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Facile construction of 1,2-*cis* glucosidic linkage using sequential oxidation–reduction route for synthesis of an ER processing α-glucosidase I substrate

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ABSTRACT

The fluorescence-labeled hexasaccharide (Glcα1-2Glcα1-3Glcα1-3Manα1-2Manα1-2Manα) was synthesized as a substrate for the processing enzyme α-glucosidase I. To construct the 1,2-*cis* glucosidic linkages, we employed an α stereoselective coupling using the mannosyl donor by assisted neighboring-group participation, followed by conversion of the stereochemistry of the C-2 hydroxyl group in the mannose residue using sequential oxidation of C-2 hydroxyl group to a 2-keto group and stereoselective reduction of the hydroxyl group to the *gluco*-configuration to provide the corresponding α-glucoside derivative. Using this strategy, the three consecutive α-glucosidic linkages were easily obtained in a stereoselective manner. Finally, the Dansyl labeled hexasaccharide derivative was used to measure the activity of processing α-glucosidase I.

Oligosaccharide parts of glycoproteins play critical roles in numerous biological events.[1] In particular, the important role of asparagine (Asn)-linked oligosaccharides in glycoprotein quality control has attracted attention recently.[2] The synthesis of Asn-linked glycoproteins is a multistep process, initiated by the *en bloc* transfer of a tetradecasaccharide ($Glc_3Man_9GlcNAc_2$) from dolichyl pyrophosphate to the asparagine residue of a protein in the lumen of the endoplasmic reticulum. Subsequent removal of the glucose residues by glucosidase I and glucosidase II delivers glycoproteins into the folding machinery called the calnexin/calreticulin cycle.[3] Additions are subsequently made to the carbohydrate structure by various glycosidases and glycosyltransferases, resulting in a wide variety of complex *N*-glycans.[4]

Processing α-glucosidase I catalyzes the first step of *N*-glycan processing which is a critical point in the regulation of glycoprotein synthesis because the processing of oligosaccharides is blocked until the removal of the terminal α-linked glucose residue.[5] Recently, homologous proteins of the processing α-glucosidase I belonging to the glycoside hydrolase family 63 (GH63) was found in bacteria, archaea, and eukaryotes, as well as a report on successful cloning and heterologous production.[6] To study the structure–function relationship of these enzymes at the molecular level, the current lack of a sufficient amount of the substrate and its derivatives presents a bottleneck. The substrate of the processing α-glucosidase I includes a tri-glucoside structure having sequential 1,2-*cis* glucosidic linkages. In order to conduct synthetic studies of this sequence, conditions for efficient construction of the 1,2-*cis* glucosidic linkages are necessary. Therefore, extensive efforts by many laboratories have been devoted to the synthesis of such oligosaccharides. The recent development of numerous diverse and innovative strategies for the synthesis of 1,2-*cis* glucosides has been reviewed.[7]

In this Letter, we describe the preparation of the hexasaccharide derivative **1**, Glcα1-2Glcα1-3Glcα1-3Manα1-2Manα1-2Man, which is the substrate for processing α-glucosidase I (Fig. 1A). Recently, we synthesized tetradecasaccharide containing 1,2-*cis* glucosidic linkages. However, glycosylation of undecasaccharide acceptor with triglucoside donor gave a tetradecasaccharide derivative which contaminated with a β-isomer (α/β = 19/1) and could not separate it at this stage.[8] To avoid this, the novel aspect of the synthesis described in this report is the efficient and facile construction of 1,2-*cis* glucosidic linkages using (1) the anomeric effect and neighboring-group participation assisted stereoselective
α-mannosylation for the construct of the α-linkages, and (2) inversion of the stereochemistry of C-2 hydroxyl groups via oxidation

