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Highly Stereoselective and Iterative Synthesis of α-(1→4)-Linked Polysaccharides Composed of 3-*O*-Methyl-D-mannose

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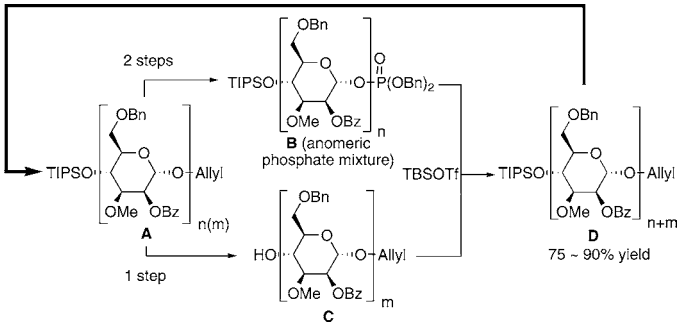
ABSTRACT



A second-generation synthesis of synthetic 3-*O*-methyl-D-mannose-containing polysaccharides (*s*MMPs) is reported. The glycosidation of donor B and acceptor C, prepared from a common precursor A in two and one steps, respectively, is effected by *t*-butyldimethylsilyl trifluoromethanesulfonate to furnish only the desired α-anomer D in high yields. Unlike the first-generation synthesis, this synthesis gives the desired product free from contamination of scrambling products. A three-step protocol is used to deprotect D to furnish *s*MMPs.

Fatty acid (FA) biosynthesis in *Mycobacterium smegmatis* is known to show a bimodal product distribution, with palmitic and tetracosanoic acids being the two dominant products. Bloch and co-workers discovered that two endogenous *M. smegmatis* 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), have profound effects on the FA biosynthesis catalyzed by *M. smegmatis* fatty acid synthase I (FAS I), most noticeably on the product distribution and stimulation.[1] We are interested in gaining mechanistic insight into 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 are isolated as complex mixtures of closely related polysaccharides.[1] Thus, we designed and used synthetic polysaccharides structurally related to natural MMP and MG(L)P for two reasons: (1) synthetic polysaccharides can be available as structurally well-defined and chemically homogeneous materials and (2) synthetic polysaccharides can be structurally tunable for the needs of our systematic investigation. Obviously, the most unique structural feature of natural MMP and MG(L)P is the polymeric form of 3-*O*-methyl mannose and 6-*O*-methyl glucose, respectively. Therefore, we incorporated this struc-

(1) For isolation and characterization of MMPs, see (a) Gary, G. R.; Ballou, C. E. *J. Biol. Chem.* **1971**, *246*, 6835. (b) 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. (c) Maitra, S. K.; Ballou, C. E. *J. Biol. Chem.* **1977**, *252*, 2459. For isolation and characterization of MGLPs, see (d) Lee, Y. C.; Ballou, C. E. *J. Biol. Chem.* **1964**, *239*, PC3602. (e) Forsberg, L. S.; Dell, A.; Walton, D. J.; Ballou, C. E. *J. Biol. Chem.* **1982**, *257*, 3555. For the revised structure of MG(L)P, see (f) Tuffal, G.; Albigot, R.; Rivière, M.; Puzo, G. *Glycobiology* **1998**, *8*, 675.

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tural feature in the synthetic polysaccharides, that is, synthetic MMPs (*s*MMPs) and synthetic MGPs (*s*MGPs) (see Figure 1).[2]

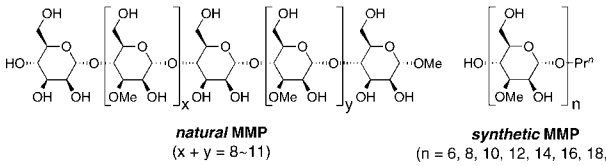
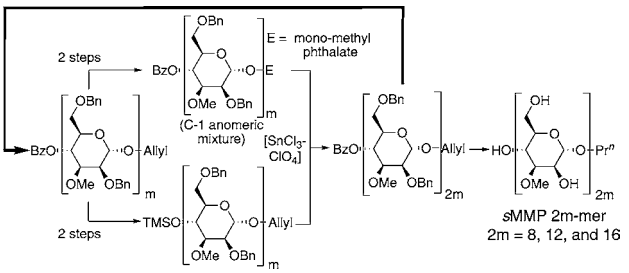


Figure 1. Structures of *natural* and *synthetic* MMPs.

