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Imidazolium Cation Supported Solution-Phase Assembly of Homolinear α(1→6)−Linked Octamannoside: An Efficient Alternate Approach for Oligosaccharide Synthesis

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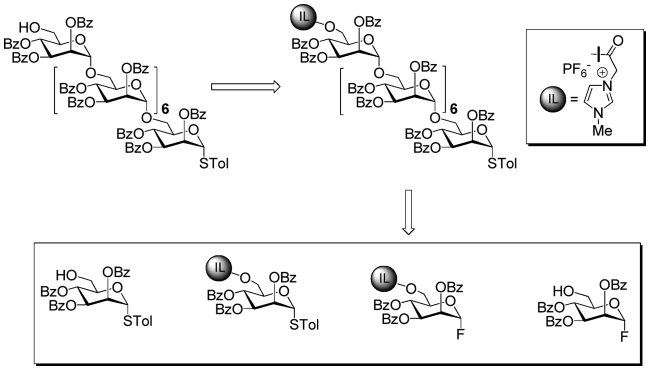

An efficient, simple convergent assembly of a homolinear α(1→6)-linked octamannosyl thioglycoside was obtained starting from imidazolium cation-tagged mannosyl fluoride and thiomannoside using block couplings. During chain elongation glycosylation reactions no column chromatographic purifications were used.

The significance of carbohydrates is now well valued as these play pivotal roles in several key biological systems and are essential parts of every cell surface that is crucial in cell-to-cell recognition and communication.[1] Access to pure well-defined oligosaccharide and glycoconjugate structures needed as biochemical tools is still a challenge to chemists. However, the synthesis of these compounds is made possible by controlled stereo- and regiospecific, chemoselective, orthogonal, and bidirectional glycosylation reactions via solution-phase as well as polymer-supported solution- or solid-phase organic synthesis.[2,3] Although highly successful, solid-phase synthesis suffers from a series of drawbacks due to heterogeneous reaction conditions and stepwise characterization. Alternate solution-phase polymer-supported methods allow efficient homogeneous conditions, but issues with loading capacity and product losses during purification are still challenging. Fluorous chemistry using a fluorous biphasic system and fluorous tag for fluorous separations have also been recently exploited for the synthesis of oligosaccharides.[4]

Access to newer, straightforward, and efficient methods to produce biologically relevant oligosaccharides is still warranted, and the use of imidazolium-type cationic species (ionic liquids, ILs)[5] as support during glycosylation processes can be approached to facilitate and condense the process of oligosaccharide synthesis. Imidazolium cationic species are reported to be used as anchors for reaction substrates in the synthesis of peptides,[6] nucleotides[7] and other types of organic molecules.[5,8] IL-linkers are real molecules and have defined structures and molecular weights in contrast to polymer linkers, which are functionalized materials with variable loading capabilities. Recently, we and others have successfully utilized imidazolium cations as phase-separation tags on glycosyl donors in glycosylation reactions.[9,10] The main feature of IL-supported substrates are their solubility properties, as solubility can be altered toward particular types of solvents by simply varying the anions. Thereafter, IL-supported species can be purified from the reaction mixture by simply washing the product with a solvent in which the IL-tagged compounds are not soluble. Whenever required, the IL support can be clipped off and purified by a simple phase-separation technique. In principle, synthesis of almost pure oligosaccharides can be achieved efficiently with minimal column chromatographic purification via this approach in homogeneous reaction conditions. In addition, IL-tagged substrates are easily characterized by conventional spectroscopic techniques. A mannosyl fluoride donor linked with imidazolium

(1) (a) Varki, A. *Glycobiology* **1993**, *3*, 97–130. (b) Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683–720. (c) Sears, P.; Wong, C.-H. *Cell. Mol. Life Sci.* **1998**, *54*, 223–152. (d) Rudd, P. M.; Elliot, T.; Cresswell, P.; Wilson, I. A.; Dwek, R. A. *Science* **2001**, *291*, 2370–2376.

(2) (a) Levy, D. E.; Fugedi, P. *The Organic Chemistry of Sugars*; CRC Press: Boca Raton, FL, 2006. (b) Hanessian, S. *Preparative Carbohydrate Chemistry*; Marcel Dekker: New York, 1996. (c) Demchenko, A. V. *Handbook of Chemical Glycosylation: Advances in Stereoselectivity and Therapeutic Relevance*; Wiley-VCH: Weinheim, 2008.