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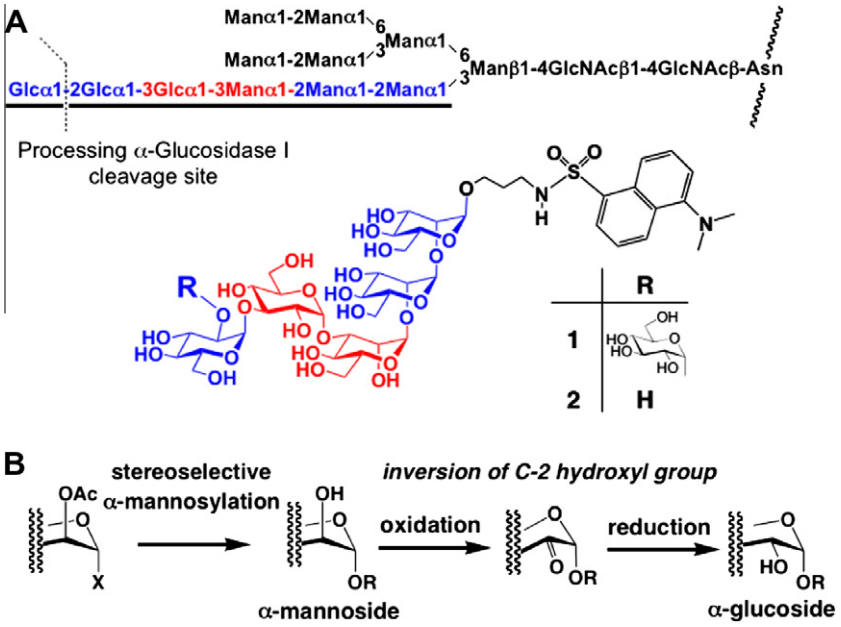
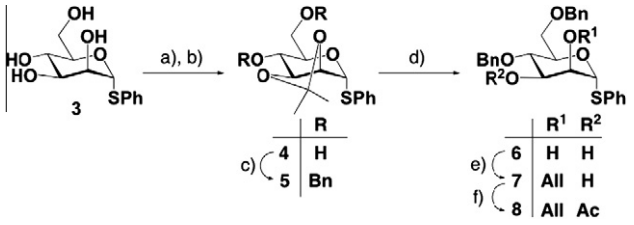


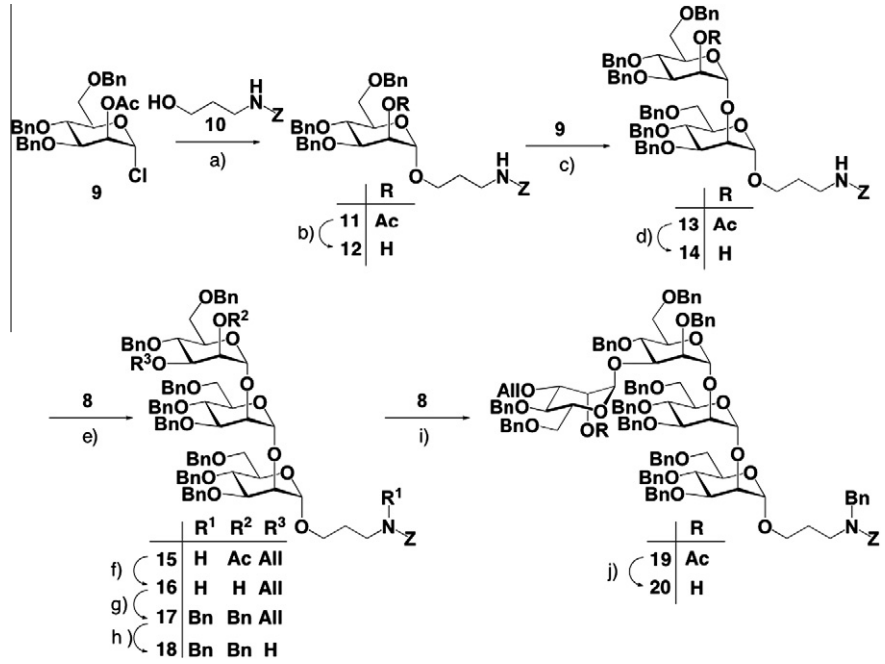
Figure 1. Structure of the target compounds (**A**) and synthetic strategy for the construction of α-glucoside linkage (**B**).



