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Concise assembly of linear α(1→6)-linked octamannan fluorescent probe

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Abstract

Synthesis of a fluorescently labelled (dansylated) linear α(1→6)-linked octamannan, using glycosyl fluoride donors and thioglycosyl acceptors is described. A selective and convergent two-stage activation progression was executed to construct di-, tetra and octa-mannosyl thioglycosides in three glycosylation steps with excellent yield. Further a 5-*N*,*N*-Dimethylaminonaphthalene-1-sulfonamidoethyl (dansyl) group was coupled to 1-azidoethyl octamannosyl thioglycoside. Global deprotection of the coupled product afforded the desired dansylated homo-linear α(1→6)-linked octamannan.

Mannans are constituents of many bacterial and fungal cell walls.[1] They are functional components of proteins and play important roles in defining the structures of proteins, their stabilization under physiological conditions, tuning of enzymatic activities, cell–cell recognition and in the adhesion of the microorganism to host cells.[2] The α(1→6)-linked oligomannans are found in the cell wall polysaccharides of yeast.[3] Polysaccharides, especially oligomannan, can be used to deliver drugs, genes and antigens through polysaccharide receptors present in cells and macrophages.[4] Macrophages are known to express high levels of specific polysaccharide receptors, e.g. mannan, glucan, and galactin receptors, on their membranes that generally bind neutral or charged polysaccharides and internalize these ligands.[5] Receptor mediated delivery of drug–polysaccharide conjugates is an approach that can deliver small and effective amounts of drugs specifically to target organisms, minimizing patient exposure and potential toxic side effects. We have recently demonstrated the selective, receptor–mediated delivery of an antibacterial drug to macrophages infected with *Mycobacterium tuberculosis* via conjugation of moxifloxacin with 1,3-β-glucan.[6]

Drug loading on commercially available high molecular weight polysaccharides is very low and it is attributed to their poor solubility in solvents during the drug conjugation reactions. Therefore, we describe here a simple and efficient method for the synthesis of a low molecular weight homo-linear α(1→6)-linked octamannan fluorescent probe **1** (figure 1) that may be utilized to study uptake by macrophages through the mannan receptor present on the plasma membrane. Further, in the future, such conjugation can be exploited for selective receptor–mediated delivery of drugs. We have previously synthesized and reported dansylated disaccharide fluorescent probes to study glycosyltransferases in the biosynthesis of *M.*

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Supplementary data
Experimental procedures, analytical and spectral data of all new compounds can be found in the online version.

tuberculosis cell wall polysaccharides.[7] Herein, the assembly of dansylated oligomannan fluorescent probe **1** was approached from synthetic homo-linear α(1→6) thiooctamanosyl glycoside **2**, 1-azidoethanol **3** and commercially available dansyl chloride **4** fragments as represented in Figure 1.

Branched oligomannan syntheses are reported in the literature using several different methodologies.[8] Specifically, the homo-linear α(1→6) oligomannans were synthesized by glycosylation using a trichloroacetimidate donor glycoside.[9] The strategy of combining the chemistry of thioglycosides with that of glycosyl fluorides[10] for the synthesis of oligosaccharides known as the two-stage activation procedure (Figure 2) was developed by Nicolaou *et. al*.[11] Briefly, in the activation stage one, the stable thioglycoside is converted to the more reactive glycosyl fluoride donor by treatment with *N*-bromosuccinimide (NBS) and diethylaminosulfur trifluoride (DAST). In stage two, the glycosyl fluoride is then reacted with a thioglycoside acceptor to produce a thiodisaccahride for further promulgation of the oligosaccharide chain. Reiteration of this process of converting thiooligosaccharide to a glycosyl fluoride and their coupling with acceptor thioglycosides straightforwardly produces chain elongation (figure 2).

