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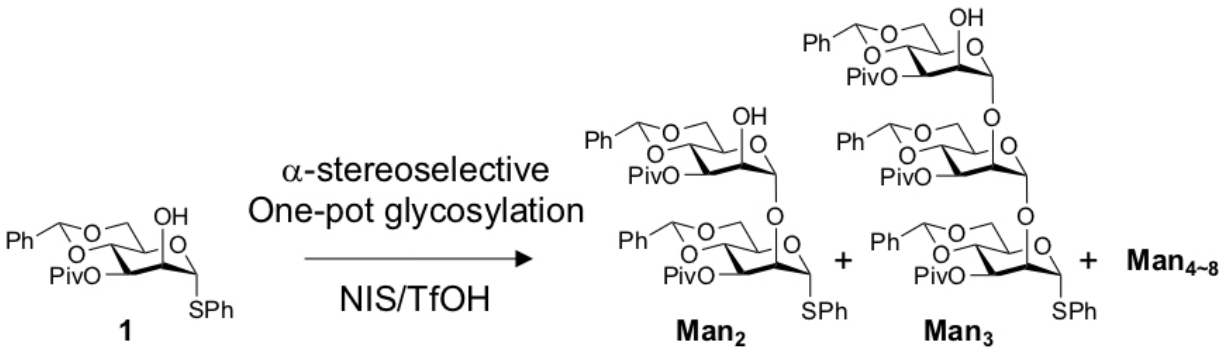

Efficient synthesis of α(1,2)-linked oligomannoside derivatives through one-pot glycosylation

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Abstract: An α(1,2)-linked oligomannoside derivative having a free C-2 hydroxyl group and a C-3 pivaloyl group was synthesized from a thiophenyl mannose derivative **1** using a one-pot self-condensation and applying a α-stereoselective procedure. The mannosylation exclusively generated α-mannoside linkages. The observed α-directing effect was rationalized by the remote participation of the pivaloyl group in C-3 position. The polymerization degree was controlled by the promoter amount providing the mannobiose derivative as a major product. Applying this method eliminated many synthetic steps. The α(1,2)-linked oligomannoside derivatives, which are key intermediates for the synthesis of oligomannose type *N*-glycans for glycoproteins, were easily prepared.

Keywords: One-pot glycosylation; α(1-2) oligomannoside; stereocontrolled synthesis; oligomannose type *N*-glycan

1. Introduction

α(1,2)-linked oligomannoside structures are present on surfaces of pathogenic microorganisms such as viruses, bacteria, fungi, and protozoan parasites. They play an important role in the pathogenesis of several pathogens and communication with the immune system [1–3]. Further, these structures are major components of oligomannose type *N*-glycans [Figure 1A] which play critical roles in the glycoprotein quality control of the endoplasmic reticulum (ER) [4]. To study the function of oligomannose type *N*-glycans and related proteins at molecular level, we synthesized these glycans using the convergent synthesis [5] and applying the top-down chemoenzymatic approach [6]. Many studies reported the attempt to synthesize these glycans [7], however, these approaches were insufficient because many synthetic steps are required to prepare the oligomannoside blocks. Recently, the fast and easy synthesis of α(1-2)-linked oligomannoside, which includes mannobiose and mannotriose as major components of oligomannose type *N*-glycans, was achieved by chain-growth polymannosylation using a C-2 acetylated thiophenyl donor [8] or 1,2-orthoester [9], followed by the self-condensation of a thiophenyl donor having a free C-2 hydroxyl group [10]. Two or more glycosylation steps are sequentially carried out without requiring deprotection processes and purification steps, eliminating the extensive synthetic effort to obtain the target oligosaccharides [11]. In this study, the stereocontrolled synthesis of α(1-2)-linked oligomannoside derivatives using a one-pot method is described to easily access ER-rerated *N*-glycans.

2. Results and discussion

2.1. Stereoselective construction of oligomannoside using a one-pot synthesis method

The synthesis of α(1-2)-linked oligomannoside derivatives was carried out using a one-pot synthesis method as depicted in Figure 1B. It started with the preparation of mannose derivative **1,** which has a thiophenyl group at the anomeric position and a free hydroxyl group at the C-2 position of the same molecule. It was hypothesized that this compound will achieve the self-condensation reactions and easily proceed with high α-stereoselectivity because of 1) an epoxide-like intermediate which is formed by the nucleophilic attack of the C-2 hydroxyl group on the oxocarbenium cation at the anomeric position, and 2) the remote

participation of the pivaloyl group in C-3 position [12, 13].

The mannose derivative **1** was synthesized according to literature [13] using a one-pot glycosylation reaction with *N*-iodosuccinimide (NIS)/trifluoromethanesulfonic acid (TfOH) as promotor [14]. When compound **1** was added slowly to the mixture of 1 equiv. of NIS and 0.3 equiv. of TfOH in CH_2Cl_2 at −78 °C, oligomannoside derivatives (**Man$_2$**–**Man$_8$**) and debenzylidenated products were detected by MALDI TOF MS analysis. Then, the order of reagent addition was changed to reduce the damage to the protecting group. Adding 0.3 equiv. of TfOH to the mixture of compound **1** (37 mM) and 1 equiv. of NIS at –78 °C, the reaction smoothly proceeded to provide the oligomannoside derivatives. The debenzylidenated products were not detected by MALDI TOF MS. To analyze the structure of the coupling products, the reaction mixture was separated by molecular weight using gel permeation chromatography (GPC) and each collected fraction was analyzed with ^{1}H NMR spectroscopy. The profiles of the GPC chromatograms and ^{1}H NMR spectra are shown in Figure 2. These data clearly indicated that all fractions contained a single isomer. The stereochemistry of the glycosidic linkages was confirmed by the $^1J_{C\text{-}H}$ coupling constants obtained from non-decoupling HSQC and 2D NMR spectra (see Supporting Information). These results verified that the newly formed glycosidic linkages were of α-configuration. The product yields were calculated from the weight ratio of the starting material **1** and the provided compounds. The one-pot mannosylation proceeded with high α-stereoselectivity and the disaccharide derivative **Man$_2$** (28%), trisaccharide derivative **Man$_3$** (23%), tetrasaccharide derivative **Man$_4$** (7.4%), pentasaccharide derivative **Man$_5$** (5.2%), hexasaccharide derivative **Man$_6$** (2.2%), heptasaccharide derivative **Man$_7$** (0.1%), and octasaccharide derivative **Man$_8$** (trace) were obtained with only one purification step.

Next, we tested the effect of NIS addition. The use of **1** with 0.5 equiv. of NIS clearly favored the formation of the disaccharide to generate **Man$_2$** in 48% yield, although some starting material **1** remained. Furthermore, we examined the addition of NIS to enrich the trisaccharide derivative, however, the desired result was not achieved. Further, the one-pot reaction of **1** was carried out on gram-scale (72 mM) using 1.0 equiv. of NIS and 0.3 equiv. of TfOH. The reaction easily provided disaccharide derivative **Man$_2$** (316 mg), trisaccharide derivative **Man$_3$** (278 mg), and other oligomannoside derivatives. Therefore, the synthesis of key

intermediates for the construction of the oligomannose type *N*-glycans was successfully accomplished by this method. To investigate the influence of the activators controlling the degree of polymerization, methyl trifluoromethanesulfonate (MeOTf) and dimethyl(methylthio)sulfonium trifluoromethanesulfonate (DMTST) were tested. With both activators, small amount of oligomannoseide derivatives were obtained. Although, methylation of the C-2 hydroxyl group was detected by MALDI TOF MS and NMR analysis. We did not examine this further.

2.2. Mechanistic analysis of the stereoselectivity

The mannosylation reaction is easily stereocontrolled by the anomeric effect and the participation of the adjacent group at the C-2 position. However, the reaction with highly reactive hydroxyl groups often results in β-mannoside as a minor product. Here, the reaction was completely stereoselective. It is suggested that this stereoselectivity is regulated by both neighboring groups, the C-2 hydroxyl and the remote C-3 pivaloyl group. To confirm this hypothesis, we prepared mannose derivative **2** [15] which has a benzyl group at the C-3 position. This compound can eliminate the influence of the remote carbonyl group at the C-3 position. The one-pot glycosylation with **2** was conducted (Figure 3). The resultant mixture separated by molecular weight using GPC. The resulting fractions were analyzed by ^{1}H NMR. Their spectra provided a complex mixture of stereo isomers (Figure 3D), revealing that the C-3 benzylated derivative exhibited low stereoselectivity. It was considered from this result that the hydroxyl group at the C-2 position does not affect the stereoselectivity. Thus, the high α-stereoselectivity of the one-pot glycosylation with **1** would be controlled by the remote participation of the pivaloyl group at the C-3 position instead of the epoxy intermediate neighboring C-2 hydroxyl group.

