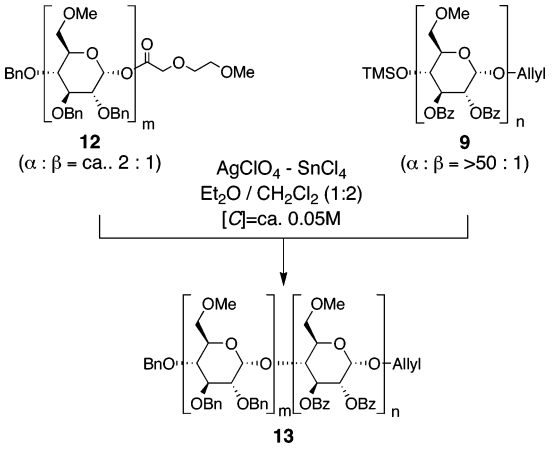
have the free hydroxyl group at C4 of the nonreducing end. Thus, unlike in the case of **6b** → **7b** (Scheme 4), it was unnecessary to face the problem associated with the selective hydrolysis.

We applied the BF_3·Et_2O/$MeOCH_2CO_2H$ 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: (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 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 cosolvent 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 nonreducing 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:

TABLE 4. Selected Examples for Screening the Catalyst Loading for the Glycosidation



12	**9**	**9/12**	equiv of Lewis acid	temp (°**C**)	time (h)	yield (%)[a]	α/*β*[b]
m = 6	*n* = 6	1.0	0.2	0	6	60	6:1
6	6	1.0	1.0	−30	24	60	6:1
7	7	1.0	0.2	0	6	38	5:1
7	7	1.0	1.0	−30	24	59	5:1
8	8	1.0	1.0	−30	24	54	5:1
8	8	2.0	2.0	−30	24	61	5:1
14	6	2.0	2.0	−30	24	41	6:1
14	6	2.0	3.0	−30	24	51	5:1

[a] Combined yield of α- and *β*-anomers. [b]Stereoselectivity estimated by ^{1}H NMR.

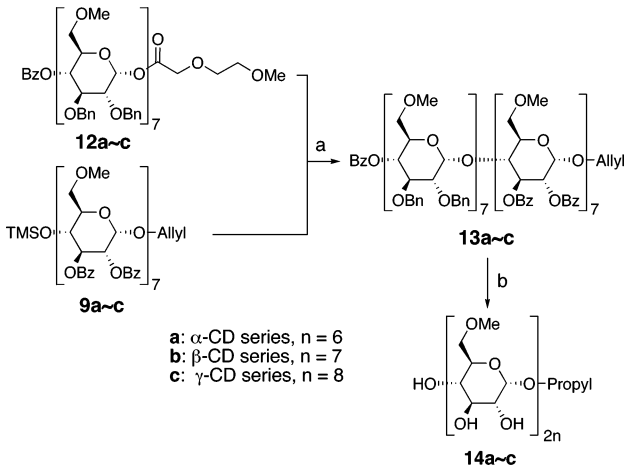
silylation of the C4 hydroxyl group at the nonreducing end, selective removal of the methoxyacetate group at C1 of the reducing end, and allylation of the resultant hemiacetal (Scheme 6). As described in the previous series, allylation of the resultant anomers in DMF furnished α-allylated products with a 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) → **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 $Et_2O-CH_2Cl_2$ mixture as the solvent, but the α/*β*-selectivity diminished from 16:1 (Et_2O) down to 6:1 (1:2 $Et_2O-CH_2Cl_2$). 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

SCHEME 7 [a]

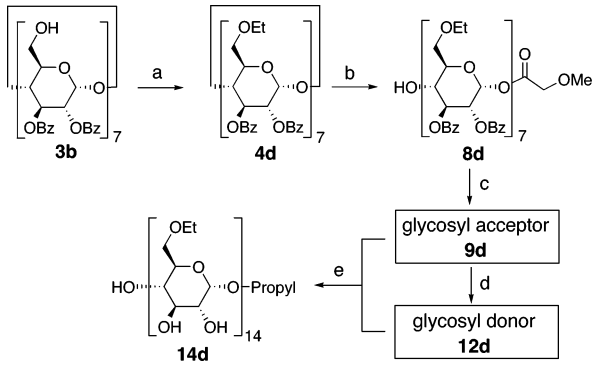


[a] Reagents and conditions: (a) 100 mol % of $[SnCl_3ClO_4]$, $CH_2Cl_2-Et_2O$ (2:1), −30 °C (**a** 49%; **b** 50%; **c** 51%); (b) (i) H_2, $Pd(OH)_2$ on C, THF, rt; (ii) NaOMe, CH_2Cl_2-MeOH (3:1), rt (**a** 86%; **b** 66%; **c** 68%).