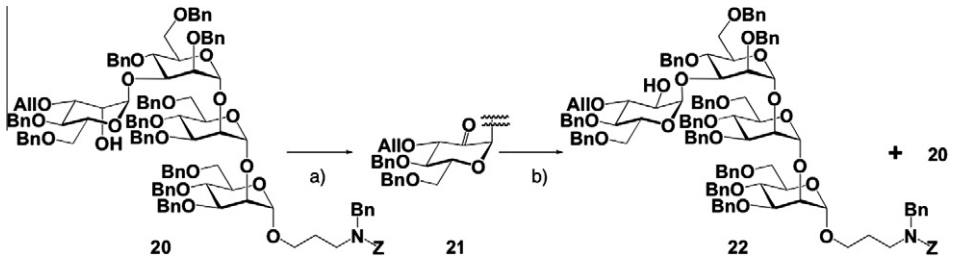
Scheme 1. Reagents and conditions: (a) 2,2-dimethoxypropane, CSA, DMF, 50 °C, 76%; (b) PPTS, MeOH, CH_2Cl_2, 79%; (c) NaH, BnBr, DMF, 94%; (d) Amberlyst, MeOH, CH_2Cl_2, 99%; (e) (i) Bu_2SnO, MeOH, 90 °C; (ii) AllBr, TBAI, toluene, 110 °C, 80% in two steps; (f) Ac_2O, pyridine, 96%.

followed by stereoselective reduction to the desired α-*gluco*-configuration (Fig. 1B). Inversion of the stereochemistry of the C-2 hydroxyl group either by the oxidation–reduction method or by direct S_N2 displacement is the most commonly used strategy for the synthesis of β-mannosides[9] but there are few reports on the construction of α-glucosides by inversion methods.[10]

In order to construct the target molecule and reduce the number of synthetic steps required, we prepared the two mannose donors **8** and **9**. Firstly the required mannose donor, a 2,3-OH masked by two orthogonal protecting groups was obtained from



Scheme 2. Reagents and conditions: (a) AgOTf, MS 4 Å, CH_2Cl_2, −20 °C, 93%; (b) NaOMe, MeOH, 86%; (c) AgOTf, MS 4 Å, CH_2Cl_2, −40 °C, 91%; (d) NaOMe, MeOH, 94%; (e) NIS, AgOTf, MS 4 Å, CH_2Cl_2, −20 °C, 70%; (f) NaOMe, MeOH, 92%; (g) NaH, BnBr, DMF, 92%; (h) (i) $[Ir(cod)(PMePh_2)_2]$ PF_6, H_2, THF; (ii) 1 M aq HCl, acetone, 77% in two steps; (i) NIS, AgOTf, MS 4 Å, CH_2Cl_2, −20 °C, 77%; (j) NaOMe, MeOH, THF, 88%.



Scheme 3. Reagents and conditions: (a) Ac_2O, DMSO; (b) $NaBH(OAc)_3$, THF, 0 °C, 78% in two steps.

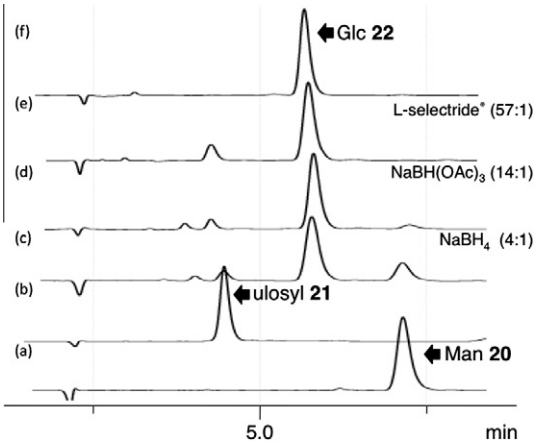
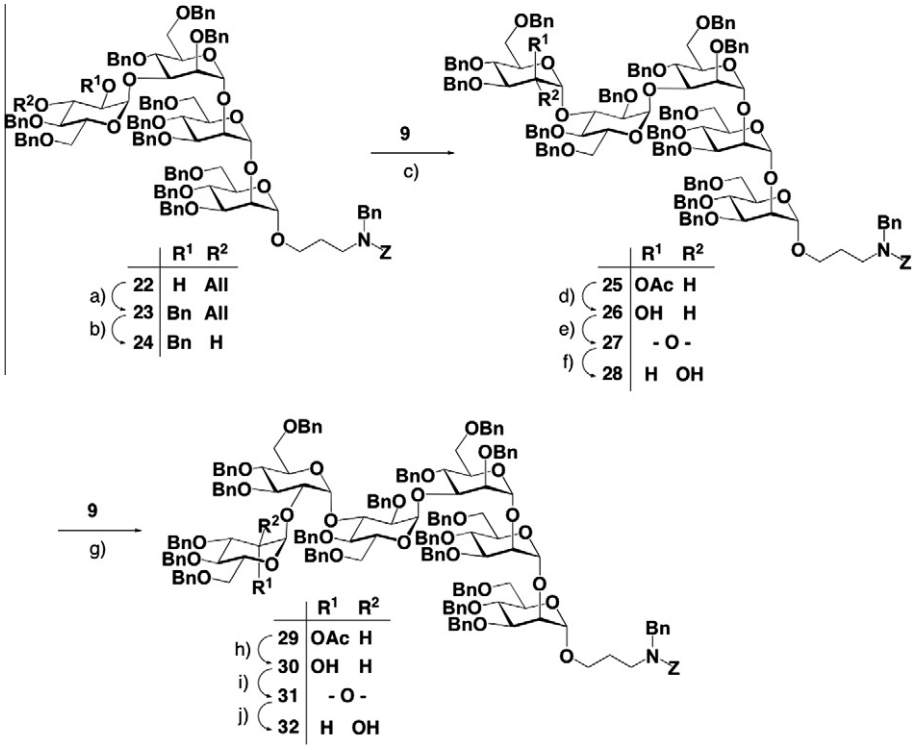