(3) (a) Seeberger, P. H. *Chem. Soc. Rev.* **2008**, *37*, 19–28. (b) Seeberger, P. H. *Solid Support oligosaccharide Synthesis and Combinatorial Carbohydrate Libraries*; John Wiley: New York, 2001, and references cited therein.

(4) Zhang, W. *Chem. Rev.* **2009**, *109*, 749–795.

(5) (a) Murugesana, S.; Linhardt, R. J. *Curr. Org. Synth.* **2005**, *2*, 437–451. (b) Miao, W.; Chan, T. H. *Acc. Chem. Res.* **2006**, *39*, 897–908. (c) El Seoud, O. A.; Koschella, A.; Fidale, L. C.; Dorn, S.; Heinze, T. *Biomacromolecules* **2007**, *8*, 2629–2647. (d) Martin, M. A. P.; Frizzo, C. P.; Moreira, D. N.; Zanatta, N.; Bonacorso, H. G. *Chem. Rev.* **2008**, *108*, 2015–2050. (e) Borikar, S. P.; Daniel, T.; Paul, V. *Tetrahedron Lett.* **2009**, *50*, 1007–1009. (f) Wang, J.; Song, G.; Peng, Y.; Zhu, Y. *Tetrahedron Lett.* **2008**, *49*, 6518–6520. (g) Galan, M. C.; Brunet, C.; Fuebsanta, M. *Tetrahedron Lett.* **2009**, *50*, 442–445.

(6) (a) Miao, W.; Chan, T. H. *J. Org. Chem.* **2005**, *70*, 3251–3255. (b) He, X.; Chan, T. H. *Org. Lett.* **2007**, *9*, 2681–2684.

(7) Donga, R. A.; Khaliq-Uz-Zaman, S. M.; Chan, T. H.; Damha, M. J. *J. Org. Chem.* **2006**, *71*, 7901–7910.

(8) (a) Legeay, J. C.; Vanden Eynde, J. J.; Bazureau, J. P. *Tetrahedron* **2008**, *64*, 5328–5335. (b) Grzyb, J. A.; Batey, R. A. *Tetrahedron Lett.* **2008**, *49*, 5279–5282.

(9) Pathak, A. K.; Yerneni, C. K.; Young, Z.; Pathak, V. *Org. Lett.* **2008**, *10*, 145–148.

(10) (a) Huang, J.-Y.; Lei, M.; Wang, Y.-G. *Tetrahedron Lett.* **2006**, *47*, 3047–3050. (b) He, X.; Chan, T. H. *Synthesis* **2006**, 1645–1651.

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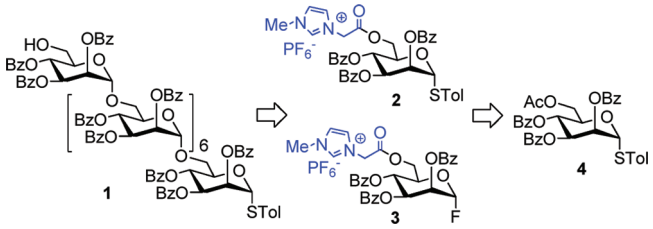
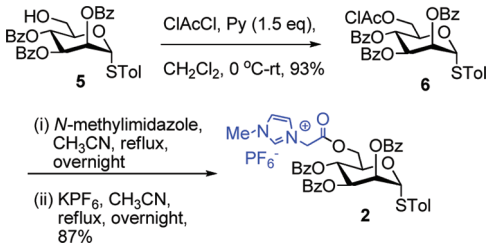


FIGURE 1. Retrosynthetic analysis of IL-supported synthesis of α(1→6)-octamannoside using imidazolium cation-tagged donors.

hexafluorophosphoacetyl was previously used by us, and iterative coupling[11] with thioglycoside acceptors led to the solution-phase synthesis of a linear α(1→6)-linked tetramannoside in an efficient manner.[9]

