Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron
Tetrahedron 64 (2008) 92–102
www.elsevier.com/locate/tet

Synergistic solvent effect in 1,2-*cis*-glycoside formation

Akihiro Ishiwata [a,b,*], Yuichi Munemura [a,b], Yukishige Ito [a,b,*]

[a] *RIKEN (The Institute of Physical and Chemical Research), 2-1 Hirosawa, Wako-shi, Saitama 351-0198, Japan*
[b] *CREST, JST, Kawaguchi 332-1102, Japan*

Received 12 September 2007; received in revised form 23 October 2007; accepted 23 October 2007
Available online 26 October 2007

Abstract

Construction of three continuous 1,2-*cis*-α-glucosidic linkages was achieved in optimized solvent system. High-throughput optimization was conducted, by using substrates protected by perdeuterated benzyl (Bn-d_7) groups. It enabled facile evaluation of yield and stereoselectivity with ^{1}H NMR and MALDI-TOF MS, respectively. We found that $CHCl_3$ and ethereal solvent had a synergetic effect to enhance the α-selectivity. The optimized solvent systems in $CHCl_3$/CPME and $CHCl_3$/Et_2O were applied to the linear synthesis of Glcα1→2Glcα1→3Glcα1→3Man (Glc_3Man_1), which was achieved in 86% overall stereoselectivity.
© 2007 Elsevier Ltd. All rights reserved.

Keywords: 1,2-*cis*-Glycosylation; α-Glucoside; Stereoselective; Solvent effect

1. Introduction

1,2-*cis*-*O*-Glycosides are widespread in nature.[1] They are important constituents of various biologically active natural products and glycoconjugates.[2] For example, α-glycosides of D-glucose (Glc), L-fucose (Fuc), and a β-glycoside of D-mannose (Man) are major constituents of asparagine-linked (N-linked) glycoproteins and an α-glycoside of *N*-acetyl-D-galactosamine (GalNAc) constitutes the core structure of serine/threonine-linked (O-linked) glycoproteins.[3] Heparin and heparan sulfate are polysulfated glycosaminoglycans, which contain large numbers of α-linked *N*-acetyl-D-gluctosamine (GlcNAc) residues in their repeating units.[4] Biomedically important glycosphingolipids such as Lewis (Le) antigens,[5] globotriaosyl ceramide (Gb_3),[6] and α-galactosyl ceramides[7] contain α-linked Fuc or D-galactose (Gal), and α-GalNAc and α-Gal are determinants of human blood types.[8]

In most cases, 1,2-*cis*-glycosides are α-anomers, which consist of axially oriented C(1)–O linkages. The formation of α-glycosides is stereoelectronically preferred over corresponding β-isomers, due to the anomeric effect.[9] However, their highly selective synthesis is generally difficult. There are a number of factors that may affect stereoselectivity and yield of glycosylation.[10,11] They include structures of substrates, promoters, solvents, and temperatures.[10] Among them, effects of solvents are particularly important. As a rule of thumb, ethereal solvents have a tendency to dictate the glycosylation in an α-(axial) selective fashion, while nitrile solvents increase the proportion of β-(equatorial) glycoside. However, the extent of selectivity is difficult to predict precisely.