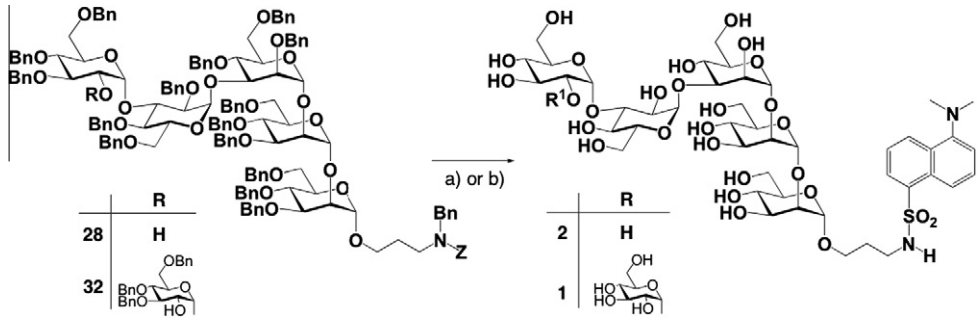
Figure 2. HPLC charts of hydride reduction of **21** using reducing agents. Analytical conditions; column: Mightysil Si 60 (4.6 × 150 mm), eluent: hexane/ethyl acetate (65/35), flow rate: 1 mL/min, detection: UV 210 nm; (a) *manno*-derivative **20**; (b) ulosyl **21**; (c) $NaBH_4$ in dioxane/H_2O = 8/1 (**22**/**20** = 4/1); (d) $NaBH(OAc)_3$ in THF (**22**/**20** = 14/1); (e) L-Selectride® in THF (**22**/**20** = 57/1); (f) *gluco*-derivative **22**.

thiophenyl mannoside **3** as shown in Scheme 1. Treatment of **3** with dimethoxypropane in the presence of CSA gave the 2,3:4,6-diisopropylidene mannose derivative and removal of the 4,6-isopropyridene group by PPTS in MeOH gave compound **4** in 60% yield (two steps). Benzylation of **4** and subsequent removal of the 2,3-isopropyridene group provided the 2,3-position di-hydroxyl derivative **6**. Regioselective allylation of the C-3 hydroxyl group **6** via the stannylene acetal provided **7** in 80% yield. The resultant was converted into the mannosyl donor **8** in 96% yield. In order to obtain the mannose donor for construction of the α-1,2-linkage, we synthesized the mannosyl chloride derivative **9** well established for α-mannosylation from D-mannose according to the literature in six steps.[11]

With all building blocks in hand, construction of the tetra-mannooligosaccharide derivative was undertaken as illustrated in Scheme 2. Glycosylation of **9** and the amino alcohol derivative **10** was conducted by first using AgOTf as a promoter, followed by deacetylation to provide **12** in 80% yield (two steps). Under similar conditions, coupling of **9** and **12** provided the corresponding disaccharide derivative **13** in 91% yield. Subsequent deacetylation of **13**



Scheme 4. Reagents and conditions: (a) NaH, BnBr, DMF, 95%; (b) (i) $[Ir(cod)(PMePh_2)_2]PF_6$, H_2, THF; (ii) 1 M aq HCl, acetone, 92% in two steps; (c) AgOTf, MS 4 Å, CH_2Cl_2, −40 °C, quant.; (d) NaOMe, MeOH, THF, 93%; (e) Ac_2O, DMSO; (f) $NaBH(OAc)_3$, THF, 64% in two steps; (g) AgOTf, MS 4 Å, CH_2Cl_2, −40 °C, quant.; (h) NaOMe, MeOH, THF, 82%; (i) Ac_2O, DMSO; (j) $NaBH(OAc)_3$, THF, 64% in two steps.