We executed the synthesis of the desired *p*-thiotolyl α(1→6) octamannoside **2** using a convergent two-stage activation and iterative orthogonal glycosylation technique starting from the coupling of thioglycoside acceptor **5** and glycosyl fluoride donor **6** (Figure 3). The synthesis of mannose building blocks **5** and **6** was achieved from thioglycoside **7** as reported earlier by us.[12] However, the synthesis of thioglycoside **7** was accomplished from the known synthon 1,6-di-*O*-acetyl-2,3,4-tri-*O*-benzoyl-α-D-mannose (**8**) starting from commercially available D-mannose in an 88% overall yield.[13] Selective de-acetylation of **7** to the acceptor glycoside **5** was achieved by the overnight reaction with an AcCl/MeOH/CH_2Cl_2 (0.1:20:20 v/v, 12.0 mL/mmol) mixture.[12]

Following the synthesis of starting mannose synthons **5** and **6**, the synthesis of **2** was carried out as illustrated in Scheme 1. In the first step of the glycosylation sequence, the *p*-thiotolyl disaccharide **9** was synthesized in high yield by selective anomeric activation of glycosyl fluoride **6** by $AgClO_4$ and $SnCl_2$ in CH_2Cl_2 and its coupling with the acceptor **5**.[12] The exclusive formation of α(1→6)-linked disaccharide **6** was supported by its analytical and spectral analysis.[12]

Next, for the assembly of α(1→6)-linked tetramannosyl thioglycoside **12**, the disaccharide **9** was converted to acceptor thiodisaccharide **10** and donor 1-fluorodisaccharide **11**. Deacetylation of **9** by the overnight reaction with an AcCl/MeOH/CH_2Cl_2 mixture produced the desired acceptor disaccharide **10** in quantitative yield. In the ^{1}H and ^{13}C NMR spectra of compound **10**, the absence of signals from the acetate group as seen in compound **9**, confirmed the selective deacetylation. The disaccharide **9** was also converted to the donor fluoride **11** in 91% yield using DAST and NBS at −20 °C. The structure of **11** was supported by the ^{1}H NMR spectra in which two characteristic peaks in thiotolyl glycosides [doublet at δ 7.20 ppm from two protons of tolyl ring and a singlet at δ 2.25 ppm from methyl] were not observed. The α-stereochemistry at the anomeric carbon was supported by a C-1 signal at δ 105.21 ppm (d, $J_{C-1,F}$ = 222.1 Hz) in the ^{13}C NMR spectrum of **11**, whereas, the signal at δ 98.11 ppm was attributable to the other anomeric carbon. In the ^{1}H NMR spectrum of **11**, the H-1 signal was found obscured with other protons in the multiplet at δ 5.92 ppm, however, the anomeric proton H-1′ was observed δ 5.15 ppm (J = 1.4 Hz).

With the acceptor disaccharide **10** and fluoride donor disaccharide **11** in hand, we next performed the [2+2] glycosylation reaction to access α(1→6)-linked tetramannosyl thioglycoside **12**. The coupling reaction between glycosides **10** and **11** was carried out at room temperature with promotors $AgClO_4$ and $SnCl_2$ in CH_2Cl_2 over 4Å molecular sieves and resulted in the desired glycoside **12** in 80% yield after purification by SiO_2 column chromatography. The ^{1}H NMR spectrum of **12** in $CDCl_3$ showed four anomeric protons at δ 5.77, 5.25, 5.02 and 4.85 ppm as singlets suggesting α-glycosylation. In the ^{13}C NMR spectrum of **12**, the four anomeric carbons were observed at δ 98.37, 97.99, 97.79 and 86.91 ppm. The final structural confirmation was obtained by FABMS analysis of **12**, which showed a peak at 2085.0 $[M+Na]^+$, corresponding to the molecular formula $C_{117}H_{98}O_{33}SNa$.

The α(1→6)-linked tetramannosyl thioglycoside **12** was further converted to thioglycosyl acceptor **13** and glycosyl fluoride donor **14** in 96% and 84% yields by similar reactions performed for the syntheses of glycosides **10** and **11** respectively. The characteristic acetate and thiotolyl groups were found absent in the ^{1}H NMR and ^{13}C NMR spectra of compounds **13** and **14** respectively. In the ^{13}C NMR spectra of glycosyl fluoride **14**, the four anomeric carbon signals were observed at δ 105.21 (d, $J_{C\text{-}1,F}$ = 223.0 Hz), 98.38, 97.92 and 97.56 ppm. Finally, the FABMS analyses of glycosides **13** and **14** confirmed the formation of these products.