We recently reported a first-generation synthesis of *s*MMPs.[2a] With slight modifications of the Mukaiyama glycosidation,[3] high α-selectivity (>50:1 to >20:1) and yields (74−79%) were achieved for the key glycosidation steps (Scheme 1). The observed, exceptionally high α-selectivity was due to the stereospecific anomerization of β- to α-anomer under the glycosidation conditions. This glycosidation was well suited for a highly convergent oligosaccharide synthesis, particularly because of excellent chemical yields even when using equal- or similar-sized donors and acceptors in an approximately 1:1 molar ratio. Thus, an iterative sequence allowed the growing oligosaccharide to double in size after each cycle and led to an efficient synthesis of *s*MMP 8-, 12-, and 16-mers. With the use of these *s*MMPs, we were able to demonstrate that *s*MMPs mimic the chemical and biological roles of *natural* MMP.[4,5]

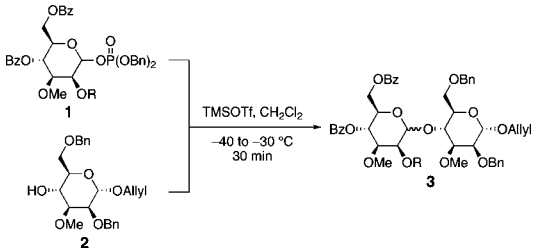
Scheme 1. First Generation of Iterative *s*MMP Synthesis



With this exciting result in hand, we then realized that it had become critically important to secure the supply of a relatively large quantity of *s*MMPs for further studies. In this connection, we were anxious to address two specific issues regarding the glycosidation in the first-generation synthesis. First, as mentioned, the exceptionally high stereoselectivity of glycosidation was due to the stereospecific anomerization of the undesired β-anomers to the desired α-anomers. This beneficial anomerization was compromised by the fact that the glycosidic bonds were susceptible to random cleavages, thereby resulting in the product being contaminated with scrambled polysaccharides.[2a] Second, the glycosidation in the presence of the Mukaiyama catalyst presented technical difficulties in terms of scalability and reproducibility, particularly for the synthesis of larger oligosaccharides.[2]

Clearly, our first priority was to identify a glycosidation without scrambling. Using the monomeric substrates shown in Table 1, we screened a variety of the glycosidation methods reported in the literature.[6] Through this screening, the glycosidation via an anomeric phosphate, originally reported by Hashimoto, Honda, and Ikegami,[7,8] emerged as the most promising candidate; in particular, we were encouraged with the mildness of the glycosidation conditions (activator, reaction temperature, and reaction time). With the donor bearing a benzoate at C2, the desired α-anomer was obtained in high yields from both α- and β-anomeric phosphates. A ^{1}H NMR analysis indicated that no undesired β-anomer was formed in the glycosidation.[9] This high

Table 1. Glycosidation of α- and β-Anomeric Phosphates



	donor (**1**)		glycosidation	
entry	R	stereochemistry (C1)	yield	selectivity (α:β)
1	Bn	α	92%	1.3:1
2	Bn	β	93%	1.2:1
3	Bz	α	90%	>20:1
4	Bz	β	91%	>20:1

(2) (a) Hsu, M. C.; Lee, J.; Kishi, Y. *J. Org. Chem.* **2007**, *72*, 1931. (b) Meppen, M.; Wang, Y.; Cheon, H.-S.; Kishi, Y. *J. Org. Chem.* **2007**, *72*, 1941.

(3) (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.

(4) Cheon, H.-S.; Wang, Y.; Ma, J.; Kishi, Y. *ChemBioChem* **2007**, *8*, 353.

(5) Papaioannou, N.; Cheon, H.-S.; Lian, Y.; Kishi, Y. *ChemBioChem*, in press.

(6) For comprehensive monographs, general reviews, and examples relevant to this work, see references 9, 10, and 11 cited in reference 2a.

(7) Hashimoto, S.; Honda, T.; Ikegami, S. *J. Chem. Soc., Chem. Commun.* **1989**, 685.