from that in the *s*MMP series. Yet, we recognized another major difference: a catalytic amount (10 mol %) of the Lewis acid $[SnCl_3ClO_4]$ was sufficient to achieve a good conversion in the *s*MMP series, whereas a higher loading of the catalysis seemed to be 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 in comparison to that in the *s*MMP series accounts for the differences and conducted an optimization study. 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[17,18] was negligible in the *s*MGP series. In this series, we noticed byproduct formation from the donors[19] but found that this byproduct formation was significantly suppressed at −30 °C. With these modifications, we were able to achieve the glycosidation with satisfactory reproducibility.

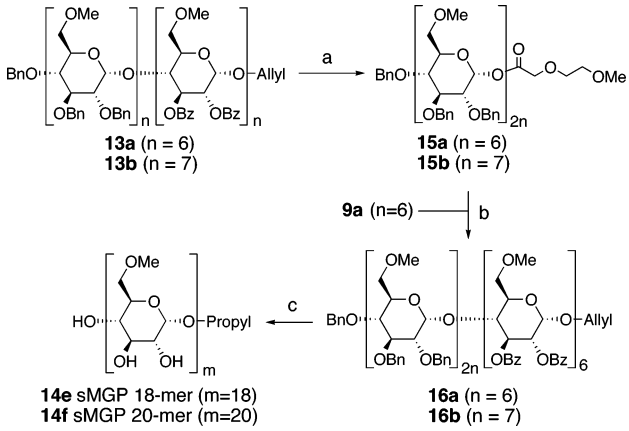
The glycosidation products were subjected to a two-step procedure of deprotection, and the *s*MGP 12-, 14-, and 16-mers were isolated by reverse-phase column chromatography on C_{18} silica gel (Scheme 7). To assess the purity of *s*MGP, extensive spectroscopic studies were conducted, thereby demonstrating

SCHEME 8 [a]



[a] Reagents and conditions: (a) iodoethane, Ag_2O, MS (4 Å), toluene, 75%; (b) follow the steps in Table 3; (c) follow step a in Scheme 6 (77% overall yield); (d) follow steps b−d in Scheme 6 (69% overall yield); (e) (1) 200 mol % of $[SnCl_3ClO_4]$, $CH_2Cl_2-Et_2O$ (2:1), −30 °C, 50%; (2) follow step b in Scheme 7 (54% overall yield). For the details, see Supporting Information.

SCHEME 9 [a]



[a] Reagents and conditions: (a) (1) (i) NaOMe, $MeOH-CH_2Cl_2$ (1:1), rt; (ii) NaH, BnBr, DMF, rt (**a** 70%; **b** 72%); (2) (i) follow steps c and d in Scheme 6 (**a** 57%, **b** 57%); (b) 300 mol % of $[SnCl_3ClO_4]$, $CH_2Cl_2-Et_2O$ (2:1), −30 °C (**a** 44%; **b** 43%); (c) follow step b in Scheme 7 (**14e** 58%; **14f** 64%).

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, to address the hydrophobic effect of polysaccharides for the complexation event with fatty acids, we pursued the synthesis of *s*MGP analogues composed of 6-*O*-ethyl-D-glucose **14d**. Gratifyingly, the same synthetic scheme as that 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, to test the size effect of *s*MGPs in the fatty acid biosynthesis catalyzed by FAS I, we wished to have a broad

(17) Following the same sequences as shown in Schemes 6 and 7, β-anomer enriched **9a**−**c** were converted to the β-anomer enriched *s*MGP **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 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) On the basis of the ^{1}H NMR analysis, the major byproduct appeared to be a benzyl glycoside of the donors.

(20) An extensive study on the ^{1}H 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 *s*EMG 14-mer series was made on the basis of the ^{1}H NMR analysis in comparison with the corresponding *s*MGP 14-mer series.