The physical properties of imidazolium cation-tagged protected sugars beyond tetrasaccharide are not clear, and we sought to further examine the solubility properties of the growing chain as well as their phase-tag purification by simple washing with solvents. To test the limits of a IL-tag assisted approach to oligosaccharide synthesis, access to a model homolinear α(1→6)-linked octamannosyl thioglycoside **1** on imidazolium cation-tagged substrates using chemoselective/orthogonal glycosylation was chosen (Figure 1). D-Mannose oligomers are profoundly present in nature and are essential substructures in many biologically important glycoconjugates such as *N*-glycans, fungal, and bacterial cell wall mannans and GPI anchors.[12] Branched oligomannan syntheses are reported in the literature using several different methodologies[13] including solution- and solid-phase polymer supports[14] and fluorous-tag[15] assisted solution phase. Specifically, the homolinear α(1→6) oligomannans were previously synthesized in the solution phase[16,17] and by an automated solid-phase[18] approach. In a tedious conventional solution-phase process, we recently synthesized a

SCHEME 1. Synthesis of IL-Tagged Thiomannopyranoside Donor 2



linear α(1→6)-octamannosyl fluorescent probe[17] via the two-stage activation procedure for the synthesis of oligosaccharides,[19] combining the chemistry of thioglycosides with that of glycosyl fluorides.

With our interest in developing newer simple and efficient approaches to synthesize oligosaccharides in small to large quantities, we report herein a convergent synthesis of octasaccharide **1** by means of conveniently accessible IL-tagged *p*-thiotolyl mannoside **2** and IL-tagged mannosyl fluoride **3** and block glycosylation. The IL-tagged mannosyl synthons **2** and **3** were synthesized from a common known *p*-thiotolyl mannoside **4**.[9,17] The thioglycoside **4** was chosen as the starting precursor because it can be prepared on a multigram scale in just two steps from D-mannose through a known precursor 1,6-di-*O*-acetyl-2,3,5-tri-*O*-benzoyl-α-D-mannose.[20] The thioglycoside **4** was converted to IL-tagged glycosyl fluoride **3** using DAST and NBS at −20 °C followed by installation of the imidazolium tag as reported in excellent yield.[9]

IL-tagged thioglycoside **2** was synthesized on large scale from **4** in three steps as shown in Scheme 1. First, the selective removal of the acetyl group at C-6 in compounds **4** was induced by an AcCl/MeOH/CH_2Cl_2 mixture (0.1:20:20 v/v, 12.0 mL/mmol) and afforded 6-OH glycosides **5** in 93% yield.[9] Glycoside **5** was then treated with chloroacetyl chloride to 6-*O*-chloroacetylated product **6**. Lastly, glycoside **6** was treated overnight with *N*-methylimidazole under reflux conditions in CH_3CN, and subsequent exchange of chloride anion with hexafluorophosphino anion (PF_6^-) was obtained in the same pot by reaction with KPF_6. The reaction was cooled to room temperature, and removal of solvent afforded a solid which was then suspended in $CHCl_3$ and filtered. The filtrates were concentrated to a solid and washed several times with diethyl ether to produce almost pure IL-tagged thioglycoside **2** on large scale, equipped with a robust participating benzoyl group at C2 to ensure α-selectivity and a removable temporary imidazolium cation-tagged *O*-acetyl protecting group at the C6 for further chemical manipulations.

A detailed IL-supported synthesis of target octasaccharide **1** is illustrated in Scheme 2. With large quantities of IL-tagged *p*-tolylthio mannoside **2** and mannosyl fluoride **3** available, we assembled IL-tagged disaccharides **8** and **9**. Glycosylation of IL-tagged mannosyl fluoride donor **3** with thiomannoside acceptor **5** using coupling reagents[21]

(11) In this process, after each coupling reaction between IL-tagged mannosyl fluoride donor and thiomannosyl acceptor, the IL-tag was removed from the glycosylated product to obtain thiomannoside acceptor. In the next glycosylation step, this thiomannoside acceptor was further coupled with another equivalent of IL-tagged mannosyl fluoride donor, and chain elongation was achieved.