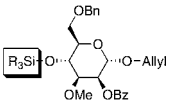
(8) Seeberger has extensively used anomeric phosphates for both solution- and solid-phase syntheses. For examples of solution-phase synthesis, see (a) Plante, O. J.; Andrade, R. B.; Seeberger, P. H. *Org. Lett*. **1999**, *1*, 211. (b) Plante, O. J.; Palmacci, E. R.; Andrade, R. B.; Seeberger, P. H. *J. Am. Chem. Soc*. **2001**, *123*, 9545. (c) Codee, J. D. C.; Seeberger, P. H. *ACS Symp. Ser*. **2007**, *960*, 150−164 and references cited therein. For recent examples of solid-phase synthesis, see (d) Plante, O. J.; Palmacci, E. R.; Seeberger, P. H. *Science* **2001**, *291*, 1523. (e) Werz, D. B.; Castagner, B.; Seeberger, P. H. *J. Am. Chem. Soc*. **2007**, *129*, 2770 and references cited therein.

(9) The stereoselectivity was determined from the ^{1}H NMR spectrum of the crude product; only the desired α-anomer and unreacted acceptor were detected. The stereochemistry of the coupled product was assigned by nuclear Overhauser effect studies.

selectivity can be attributed to a neighboring group participation of the C2 benzoate during glycosidation.[10] Thus, unlike the previous synthesis, it is unnecessary to rely on the anomerization to enhance the overall stereoselectivity of glycosidation.

In order to adopt the phosphate-based glycosidation for the iterative synthesis of *s*MMPs, we needed to use orthogonal protecting groups for the C2, C4, and C6 hydroxyl groups. To achieve a highly stereoselective glycosidation, we chose to use a benzoyl (or an acyl) group for the C2 protecting group of the donor. We also recognized two benefits of protecting the C2 hydroxyl group of the acceptor with the same acyl group. First, because of the electronic effect, we anticipated that the C2 acyl group should suppress the formation of an oxonium ion, leading to the undesired process of scrambling. Second, except for *n*(*m*), the structure of the product after each iteration is identical to the structure of the starting material, and therefore it is not necessary to adjust the protecting groups after each iteration. Because of its stability and accessibility, we decided to use the benzyl group for the protection at the C6 hydroxyl. Considering that these protecting groups should be orthogonal, we focused on a silyl ether for the C4 protecting group and conducted a stability test for *t*-butyldimethylsilyl (TBS), triisopropylsilyl (TIPS), and *t*-butyldiphenylsilyl (TBDPS) under the deallylation and glycosidation conditions. As seen from the summary given in Table 2, a TIPS group meets our needs

Table 2. Screening for C4-OH Protecting Group



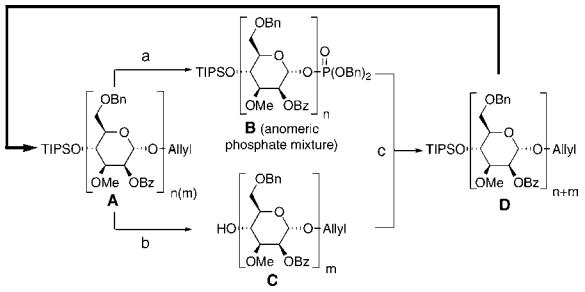
A

reaction conditions		R$_3$Si: ^{t}BuMe$_2$Si (TBS)	^{i}**Pr$_3$Si** (TIPS)	^{t}BuPh$_2$Si (TBDPS)
deallylation[a]	H_3O^+, heat	desilylation	desilylation	desilylation
	$HgCl_2$−HgO	stable	stable	n.d.[b]
	I_2	stable	**stable**	n.d.[b]
glycosidation	TMSOTf	desilylation	desilylation	desilylation
(CH_2Cl_2, −40 °C)	TESOTf	desilylation	desilylation	n.d.[b]
	TBSOTf	desilylation	**stable**	n.d.[b]

[a] Deallylation was performed in two steps, i.e., (1) Rh-catalyzed double-bond isomerization and (2) hydrolysis of the resulting enol ether. The reaction conditions indicated are concerned with the second step. [b] n.d.: not determined.

