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Versatile Set of Orthogonal Protecting Groups for the Preparation of Highly Branched Oligosaccharides

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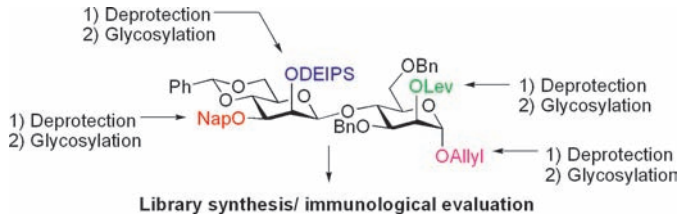
ABSTRACT



A new set of orthogonal protecting groups has been developed based on the use of a diethylisopropylsilyl (DEIPS), methylnaphthyl (Nap), allyl ether, and levulinoyl (Lev) ester. The protecting groups are ideally suited for the preparation of highly branched oligosaccharides and their usefulness has been demonstrated by the chemical synthesis of a *β*-D-Man-(1→4)-D-Man disaccharide, which is appropriately protected for making a range of part-structures of the unusual core region of the lipopolysaccharide of *Francisella tularensis*.

F. tularensis is a gram-negative bacterium that can cause tularemia (rabbit fever) in animals and humans[1] and has been classified by the CDC as a top-priority (Class A) bioterrorism agent.[2] Common to all class-A agents, tularemia transmits easily, has the capacity to inflict substantial morbidity and mortality on a large number of people and can induce widespread panic. To prevent tularemia, an attenuated life vaccine strain (LVS) was developed in the 1950s, but was not licensed for use as a human vaccine because the nature of its attenuation was not known and may not be stable. Diagnoses are based on time-consuming culture, serology or sophisticated molecular techniques. Therefore improved vaccine candidates and rapid diagnostic tests are needed for this pathogen.

The structure of the lipopolysaccharide (LPS) of *F. tularensis* has been determined (Figure 1)[3] and it was established that it has an unusual core structure. The core is linked to the lipid A region by only one 3-deoxy-D-manno-2-octulosonic acid (KDO) moiety instead of the usual two KDO residues. It does not contain heptosyl residues but contains two mannosyl moieties. One of the mannosides is *β*-linked to another mannoside, and this disaccharide fragment is further substituted at C-2, C-2′ and C-3′ by a *β*-glucoside, an α-galactosamine and a α-glucoside, respectively. It has been proposed that the lipopolysaccharide of *F. tularensis* is an attractive candidate for vaccine and diagnostic test development.[4] However, isolation of saccharides from a Class A bioterrorism agent is highly undesirable. Furthermore, it is difficult to conjugate short oligosaccharides

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(1) Elkins, K. L.; Cowley, S. C.; Collazo, C. M. *Expert. Rev. Vaccines* **2004**, *2*, 735.
(2) Dennis, D. T.; Inglesby, T. V.; Henderson, D. A.; Bartlett, J. G.; Ascher, M. S.; Eitzen, E.; Fine, A. D.; Friedlander, A. M.; Hauer, J.; Layton, M.; Lillibridge, S. R.; McDade, J. E.; Osterholm, M. T.; O'Toole, T.; Parker, G.; Perl, T. M.; Russell, P. K.; Tonat, K. *J. Am. Med. Assoc.* **2001**, *285*, 2763.
(3) Vinogradov, E.; Perry, M. B.; Conlan, J. W. *Eur. J. Biochem.* **2002**, *269*, 6112.
(4) Conlan, J. W.; Shen, H.; Webb, A.; Perry, M. B. *Vaccine* **2002**, *20*, 3465.