(12) Few selected articles, see: (a) Kobayashi, H.; Mitobe, H.; Takahashi, K.; Yamamoto, T.; Shibata, N.; Suzuki, S. *Arch. Biochem. Biophys*. **1992**, *294*, 662–669. (b) Mandal, D. K.; Bhattacharya, L.; Koenig, S. H.; Brown R. D.; Oscarson, S.; Brewer, C. F. *Biochemistry* **1994**, *33*, 1157–1162. (c) Brennan, P. J. *Annu. Rev. Biochem*. **1995**, *64*, 29–63. (d) Chatterjee, D.; Khoo, K. H. *Glycobiology* **1998**, *8*, 113–120. (e) Hadar, F.; Daniel, A. M.; Kurt, D.; William, I. W. *Science* **2001**, *294*, 2163–2166. (f) Carole, A. B.; Shigeki, K.; Hamachi, I. *J. Mol. Biol*. **2002**, *322*, 881–889. (g) Botos, I.; O'Keefe, B. R.; Shenoy, S. R.; Cartner, L. K.; Ratner, D. M.; Seeberger, P. H.; Boyd, M. R.; Wlodawer, A. *J. Biol. Chem*. **2002**, *277*, 34336–34342.

(13) Few selected articles, see: (a) Heng, L.; Ning, J.; Kong, F. *J. Carbohydr. Chem*. **2001**, *20*, 285–296. (b) Zhu, Y.; Chen, L.; Kong, F. *Carbohydr. Res*. **2002**, *337*, 207–215. (c) Ning, J.; Heng, L.; Kong, F. *Tetrahedron Lett*. **2002**, *43*, 673–675. (d) Xing, Y.; Ning, J. *Tetrahedron: Assymetry* **2003**, *14*, 1275–1283. (e) Ratner, D. M.; Plante, O. J.; Seeberger, P. H. *Eur. J. Org. Chem*. **2002**, 826–833. (f) Crich, D.; Banerjee, A.; Yao, Q. *J. Am. Chem. Soc*. **2004**, *126*, 14930–14934. (g) López, J. C.; Agocs, A.; Uriel, C.; Gómeza, A. M.; Fraser-Reid, B. *Chem. Commun*. **2005**, 5088–5090. (h) Jayaprakash, K. N.; Chaudhuri, S. R.; Murty, C. V.; Fraser-Reid, B. *J. Org. Chem*. **2007**, *72*, 5534–5545.

(14) (a) Douglas, S. P.; Whitfield, D. M.; Krepinsky, J. J. *J. Am. Chem. Soc*. **1995**, *117*, 2116–2117. (b) Andrade, R. B.; Plante, O. J.; Melean, L. G.; Seeberger, P. H. *Org. Lett*. **1999**, *1*, 1811–1814.

(15) Jaipuri, F. A.; Pohl, N. L. *Org. Biomol. Chem*. **2008**, *6*, 2686–2691.

(16) Zhu, Y.; Kong, F. *Carbohydr. Res*. **2001**, *332*, 1–21.

(17) Aqueel, M. S.; Pathak, V.; Pathak, A. K. *Tetrahedron Lett*. **2008**, *49*, 7157–7160.