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, the amount of the Mukaiyama acid required in the *s*MGP series increased with an increase of substrate size, so 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 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. A. Transformation of 1a to 2a. To a solution of α-CD (**1a**, 10.0 g, 10.3 mmol), dried at 90 °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 °C. The mixture was stirred at room temperature, and the reaction was monitored by TLC every 12 h. Additional TBSCl (up to 0.2 × 6 equiv) 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 1 N HCl, aqueous $NaHCO_3$, 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.

B. Transformation of 2a to 3a. For the α-CD series, the following procedure of benzoylation 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 the reaction was monitored by NMR. The mixture was concentrated down to ca. 300 mL of pyr, to which was added aqueous $NaHCO_3$ (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 CH_2Cl_2 (300 mL) and was washed with 1 N HCl, aqueous $NaHCO_3$, and brine. After concentration, the product was recrystallized from EtOH (23.5 g, 95%).

C. Transformation of 2b,c to 3b,c. For the β- and γ-CDs series, the following procedure of benzoylation 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 the reaction was monitored by NMR. The mixture was concentrated by evaporation of pyr (400 mL), to which was added aqueous $NaHCO_3$ (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 CH_2Cl_2 (300 mL) and was washed with 1 N HCl, aqueous $NaHCO_3$, and brine. After concentration, the product was recrystallized from EtOH (26 g, 93%).

For the desilylation, the following procedure was 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 $CH_3CN-CH_2Cl_2$ (20 mL, 3:1) in a plastic reactor equipped with a stirring bar. To this mixture was added 48% aqueous HF (2.0 mL, 28 mmol) slowly at room temperature, and it was stirred for 2 h. The reaction mixture was diluted with CH_2Cl_2 (25 mL) and was poured into aqueous $NaHCO_3$ (100 mL). The aqueous phase was further extracted with CH_2Cl_2 (15 mL × 2). The combined organic phase was washed with brine and dried over Na_2SO_4. The product was purified by flash chromatography (eluent: CH_2Cl_2/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.

D. Transformation of 3a to 4a. To a solution of 2,3-di-*O*-benzoyl-α-CD **3a** (890 mg, 0.40 mmol) in toluene−CH_2Cl_2 (40 mL, 3:1) were added MeI (1.2 mL, 19 mmol), Ag_2O (1.7 g, 7.2 mmol), $NaHCO_3$ (0.20 g, 2.4 mmol), and crushed 4 Å molecular sieves (3.3 g, dried). The resulting slurry was sonicated for 12 h and stirred for 8 h, after which an additional portion of Ag_2O (0.6 g) and 4 Å molecular sieves (1 g) were added. Sonication was resumed for a further 8 h. The slurry was filtered over Celite, rinsing with CH_2Cl_2 and CH_2Cl_2−EtOAc (2:1) successively. The filtrate was reduced in vacuo and purified by flash chromatography on silica gel (eluent: CH_2Cl_2/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.

E. Spectroscopic Data for 4a: ^{1}H NMR δ 3.56 (s, 3 × 6 H), 3.85 (d, J = 10.0 Hz, 1 × 6 H), 4.16 (dd, J = 11, 4.0 Hz, 1 × 6 H), 4.19 (dd, J = 8.5, 8.5 Hz, 1 × 6 H), 4.50 (dd, J = 9.5, 3.5 Hz, 1 H), 5.04 (dd, J = 11.0, 4.0 Hz, 1 × 6 H), 5.44 (d, J = 3.5 Hz, 1 × 6 H), 6.16 (t, J = 8.5 Hz, 1 H), 6.83 (t, J = 8.0 Hz, 2 × 6 H), 6.90 (t, J = 7.5 Hz, 2 × 6 H), 7.11 (t, J = 8.0 Hz, 1 × 6 H), 7.15 (t, J = 8.0 Hz, 1 × 6 H); ^{13}C 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) calcd for ($C_{126}H_{120}O_{42}$-Na^+) 2327.71, found 2327.9; $[\alpha]^{25}_D$ +94.8 (*c* 0.26, $CHCl_3$).