3. Conclusions

The stereoselective synthesis of α(1,2)-linked oligomannoside derivatives was successfully achieved using a one-pot method. The observed α-directing effect on mannose derivative **1** would be caused by the remote participation of the pivaloyl group in C-3 position. The α(1,2)-linked mannobioside and mannotrioside

derivatives, which correspond to key intermediates for the synthesis of oligomannose type *N*-glycans, were easily prepared. Using these oligosaccharide derivatives, the systematic synthesis of oligomannose type *N*-glycans is planned to analyze the biosynthetic process of asparagine-linked glycan related enzymes and cytosolic free oligosaccharide functions.

4. Experimental section

4.1. General methods

All reactions were carried out under an argon atmosphere. Dry CH_2Cl_2 was purchased from Kanto Kagaku Co., Ltd, Japan. Molecular sieves (4Å) were activated at 170–200 °C for 2–3 h under vacuo prior to application. Analytical thin layer chromatography was developed on a silica gel 60 F plate (Merck). HPLC was performed on a Recycling Preparative HPLC system (LaboACE LC-5060) equipped with a UV detector (Japan Analytical Industry Co., Ltd., Tokyo, Japan). The gel permeation column for HPLC (JAIGEL-HR) was a product of Japan Analytical Industry Co., Ltd. Gel permeation chromatography for the preparative scale was performed on a SX-1 (Bio-Rad) column (100 mm ϕ × 800 mm). NMR spectra were recorded with a JEOL ECS-400 and an ECA-600 spectrometer. ^{1}H NMR spectra were referenced to TMS at 0.00 ppm. ^{13}C NMR spectra were referenced to the central peak of $CDCl_3$ at 77.0 ppm. Assignments were made by standard pfg COSY, pfg TOCSY and pfg HSQC spectra. MALDI-TOF MS was recorded in the high-resolution mode with positive ion mode on an AXIMA-Performance (Shimadzu, Kyoto, Japan).

4.2 General procedures

4.2.1. One-pot mannosylation using 1

The thiophenyl mannoside **1** (50.0 mg, 0.112 mmol) and *N*–iodosuccinimide (1.0 eq, 25 mg, 0.112 mmol) in 3 mL of CH_2Cl_2 were stirred at –78 °C in the presence of MS 4A. Then, trifluoromethane sulfonic acid (3 µL, 34 µmol) was added. The reaction mixture was stirred at –78 °C for 4 hours. The reaction was added to triethylamine. The mixture was then diluted with EtOAc and filtered using Celite. The filtrate was washed with saturated aqueous (sat. aq) $Na_2S_2O_3$, sat. aq $NaHCO_3$, sat. aq NaCl, and dried over $MgSO_4$. The organic

layer was filtered and concentrated *in vacuo*. The resultant residue was purified by HPLC and fitted on a gel filtration column (JAIGEL-2HR and 2.5 HR) to obtain mannobiose **Man$_2$** (13.9 mg, 28%), mannotriose **Man$_3$** (11.3mg, 23%), mannotetraose **Man$_4$** (3.7 mg, 7.4%), mannopentaose **Man$_5$** (2.6 mg, 5.2%), mannohexose **Man$_6$** (1.1 mg, 2.2%), mannoheptaose **Man$_7$** (0.1 mg, 0.1%) and mannooctaose **Man$_8$** (trace), respectively.

Phenyl 4,6-*O*-benzylidene-3-*O*-pivaloyl-α-D-mannopyranosyl-(1→2)-4,6-*O*-benzylidene-3-*O*-pivaloyl-1-thio-α-D-mannopyranoside (**Man$_2$**)

R*f*= 0.36 (Toluene/AcOEt =6/1, v/v); ^{1}H-NMR (600 MHz, $CDCl_3$): δ 7.46-7.27 (m, 15H; aromatic *H*), 5.46, 5.56 (2s, 2H; PhC*H*), 5.33 (bs, 1H; H-1^I), 5.43 (dd, 1H, $J_{2,3}$ = 3.0 Hz, $J_{3,4}$ = 9.6 Hz; H-3^{II}), 5.36 (dd, 1H, $J_{2,3}$ = 3.0 Hz, $J_{3,4}$ = 10.2 Hz; H-3^I), 4.97 (d, 1H, $J_{1,2}$ = 1.2 Hz; H-1^{II}), 4.42 (dd, 1H, $J_{1,2}$ = 1.2 Hz, $J_{2,\,OH}$ = 3.0 Hz; H-2^I), 4.37 (td, 1H, $J_{4,5}$ = $J_{5,6a}$ = 10.2 Hz, $J_{5,6e}$ = 5.4 Hz; H-5^I), 4.24 (m, 2H, H-2^{II}, H-6^I), 4.19 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 10.2 Hz; H-4^I), 4.10(m, 2H; H-4^{II}, H-6^{II}), 4.01 (td, 1H, $J_{4,5}$ = $J_{5,6a}$ = 9.6 Hz, $J_{5,6e}$ = 4.8 Hz; H-5^{II}), 3.90 (t, 1H, $J_{5,6a}$ = 10.2 Hz; H-6a^I), 3.78 (t, 1H, $J_{5,6a}$ = 10.2 Hz; H-6^{II}), 2.11 (bs, 1H; O*H*), 1.26, 1.23 ppm (2s, 18H; COC(C*H*$_3$)$_3$). ; ^{13}C NMR (150 MHz, $CDCl_3$): δ 177.63, 177.49 (2C; *C*O), 137.33-125.85 (aromatic *C*), 102.82 (C-1^I), 104.41, 101.37 (2C, Ph*C*H), 88.32 (C-1^I), 79.24 (C-2^I), 76.24 (C-4^I), 76.10 (C-4^{II}), 70.31 (C-3^{II}), 70.17 (C-3^I), 70.17 (C-2^{II}), 68.47 (2C; C-6^I, C-6^{II}), 65.57 (C-5^I), 64.42 (C-5^{II}), 39.23, 39.08 (2C; CO*C*(CH$_3$)$_3$), 27.25, 27.17 (2C; COC(*C*H$_3$)$_3$) ppm.; $^1J_{CH}$(I)= 173 Hz, $^1J_{CH}$(II)= 177 Hz,; MALDI-TOF MS: *m*/*z* calcd for $C_{42}H_{50}O_{12}Na^+$: 801.292 $[M+Na]^+$; found: 801.289.

Phenyl 4,6-*O*-benzylidene-3-*O*-pivaloyl-α-D-mannopyranosyl-(1→2)-4,6-*O*-benzylidene-3-*O*-pivaloyl-α-D-mannopyranosyl-(1→2)-4,6-*O*-benzylidene-3-*O*-pivaloyl-1-thio-α-D-mannopyranoside (**Man$_3$**)

R*f*= 0.49 (Toluene/AcOEt =6/1, v/v); ^{1}H-NMR (600 MHz, $CDCl_3$): δ 7.48-7.27 (m, 20H; aromatic *H*), 5.64, 5.61, 5.58 (3s, 3H; PhC*H*), 5.57 (d, 1H; $J_{1,2}$ = 1.2 Hz; H-1^I), 5.40 (m, 2H; H-3^{II}, H-3^{III}), 5.23 (dd, 1H, $J_{2,3}$ = 3.6 Hz, $J_{3,4}$ = 10.8 Hz; H-3^I) 4.92 (d, 1H, $J_{1,2}$ = 1.2 Hz; H-1^{II}), 4.88 (d, 1H, $J_{1,2}$ = 1.8 Hz; H-1^{III}), 4.43 (dd,