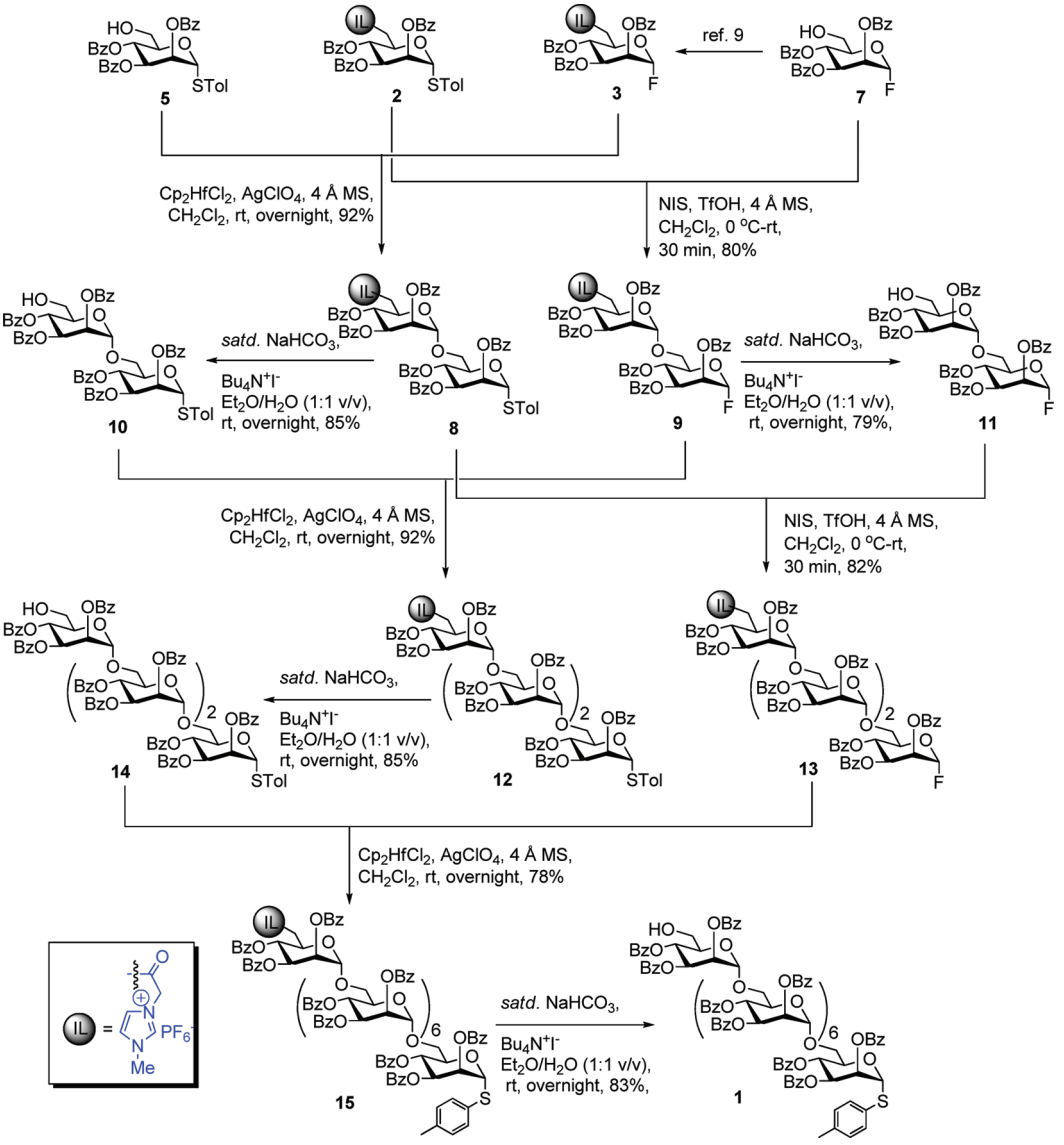
(18) Liu, X.; Wada, R.; Boonyarattanakalin, S.; Castagner, B.; Seeberger, P. H. *Chem. Commun*. **2008**, 3510–3512.

(19) Nicolaou, K. C.; Ueno. H. In *Preparative Carbohydrate Chemistry*; Hanessian, S., Ed.; Marcel Dekker: New York, 1996; pp 313−338 and references cited therein.

(20) Heng, L.; Ning, J.; Kong, F. *Carbohydr. Res*. **2001**, *331*, 431–437.

(21) Suzuki, K.; Maeta, H.; Matsumoto, T.; Tsuchihashi, G.-I. *Tetrahedron Lett*. **1988**, *29*, 3571–3574.

SCHEME 2. Synthesis of α(1→6)-Linked Octamannan 1



Cp_2HfCl_2 and $AgClO_4$ in CH_2Cl_2 progressed as expected to give almost pure IL-tagged thiodisaccharide **8** after the product was washed with diethyl ether and the product was analytically compared with the previously prepared sample by us.[9] On the other hand, glycosylation of IL-tagged thiomannosyl donor **2** with fluoride acceptor **7** was carried out in the presence of promoter NIS and triflic acid. The workup and simple washing purification protocol with diethyl ether produced almost pure IL-tagged dimannosyl fluoride **9** in 80% yield. The ^1H and ^{13}C NMR and ESIMS spectral studies of **9** supported the structure, and the reducing end anomeric carbon was observed as a doublet at δ 105.39 ($J_{C-F} = 223.3$ Hz) in the ^{13}C NMR spectrum.