Scheme 5. Reagents and conditions: (a) (i) $Pd(OH)_2/C$, H_2, THF, H_2O, 59%; (ii) Dansyl Cl, 1 M aq $NaHCO_3$, 85% (**1**); (b) (i) $Pd(OH)_2/C$, H_2, THF, H_2O, 66%; (ii) Dansyl Cl, 1 M aq $NaHCO_3$, 66% (**2**).

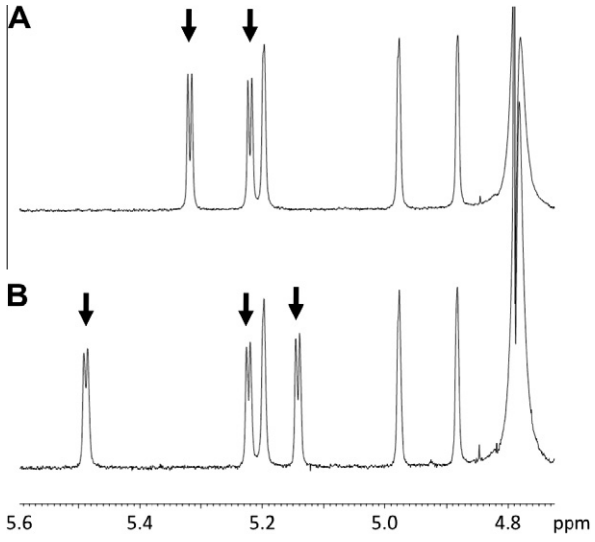


Figure 3. ^1H NMR spectra (600 MHz, D_2O, 18 °C, referenced to HOD adjusted to 4.79 ppm) of pentasaccharide **2** (A) and hexasaccharide **1** (B). Anomeric signals derived from α-Glc are indicated by arrows.

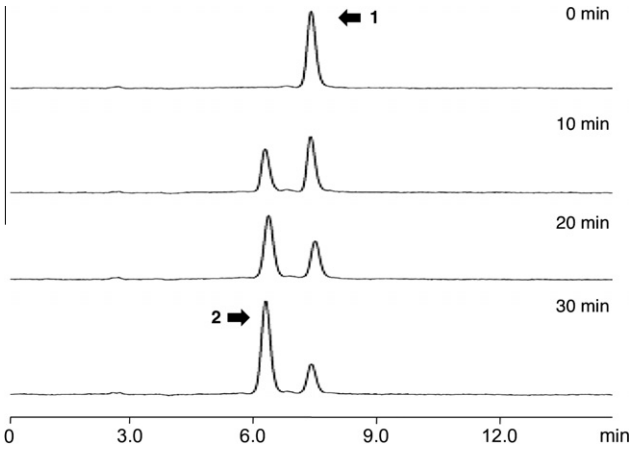


Figure 4. HPLC analysis of the hydrolysates of **1** treated with processing α-glucosidase I. Analytical conditions; column: Shodex Asahipak NH_2P-50 4E (4.6 × 250 mm), eluent: solvent A consisted of 97% acetonitrile, 0.3% acetate buffer (pH 7.0); solvent B consisted of 30% acetonitrile, 0.3% ammonium acetate buffer (pH 7.0). The samples were eluted with a 20 min linear gradient from 30% to 65% solvent B at a flow rate of 0.8 mL min^{-1} and a column temperature of 40 °C. The Dansyl-oligosaccharide elutions were monitored via their fluorescence signals (excitation wavelength, 310 nm; emission wavelength, 380 nm).