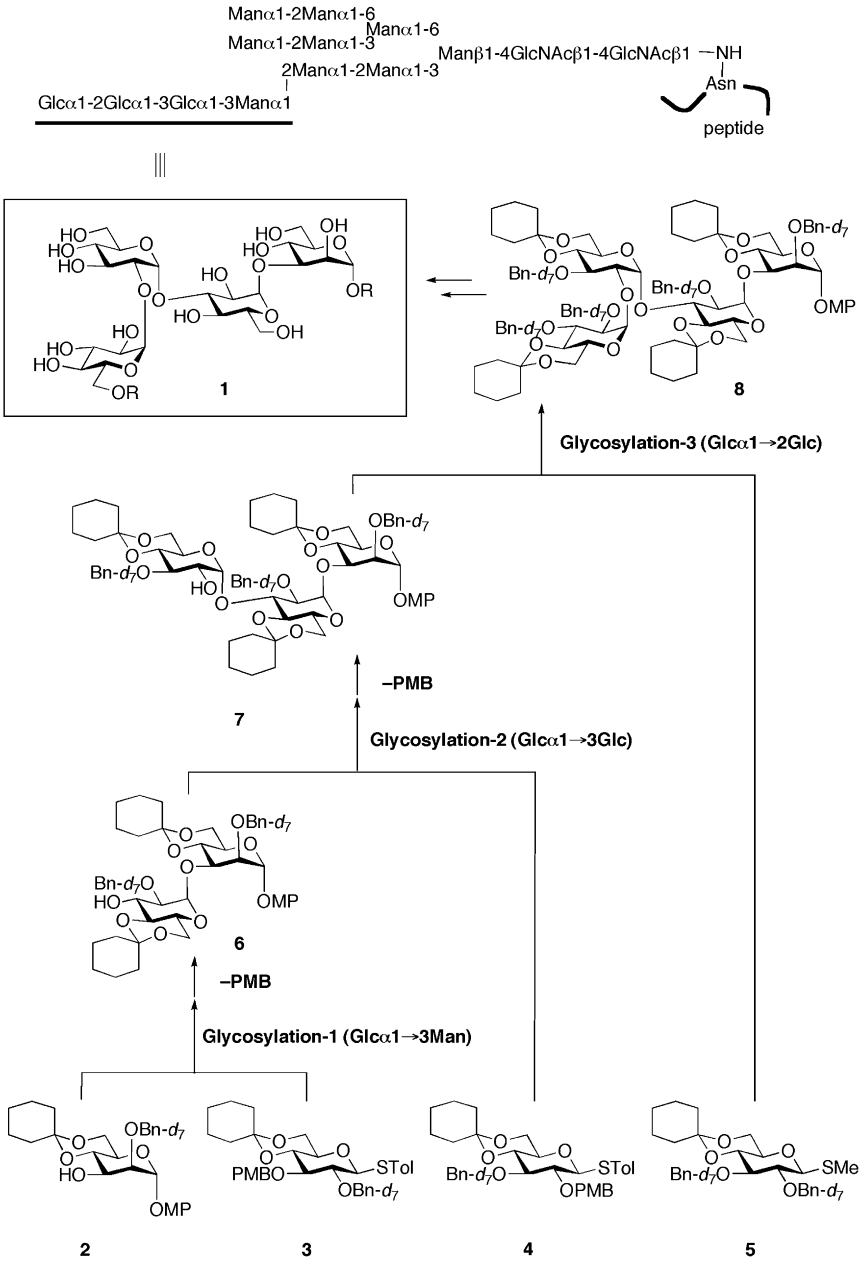
We recently developed a high-throughput screening (HTS) system, which enabled rapid and quantitative evaluation of glycosylation conditions.[12] Herein, we describe the implementation of this system to the synthesis of the tetrasaccharide **1**, which consists of three continuous 1,2-*cis*-α-glycosidic linkages.[13–15] This tetrasaccharide corresponds to the non-reducing terminal structure of tetradecasaccharide $Glc_3Man_9GlcNAc_2$, a common precursor of all N-linked glycans (Scheme 1).[2,16]

2. Results and discussions

The key in our HTS system is the use of isotopically labeled protective group, perdeuterated benzyl ether (Bn-d_7) (Fig. 1).[17] Benzyl (Bn) ether[18] is one of the most widely used hydroxy protective groups in carbohydrate chemistry.[19]

* Corresponding authors. Fax: +81 48 462 4680 (Y.I.).
E-mail addresses: aishiwa@riken.jp (A. Ishiwata), yukito@riken.jp (Y. Ito).