F. Spectroscopic Data for 4b: ^{1}H NMR δ 3.55 (s, 3 × 7 H), 3.81 (d, J = 11.0 Hz, 1 × 7 H), 4.18 (dd, J = 9.5 Hz, 1 × 7 H), 4.22 (dd, J = 11.0, 3.5 Hz, 1 × 7 H), 4.44 (m, 1 × 7 H), 5.04 (dd, J = 11.0, 3.5 Hz, 1 × 7 H), 5.50 (d, J = 3.5 Hz, 1 × 7 H), 5.95 (dd, J = 9.5, 9.5 Hz, 1 × 7 H), 6.96 (m, 4 × 7 H), 7.22 (m, 4 × 7 H), 7.46 (m, 4 × 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) calcd for ($C_{147}H_{140}O_{49}$-Na^+) 2711.84, found 2711.8; $[\alpha]^{25}_D$ +70.5 (*c* 0.33, $CHCl_3$).

G. Spectroscopic Data for 4c: ^{1}H NMR δ 3.52 (s, 3 × 8 H), 3.78 (d, J = 10.0 Hz, 1 × 8 H), 4.10 (m, 1 × 8 H), 4.27 (dd, J = 9.5, 9.5 Hz, 1 × 8 H), 4.32 (m, 1 × 8 H), 5.07 (dd, J = 10.5, 3.5 Hz, 1 × 8 H), 5.54 (d, J = 3.5 Hz, 1 × 8 H), 5.90 (dd, J = 8.5, 8.5 Hz, 1 × 8 H), 6.99 (t, J = 8.0 Hz, 2 × 8 H), 7.07 (t, J = 8.0 Hz, 2 × 8 H), 7.17 (t, J = 7.15 Hz, 1 × 8 H), 7.30 (d, J = 7.5 Hz,

(23) With use of the reported synthetic route, *s*MGP 6-, 7-, 8-, 10-, 12-, 14-, 16-, 18-, and 20-mers were synthesized. For details, see Supporting Information.

(24) (a) Cheon, H. S.; Wang, Y.; Ma, J.; Kishi, Y. *ChemBioChem.*, in press. (b) Papaioannou, N.; Cheon, H.-S.; Kishi, Y., in preparation.

(25) Cheon, H.-S.; Kishi, Y., in preparation.

1 × 8 H), 7.56 (m, 4 × 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) calcd 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. A.Transformation of 4a to 8a. To a solution of **4a** (5.20 g, 2.24 mmol) in CH_2Cl_2 (210 mL) at room temperature were added methoxyacetic acid (33 mL) and BF_3·OEt_2 (33 mL), and the mixture was warmed to 40 °C and stirred for 1 h. The mixture was diluted with CH_2Cl_2 and poured into aqueous $NaHCO_3$ (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%, α/β = ca. 10): ^{1}H 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) calcd for ($C_{129}H_{126}O_{45}Na^+$) 2417.74, found 2418.0; $[\alpha]^{25}_D$ +78.1 (*c* 0.43, $CHCl_3$).

B. 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·OEt_2 (47 mL) at room temperature. After approximately 2 h of stirring, the starting material dissolved completely, followed by the 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 × 100 mL). The solid was taken up with CH_2Cl_2 (200 mL) and washed with aqueous $NaHCO_3$ (2 × 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%, α/β = ca. 3): ^{1}H 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) calcd for ($C_{150}H_{146}O_{52}Na^+$) 2801.87, found 2801.9; $[\alpha]^{25}_D$ +75.6 (*c* 0.31, $CHCl_3$).

C. Transformation of 4c to 8c. Following the procedure given for **4b** → **8b**, **4c** (3.80 g, 12.5 mmol) was converted to **8c** (3.46 g, 88%, α/β = ca. 2): ^{1}H 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) calcd 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. A. Transformation of 8a to 9a. To a solution of **8a** (4.50 g, 1.89 mmol) and Et_3N (1.7 mL, 11.4 mmol) in CH_2Cl_2 (100 mL) at 0 °C was added TMSOTf (1.05 mL, 5.67 mmol) slowly. The solution was gradually warmed to room temperature and was stirred for 1 h. Workup with CH_2Cl_2/aqueous $NaHCO_3$ was done. 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 CH_2Cl_2 (60 mL) was added ethanolamine (1.04 mL, 17.3 mmol) at room temperature, and the mixture was stirred for 5 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL), poured into water (100 mL), and partitioned. The organic phase was washed with brine and dried over Na_2SO_4. Flash chromatography (eluent: CH_2Cl_2/EtOAc) yielded the glycoside (3.85 g, 92%, α/β = ca. 2).