1H, $J_{1,2}$ = 1.2 Hz, $J_{2,OH}$ = 3.0 Hz; H-2^{I}), 4.39 (td, 1H, $J_{4,5}$ = 4.8 Hz, $J_{5,6a}$ = $J_{5,6e}$ = 9.6 Hz; H-5^{I}), 4.23 (m, 3H; H-6^{I}, H-2^{III}, H-6^{III}), 4.14 (m, 4H; H-4^{I}, H-2^{II}, H-4^{II}, H-4^{III}), 3.98 (m, 3H; H-5^{II}, H-6^{II}, H-5^{III}), 3.90 (t, 1H, $J_{5,6a}$ = 10.2 Hz; H-6^{I}), 3.81 (m, 2H; H-6^{II}, H-6^{III}), 2.13 (d, 1H, $J_{2,OH}$ = 3.6 Hz; O*H*), 1.27, 1.24, 1.23 (3s, COC(C*H*$_3$)$_3$) ppm.; ^{13}C NMR (150 MHz, $CDCl_3$): δ 177.90, 177.81, 177.50 (3C; *C*O), 137.35-125.61 (aromatic *C*), 102.92, 102.85 (2C; C-1^{II}, C-1^{III}), 101.39, 101.22 (3C; Ph*C*H), 88.62 (C-1^{I}), 81.16 (C-2^{I}), 77.90, 76.17, 76.00 (4C; H-4^{I}, H-2^{II}, H-4^{II}, H-4^{III}), 70.28, 70.00, 69.84 (3C; C-3^{I}, C-3^{II}, C-3^{III}), 68.49, 68.38, 68.22 (3C; C-6^{I}, C-6^{II}, C-6^{III}), 65.47 (C-5^{I}), 64.58, 64.53 (2C; C-5^{II}, C-5^{III}), 39.25, 39.13, 39.10 (3C; CO*C*(CH$_3$)$_3$), 27.25, 27.23, 27.17 (3C, COC(*C*H$_3$)$_3$) ppm. ;$^{1}J_{CH}$(I) = 173 Hz, $^{1}J_{CH}$(II) = 175 Hz, $^{1}J_{CH}$(III) = 173 Hz; MALDI-TOF MS: *m*/*z* calcd for $C_{60}H_{72}O_{18}Na^{+}$: 1135.434 [M+Na]$^{+}$; found: 1135.444.

Phenyl

4,6-*O*-benzylidene-3-*O*-pivaloyl-α-D-mannopyranosyl-(1→2)-4,6-*O*-benzylidene-3-*O*-pivaloyl-α-D-mannopy ranosyl-(1→2)-4,6-*O*-benzylidene-3-*O*-pivaloyl-α-D-mannopyranosyl-(1→2)-

4,6-*O*-benzylidene-3-*O*-pivaloyl-1-thio-α-D-mannopyranoside (**Man$_4$**)

R*f*= 0.49 (Toluene/AcOEt =6/1, v/v); ^{1}H-NMR (600 MHz, $CDCl_3$): δ 7.48-7.28 (m, 25H; aromatic *H*), 5.64, 5.63, 5.62, 5.61 (4s, 4H; PhC*H*), 5.51 (d, 1H; $J_{1,2}$ = 1.2 Hz; H-1^{I}), 5.41 (dd, 1H, $J_{2,3}$ = 3.0 Hz, $J_{3,4}$ = 9.6 Hz; H-3), 5.36 (dd, 1H, $J_{2,3}$ = 3.0 Hz, $J_{3,4}$ = 10.2 Hz; H-3), 5.26 (dd, 1H, $J_{2,3}$ = 3.6 Hz, $J_{3,4}$ = 10.8 Hz; H-3), 5.24 (dd, 1H, $J_{2,3}$ = 3.0 Hz, $J_{3,4}$ = 10.8 Hz; H-3), 4.91 (d, 1H, $J_{1,2}$ = 1.8 Hz; H-1), 4.85 (d, 1H, $J_{1,2}$ = 1.2 Hz; H-1), 4.79 (d, 1H, $J_{1,2}$ =1.8 Hz; H-1^{IV}), 4.39 (td, 1H, $J_{5,6e}$ = 4.8 Hz, $J_{5,6a}$ = $J_{6a,\,6e}$ = 9.6 Hz; H-5^{I}), 4.36 (dd, 1H, $J_{2,OH}$ =3.6 Hz, $J_{1,2}$ =1.2 Hz; H-2^{I}), 4.12 (m, 10H), 3.91 (m, 8H) 1.23 (s, 9H; COC(C*H*$_3$)$_3$), 1.24 (s, 18H; COC(C*H*$_3$)$_3$), 1.73 (s, 9H; COC(C*H*$_3$)$_3$) ppm.; ^{13}C NMR (150 MHz, $CDCl_3$): δ 178.33, 178.04, 177.90, 177.59 (4C; *C*O), 137.34-125.86 (aromatic *C*), 103.18, 103.03 (3C, C-1$^{II,\,III,\,IV}$), 101.36, 101.26, 101.19 (4C, Ph*C*H), 88.75 (C-1^{I}), 81.56 (C-2^{I}) 79.80, 77.99, 76.23, 75.90, 75.78, 70.31, 69.91, 69.78, 69.67, 68.48, 68.24, 68.14, 68.04, 65.41 (C-5^{I}), 64.66, 64.55, 64.38, 39.26, 39.21, 39.15, 39.07 (4C, CO*C*(CH$_3$)$_3$), 27.26, 27.24, 27.18, 26.93 (4C, COC(*C*H$_3$)$_3$) ppm.; $^{1}J_{CH}$(I) = 172 Hz, $^{1}J_{CH}$(II, III, IV) = 176 Hz, 176 Hz, 176 Hz; MALDI-TOF MS: *m*/*z* calcd for $C_{78}H_{94}O_{24}Na^{+}$: 1469.575 [M+Na]$^{+}$; found: 1469.934.

Phenyl

4,6-*O*-benzylidene-3-*O*-pivaloyl-α-D-mannopyranosyl-(1→2)-4,6-*O*-benzylidene-3-*O*-pivaloyl-α-D-mannopyranosyl-(1→2)-4,6-*O*-benzylidene-3-*O*-pivaloyl-α-D-mannopyranosyl-(1→2)-4,6-*O*-benzylidene-3-*O*-pivaloyl-α-D-mannopyranosyl-(1→2)-4,6-*O*-benzylidene-3-*O*-pivaloyl-1-thio-α-D-mannopyranoside (**Man$_5$**)

R*f*= 0.49 (Toluene/AcOEt =6/1, v/v); ^{1}H-NMR (600 MHz, $CDCl_3$): δ 7.49-7.27 (m, 30H; aromatic *H*), 5.64, 5.63, 5.63, 5.62 (4s, 4H; PhC*H*), 5.49 (d, 1H; $J_{1,2}$ = 1.2 Hz; H-1^I), 5.41 (dd, 1H, $J_{2,3}$ = 3.0 Hz, $J_{3,4}$ = 9.6 Hz; H-3), 5.33 (dd, 1H, $J_{2,3}$ = 3.6 Hz, $J_{3,4}$ = 10.8 Hz; H-3), 5.28 (dd, 1H, $J_{2,3}$ = 3.6 Hz, $J_{3,4}$ = 10.8 Hz; H-3), 5.21 (m, 3H, H-3), 4.85 (d, 1H, $J_{1,2}$ = 1.2 Hz; H-1), 4.83 (d, 1H, $J_{1,2}$ = 1.2 Hz; H-1), 4.82 (bs, 1H; H-1), 4.78 (d, 1H, $J_{1,2}$ = 0.6 Hz; H-1), 4.39 (m, 2H; H-2I, H-5^I), 4.01 (m, 23H), 1.27, 1.25, 1.20, 1.19 (5s, 45H; COC(CH_3)$_3$) ppm.; ^{13}C NMR (150 MHz, $CDCl_3$): δ 178.48, 178.45, 178.08, 177.97, 177.65(5C; *C*O), 137.34-125.86 (aromatic *C*), 103.28, 103.21 (4C, C-1$^{II, III, IV, V}$), 101.38, 101.23, 101.16, 101.11 (5C, Ph*C*H), 88.81 (C-1I), 81.62 (C-2I), 80.18, 79.65, 78.16, 77.15, 76.18, 75.87, 75.83, 75.73, 75.60, 70.30, 69.91, 69.85, 69.56, 68.48, 68.18, 68.08, 67.96, 67.75, 65.40, 64.62, 64.55, 64.42, 64.31, 39.27, 39.23, 39.15, 39.08 (5C, CO*C*(CH$_3$)$_3$), 27.26, 27.23, 27.17, 26.92 (15C, COC(*C*H$_3$)$_3$) ppm.; $^1J_{CH}$(I) = 172 Hz, $^1J_{CH}$(II, III, IV, V) = 179 Hz, 179 Hz, 179 Hz, 179 Hz; MALDI-TOF MS: *m*/*z* calcd for $C_{96}H_{116}O_{30}Na^+$: 1803.716 $[M+Na]^+$; found: 1803.570.