0040-4020/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2007.10.087



Scheme 1.

However, direct ^{1}H NMR analysis of oligosaccharides having multiple *O*-Bn groups is problematic. This is because benzylic methylene signals appear at 4–5 ppm as AB-quartets and obscure the signals derived from anomeric protons. By employing Bn-d_7 instead, all of these signals disappear and isomeric ratios of glycosylated products can be estimated readily by relative intensities of anomeric signals. In addition, the introduction of Bn-d_7 ethers enables facile evaluation of yield by MALDI-TOF MS (Fig. 1). Each Bn-d_7 contributes to increase the molecular weight (M.W.) with +7 Da compared to non-labeled Bn. Therefore, measurement of MS-spectrum of aliquot of each reaction, which was mixed with a defined amount of non-labeled substrates and product should provide the quantitative estimates[12] of yield and substrate recovery. Therefore, combined use of MALDI-TOF MS and high-field NMR enables the facile analyses of reactions performed in small (μmol) scales. It allows for the systematic screening of various conditions in a parallel fashion.

In this study, application of the HTS system to the synthesis of oligosaccharide having multiple 1,2-*cis*-glycosidic linkages was investigated. We selected the tetrasaccharide Glc_3Man_1 (**1**) as our target, which consists of Glcα1→2Glc (Linkage 1), Glcα1→3Glc (Linkage 2), and Glcα1→3Man (Linkage 3) substructures. It was planned to synthesize **1** from mannose derivative **2**[20] through consecutive couplings with selectively protected glucosyl donors **3**, **4**, and **5** (Scheme 1).

For screening of reaction conditions, 2,3-*O*-Bn-d_7 protected donor **5D** was employed and reacted with acceptors **9**, **10**, and **2D** (Scheme 2), which were synthesized as shown in Scheme 3.[20] Reactions were conducted in a parallel manner with