Next, we pursued the [4+4] glycosylation reaction to access α(1→6)-linked octamannosyl *p*-tolylthioglycoside **2**. The glycosyl donor **14** was treated with glycosyl acceptor **13** in the presence of promoters $AgClO_4$ and $SnCl_2$ in CH_2Cl_2. Even after 48 hrs at room temperature, only 20% yield of the desired octasaccharide **10** was achieved along with the recovery of unreacted acceptor glycoside **13**. The glycosylation reaction between **13** and **14** was next performed with coupling reagents Cp_2HfCl_2 and AgOTf in CH_2Cl_2 overnight at room temperature. Octamannoside **2** was produced in 61% yield after purification by SiO_2 column chromatography. The ^{1}H NMR spectrum of **2** in $CDCl_3$ showed the anomeric protons at δ 5.78, 5.24, 5.06, 5.01, 5.00, 4.99, 4.82 ppm as singlets and at δ 4.96 ppm ($J_{1,2}$ = 1.4 Hz) as a doublet supporting the 1,2-*trans* glycosylation. In the ^{13}C NMR spectrum of **2**, the anomeric carbons were not well resolved and appeared as three signals at δ 98.41, 97.99 and 86.89 ppm. Lastly, the final structural confirmation was obtained by MALDI-TOF mass analysis of **2**, which showed a peak at 3983.9 $[M+Na]^+$ corresponding to the molecular formula $C_{225}H_{186}O_{65}SNa$.

After the successful synthesis of **2**, dansylated octamannan fluorescent probe **1** was synthesized in four steps (Scheme-2). In step one, α(1→6)-linked octamannosyl thioglycoside **2** was reacted with 1-azidoethanol **3**[14] in presence of activator NIS and Lewis acid AgOTf at −4 °C for 45 minutes. Usual workup of the coupling reaction followed by purification of the reaction product by column chromatography gave the desired 1-azidoethyl α(1→6)-linked octamannosyl glycoside **15** in 89% yield. In the ^{1}H NMR and ^{13}C NMR spectra of **15**, the signals from *p*-thiotolyl group were found absent. The ^{13}C NMR spectrum of **15** clearly indicated the attributable to CH_2N_3 and OCH_2 carbons respectively. The MALDI-TOF mass analysis of **15** showed a peak at 3947.1 $[M+Na]^+$ corresponding to the molecular formula $C_{220}H_{183}N_3O_{66}Na$.

In step two, the azido functionality in **15** was reduced using ammonium formate (HCO_2NH_4) over Pd/C in CH_2Cl_2-MeOH (9:1) and was monitored by TLC. Upon completion of the reaction, concentration and the usual workup produced 1-aminoethyl α(1→6)-linked octamannosyl glycoside **16**. Without further purification, the crude **16** was reacted overnight at 0 °C in the dark with dansyl chloride **3** and *N*-methylimidazole. TLC showed a major fluorescent spot in long wave (365 nm) UV lamp and purification by column chromatography gave the desired blocked dansylated α(1→6)-linked octamannosyl glycoside **17** in 69% yield. The ^{1}H NMR spectrum of **17** indicated formation of the desired product as it showed signals due to a dansyl group in the aromatic region as well as ppm $[N(CH_3)_2]$. In the APT ^{13}C NMR spectrum of **17** in $CDCl_3$, the carbons of CH_2NH and $N(CH_3)_2$ were observed at δ 42.67 and

45.39 ppm respectively. In the MALDI-TOF mass analysis of **17** gave a peak at 4155.8 $[M+Na]^+$ corresponding to the molecular formula $C_{232}H_{196}N_2O_{68}SNa$.