In the present process, the IL-tagged glycosylated products, after purification by simple washing, were split into two portions. In the subsequent glycosylation reaction, one portion was used as a glycosyl donor and the other portion was utilized as a glycosyl acceptor after removal of the IL-tag and purification by a biphasic separation technique. In comparison to our previous iterative glycosylation approach[11] for the IL-supported synthesis of linear tetramannoside in which the monosaccharide block was added one at a time, this block glycosylation efficiently produced the target octasaccharide in fewer steps.

Now, to proceed further, each IL-tagged disaccharide **8** and **9** was divided into two portions. On one portion of each disaccharide, the IL-tag was conveniently detached by stirring with aqueous saturated $NaHCO_3$ in a H_2O/ether (1:1) biphasic solvent mixture in the presence of a phase-transfer catalyst $Bu_4N^+I^-$. Concentration of the ether layer provided almost pure acceptor disaccharides **10** and **11** as shown by their ^1H and ^{13}C NMR spectra, and they were further used as such in glycosylation reactions. With the acceptor thiodisaccharide **10** and glycosyl fluoride **11** in hand, we next executed the [2 + 2] block glycosylation reaction and accessed IL-supported α(1→6)-tetramannose thioglycoside **12** and α(1→6)-tetramannosyl fluoride **13**. In a glycosylation process similar to that for the synthesis of disaccharides **8** and **9**, the IL-tagged thioglycoside **8** was reacted with mannosyl fluoride acceptor **11** and IL-tagged

mannosyl fluoride **9** was reacted with thioglycoside acceptor **11** in the presence of coupling promoters NIS−TfOH and Cp_2HfCl_2−$AgClO_4$, respectively. The workup and simple washing purification protocol with diethyl ether produced almost pure IL-tagged tetrasaccharides **12** and **13** for next step usage.

Again, a portion of synthesized IL-tagged thiomannosyl tetrasaccharide **12** was treated with an aqueous saturated $NaHCO_3$ in a H_2O/ether (1:1) biphasic solvent mixture in the presence of $Bu_4N^+I^-$. After concentration of the ether layer, almost pure acceptor thiomannosyl tetrasaccharide **14** was obtained and further utilized in glycosylation reaction without purification. To facilitate the synthesis of IL-tagged α(1→6)-linked octamannosyl thioglycoside **15**, a convergent block coupling of [4 + 4] was commenced with the overnight coupling reaction of donor IL-tagged mannosyl fluoride **13** and acceptor thiomannoside **14** in CH_2Cl_2 using coupling reagents Cp_2HfCl_2 and $AgClO_4$. The usual workup and simple washing purification protocol with diethyl ether produced almost pure IL-tagged octasaccharides **15**. Finally, the IL support from the octasaccharide **15** was removed, as described for **14**, and resulted in almost pure homolinear α(1→6)-linked octamannosyl thioglycoside **1** in 83% yield. The structure of **1** was supported by its ^1H and ^{13}C NMR spectra, and the final structural confirmation was obtained by ESIMS analysis of **1**, which showed a peak at 3942.8 $[M+Na]^+$ corresponding to the molecular formula $C_{223}H_{184}O_{64}SNa$.

In conclusion, we demonstrate a successful, orthogonal block synthesis of an octasaccharide on an imidazolium cation support with minimal column chromatographic purification as an alternative to existing oligosaccharide synthetic approaches on support materials. The IL-tagged thioglycosides and IL-tagged glycosyl fluorides were chemoselectively activated in presence of each other by coupling reagents NIS−TfOH and Cp_2HfCl_2−$AgClO_4$ respectively. All intermediate products were analyzed by ^1H and ^{13}C NMR, and MS spectral techniques. Further development of IL-supported solution-phase techniques will provide carbohydrate chemists a new tool to achieve their synthetic goals in an efficient and cost-effective approach and will broaden the diversity of targets for glycobiology.