B. 9a (α-Anomer Enriched). To a solution of the glycoside thus obtained (2.00 g, 0.842 mmol) in DMF (60 mL) were added Ag_2O (1.56 g, 6.47 mmol), crushed 4 Å MS (activated, 2.0 g), allyl bromide (0.73 mL, 8.42 mmol), and $NaHCO_3$ (0.42 g, 5.05 mmol). The dark suspension was stirred for 18 h. The slurry was filtered over Celite, rinsing with CH_2Cl_2 and CH_2Cl_2−EtOAc (2:1) successively. The filtrate was reduced in vacuo and purified by flash chromatography on silica gel (eluent: CH_2Cl_2/EtOAC) to give the product **9a** as a white solid (2.03 g, 92%, α/β = ca. 7). The anomeric mixture (1.5 g) was loaded on a Biotage column (40 + M, ϕ = 4.0 cm) and eluted by EtOAc/$CHCl_3$ with a linear gradient from 4% to 20% (1.2 L), to furnish the α-anomer (1.0 g, α/β = ca. 100:1) and the β-enriched anomeric mixture (0.34 g, α/β = ca. 1:2). Spectroscopic data for **9a** (α/β = ca. 100:1): ^{1}H 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 × 2 H), 3.56 (s, 3 H); the reducing end anomeric-*H* 5.26 (d, *J* = 3.4 Hz, 1 H); MS (MALDI-TOF) calcd for ($C_{132}H_{134}O_{43}SiNa^+$) 2457.80, found 2457.9; $[\alpha]^{25}_D$ +68.8 (*c* 0.28, $CHCl_3$).

C. 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** (α/β = ca. 100:1) were obtained by using the same chromatographic method.

D. Spectroscopic Data for 9b (α-Anomer Enriched): TMS-*H* δ 0.00 (s, 9 H); seven methoxy-*H* 3.30 (s, 3 × 3 H), 3.31 (s, 3 H), 3.34 (s, 3 H), 3.44 (s, 3 × 2 H), 3.56 (s, 3 H); the reducing end anomeric-*H* 5.26 (d, *J* = 3.4 Hz, 1 H); MS (MALDI-TOF) calcd for ($C_{153}H_{154}O_{50}SiNa^+$) 2841.92, found 2881.9; $[\alpha]^{25}_D$ +76.4 (*c* 0.38, $CHCl_3$).

E. Spectroscopic Data for 9c (α-Anomer Enriched): ^{1}H 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 × 3 H), 3.34 (s, 3 H), 3.44 (s, 3 × 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) calcd for ($C_{153}H_{154}O_{50}SiNa^+$) 3226.04, found 3226.0; $[\alpha]^{25}_D$ +78.0 (*c* 0.45, $CHCl_3$).

F. 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 a mixture of 1 N HCl/acetone (40 mL, 1:9), and the mixture was stirred at room temperature for 1 h. Then the mixture was reduced in vacuo, and the residue was taken with CH_2Cl_2 (50 mL) and washed with $NaHCO_3$ and brine successively. After drying over Na_2SO_4, solvents of the organic phase were exchanged with CH_2Cl_2−CH_3OH (30−15 mL).

To the resultant residue was added NaOMe (400 mg, 7.40 mmol). The mixture was stirred at room temperature 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 room temperature for 30 min before benzyl bromide (3.13 mL, 26.4 mmol) was added. The mixture was stirred at room temperature for 21 h. After quenching by 5 mL of methanol at 0 °C, the mixture was taken with CH_2Cl_2 (100 mL) and rinsed with 1 N HCl, aqueous $NaHCO_3$, and brine. Flash chromatography on silica gel (eluent: benzene/EtOAc) afforded **10a** (1.20 g, 77%): ^{1}H 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-C*H*$_2$ 5.22 (dd, *J* = 11.0, 1.5 Hz, 1 H), 5.36 (dd, *J* = 17.0, 1.5 Hz, 1 H); six a-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) calcd for ($C_{136}H_{160}O_{34}Na^+$) 2308.05, found 2307.9; $[\alpha]^{25}_D$ +72.0 (*c* 0.23, $CHCl_3$).