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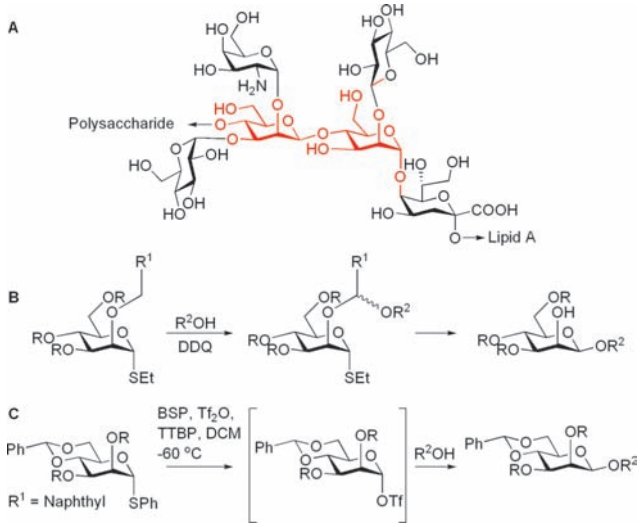


Figure 1. Target molecule and synthetic strategy. (A) Highly branched hexasaccharide fragment isolated from *F. tularensis* LVS lipopolysaccharide, target β-D-Man-(1→4)-α-D-Man disaccharide highlighted in red. (B) Intramolecular aglycon delivery through acetal tethering. (C) 4,6-Benzylidene mediated α-triflate formation followed by S_N2-like displacement.

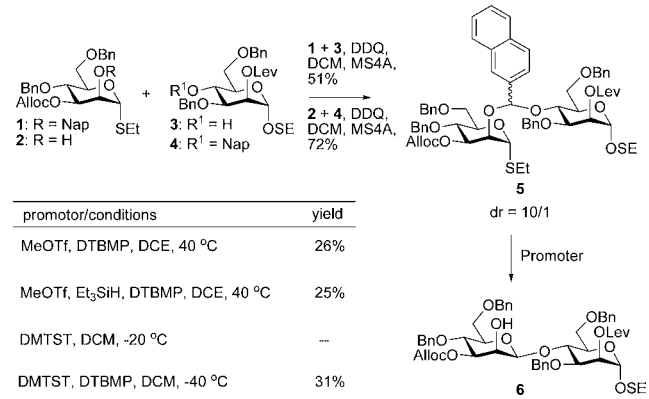
to carrier proteins without destroying vital immunological domains. Synthetic chemistry can address these issues since it makes it possible to incorporate an artificial linker for controlled conjugation to proteins. In addition, substructures can be prepared to determine the minimal epitope required to elicit protective immune responses.

Herein we report the chemical synthesis of a β-D-Man-(1→4)-α-D-Man disaccharide that is functionalized with a set of orthogonal protecting groups at C-1, C-2, C-2′ and C-3′. The orthogonal protecting groups make it possible to selectively introduce glycosides for the synthesis of a library of *F. tularensis* oligosaccharides.[5−8]

To this end, two β-mannosylation strategies were explored as well as a variety of orthogonal protecting group combinations. β-Mannosides, which are an important class of 1,2-*cis* glycosides, are difficult to introduce due to the axial C-2 substituent, which sterically blocks incoming nucleophiles from the β-face and the Δ-anomeric effect, which provides additional stabilization of the α-anomer.[9,10] An elegant methodology for the construction of β-mannosidic linkages is based on intramolecular aglycon delivery (IAD), which usually gives absolute β-anomeric selectivity. In this two-step protocol, the glycosyl donor and acceptor are tethered through a mixed acetal by for example oxidative coupling of a 4-methoxybenzyl- or methylnaphthyl (Nap) ether using 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ).[11−15] The ether can be present on the glycosyl donor or acceptor, the latter being referred to as reverse tethering. In the second step, the glycosyl donor is activated and the glycosyl acceptor is forced to attack from the same face as the C-2′ tether leading to the introduction of a 1,2-*cis*-glycoside with concomitant loss of the C-2′ protecting group.