Phenyl

4,6-*O*-benzylidene-3-*O*-pivaloyl-α-D-mannopyranosyl-(1→2)-4,6-*O*-benzylidene-3-*O*-pivaloyl-α-D-mannopyranosyl-(1→2)-4,6-*O*-benzylidene-3-*O*-pivaloyl-α-D-mannopyranosyl-(1→2)-4,6-*O*-benzylidene-3-*O*-pivaloyl-α-D-mannopyranosyl-(1→2)-4,6-*O*-benzylidene-3-*O*-pivaloyl-1-thio-α-D-mannopyranoside (**Man$_6$**)

R*f*= 0.49 (Toluene/AcOEt =6/1, v/v); ^{1}H-NMR (600 MHz, $CDCl_3$): δ 7.49-7.28 (m, 35H; aromatic *H*), 5.64, 5.63, 5.62, 5.61, 5.59, 5.58 (6s, 6H; PhC*H*), 5.48 (d, 1H; $J_{1,2}$ = 1.2 Hz; H-1^I), 5.41 (dd, 1H, $J_{2,3}$ = 3.0

Hz, $J_{3,4}$ = 10.2 Hz; H-3), 5.32 (dd, 1H, $J_{2,3}$ = 3.6 Hz, $J_{3,4}$ = 10.8 Hz; H-3), 5.22 (m, 4H; H-3), 4.82, 4.81, 4.76 (bs, 5H; H-$1^{II,III,IV,V,VI}$), 4.39 (m, 2H; H-2^{I}, H-5^{I}), 4.04 (m, 28H), 1.28, 1.25, 1.25, 1.21, 1.20, 1.19 (6s, 54H; COC(CH_3)$_3$) ppm.; ^{13}C NMR (150 MHz, $CDCl_3$): δ 178.51, 178.08, 178.03, 177.65, 177.65 (6C; *C*O), 137.33-125.85 (aromatic *C*), 103.29, 103.25 (5C, C-$1^{II,III,IV,V,VI}$), 101.37, 101.19, 101.07, (6C, Ph*C*H), 88.80 (C-1I$^{)}$, 81.71 (C-2^{I}), 80.14, 79.96, 79.63, 78.12, 76.18, 75.83, 75.69, 75.53, 70.30, 69.91, 69.87, 69.83, 69.62, 69.53, 69.50, 68.47, 68.17, 68.03, 67.92, 67.69, 65.35, 64.55, 64.40, 64.33, 69.30, 39.27, 39.22, 39.15, 39.07, (6C, CO*C*(CH$_3$)$_3$), 27.26, 27.22, 27.17, 26.889 (18C, COC(*C*H$_3$)$_3$) ppm.; $^{1}J_{CH}$(I) = 173 Hz, $^{1}J_{CH}$(II, III, IV, V) = 176 Hz, 176 Hz, 176 Hz, 176 Hz., 176 Hz; MALDI-TOF MS: *m*/*z* calcd for $C_{114}H_{138}O_{36}Na^{+}$: 2137.858 $[M+Na]^{+}$; found: 2137.489.

Phenyl

4,6-*O*-benzylidene-3-*O*-pivaloyl-α-D-mannopyranosyl-(1→2)-4,6-*O*-benzylidene-3-*O*-pivaloyl-α-D-mannopyranosyl-(1→2)-4,6-*O*-benzylidene-3-*O*-pivaloyl-α-D-mannopyranosyl-(1→2)-4,6-*O*-benzylidene-3-*O*-pivaloyl-α-D-mannopyranosyl-(1→2)-4,6-*O*-benzylidene-3-*O*-pivaloyl-α-D-mannopyranosyl-(1→2)-4,6-*O*-benzylidene-3-*O*-pivaloyl-α-D-mannopyranosyl-(1→2)-4,6-*O*-benzylidene-3-*O*-pivaloyl-1-thio-α-D-mannopyranoside (**Man$_7$**)

R*f*= 0.49 (Toluene/AcOEt =6/1, v/v); ^{1}H-NMR (600 MHz, $CDCl_3$): δ 7.49-7.23 (m, 40H; aromatic *H*), 5.60 (m, 7H; PhC*H*), 5.48 (s,1H; H-1^{I}), 5.41 (dd, 1H, $J_{2,3}$ = 3.6 Hz, $J_{3,4}$ = 10.8 Hz; H-3), 5.25 (m, 6H; H-3), 4.80 (m, 6H; H-$1^{II-IVII}$), 4.39 (m, 2H), 4.04 (m, 33H), 1.23 (7s, 63H; COC(CH_3)$_3$) ppm.; MALDI-TOF MS: *m*/*z* calcd for $C_{132}H_{160}O_{42}Na^{+}$: 2472.000 $[M+Na]^{+}$; found: 2471.963.

Phenyl

4,6-*O*-benzylidene-3-*O*-pivaloyl-α-D-mannopyranosyl-(1→2)-4,6-*O*-benzylidene-3-*O*-pivaloyl-α-D-mannopyranosyl-(1→2)-4,6-*O*-benzylidene-3-*O*-pivaloyl-α-D-mannopyranosyl-(1→2)-4,6-*O*-benzylidene-3-*O*-pivaloyl-α-D-mannopyranosyl-(1→2)-4,6-*O*-benzylide

ne-3-*O*-pivaloyl-α-D-mannopyranosyl-(1→2)- 4,6-*O*-benzylidene-3-*O*-pivaloyl-α-D-mannopyranosyl-(1→2)-4,6-*O*-benzylidene-3-*O*-pivaloyl-1-thio-α-D-mannopyranoside (**Man_8**)

R*f*= 0.49 (Toluene/AcOEt =6/1, v/v); ^{1}H-NMR (600 MHz, $CDCl_3$): δ 7.49-7.23 (m, 45H; aromatic *H*), 5.60 (m, 8H; PhC*H*), 5.50 (s,1H; H-1^I), 5.41 (dd, 1H, $J_{2,3}$ = 3.6 Hz, $J_{3,4}$ = 10.2 Hz; H-3), 5.26 (m, 7H; H-3), 4.87 (m, 7H; H-1II-IVIII), 4.39 (m, 2H), 4.04 (m, 38H), 1.23 (8s, 72H; COC(CH_3)$_3$) ppm.; MALDI-TOF MS: *m*/*z* calcd for $C_{150}H_{186}O_{48}Na^+$: 2806.141 $[M+Na]^+$; found: 2806.439.

4.2.2. Synthesis of mannobiose

The thiophenyl mannoside **1** (50 mg, 0.112 mmol) and NIS (0.5 eq, 13 mg, 0.056 mmol) in 3 mL of CH_2Cl_2 were stirred at –78 °C in the presence of MS 4A. Then TfOH (3 µL, 34 µmol) was added. The reaction mixture was stirred at –78 °C for 5 hours and triethylamine was added. The reaction mixture was diluted with EtOAc and filtered using Celite. The filtrate was washed with sat. aq $Na_2S_2O_3$, sat. aq $NaHCO_3$, sat. aq NaCl, and dried over $MgSO_4$. The organic layer was filtered and concentrated *in vacuo*. The organic solvent was evaporated under vacuum. The resultant residue was purified by HPLC and fitted on a gel filtration column (JAIGEL-HR) to provide mannobiose **Man_2** (23.9 mg) in 48% yield.