Finally, global de-protection of **17** was accomplished by overnight reaction with satd. NH_3 solution in MeOH at room temperature. TLC in $CHCl_3$:MeOH:H_2O (65:35:10, lower layer) showed one fluorescent spot under a long wave UV lamp. The reaction mixture was concentrated to dryness and the syrup was washed several times with CH_2Cl_2 and finally with EtOAc. The syrup was purified by column chromatography on Sephadex LH-20 using MeOH as the mobile phase to furnish the desired fluorescent probe α(1→6)-linked octamannosyl glycoside **1** in 71% yield. The signals in the ^{1}H NMR spectrum of **1** in D_2O were not well resolved at 300 MHz, but showed protons in aromatic and sugar regions along with a singlet at δ 3.11 PPM (CH_2NH) and δ 2.29 [$N(CH_3)_2$]. Similarly, in the 75 MHz ^{13}C NMR spectrum of **1** in D_2O, the presence of signals at δ 46.82 ppm [$N(CH_3)_2$] and δ 43.56 ppm (CH_2NH) supported the structure. The final structural confirmation was obtained by FABMS analysis of **1**, which showed a peak at 1613.1 $[M+Na]^+$, corresponding to the molecular formula $C_{62}H_{98}N_2O_{43}SNa$.

In conclusion, we have reported a successful and efficient synthesis of a dansylated homolinear α(1→6)-linked octamannan fluorescent probe for its future biochemical potential use as a delivery vehicle for drugs via mannan receptors present in the infected macrophages.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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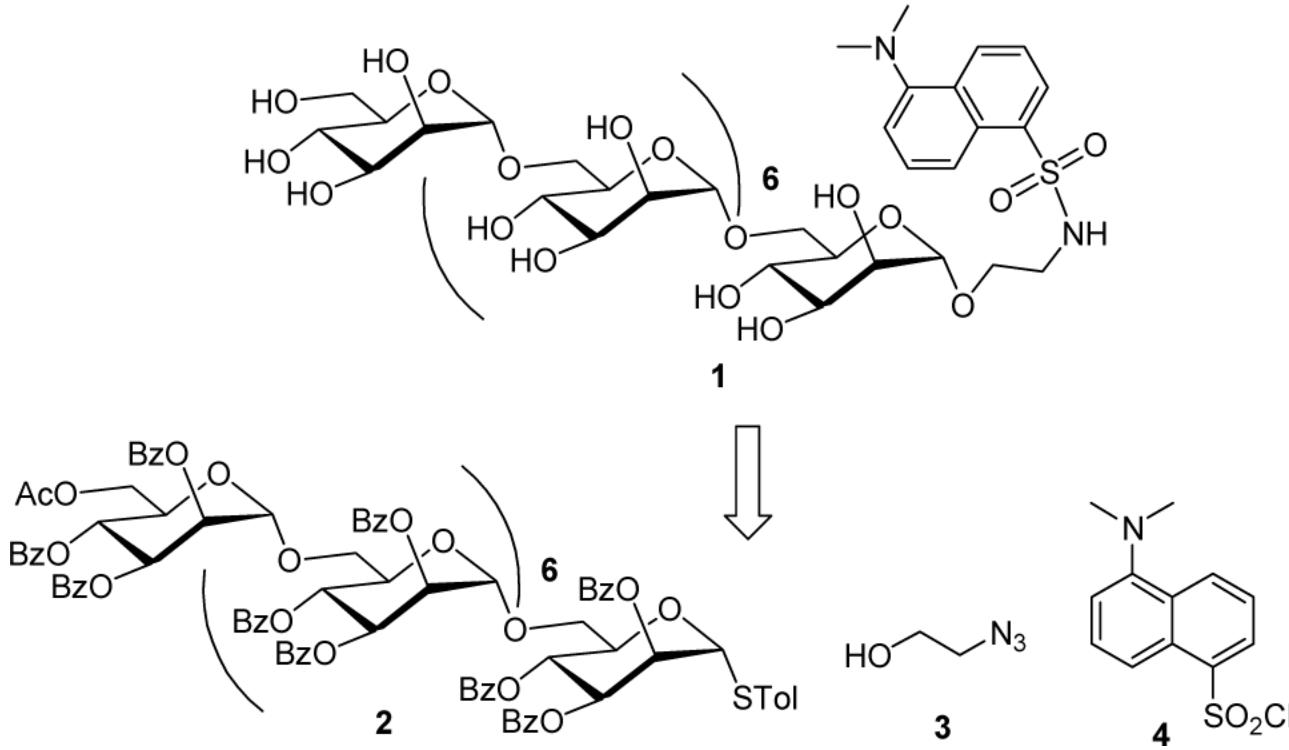

Figure 1.
Target α(1→6)-linked octamannan fluorescent probe **1** and its precursors.