Monosaccharide building blocks **1**-**4** were prepared (see Supporting Information) to explore the utility of IAD for the synthesis of an orthogonal protected β-D-Man-(1→4)-α-D-Man disaccharide (Scheme 1). It was envisaged that

Scheme 1. Intramolecular Aglycon Delivery using Acetal Tethering



promotor/conditions	yield
MeOTf, DTBMP, DCE, 40 °C	26%
MeOTf, Et_3SiH, DTBMP, DCE, 40 °C	25%
DMTST, DCM, -20 °C	---
DMTST, DTBMP, DCM, -40 °C	31%

compound **1**, which is equipped with a methylnapthyl ether, would provide a useful starting material to make tethered derivative **5**, which upon IAD should provide disaccharide **6**. The resulting free hydroxyl at C-2′ of **6** can then immediately be used for the introduction of the GalN of the core region of *F. Tularensis*. It was expected that the allyloxycarbonyl (Alloc), levulinoyl ester (Lev)[16] and the 2-(trimethylsilyl)ethyl ether (SE)[17] would provide an attractive set of orthogonal protecting groups for further glycosylations.

Thus, a mixture of **1** and **3** in CH_2Cl_2 in the presence of molecular sieves was treated with DDQ to afford mixed acetal **5** in a moderate yield of 51% as a 10/1 mixture of diastereoisomers (Scheme 1). The yield was significantly improved when reverse tethering was employed using **2** and **4** to give **5** in 72% yield. Presumably, a higher yield is obtained due to the higher nucleophilicity of the C-2′ alcohol. Next, mixed acetal **5** was activated with methyl triflate in the presence of 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP)

(5) Wong, C. H.; Ye, X. S.; Zhang, Z. Y. *J. Am. Chem. Soc.* **1998**, *120*, 7137.
(6) Zhu, T.; Boons, G. J. *Tetrahedron: Asymmetry* **2000**, *11*, 199.
(7) Markad, S. D.; Schmidt, R. R. *Eur. J. Org. Chem.* **2009**, 5002.
(8) Wang, C. C.; Lee, J. C.; Luo, S. Y.; Kulkarni, S. S.; Huang, Y. W.; Lee, C. C.; Chang, K. L.; Hung, S. C. *Nature* **2007**, *446*, 896.
(9) Gridley, J. J.; Osborn, H. M. I. *J. Chem. Soc., Perkin Trans. 1* **2000**, 1471.
(10) Cai, F.; Wu, B. L.; Crich, D. *Adv. Carbohydr. Chem. Biochem.* **2009**, *62*, 251.
(11) Jung, K. H.; Muller, M.; Schmidt, R. R. *Chem. Rev.* **2000**, *100*, 4423.
(12) Lee, Y. J.; Ishiwata, A.; Ito, Y. *J. Am. Chem. Soc.* **2008**, *130*, 6330.
(13) Boltje, T. J.; Buskas, T.; Boons, G. J. *Nat. Chem.* **2009**, *1*, 611.
(14) Cumpstey, I. *Carbohydr. Res.* **2008**, *343*, 1553.
(15) Zhu, X. M.; Schmidt, R. R. *Angew. Chem., Int. Ed.* **2009**, *48*, 1900.
(16) van Boom, J. H.; Burgers, P. M. J. *Tetrahedron Lett.* **1976**, *52*, 4875.
(17) Jansson, K.; Frejd, T.; Kihlberg, J.; Magnusson, G. *Tetrahedron Lett.* **1986**, *27*, 753.