4.2.3. Large-scale synthesis of mannobiose and mannotriose

The thiophenyl mannoside **1** (1.0 g, 2.24 mmol) and NIS (1 eq, 500 mg, 2.24 mmol) in 30 mL of CH_2Cl_2 were stirred at –78 °C in the presence of MS 4A. TfOH (60 µL, 680 µmol) was slowly added. The reaction mixture was further stirred at –78 °C for 4 hours. The reaction mixture was added triethylamine and stirred at –78 °C for 1 day. The reaction mixture was diluted with EtOAc and filtered using Celite. The filtrate was washed with sat. aq $Na_2S_2O_3$, sat. aq $NaHCO_3$, sat. aq NaCl, and dried over $MgSO_4$. The organic layer was filtered and concentrated *in vacuo*. The resultant residue was applied to gel filtration chromatography (SX1, toluene) to obtain mannobiose **Man_2** (316 mg, 32%) and mannotriose (278 mg, 28%) mannotetraoase **Man_4** (45 mg, 4.5%), mannopentaose **Man_5** (40 mg, 4.0%), mannohexose **Man_6** (17 mg, 1.7%), mannoheptaose **Man_7** (5.2 mg, 0.5%) and mannooctaose **Man_8** (5.1 mg, 0.5%), respectively.

4.2.4. One-pot mannosylation using 2

The thiophenyl mannoside **2** (101 mg, 0.224 mmol) and NIS (50 mg, 0.244 mmol) in 6 mL of CH_2Cl_2 were stirred at –78 °C in the presence of MS 4A. Then TfOH (6 µL, 0.067 mmol) was added. The reaction was stirred at –78 °C for 49 h. The reaction mixture was added to triethylamine. The mixture was diluted with EtOAc and filtered using Celite. The filtrate was washed with sat. aq $Na_2S_2O_3$, sat. aq $NaHCO_3$, sat. aq NaCl, and dried over $MgSO_4$. The organic layer was filtered and concentrated *in vacuo*. The resultant residue was purified by HPLC and fitted on a gel filtration column (JAIGEL-2HR and 2.5 HR) obtaining **3-BnMan$_2$**, **3-BnMan$_3$**, and **3-BnMan$_4$**. Each fraction was purified by silica-gel column chromatography receiving mannobiose derivative **Manα1-2Man** (2.0 mg, 1.1%), mannobiose derivative **Manβ1-2Man** (12 mg, 6.6%), mannotriose derivative **Manβ1-2Manα1-2Man** (5.8 mg, 2.3%), mannotriose derivative **Manβ1-2Manβ1-2Man** (5.5 mg, 2.2%), and **Man-NS** (39 mg, 40%), respectively.

Phenyl 3-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranyl-(1-2)-3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (**Manβ1-2Man**)

R*f*= 0.29 (Toluene/AcOEt =5/1, v/v); ^{1}H-NMR (600 MHz, $CDCl_3$): δ 7.52-7.24 (25H; aromatic *H*), 5.55 (s, 1H; PhC*H*), 5.49 (d, 1H, $J_{1,2}$ = 1.2 Hz; H-1^I), 5.47 (s, 1H; PhC*H*), 4.80 (m, 4H; PhCH_2), 4.73 (d, 1H; $J_{1,2}$ = 0.6 Hz; H-1^{II}), 4.62 (bd, 1H, $J_{1,2}$ = 2.4 Hz; H-2^I), 4.27 (m, 3H; H-5^I, H-4^{II}, H-6^{II}), 4.19 (m, 2H; H-4^I, H-6^I), 4.15 (bd, 1H, $J_{1,2}$ = 3.0 Hz; H-2^{II}), 3.98 (dd, 1H, $J_{2,3}$ = 3.0 Hz, $J_{3,4}$ = 9.6 Hz; H-3^I), 3.77 (m, 2H; H-6^I, H-6^{II}), 3.68 (dd, 1H, $J_{2,3}$ =3.6 Hz, $J_{3,4}$ = 9.0 Hz; H-3^{II}), 3.39 (td, 1H, $J_{5,6e}$ = 4.8 Hz, $J_{4,5}$ = $J_{5,6a}$ = 9.6 Hz; H-5^{II}), 3.16 (bs, 1H; O*H*) ppm.; ^{13}C NMR (150 MHz, $CDCl_3$): δ138.17-126.12 (aromatic *C*), 101.62 (Ph*C*H), 101.43 (Ph*C*H), 97.62 (C-1^{II}), 86.69 (C-1^I), 78.75 (C-4^I), 78.63 (C-4^{II}), 76.24 (C-3^{II}), 74.63 (C-3^I), 74.54 (C-2^I), 72.64 (Ph*C*H$_2$), 72.52 (Ph*C*H$_2$), 69.65 (C-2^{II}), 68.74(C-6^{II}), 68.47 (C-6^I), 66.96 (C-5^{II}), 65.36 (C-5^I) ppm.; MALDI-TOF MS: *m*/*z* calcd for $C_{46}H_{46}O_{10}SNa^+$: 813.270 $[M+Na]^+$; found: 813.264. NMR data are in accordance with those published previously [16].

Phenyl

3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranyl-(1-2)-3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (**Manα1-2Man**)

R*f*= 0.50 (Toluene/AcOEt =5/1, v/v); ^{1}H-NMR (600 MHz, $CDCl_3$) δ 7.53-7.22 (m, 15H), 5.63 (s, 2H; PhC*H*), 5.60 (s, 2H; H-1^{I}, H-1^{II}), 4.88 (m, 2H; PhCH_2), 4.76-4.69 (m, 2H; PhCH_2), 4.22 (m, 8H), 4.00 (m, 2H), 3.85 (m, 3H) ppm.; ^{13}C-NMR (150 MHz, $CDCl_3$) δ 138.1-126.1 (aromatic *C*), 101.72 (Ph*C*H, Ph*C*H, C-1^{II}), 10157 (C-1^{I}), 87.85, 79.08, 78.73, 77.33, 77.11, 76.90, 75.78, 73.30, 71.50, 68.62, 64.64 ppm. ; MALDI-TOF MS: *m*/*z* calcd for $C_{46}H_{46}O_{10}SNa^{+}$: 813.270 $[M+Na]^{+}$; found: 813.453.

Phenyl

3-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranyl-(1-2)-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranyl-(1-2)-3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (**Manβ1-2Manα1-2Man**)

R*f*= 0.41 (Toluene/AcOEt =6/1, v/v); ^{1}H-NMR (600 MHz, $CDCl_3$): δ 7.51-7.04 (m, 30H; aromatic *H*), 5.64 (s, 1H; PhC*H*), 5.62 (bs,1H; H-1^{II}), 5.60 (s, 1H; PhC*H*), 5.51 (bs, 1H; H-1^{I}), 5.28 (s, 1H; PhC*H*), 4.76 (m, 5H, PhCH_2), 4.59 (d, 1H, $J_{1,2}$ = 0.6 Hz; H-1^{III}), 4.48 (m, 2H; H-2^{I}, PhCH_2), 4.41 (dd, 1H, $J_{6a,6e}$ = 5.4 Hz, $J_{5,6}$ = 10.2 Hz; H-6^{II}), 4.29 (m, 8H; H-4^{I}, H-5^{I}, H-2^{II}, H-4^{II}, H-5^{II}, H-2^{III}, H-4^{III}, H-6^{III}), 3.99 (dd, 1H, $J_{6a,6e}$ = 4.8 Hz, $J_{5,6a}$ = 10.8 Hz; H-6^{I}), 3.88 (m, 4H, H-3^{I}, H-6^{I}, H-3^{II}, H-6^{II}), 3.78 (t, 1H, $J_{5,6a}$ = $J_{6a,6e}$ = 10.2 Hz; H-6^{III}), 3.69 (dd, 1H; $J_{2,3}$ = 2.4 Hz, $J_{3,4}$ = 9.6 Hz; H-3^{III}), 3.34 (td, 1H, $J_{5,6e}$ = 4.8 Hz, $J_{4,5}$ =$J_{5,6a}$ = 9.6 Hz; H-5^{III}), 2.36 (s, 1H; OH) ppm.; ^{13}C NMR (150 MHz, $CDCl_3$): δ 138.89-125.94 (aromatic *C*), 101.78, 101.64, 100.59 (C-1^{II}), 100.22, 99.26 (C-1^{III}), 86.88 (C-1^{I}), 79.26, 78.68, 78.62, 77.52, 75.94, 74.37, 73.52 (Ph*C*H$_2$), 72.99 (Ph*C*H$_2$), 72.34, 71.04 (Ph*C*H$_2$), 70.40, 68.77 (C-6), 68.41 (C-6), 67.77 (C-5^{III}), 67.66 (C-6), 65.65, 63.66 ppm.;$^{1}J_{CH}$(I) = 168 Hz, $^{1}J_{CH}$(II) = 168 Hz, $^{1}J_{CH}$(III) = 168 Hz; MALDI-TOF MS: *m*/*z* calcd for $C_{66}H_{66}O_{15}SNa^{+}$: 1153.402 $[M+Na]^{+}$; found: 1153.411.