G. Spectroscopic Data for 10b: ^{1}H 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-C*H*$_2$ 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) calcd for ($C_{157}H_{180}O_{36}Na^+$) 2664.21, found 2664.3; $[\alpha]^{25}_D$ +75.3 (*c* 0.15, $CHCl_3$).

H. Spectroscopic Data for 10c: ^{1}H 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-C*H*$_2$ 5.21 (dd, *J* = 11.0, 1.5 Hz, 1 H), 5.36 (dd, *J* = 17.0, 1.5 Hz, 1 H); eight a-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) calcd for ($C_{178}H_{204}O_{41}Na^+$) 3020.38, found 3020.1; $[\alpha]^{25}_D$ +68.3 (*c* 0.43, $CHCl_3$).

I. 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 $(Ph_3P)_3RhCl$ (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 1 N 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 CH_2Cl_2 and washed with aqueous $NaHCO_3$ and brine. Flash chromatography on silica gel (eluent: CH_2Cl_2/EtOAc) afforded **11a** (0.65 g, 82%, α/β = ca. 2:1): ^{1}H NMR (500 MHz) characteristic peaks for α-anomer, the reducing end C1 O*H* 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) calcd for ($C_{133}H_{152}O_{31}$-Na^+) 2268.02, found 2268.4; $[\alpha]^{25}_D$ +57.5 (*c* 0.40, $CHCl_3$).

J. Spectroscopic Data for 11b (α/β = ca. 2:1): ^{1}H NMR (500 MHz) characteristic peaks for α-anomer, the reducing end C1 O*H* 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) calcd for ($C_{154}H_{176}O_{36}$-Na^+) 2624.18, found 2624.2; $[\alpha]^{25}_D$ +79.1 (*c* 0.18, $CHCl_3$).

K. Spectroscopic Data for 11c (α/β = ca. 2:1): ^{1}H NMR (500 MHz) characteristic peaks for α-anomer, the reducing end C1 O*H* 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) calcd for ($C_{175}H_{200}O_{41}Na^+$) 2980.35, found 2980.2; $[\alpha]^{25}_D$ +79.3 (*c* 0.19, $CHCl_3$).

L. 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 CH_2Cl_2 (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 room temperature for 2 h. The mixture was diluted with CH_2Cl_2 (20 mL) and washed with aqueous $NaHCO_3$ and brine. Flash chromatography on silica gel (eluent: CH_2Cl_2/EtOAc) afforded **12a** as a colorless solid (0.50 g, 96%, α/β = ca. 2): ^{1}H 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 × 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) calcd for ($C_{138}H_{160}O_{34}Na^+$) 2384.07, found 2384.2; $[\alpha]^{25}_D$ +80.1 (*c* 0.23, $CHCl_3$).

M. Spectroscopic Data for 12b (α/β = ca. 2:1): ^{1}H 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 × 2 H), 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) calcd for ($C_{159}H_{184}O_{39}Na^+$) 2740.23, found 2740.0; $[\alpha]^{25}_D$ +81.2 (*c* 0.26, $CHCl_3$).

N. Spectroscopic Data for 12c (α/β = ca. 2:1): ^{1}H 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 × 2 H), 3.41 (s, 3 H); eight a-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) calcd for ($C_{180}H_{208}O_{44}Na^+$) 3096.39, found 3095.8; $[\alpha]^{25}_D$ +83.2 (*c* 0.25, $CHCl_3$).

Glycosidations Summarized in Scheme 7. A. General Note on Glycosidation Reactions. Precautions: for handling of anhydrous $AgClO_4$, see refs 26a,b.[b] All solvents were freshly distilled prior to use (CH_2Cl_2 from CaH_2; Et_2O from $LiAlH_4$) and transferred via cannula into the solvent flasks. All flasks were flame dried just before use. Syringes, septa, needles, $AgClO_4$ (placed in a pear-shaped flask equipped with a stirrer bar, septum, and needle), aluminum foil, stoppers, and plastic bags were dried over P_2O_5/Drierite in a desiccator in vacuo overnight. The starting materials were also dried over P_2O_5 under reduced pressure overnight. After all the reagents, solvents, and the desiccator were placed in a glove bag, the atmosphere was exchanged three times with nitrogen (introduced through Drierite plug).