gave the disaccharide acceptor **14** in 94% yield. Coupling of the thioglycoside donor **8** to the disaccharide acceptor **14** in the presence of NIS/AgOTf[12] in CH_2Cl_2 at −20 °C gave the tri-saccharide derivative **15** in moderate yield (70%). Conversion of the trisaccharide **15** into the acceptor **18** was performed by deacetylation, followed by benzylation of the C-2 hydroxyl group with an amino group to give **17**. Deallylation of **17** was cleanly achieved by an iridium-catalyzed process[13] to provide the trisaccharide accepter **18** in 65% yield (four steps). Construction of the tetrasaccharide was performed using **8** and the acceptor **18** using NIS/AgOTf as a promoter, providing the tetrasaccharide **19** in 77% yield.[14] Subsequent deacetylation of the tetrasaccharide **19** gave the mannotetraose derivative **20** in 88% yield.

Having obtained the mannotetraose derivative **20** possessing a C-2 free hydroxyl group at non-reducing end of the mannose residue, we turned out our attention to inverting the stereochemistry of C-2 hydroxyl group (Scheme 3).

First, we examined a procedure reported by Lemieux.[10] Oxidation of the C-2 hydroxyl group into the ulosyl derivative **21** was done using DMSO and Ac_2O. The reaction was monitored by HPLC analysis as shown in Figure 2. After consumption of the *manno*-derivative **20**, the resultant 2-keto-derivative **21** was obtained by reduction using $NaBH_4$ in aqueous dioxane, to give the corresponding α-glucoside derivative **22** as the major product. The ratio of *gluco*-derivative **22** and *manno*-derivative **20** was 4:1, as determined by HPLC. The epimeric *gluco* **22** and *manno*-derivative **20** were readily separated by column chromatography to afford pure compounds (**20**; R_f 0.24, **22**; R_f 0.43, toluene/EtOAc, 5:1) and the *manno* epimer **20** could be recycled. In order to evaluate the stereoselectivity of the reduced product, we carried out the procedure using different reducing agents and reaction solvent(s). The conversion ratio of *gluco* **22**: *manno* **20** was determined by HPLC. Some of the results are shown in Figure 2. The *gluco* isomer **22** was obtained as major product using the sterically bulky reducing agent. In the case of $NaBH(OAc)_3$ reduction was obtained to give the *gluco* derivative **22** and *manno* derivative **20** in 78% and 8% yield, respectively. The reduction of **21** using L-Selectride® yielded the corresponding α-glucoside **22** selectively, but the isolated yield was low (47%) because of the production of highly polar products.

Encouraged by our results with this new method for stereoselective reduction to the *gluco* derivative, we applied it to construction of a triglucoside sequence (Scheme 4). Benzylation of **22** and subsequent removal of the allyl group provided the tetrasaccharide acceptor **24** in 87% yield (three steps).

Stereoselective coupling of **9** and **24** assisted by neighboring-group participation gave pentasaccharide **25** as the sole product. The subsequent deacetylation of **25** provided **26** in 93% yield. The resultant **26** was converted into the ulosyl derivative **27** using $DMSO/Ac_2O$ and subsequently reduced by $NaBH(OAc)_3$ to give

the glucose derivative **28** in good yield and the *manno*-derivative **26**, 64% and 15% yields, respectively (**26**; R_f 0.23, **28**; R_f 0.30, hexane/EtOAc, 7:3). The *manno* derivative was recycled. Finally, we proceeded to the assembly of the hexasaccharide derivative. The coupling of **9** with **28** was achieved by the action of AgOTf to afford the hexasaccharide **29**, which was isolated as a single isomer in quantitative yield. Removal of the acetyl group afforded **30**. Inversion of the C-2 hydroxyl group of **30** to *gluco*-configuration using oxidation–reduction procedure preferentially gave the glucose derivative **32** (the ratio of Glc:Man, 7:1). The reaction mixture was purified by conventional column chromatography (**30**; R_f 0.28, **32**; R_f 0.46, toluene/EtOAc, 7:1) to give **32** in 64% yield (two steps).