Experimental Section

General Procedure for Coupling with IL-Tagged Thioglycoside. IL-supported donor thioglycoside (1.0 equiv), acceptor fluoride glycoside (1.2 equiv), and activated powdered 4 Å molecular sieves in CH_2Cl_2 were cooled at −20 °C under argon atmosphere. The mixture was stirred for 10 min, and NIS (1.2 equiv) followed by TfOH (0.1 equiv) was added to initiate coupling. The reaction mixture was allowed to stir for 30 min, and the reaction was quenched by the addition of saturated aqueous $NaHCO_3$. It was diluted with $CHCl_3$, and the organic phase was filtered through a Celite pad. The filtrate was washed with 10% $Na_2S_2O_3$ (10 mL), and the organic layer was dried over anhydrous Na_2SO_4. It was concentrated under vacuum to a solid which was purified by repeated washing with diethyl ether, and the solvent was removed by decantation. The solid residue was then dried under vacuum to afford IL-supported fluoride saccharide.

General Procedure for Coupling with IL-Tagged Fluoride Donor. The IL-tagged fluoride (1.0 equiv) and the acceptor thioglycosides (1.1 equiv) were dissolved in CH_2Cl_2 under Ar atmosphere in the presence of activated 4 Å molecular sieves. The reaction mixture was cooled to 0 °C, and coupling reagents $AgClO_4$ (1.5 equiv) and Cp_2HfCl_2 (1.1 equiv) were added. The reaction mixture was stirred overnight, filtered, and concentrated under vacuum. The residue was washed with diethyl ether several times, and the solvent was removed by decantation. The residue was finally dried under vacuum to give pure IL-tagged thioglycoside.

***p*-Tolyl 2,3,4-Tri-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl-2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-1-thio-2,3,4-tri-*O*-benzoyl-α-D-mannopyranoside (1).** IL-tagged octaasaccharide **15** (500 mg, 0.12 mmol) was dissolved in biphasic solvent mixture diethyl ether−water (15 mL, 1:1 v/v). To it were added saturated aqueous $NaHCO_3$ solution (10 mL) and $Bu_4N^+I^-$ (70 mg). The reaction mixture was stirred for 8 h at room temperature; the ether layer was separated and washed with brine (2 × 10 mL). The ether layer was dried over anhydrous Na_2SO_4 and concentrated, and the residue was dried under high vacuum to produce octasaccharide **1** as a colorless solid (388 mg, 83% yield): mp 110 °C; R_f = 0.2 (2:1 cyclohexane−EtOAc); ^1H NMR (300 MHz, $CDCl_3$) δ 8.20 (m, 12H), 8.08 (m, 20H), 7.88 (m, 18H), 7.54 (m, 30H), 7.43 (m, 22H), 7.28 (m, 34H), 7.14 (d, 2H, J = 8.0 Hz), 6.42 (t, 1H, J = 10.2 Hz), 6.22 (m, 5H), 6.10 (m, 4H), 6.00 (m, 7H), 5.90 (m, 4H), 5.74 (m, 4H), 5.23 (s, 1H), 5.14 (d, 1H, J = 8.3 Hz), 5.05 (s, 1H), 4.97 (m, 5H), 4.81 (s, 1H), 4.38 (dd, 1H, J = 3.9, 11.4 Hz), 4.18 (m, 7H), 3.96 (m, 2H), 3.81 (m, 5H), 3.65 (d, 1H, J = 8.3 Hz), 3.55 (d, 1H, J = 10.1 Hz), 3.35 (m, 7H), 2.22 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.9, 165.8, 165.7, 165.5, 165.5, 165.3, 165.2, 138.5, 133.8, 133.6, 133.4, 133.2, 133.1, 132.9, 130.2, 130.0, 129.8, 129.5, 129.5, 129.4, 129.3, 129.1, 128.9, 128.8, 128.6, 128.43, 98.4, 98.2, 97.9, 86.9, 72.1, 71.1, 70.9, 70.6, 70.2, 69.7, 69.6, 69.3, 67.2, 66.9, 66.6, 66.5, 66.3, 65.9, 65.8, 60.8, 21.2; ESIMS m/z 3942.8737 $[M + Na]^+$ calcd for $C_{223}H_{184}O_{64}SNa$, found 3942.7.

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Supporting Information Available: Complete experimental procedures and characterization (^1H and ^{13}C NMR spectra). This material is available free of charge via the Internet at http://pubs.acs.org.