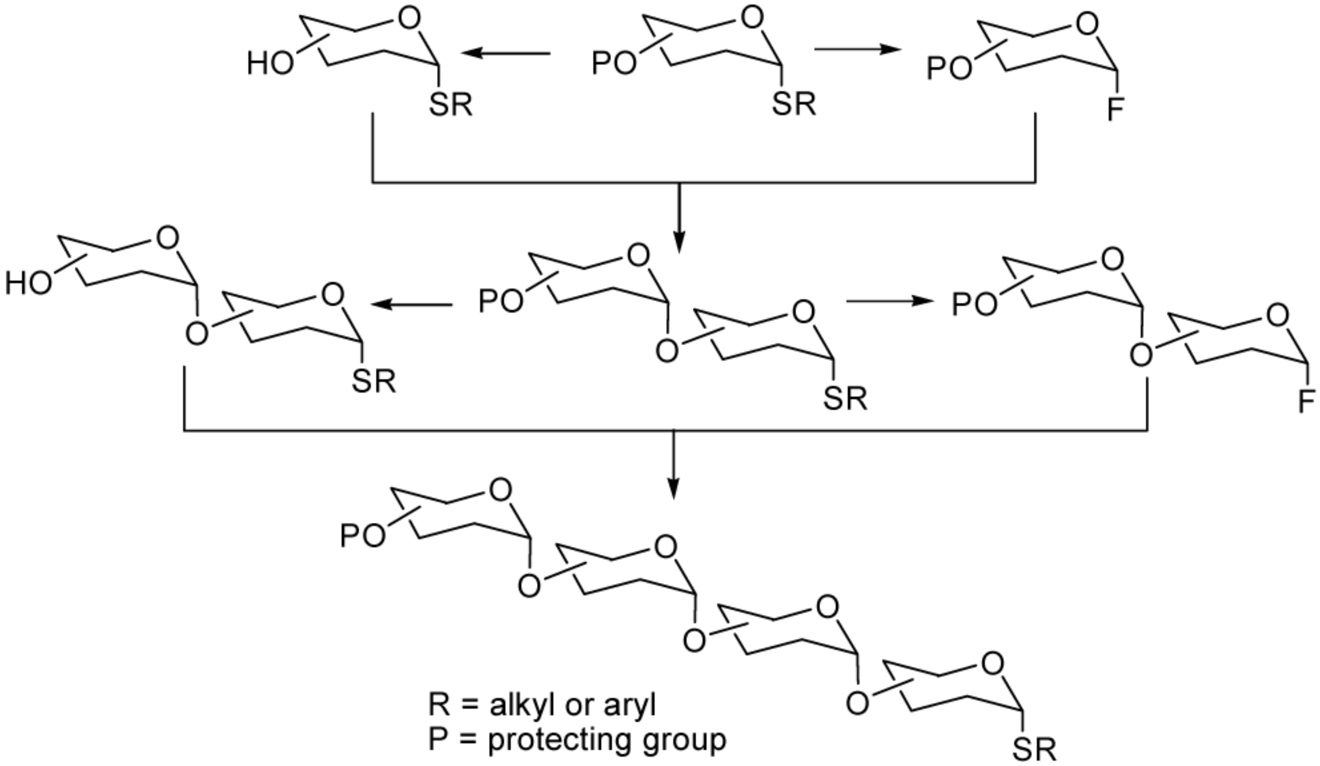

Figure 2.
Two stage activation of thioglycoside for glycoside bond formation. Other protected OH's on sugars are omitted for simplification.