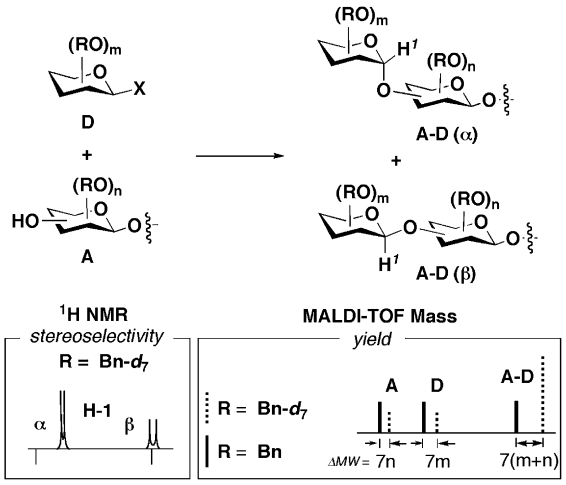


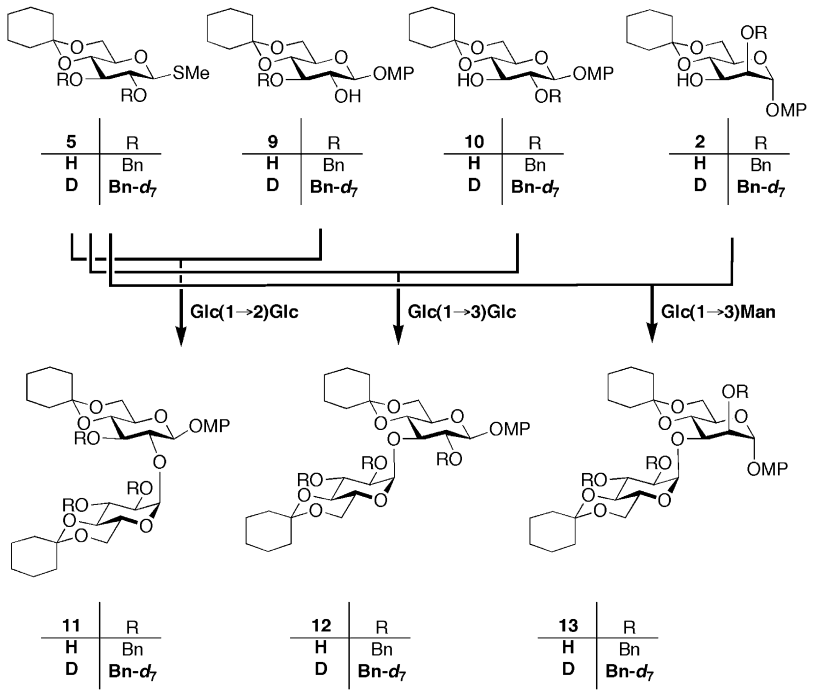
Figure 1. HTS system. This system exploits perdeuterated benzyl (Bn-d_7) ether, and stereoselectivity and yield are evaluated by ^{1}H NMR and MALDI-TOF MS, respectively. And the systematic screening was conducted in a parallel setting; reactions can be performed in ~5 μmol scale with ~2 mg of substrates. MALDI-TOF MS of the crude mixtures was measured with stock solutions of non-labeled compounds and yields were calculated from relative peak heights. Anomeric ratios were estimated by ^{1}H NMR from relative intensities of H-1 signals (in C_6D_6) of α- and β-isomer.

~5 μmol (~2 mg) of acceptors, using 1.2 equiv of **5D**, 4.2 equiv of methyl trifluoromethanesulfonate (MeOTf), and 1.5 equiv of 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) in various solvents.[21,22] A part of the results of glycosylation with an acceptor **9** is listed in Table 1. Somewhat unexpectedly, halogenated solvents, $CHCl_3$, or $(CH_2Cl)_2$ gave higher selectivity than ethereal solvents such as Et_2O, cyclopentyl methyl ether (CPME),[23] dioxane, and 1,2-dimethoxyethane (DME). Aromatic hydrocarbons (e.g., benzene and toluene) exhibited poor selectivity.