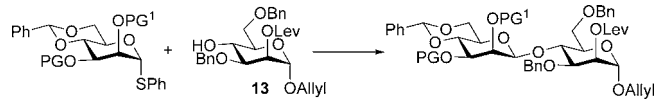
in 1,2-dichloroethane (DCE) at 40 °C. Despite the relatively high reaction temperature, the glycosylation was rather sluggish, and after a reaction time of 16 h, disaccharide **6** was isolated in a disappointing yield of 26%. The low yield was due to the formation of several byproducts including C-2′ methylated disaccharide. To address the problem of methylation, the methyl triflate promoted glycosylation was performed in the presence of triethyl silane. Under these conditions, the intermediate naphthylic cation is trapped providing a C-2′ Nap ether instead of a hydroxyl. Unfortunately, these modified reaction conditions did not improve the yield of β-mannoside **6**. Dimethyl(thiomethyl)sulfonium triflate (DMTST)[18] is also commonly employed as a thiophilic promoter for IAD.[19] However, the use of this promoter in the absence of a base did not lead to product formation. Nevertheless, the application of DMTST in combination with the base DTBMP gave **6** in a somewhat improved yield of 31%. Other promoter systems such as *N*-iodosuccinimide (NIS) could not be employed because of incompatibility with the Alloc function. Although other orthogonal protecting group pairs could be examined, attention was focused on an alternative approach for β-mannoside synthesis.

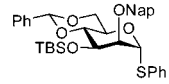
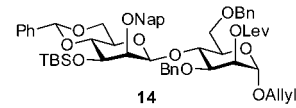
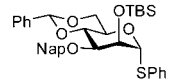
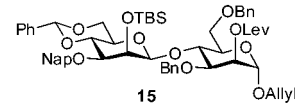
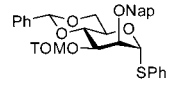
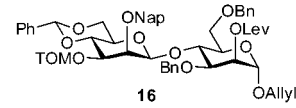
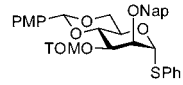
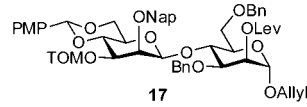
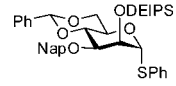
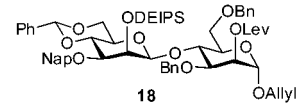
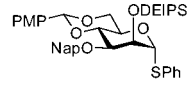
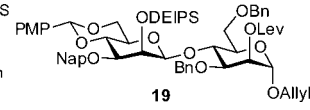
Crich and co-workers have pioneered an attractive approach for the introduction of β-mannosides by *in situ* formation of an α-anomeric triflate because of a strong endoanomeric effect.[20,21] An S_N2 like-displacement of the α-triflate by a sugar hydroxyl then results in the formation of a β-mannoside. A prerequisite of β-mannoside formation is that the donor is protected by a 4,6-*O*-benzylidene acetal. It has been proposed that this protecting group opposes oxacarbenium formation (S_N1 glycosylation) due to the torsional strain engendered by the half chair or boat conformation of this intermediate and a destabilizing electronic effect caused by placing the O-6 dipole antiparallel to the oxacarbenium ion.[22]

Glycosyl donors **7**−**12** were therefore prepared (see Supporting Information) and examined in glycosylations with glycosyl acceptor **13** using trifluoromethanesulfonic anhydride (Tf_2O), 1-benzenesulfinylpiperidine (BSP) as the promoter system (Table 1).[23] The 4,6-diol of the glycosyl donors is protected as a benzylidene- or *p*-methoxybenzylidene acetal and the C-2 and C-3 hydroxyls by different sets of orthogonal protecting groups. The latter was deemed important because previous studies have indicated that steric and electronic features of the C-2 and C-3 protecting groups can influence the β-anomeric selectivity of mannosylations.[25] It was anticipated that the Nap,[24] TBS, Lev and allyl would provide an attractive set of orthogonal protecting groups. Glycosyl donor **8** has a similar structure as **7**; however, the bulky TBS group is used for the protection of the C-2 hydroxyl and the Nap ether for the C-3 position. Low temperature (−78 °C) activation of glycosyl donor **7** was achieved with BSP/Tf_2O in the presence of DTBMP in CH_2Cl_2.[23] Addition of the acceptor and slow warming to −35 °C afforded the mannoside in a disappointing yield of 31% as a 1/1.5 mixture of β/α anomers. It has been observed that a bulky TBS groups at C-3 gives poor β/α ratios due to a so-called buttressing effect.[25] Indeed, when donor **8** was used, the stereoselectivity improved dramatically to β/α>20/1, however the yield was still moderate (40%). Attempts to remove the TBS ether using TBAF led to partial Lev cleavage and buffering with AcOH made the desilylation impractically slow. Hence, glycosyl donors carrying more labile silyl protecting groups were evaluated.