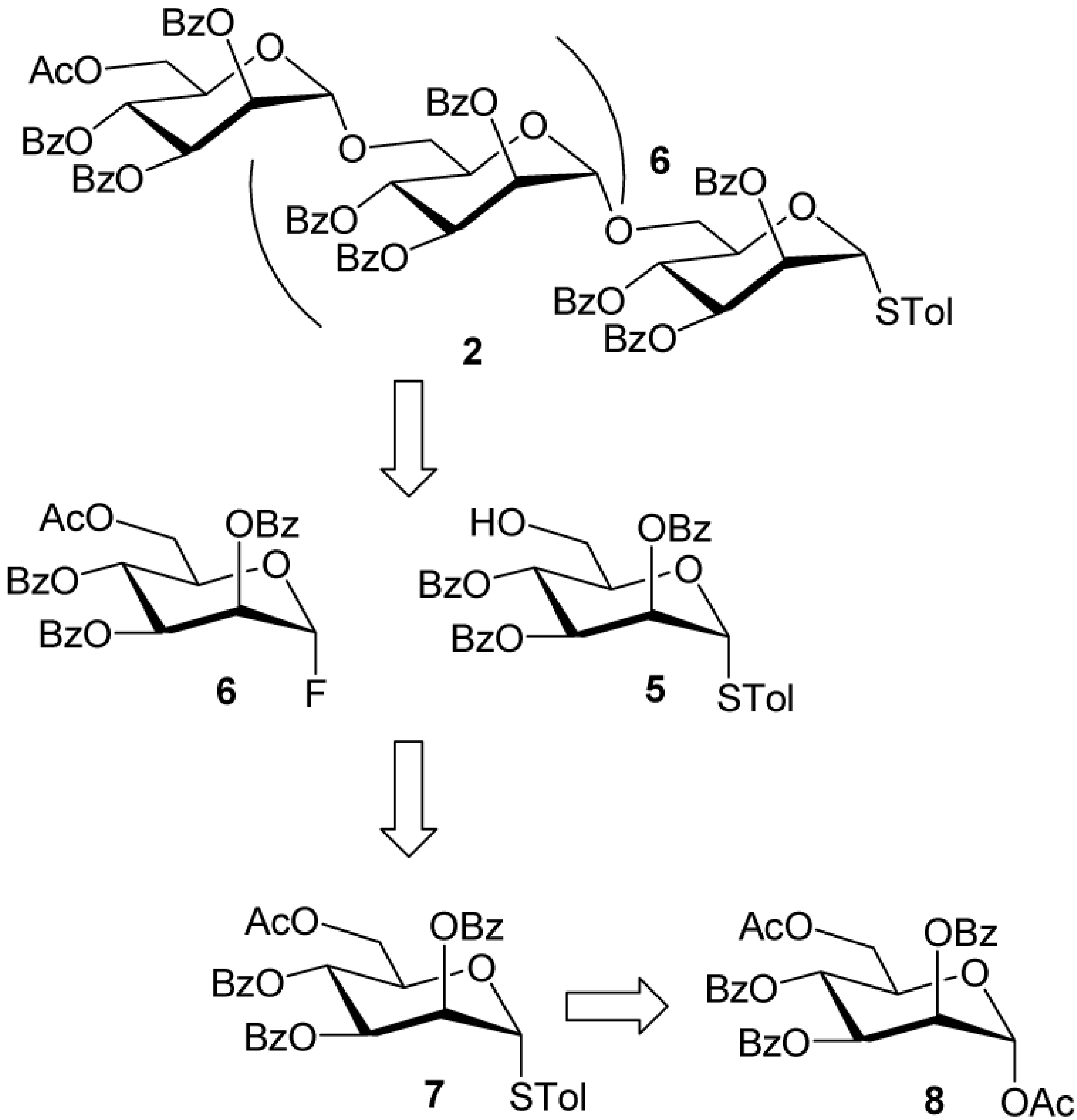

Figure 3.
Synthetic plan for the synthesis of α(1→6)-linked octamannosyl thioglycoside **2**