well; it is stable under the deallylation (Rh-catalyzed double-bond isomerization, followed by I_2 treatment[11]) and the glycosidation (using *t*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf),[12] instead of trimethylsilyl trifluoromethanesulfonate (TMSOTf), as the activator). We anticipated TBDPS to meet our needs as well, but did not see an apparent advantage in using TBDPS over a TIPS group. With these analyses, we decided to use the substrate **A** with R_3Si = TIPS as the starting material, which was prepared in six straightforward steps from D-mannose.[13]

Table 3. Synthesis of Protected *s*MMPs via Iterative Elongation[a]



n	m	$n+m$	glycosidation[b] isolated yield (α only)
1	1	2	90%
2	2	4	87%
4	2	6	85%
4	4	8	83%
8	2	10	80%
8	4	12	78%
8	6	14	83%
8	8	16	75%
12	6	18	80%
12	8	20	76%

[a] Reagents and conditions: (a) (1) $(Ph_3P)_3RhCl$, dabco, EtOH−toluene, reflux, followed by treatment with I_2, THF−CH_2Cl_2−H_2O, 80−88%. (2) $Et_2NP(OBn)_2$, 1*H*-1,2,4-triazole, $NaHCO_3$, CH_2Cl_2, followed by workup with 30% H_2O_2, THF−CH_2Cl_2, 83−89%. (b) TBAF, THF, 83∼92%; (c) TBSOTf, −40 → −30 °C, 30 min, CH_2Cl_2. [b] For all the cases, the α/β-selectivity of glycosidation was found to be >20:1.

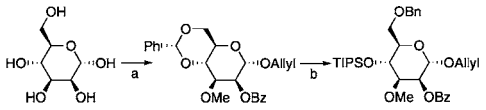
Table 3 summarizes the iterative synthesis of various sizes of the protected *s*MMPs. To our delight, transformations to donors[14] and acceptors, and their glycosidation at each iteration, proceeded smoothly, thereby demonstrating the generality of this synthetic approach. All the protecting groups worked as designed and behaved orthogonally during functional group manipulations. Donor **B** and acceptor **C**,

(10) There are a number of examples for showing the high glycosidation selectivity of mannosyl donors with C2 participating groups. For examples, see (a) reference 8b. (b) Lemanski, G.; Ziegler, T. *Helv. Chim. Acta* **2000**, *83*, 2655. (c) Ning, J.; Kong, F. *Tetrahedron Lett.* **1999**, *40*, 1357.

(11) For deallylation, see (a) Corey, E. J.; Suggs, W. J. *J. Org. Chem.* **1973**, *38*, 3224. (b) Nashed, M. A.; Anderson, L. *J. Chem. Soc., Chem. Commun.* **1982**, 1274.

(12) Seeberger used TBSOTf as an activator. For examples, see (a) reference 8b. (b) Bosse, F.; Marcaurelle, L. A.; Seeberger, P. H. *J. Org. Chem.* **2002**, *67*, 6659.

(13) This substance was prepared as shown below.



(a) (1) allyl alcohol, $Sc(OTf)_3$, reflux, 80%. (2) $PhCH(OMe)_2$, HBF_4 (54% in Et_2O), DMF, 83%. (3) (i)Bu_2SnO, MeOH, (ii) MeI, 74%. (4) BzCl, DMAP (cat.), pyridine–CH_2Cl_2, 70%. (b) (1) TFA, Et_3SiH, CH_2Cl_2, 83%. (2) TIPSOTf, 2,6-lutidine, CH_2Cl_2, 91%.

(14) There are numerous examples known for the preparation of anomeric phosphates. With two slight modifications—(1) $NaHCO_3$ added to accelerate the phosphitylation reaction and (2) *1-H* 1,2,4-triazole used instead of *1-H* tetrazole—we adopted the protocol reported by Wong; see (a) Pederson, R. L.; Esker, J.; Wong, C.-H. *Tetrahedron* **1991**, *47*, 2643. (b) Sim, M. M.; Kondo, H.; Wong, C.-H. *J. Am. Chem. Soc.* **1993**, *115*, 2260.

prepared from the common intermediate **A** in two and one steps, respectively, were coupled via TBSOTf-promoted, phos-
phate-based glycosidation to give the larger oligosaccharide **D**, which, in turn, served as the starting material for the next round of iteration. In practice, we chose donors as limiting substrates, because the acceptors were available from the common intermediate **A** more readily than the donors. For the case of $n + m = 2$ glycosidation, the optimal donor/acceptor ratio was found to be around 1:1.2. Although fine-tunings were required for each case, the optimal ratio was found to always be less than 1:1.4.