The hexasaccharide **32** and the pentasaccharide **28** were deprotected by conventional means and the hexasaccharide derivative was obtained in 59% yield (Scheme 5). A Dansyl group was then introduced for use in the detection of enzyme activities providing **1** in 85% yield. The pentasaccharide **28** was converted into **2** in 44% yield (two steps). The ^{1}H NMR spectrum of **1** and **2** were in good agreement with the data reported for closely related compounds (Fig. 3).[8,15]

The enzymatic activity of processing α-glucosidase I from *Aspergillus brasiliensis* ATCC 9642[6a] was measured using **1** as shown in Figure 4. A reaction mixture containing 2 μg of α-glucosidase I, 46 nmol of **1**, in PBS buffer (pH 7.0) was incubated for 30 min. The HPLC profile of the hydrolysis reaction showed that 80% of **1** had been hydrolyzed to diglucosylated pentasaccharide **2** as indicated by a shift in retention time.

In conclusion, facial construction of the tri-glucoside residue was established using (1) the anomeric effect and neighboring-group participation assisted stereoselective α-mannosylation, (2) oxidation of the C-2 hydroxyl group and (3) reduction of the corresponding 2-keto group to the desired α-*gluco*-configuration. Regarding the stereoselectivity of the hydride reduction toward ulosyl derivatives, our results have been inconsistent to previous reports.[9a–d] A more systematic study of the reduction is in progress. Using our strategy, the fluorescence-labeled hexasaccharide substrate for processing α-glucosidase I was successfully synthesized. In addition, the Dansyl group labeled hexasaccharide derivative was demonstrated to act as a substrate for processing α-glucosidase I from *A. brasiliensis* ATCC 9642. A detailed investigation of the relative rates of cleavage of processing α-glucosidase I is currently underway, and results will be reported in due course.

Acknowledgments

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14. Reaction of using tetra-*O*-benzyl thioglycoside derivative under MeOTf conditions afforded a mixture of α and β isomer (α/β = 3/1): Matsuo, I.; Wada, M.; Manabe, S.; Yamaguchi, Y.; Otake, K.; Kato, K.; Ito, Y. *J. Am. Chem. Soc.* **2003**, *125*, 3402–3403.
15. Physical data for **1** and **2** are given below. ^{1}H NMR spectra were measured on a JEOL ECS-600 spectrometer. MALDI-TOF MS spectra were recorded in the positive ion mode on an AXIMA Performance (Shimazu/KRATOS) equipped with nitrogen laser with an emission wavelength of 337 nm. 2,5-Dihydroxybenzoic acid (10 mg dissolved in 1 mL of 50/50 (v/v) acetonitrile/water with 0.1% TFA) was used as matrix; **1**: ^{1}H NMR (600 MHz, D_2O, 18 °C): δ 8.49 (d, 1H, J = 8.7 Hz), 8.27 (d, 1H, J = 8.7 Hz), 8.23 (d, 1H, J = 7.2 Hz), 7.68 (dd, 2H, J = 8.0 Hz, J = 15.8 Hz), 7.41 (d, 1H, J = 7.6 Hz), 5.49 (d, 1H, J = 3.9 Hz), 5.22 (d, 1H, J = 3.9 Hz), 5.20 (s, 1H), 5.14 (d, 1H, J = 3.8 Hz), 4.98 (s, 1H), 4.88 (s, 1H), 4.19 (br s, 1H), 4.04 (br s, 1H), 3.30 (m, 2H), 2.96 (m, 2H), 2.86 (s, 6H, $CH_3 \times 2$), 1.56 (m, 2H); MALDI-TOF MS calcd for $C_{51}H_{80}N_2O_{33}SNa$ $(M+Na)^+$ m/z: 1303.2, found: 1303.1; **2**: ^{1}H NMR (600 MHz, D_2O, 18 °C): δ 8.49 (d, 1H, J = 7.9 Hz), 8.27 (d, 1H, J = 8.5 Hz), 8.23 (d, 1H, J = 8.3 Hz), 7.68 (dd, 2H, J = 8.3 Hz, J = 16.7 Hz), 7.41 (d, 1H, J = 8.1 Hz), 5.31 (d, 1H, J = 3.8 Hz), 5.22 (d, 1H, J = 3.9 Hz), 5.20 (s, 1H), 4.98 (s, 1H), 4.88 (br s, 1H), 4.19 (m, 1H), 4.03 (m, 1H), 3.97 (m, 1H), 3.30 (m, 2H), 2.96 (m, 2H), 2.86 (s, 6H, $CH_3 \times 2$), 1.55 (m, 2H); MALDI-TOF MS calcd for $C_{45}H_{70}N_2O_{28}SNa$ $(M+Na)^+$ m/z: 1141.4, found: 1140.9.