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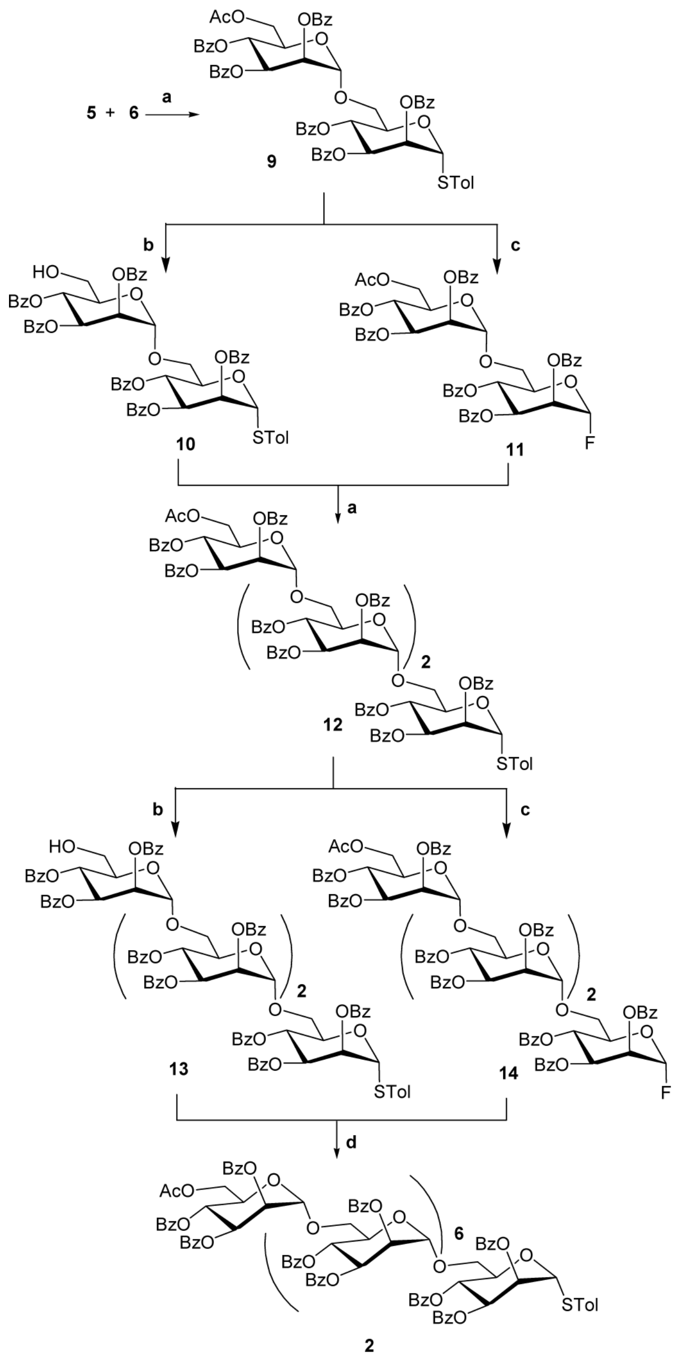
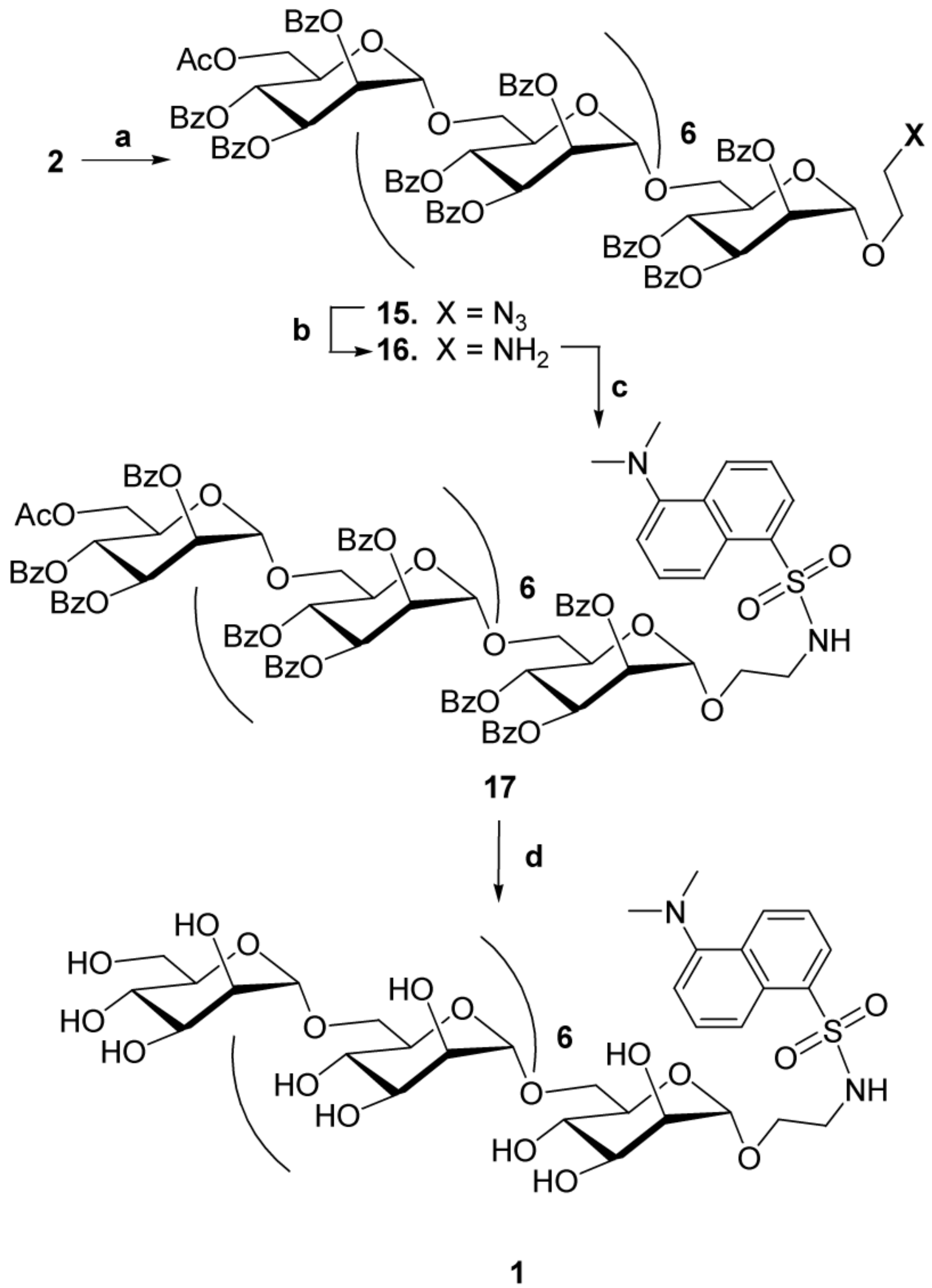


Scheme 1.

Reactions and Reagents. (a) $AgClO_4$, $SnCl_2$, dry CH_2Cl_2, 4Å Mol sieves, rt, overnight, **9:** 97%, **12:** 80%. (c) $AcCl/MeOH/CH_2Cl_2$ (1:20:20 v/v), rt, overnight, **10:** quantitative yield, **13:** 86%. (b) DAST, NBS, dry CH_2Cl_2, −20 °C, 4 h, **11:** 91%, **14:** 84%. (d) Cp_2HfCl_2, AgOTf, dry CH_2Cl_2, 4A Mol sieves, rt, overnight, 61%.



Scheme 2.

Reactions and Reagents. (a) $HOCH_2CH_2N_3$, NIS, AgOTf, dry CH_2Cl_2, 4A Mol sieves, −4 °C, 45 min, 89%. (b) Pd/C, HCO_2NH_4, dry MeOH–CH_2Cl_2 (9:1), rt, 4 h, 82%. (c) Dansyl chloride, *N*-methylimidazole, dry CH_2Cl_2, 0 °C, overnight, 69%. (d) satd. NH_3 in MeOH, dry CH_2Cl_2, rt, overnight, 71%.