Phenyl

3-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranyl-(1-2)-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranyl-(1-2)-3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside **(Manβ1-2Manβ1-2Man)**

R*f*= 0.29 (Toluene/AcOEt = 5/1, v/v); ^{1}H-NMR (600 MHz, $CDCl_3$): δ 7.50-7.14 (m, 30H; aromatic *H*), 5.59, 5.58, 5.53 (3s, 3H; PhC*H*), 5.49 (d, 1H; $J_{1,2}$ = 1.2 Hz; H-1^{I}), 5.08 (s, 1H; H-1^{II}), 4.68 (m, 7H; PhCH_2, H-1^{III}) 4.54 (dd, 1H, $J_{1,2}$ = 1.2 Hz, $J_{2,\ OH}$ = 3.6 Hz; H-2^{I}), 4.88 (d, 1H, $J_{1,2}$ = 1.8 Hz; H-1^{III}), 4.43 (dd, 1H, $J_{1,2}$ = 1.2 Hz, $J_{2,OH}$ = 3.0 Hz; H-2^{I}), 4.28 (m, 8H; H-5^{I}, H-6^{I}, H-2^{II}, H-4^{II}, H-6^{III}, H-2^{III}, H-4^{III}, H-6^{III}), 3.96 (m, 4H; H-3^{I}, H-4^{I}, H-6^{II}, H-6^{III}), 3.75 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz; H-4^{I}), 3.66 (dd, 1H, $J_{2,3}$ = 3.0 Hz, $J_{3,4}$ = 9.6 Hz; H-3^{III}), 3.53 (dd, 1H, $J_{2,3}$ = 3.0 Hz, $J_{3,4}$ = 9.0 Hz; H-3^{II}), 3.40 (td, 1H, $J_{5,6e}$ = 4.8 Hz, $J_{4,5}$ =$J_{5,6a}$ = 9.6 Hz; H-5^{III}), 3.34 (td, 1H, $J_{5,6e}$ = 4.8 Hz, $J_{4,5}$ =$J_{5,6a}$ = 9.6 Hz; H-5^{II}) 2.35 (s, 1H; O*H*) ppm.;^{13}C NMR (150 MHz, $CDCl_3$): δ 138.23-126.12 (aromatic *C*), 101.94, 101.62, 101.50, 100.95 (C-1^{II}), 98.40 (C-1^{III}), 85.76 (C-1^{I}), 78.86, 78.37, 78.12, 77.55, 76.21, 75.06, 74.08, 74.00, 72.33 (Ph*C*H_2), 71.81 (Ph*C*H_2), 71.71 (Ph*C*H_2), 69.00, 68.83, 67.80 (C-5^{II}), 67.38 (C-5^{III}), 65.28 ppm.;$^{1}J_{CH}$(I) = 168 Hz, $^{1}J_{CH}$(II) = 163 Hz, $^{1}J_{CH}$(III) = 156 Hz ; MALDI-TOF MS: *m*/*z* calcd for $C_{66}H_{66}O_{15}SNa^{+}$: 1153.402 $[M+Na]^{+}$; found: 1153.417.

3-*O*-benzyl-4,6-*O*-benzylidene-1-*N*-succinimido-D-mannopyranose (**Man-NS**)

^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.51-7.29 (m, 30H), 6.74 (s, 1H), 6.59 (s, 1H), 5.61 (d, *J* = 8.0 Hz, 3H), 5.32 (d, *J* = 3.0 Hz, 2H), 5.28 (d, *J* = 3.0 Hz, 1H), 4.93 (m, 2H), 4.83 (m, 2H), 4.31 (m, 4H), 4.24 (d, 1H),4.09 (m, 3H), 3.81 (m, 5H), 3.34 (m, 2H), 2.52 (m, 11H), 1.59 (s, 5H) ppm.; MALDI-TOF MS: *m*/*z* calcd for $C_{24}H_{25}O_7NNa^{+}$: 462.152 $[M+Na]^{+}$; found: 462.064.

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References

[1] R. Fonfeld, S. Konfeld, Assembly of asparagine-linked oligosaccharide, Annu. Rev. Biochem. 54 (1985) 631-664. doi.org/ 10.1146/annurev.bi.54.070185.003215.

[2] C. Fagioli, R. Sitia, Glycoprotein quality control in the endoplasmic reticulum - Mannose trimming by endoplasmic reticulum mannosidase I times the proteasomal degradation of unassembled immunoglobulin subunits, J. Biol. Chem. 276 (2001) 12885-12892. doi.org/10.1074/jbc.M009603200.

[3] J. Roth, Protein *N*-glycosylation along the secretory pathway: Relationship to organelle topography and function, protein quality control, and cell interactions, Chem. Rev. 102 (2002) 285-303. doi.org/10.1021/cr000423j.

[4] A. Helenius, M. Aebi, Roles of N-linked glycans in the endoplasmic reticulum, Annu. Rev. Biochem. 73 (2004) 1019-1049. doi.org/10.1146/annurev.biochem.73.011303.073752.

[5] (a) I. Matsuo, K. Totani, A. Tatami, Y. Ito, Comprehensive synthesis of ER related high-mannose-type sugar chains by convergent strategy, Tetrahedron 62 (2006) 8262-8277. doi.org/10.1016/j.tet.2006.06.045.
(b) I. Matsuo, T. Kashiwagi, K. Totani, Y. Ito, First chemical synthesis of triglucosylated tetradecasaccharide ($Glc_3Man_9GlcNAc_2$), a common precursor of asparagine-linked oligosaccharides, Tetrahedron Lett. 48 (2005) 4197-4200. doi:10.1016/j.tetlet.2005.04.056.
(c) I. Matsuo, Y. Ito, Synthesis of an octamannosyled glycan chain, the key oligosaccharide structure in ER-associated degradation, Carbohydr. Res. 338 (2003) 2163-2168.
(d) I. Matsuo, M. Wada, S. Manabe, Y. Yamaguchi, K. Otake, K. Kato, Y. Ito, Synthesis of Monoglucosylated High-mannose Type Dodecasaccharide, a Putative Ligand for Molecular Chaperone, Calnexin and

Calreticurin, J. Am. Chem. Soc., 125 (2003) 3402-3403. doi.org/10.1021/ja021288q.

(e) I. Matsuo, T. Miyazaki, M. Isomura, T. Sakakibara, K. Ajisaka, A short, efficient synthesis of the octamannan residue of high-mannose type Sugar Chains, J. Carbohydr. Chem. **17** (1998) 1249-1258. doi.org/10.1080/07328309808001897.

(f) I. Matsuo, M. Isomura, T. Miyazaki, T. Sakakibara, K. Ajisaka, Chemoenzymatic synthesis of the branched oligosaccharides which correspond to the core structures of N-Linked Sugar Chains, Carbohydr. Res. **305** (1997) 401-413. doi.org/10.1016/S0008-6215(97)10001-5.

[6] (a)A. Koizumi, I Matsuo, M. Takatani, A. Seko, M. Hachisu, Y. Takeda, Y. Ito, Top-Down Chemoenzymatic Approach to a High-Mannose-Type Glycan Library: Synthesis of a Common Precursor and Its Enzymatic Trimming, Angew. Chem. Int. Ed. 52 (2013) 7426-7431. doi.org/ 10.1002/anie.201301613.

(b) K. Fujikawa, K. Koizumi, M. Hachisu. A. Seko, Y. Takeda, Y. Ito, Construction of a High-Mannose-Type Glycan Library by a Renewed Top-Down Chemo-Enzymatic Approach, Chem. Euro. J. 21 (2015) 3224-3233. https://doi.org/10.1002/chem.201405781.

[7] (a) H. Paulsen, Syntheses, Conformations and X‐Ray Structure Analyses of the Saccharide Chains from the Core Regions of Glycoproteins Angew. Chem. Int. Ed. 29 (1990) 823-839. doi.org/10.1002/anie.199008233.