The triisopropylsiloxymethyl (TOM) ether was selected since it is less sterically demanding than the TBS ether and can thus be used as a C-3 protecting group and is also readily

Table 1. Stereoselective β-Mannosylations

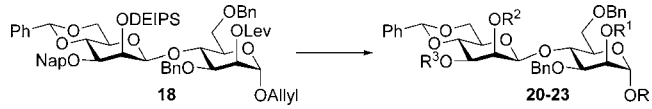


donor	product	yield[a]	ratio β/α[d]
 7	 14	31%[b]	1/1.5
 8	 15	40%[b]	>20/1
 9	 16	53%[b]	6/1
 10	 17	34%[b] 63%[c]	6/1[e]
 11	 18	41%[b] 73%[c]	>20/1[e]
 12	 19	40%[b] 73%[c]	>20/1[e]

[a] Isolated yields of the pure β anomer. [b] BSP/Tf_2O promotor system. [c] *p*-NO_2−C_6H_4SCl/AgOTf promotor system. [d] β/α ratio was determined by intergration of key signals in the ^1H NMR of the reaction mixture purified by size exclusion chromotography. The β-anomeric configuration was confirmed by the C_1'-H_1' heteronuclear coupling constant (158−162 Hz) and the chemical shift of H-5′ (~3 ppm). [e] β/α ratio was the same regardless of the promotor system used.

(18) Fugedi, P.; Garegg, P. J. *Carbohydr. Res.* **1986**, *149*, C9.
(19) Krog-Jensen, C.; Oscarson, S. *J. Org. Chem.* **1998**, *63*, 1780.
(20) Crich, D.; Sun, S. X. *Tetrahedron* **1998**, *54*, 8321.
(21) Crich, D.; Sun, S. X. *J. Am. Chem. Soc.* **1997**, *119*, 11217.
(22) Jensen, H. H.; Nordstrom, L. U.; Bols, M. *J. Am. Chem. Soc.* **2004**, *126*, 9205.
(23) Crich, D.; Smith, M. *J. Am. Chem. Soc.* **2001**, *123*, 9015.
(24) Gaunt, M. J.; Yu, J. Q.; Spencer, J. B. *J. Org. Chem.* **1998**, *63*, 4172.
(25) Crich, D.; Jayalath, P. *Org. Lett.* **2005**, *7*, 2277.

Table 2. Selective Removal of the Orthogonal Protecting Groups



entry	product	conditions	yield
1	**20**: R = H, R^1 = Lev, R^2 = DEIPS, R^3 = Nap	$PdCl_2$, NaOAc, AcOH, H_2O	72%
2	**21**: R = Allyl, R^1 = H, R^2 = DEIPS, R^3 = Nap	NH_2NH_2•AcOHTol/EtOH	99%
3	**22**: R = Allyl, R^1 = Lev, R^2 = H, R^3 = Nap	TBAF, AcOH, THF	98%
4	**23**: R = Allyl, R^1 = Lev, R^2 = DEIPS, R^3 = H	DDQ, CH_2Cl_2, H_2O	93%

cleaved using TBAF buffered with AcOH.[26] Glycosyl donor **9** and its *p*-methoxybenzylidene derivative **10** showed good β-selectivity and moderate yields (53 and 34%, respectively). In each case, the activation of the donor was exceptionally clean but when the glycosyl acceptor was added some byproduct formation was observed. Sulfoxide promoter systems such as BSP/Tf_2O are known to generate electrophilic byproducts and since glycosyl acceptor **13** is relatively unreactive, it was assumed that it partly reacted with these electrophilic byproducts thus lowering the overall yield.[27]