B. Glycosidation to Form 13a. To $AgClO_4$ (52 mg, 0.25 mmol) in a 10 mL pear-shaped flask equipped with a stirring bar was added Et_2O (5 mL). This suspension was stirred for 20 min, and the flask was wrapped with aluminum foil to avoid light. To the $AgClO_4$ suspension was added $SnCl_4$ (30 mL, 0.25 mmol) slowly, and the mixture was stirred for an additional 20 min. The suspension was then left to stand for 5 min 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 CH_2Cl_2 (0.36 mL). 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 the outer atmosphere. They were cooled to −30 °C in a cryobath. After allowing for thermal equilibration, the starting material solution was added slowly over 10 min and the solution was stirred for 24 h. The reaction mixture was quenched with aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (2 mL), dried over Na_2SO_4, and reduced in vacuo. The crude mixture was stirred in 1 N HCl/acetone (10 mL, 1:9) for 1 h at room temperature to desilylate the unreacted **9a** (20 mg, 43% recovered). The product (α/β selectivity of glycosidation = ca. 5.5:1 by the ^{1}H NMR spectrum) was purified by two successive flash chromatographies on silica gel. The first chromatography (hexanes/THF) was used to remove the byproduct, and the second chromatography (CH_2Cl_2/EtOAc) was used to isolate the desired α-glycosidation product (isolated α-isomer: 43 mg; 48%): ^{1}H 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 × 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) calcd for ($C_{262}H_{276}O_{73}Na^+$) 4612.78, found 4612.3; $[\alpha]^{25}_D$ +72.9 (*c* 0.48, $CHCl_3$).

C. 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%): ^{1}H 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 × 2 H), 3.316 (s, 3 H), 3.321 (s, 3 H), 3.327(s, 3 H), 3.358 (s, 3 × 3H), 3.364 (s, 3 H), 3.408 (s, 3 H), 3.483 (s, 3 H), 3.647 (s, 3 H); MS (MALDI-TOF) calcd for ($C_{304}H_{320}O_{85}Na^+$) 5353.06, found 5353.1; $[\alpha]^{25}_D$ +76.3 (*c* 0.66, $CHCl_3$).

D. 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%): ^{1}H 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 × 7 H), 3.360 (s, 3 × 5 H), 3.407 (s,

(26) (a) Perrin, D. D.; Armarego, W. L. F. *Purification of Laboratory Chemicals*, 3rd ed.; Pergamon: New York, NY, 1988. (b) *Encyclopedia of Reagents for Organic Synthesis*; Paquette, L. A., Ed.; John Wiley & Son: New York, NY, 1995.

3 H), 3.481 (s, 3 H), 3.646 (s, 3 H); MS (MALDI-TOF) calcd for ($C_{346}H_{364}O_{97}Na^+$) 6093.34, found 6093.6; $[\alpha]^{25}_D$ +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. A. 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% $Pd(OH)_2$ on carbon (10 mg). The mixture was equipped with a H_2 balloon and 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 room temperature for 12 h and neutralized by adding 1 N HCl (0.1 mL, 0.1 mmol). After evaporation of solvents, the residue was subjected to reverse-phase C_{18}-column chromatography (eluent: H_2O/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: MeOH/H_2O): ^{1}H NMR (D_2O, 500 MHz) δ 0.90 (t, J = 7.5 Hz, 3 H), 1.61 (m, 2 H), 3.38 (s, 3 × 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) calcd for ($C_{87}H_{152}O_{61}Na^+$) 2195.87, found 2196.0; $[\alpha]^{25}_D$ +213 (*c* 0.28, H_2O).

B. 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%)): ^{1}H NMR (D_2O, 500 MHz) δ 0.89 (t, J = 7.5 Hz, 3 H), 1.60 (m, 2 H), 3.37 (s, 3 × 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) calcd for ($C_{101}H_{176}O_{71}Na^+$) 2548.01, found 2548.2; $[\alpha]^{25}_D$ +203 (*c* 0.29, H_2O).

C. 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%)): ^{1}H NMR (D_2O, 500 MHz) 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) calcd 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 *s*MGP 12-, 14-, and 16-mers were prepared. For details, see Supporting Information.

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

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Supporting Information Available: Synthetic procedures and spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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