(b) J. R. Merritt, E. Naisang, B. Fraser-Reid, n-Pentenyl Mannoside Precursors for Synthesis of the Nonamannan Component of High Mannose Glycoproteins, J. Org. Chem. 59 (1994) 4443-4449. doi.org/10.1021/jo00095a020.

(c) Y. Nakahara, S. Shibayama, Y. Nakahara, T. Ogawa, Rationally designed syntheses of high-mannose and complex type undecasaccharides, Carbohydr. Res. 280 (1996) 67-84. DOI: 10.1016/0008-6215(95)00262-6.

(d) J. Zhang, Z. Ma, F. Kong, Efficient synthesis of a 3,6-branched mannose hepta- and octasaccharide, Carbohydr. Res. 338 (2003) 2039-2046. doi:10.1016/S0008-6215(03)00351-3.

(e) B. Meng, J. Wang, Q. Wang, A. S. Serianni, Q. Pan, Synthesis of high-mannose oligosaccharides

containing mannose-6-phosphate residues using regioselective glycosylation, Carbohydr. Res. 467 (2018) 23-32. doi.org/10.1016/j.carres.2018.07.005.

[8] H. J. Schuster, B. Vijayakrishnan, B. G. Davis, Chain-growth polyglycosylation: synthesis of linker-equipped mannosyl oligomers, Carbohydr. Res. 403 (2015) 135-141. https://doi.org/ 10.1016/j.carres.2014.06.021.

[9] W. Zhang, J. Wang, A.S. Serianni, Q. Pen, A convenient synthesis of short-chain α-(1 → 2) mannopyranosyl oligosaccharides, Carbohydr. Res. 489 (2020) 107897. doi.org/10.1016/j.carres.2019.107897.

[10] (a) J. J. Reina, A. Di Maio, J. Ramos-Soriano, R. C. Figueiredo, J. Rojo, Rapid and efficient synthesis of alpha(1-2) mannobiosides, Org. Biomol. Chem. 14 (2016) 2873-2882. https://doi.org/10.1039/c6ob00083e. (b) J. Ramos-Soriano, M. C. de la Fuente, N. de la Cruz, R. C. Figueiredo, J. Rojo, J. J. Reina, Straightforward synthesis of Man (9), the relevant epitope of the high-mannose oligosaccharide, Org. Biomol. Chem. 15 (2017) 8877-8882. https://doi.org/ 10.1039/c7ob02286g.

[11] H. Tanaka, H. Yamada, T. Takahashi, Rapid Synthesis of Oligosaccharides Based on One-Pot Glycosylation Trends Glycosci. Glycotechnol. 19 (2007) 183-193. doi.org/10.4052/tigg.19.183.

[12] (a) B. S. Komarova, M. V. Orekhova, Y. E. Tsvetkov, N. E. Nifantiev, Is an acyl group at O-3 in glucosyl donors able to control a-stereoselectivity of glycosylation? The role of conformational mobility and the protecting group at O-6, Carbohydr. Res. 384, (2014) 70-86. DOI: 10.1016/j.carres.2013.11.016. (b) H. S. Hahm, M. Hurevich, P. H. Seeberger, Automated assembly of oligosaccharides containing multiple cis-glycosidic linkages, Nat. Commun. 7 (2016), 12482. doi.org/10.1038/ncomms12482.

[13] K. Sano, T. Kuribara, N. Ishii, A. Kuroiwa, T. Yoshihara, S. Tobita, K. Totani, I. Matsuo, Fluorescence

quenching-based assay for measuring Golgi endo-α-mannosidase, Chem. Asian. J., 14 (2019) 1965-1969. doi.org/10.1038/s41467-019-10319-5.

[14] P. Konradsson, U. E. Udodong, B. Fraser-Reid, Iodonium promoted reactions of disarmed, thioglycosides, Tetrahedron Lett. 31 (1990) 4313-4316. doi.org/10.1016/S0040-4039(00)97609-3.

[15] D. Crich, P. Jayalath, T. K. Hutton, Enhanced Diastereoselectivity in β-Mannopyranosylation through the Use of Sterically Minimal Propargyl Ether Protecting Groups J. Org. Chem. 71 (2006) 3064-3070. doi.org/10.1021/jo0526789.

[16] J. Rahkila, F. S. Ekholm, R. Panchadhayee, A. Ardá, F. J. Cañada, J Jiménez-Barbero, R. Leino, Synthesis and conformational analysis of phosphorylated β-(1→2) linked mannosides, Carbohydr. Res. 383 (2014) 58-68. doi.org/10.1016/j.carres.2013.10.017.

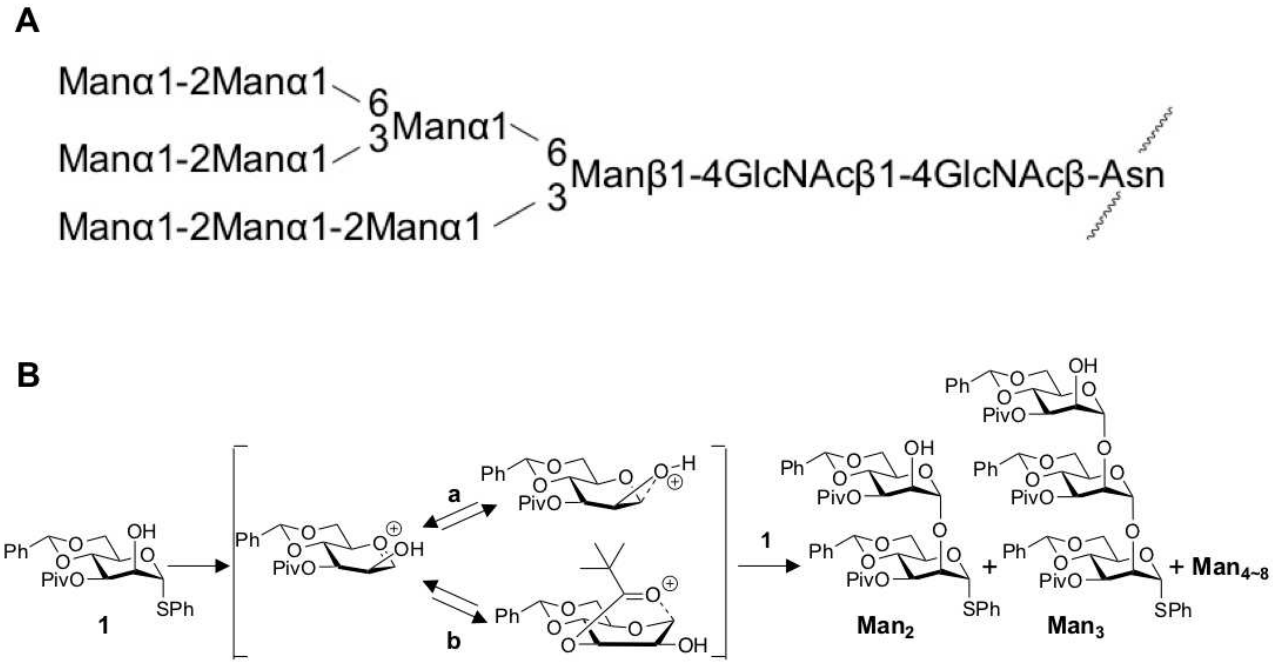

Figure 1. **A**: Structure of high-mannose type sugar chain, **B**: Synthetic scheme of oligomannosie derivatives and putative mechanism for the stereo-selective glycosylation via (**a**) C-2 hydroxyl group assisted epoxide-like intermediate, or (**b**) remote participation from the C-3 position of the pivaloyl group.