This assumption was supported by the fact that all the glycosyl acceptor was consumed even though it was used in excess. Benzenesulfenyl triflate (generated *in situ* from benzenesulfenyl chloride and silver trifluoromethane sulfonate) is known to be a powerful activator of thioglycosides that leads only to an inert disulfide as byproduct when successful. Thus, commercially available *p*-$NO_2C_6H_4SCl$ was used in combination with AgOTf in the presence of DTBMP for activation of **10**.[28] Indeed, when **10** was activated with these reagents, and reacted with **13**, disaccharide **17** was obtained in a much-improved yield of 63%. Although removability of the TOM was not an issue the stereoselectivity was moderate. Hence, the diethylisopropylsilyl ether (DEIPS) was evaluated as an orthogonal protecting group since it has a similar structure to the TBS ether but can more easily be removed.[29,30] Glycosyl donor **11** and it *p*-methoxybenzylidene derivative **12** showed excellent stereoselectivity (β/α>20/1). The yields with BSP/Tf_2O were moderate (41 and 40%, respectively) but could be improved to 73% using *p*-$NO_2C_6H_4SCl$/AgOTf. Disaccharide **18** was expected to be an excellent candidate for the preparation of the core region of *F. tularensis*.

Having established conditions for a high yielding and stereoselective β-mannosylation, attention was focused on the selective removal of the temporary protecting groups (Table 2). The anomeric allyl ether of **18** could be removed using $PdCl_2$ in high yield without affecting the other protecting groups. It is to be expected that lactol **20** can easily be converted into a trichloroacetimidate and used as a glycosyl donor in an ensuing glycosylation. The Lev ester was removed in near quantitative yield using hydrazinium acetate in a mixture of toluene and ethanol to afford **21**. DEIPS removal was performed under buffered conditions (TBAF/AcOH) to prevent the aforementioned Lev cleavage and gave **22** in 98% yield. Finally, the Nap ether was cleaved by DDQ oxidation in wet CH_2Cl_2 to provide compound **23** in high yield (93%).

In conclusion, the disaccharide β-D-Man-(1→4)-D-Man, modified with four orthogonal protecting groups, was prepared in high yield with excellent anomeric selectivity. It was found that the protecting group pattern was critical for achieving high β-anomeric selectivity and the best results were achieved with a mannosyl donor having a C-2 DEIPS, and a C-3 Nap ether and an acceptor modified with an anomeric allyl ether and a C-2 Lev ester. High yields of disaccharide were obtained when *p*-$NO_2C_6H_4SCl$/AgOTf was used as the promoter system for activating an anomeric thioglycoside. Each temporary protecting group could be removed in high yield without affecting the other protecting groups. The new set of orthogonal protecting groups is expected to be suited for the preparation of a library of *F. tularensis* inner-core oligosaccharides and will facilitate the preparation of other highly branched oligosaccharides.

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Supporting Information Available: ^1H and ^{13}C NMR spectra and experimental procedures for the preparation of compounds **1**−**23**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(26) Codee, J. D. C.; Krock, L.; Castagner, B.; Seeberger, P. H. *Chem.−Eur. J.* **2008**, *14*, 3987.
(27) Codee, J. D. C.; van den Bos, L. J.; Litjens, R.; Overkleeft, H. S.; van Boeckel, C. A. A.; van Boom, J. H.; van der Marel, G. A. *Tetrahedron* **2004**, *60*, 1057.
(28) Crich, D.; Cai, F.; Yang, F. *Carbohydr. Res.* **2008**, *343*, 1858.
(29) Zhu, T.; Boons, G. J. *J. Am. Chem. Soc.* **2000**, *122*, 10222.
(30) Toshima, K.; Mukaiyama, S.; Kinoshita, M.; Tatsuta, K. *Tetrahedron Lett.* **1989**, *30*, 6413.