It is worth mentioning several aspects of the improved glycosidation. First, regardless of the size of the oligosaccharides, the glycosidation between donors and acceptors was highly stereoselective to give only the α-linked isomer. No undesired β-anomer was detected in either the crude or isolated product of each glycosidation (^{1}H NMR).[15] Second, no scrambled product was detected in the crude product of each glycosidation. This was unambiguously demonstrated by a high-performance liquid chromatography (HPLC) analysis; namely, each oligosaccharide has a characteristic retention time and is readily distinguishable from the other oligosaccharides (Figure 2). Third, the glycosidation of

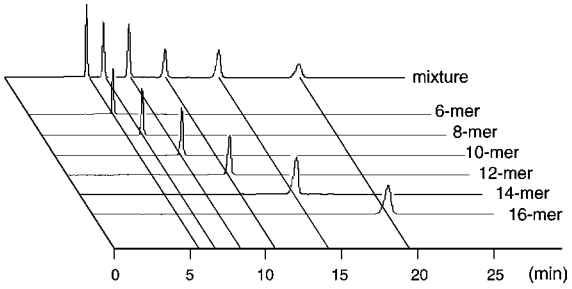
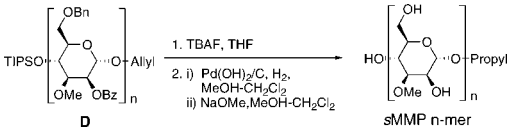


Figure 2. HPLC traces of the polysaccharides **D**. Conditions: column: Hypersil (Keystone Scientific); eluent: 30% EtOAc−hexanes (isocratic); detector: UV at 254 nm; rt.

oligosaccharides was achieved without significant reduction in yield upon scale-up. In addition, the phosphate-based approach was found to be equally effective for larger substrates;[16] indeed, *s*MMP 18-mer and 20-mer were synthesized by this method.

From the protected polysaccharides **D**, *s*MMPs were obtained by a three-step sequence of deprotection (Table 4).

Table 4. Deprotection to Form *s*MMPs



n	step 1	step 2
6	92%	87%
8	90%	82%
10	91%	78%
12	88%	81%
14	86%	66%
16	84%	71%
18	89%	69%
20	83%	73%

Overall, this improved synthesis enabled us to secure *s*MMPs ranging from 6-mer through 20-mer in relatively large quantities.

In conclusion, we have developed a second-generation synthesis of *s*MMPs. Glycosidation donor **B** and acceptor **C** were prepared from a common precursor **A** in 2 and 1 steps, respectively (Table 3). The glycosidation of **B** with **C** was effected by TBSOTf in CH_2Cl_2 to furnish only the desired α-anomer **D** in high yields. Except for the number of hexoses, the product **D** is structurally identical to the starting material **A**, thereby allowing one to iterate the synthetic sequence. Unlike the first generation synthesis, this method yielded the desired product free from contamination of the scrambling products. The three-step protocol was then used to deprotect **D** to furnish *s*MMP 6-, 8-, 10-, 12-, 14-, 16-, 18-, and 20-mers. This synthesis is easy to scale and applicable to the synthesis of analogs of *s*MMPs. For example, this iterative synthesis has been used for the synthesis of synthetic 3-*O*-ethyl-D-mannose-containing polysaccharides.[5]

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Supporting Information Available: Experimental details and ^{1}H NMR spectra (29 pages). This material is available free of charge via the Internet at http://pubs.acs.org

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(15) The stereochemistry of the product was further confirmed after the deprotection to *s*MMPs. No β-linked anomeric proton signals were detected in their ^{1}H NMR spectra; β-linked anomeric protons are known to give resonances shifted to upfields compared to those of the corresponding α-linked anomeric protons.

(16) Although reaction conditions for each glycosidation were similar for all the substrates, it was found that the amount of TBSOTf required to complete the glycosidation largely depends on both the size of oligosaccharides and the concentration of the reaction. Generally, for larger oligomers and reactions at lower concentrations, it was required to use slightly more TBSOTf. For details, see Supporting Information.