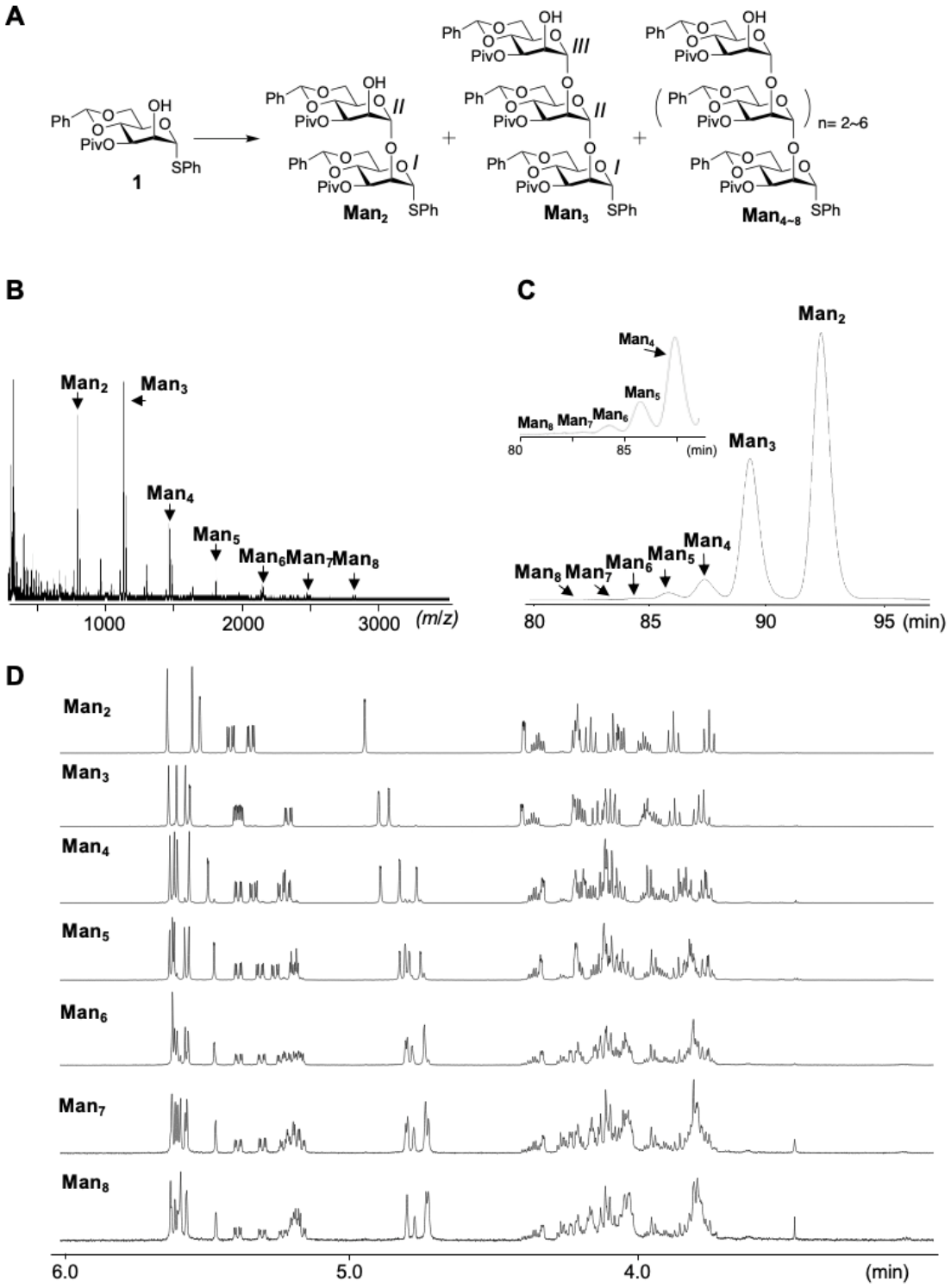

Figure 2. **A**: One-pot reaction with mannose derivative **1** (Regents and conditions; NIS, TfOH, CH_2Cl_2, -78 ^{o}C), **B**: MALDI TOF MS spectra of the reaction mixture, **C**: HPLC chromatogram of the reaction mixture (conditions; column; JAIGEL2HR-2.5HR, flow rate; 10 mL/min, solvent; chloroform, detection; UV 254 nm), **D**: NMR spectra ($CDCl_3$).

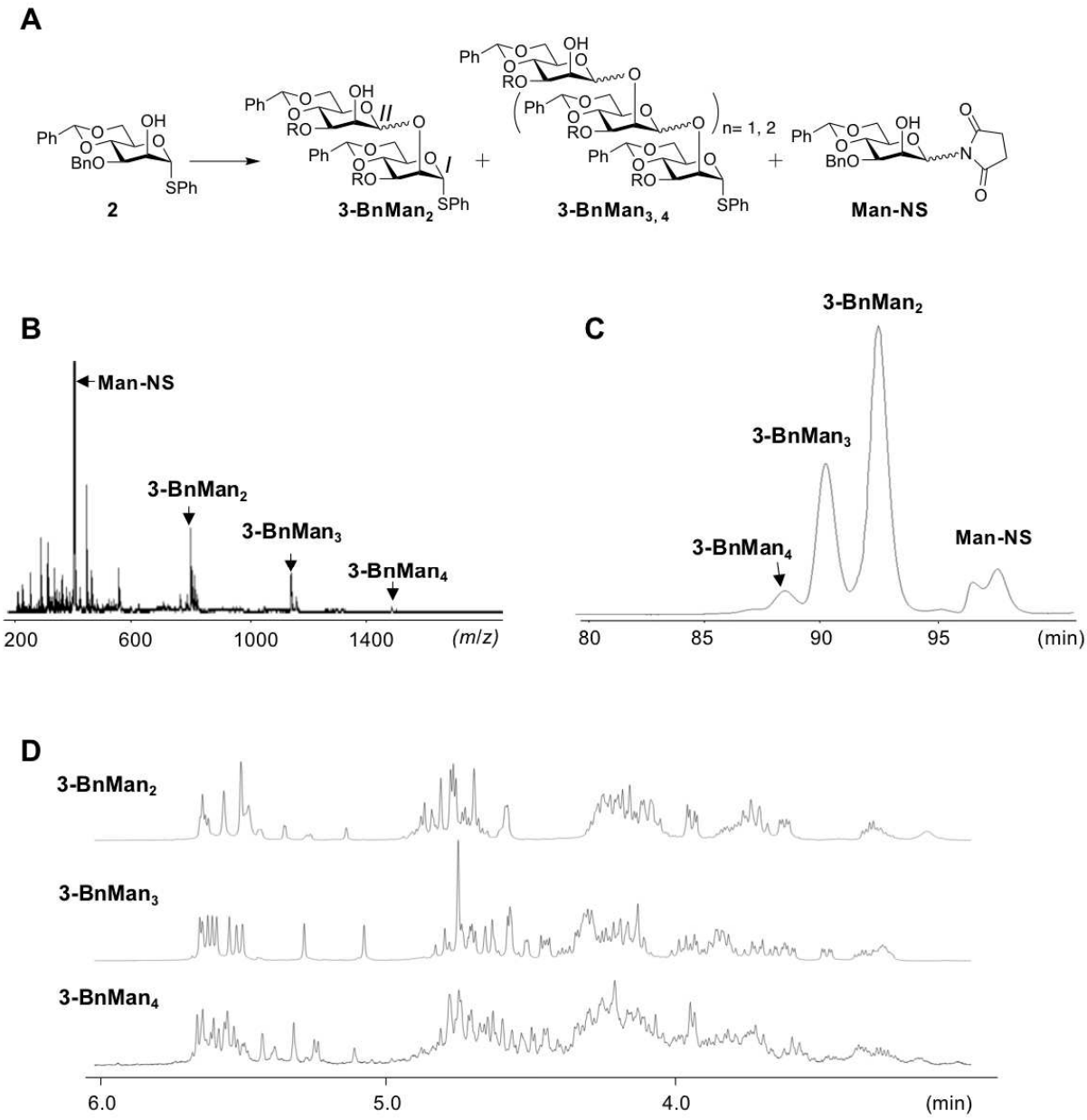

Figure 3. **A**: One-pot reaction with mannose derivative **2** (Regents and conditions; NIS, TfOH, CH_2Cl_2, -78 ^{o}C), **B**: MALDI TOF MS spectra of the reaction mixture, **C**: HPLC chromatogram of the reaction mixture (conditions; column; JAIGEL2HR-2.5HR, flow rate; 10 mL/min, solvent; chloroform, detection; UV 254 nm), **D**: NMR spectra ($CDCl_3$).

Highlights

- α(1,2)-linked oligomannoside derivatives were synthesized using a one-pot method
- The mannosylation exclusively generated α-mannoside linkages
- α(1,2)-linked mannobiose and mannotriose derivatives were also easily prepared
- The α-directing effect was caused by the remote pivaloyl group in C-3 position

To the best of our knowledge, the named authors have no conflict of interest, financial or otherwise.