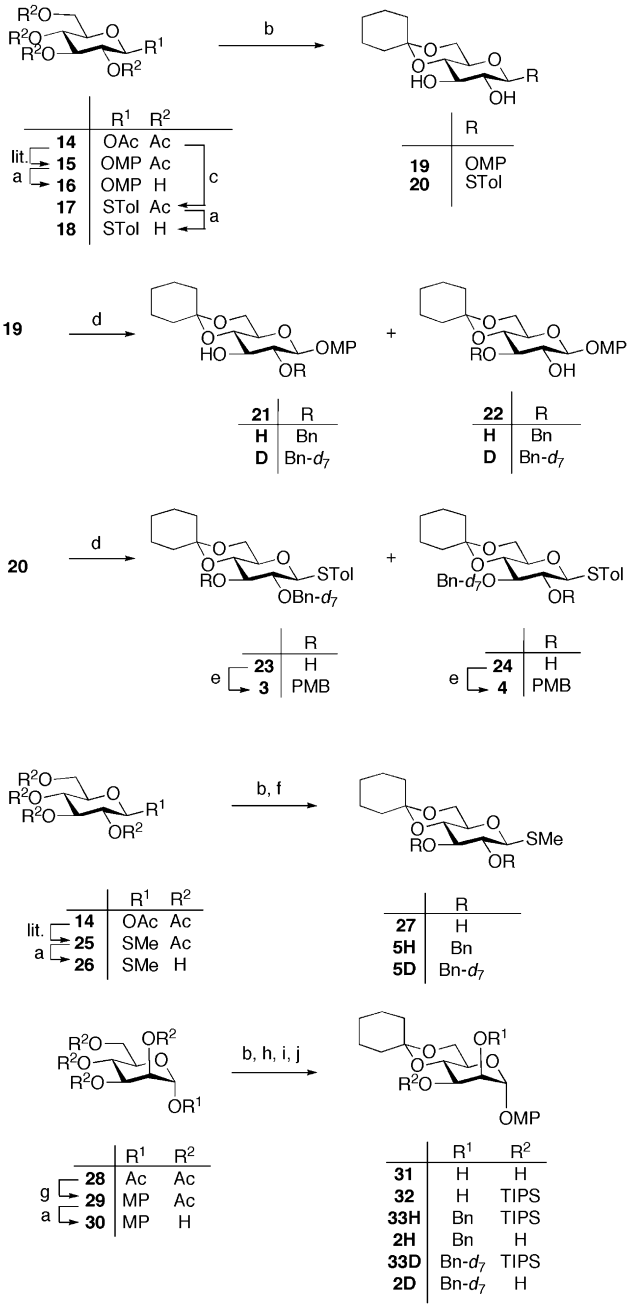
Investigation of mixed solvent systems revealed that the α-selectivity was markedly enhanced when halogenated and ethereal solvents were mixed. For example, anomeric ratios (α/β) in Et_2O, CPME, $CHCl_3$, and CH_2Cl_2 were 3.9:1, 4.6:1, 5.3:1, and 5.2:1, respectively, while those in 1:1 mixtures of $CHCl_3/Et_2O$, $CHCl_3$/CPME, and CH_2Cl_2/Et_2O were 9.3:1, 8.7:1, and 8.2:1, respectively. These results suggest that coexistence of $CHCl_3$ or CH_2Cl_2 and Et_2O or CPME has a synergistic effect in enhancing the α-selectivity.[24,25] Somewhat unexpectedly, dioxane was less effective than Et_2O or CPME as an ethereal component (entry 24). Interestingly, the selectivity was sensitive to the ratio of two components, being significantly lower when the proportion deviated from 1:1. Namely, α/β ratios were 3.9:1, 4.4:1, 4.7:1, 9.3:1, 5.3:1, 4.3:1, and 5.3:1, when the Et_2O content in $CHCl_3$ was 100%, 90%, 75%, 50%, 25%, 10%, and 0%, respectively.

These results are intriguing as it is generally perceived that ethereal solvents have a participating ability to coordinate carbocationic species (Fig. 2), while halogenated solvents such as $CHCl_3$ are more or less neutral. Our results showed that subtle tuning of solvent composition, not only the mere presence of an ethereal component, is important in maximizing the selectivity.

A 1:1 mixture of $CHCl_3$ and ethereal solvents exhibited the highest α-selectivity (11.4:1) for Glcα1→3Glc linkage as well (Linkage 2) (Table 2, entries 11 and 12).[12] Again, the α-selectivity was markedly higher than the cases where Et_2O (3.8:1) or CPME (6.9:1) was used alone. The use of $CHCl_3$ alone (entry 1) gave equally high selectivity (10.9:1), while other halogenated solvents such as CH_2Cl_2, $(CH_2Cl)_2$, and $CHBr_3$



Scheme 2.



Scheme 3. Reagents and conditions: (a) NaOMe, MeOH, quant. (b) 1,1-dimethoxycyclohexane, CSA, or TsOH·H_2O, 90% (**19**), 71% (**20**), 77% (**27**), 60% (**31**). (c) TolSH, $BF_3{\cdot}OEt_2$, 91%, β. (d) Ag_2O (1.5 equiv), BnBr or Bn-d_7Br (1.1 equiv), KI (0.2 equiv), CH_2Cl_2/DMF (10:1), rt, 24 h, 47% (**21H/22H**=2.92:1), 66% (**21D/22D**=1.88:1), 60% (**23/24**=2.0:1). (e) NaH, PMBCl (1.2 equiv), DMF, 96% (**3**), 88% (**4**). (f) NaH, BnBr or Bn-d_7Br (2.2 equiv), DMF, 99% (**5H**), 70% (**5D**). (g) MPOH, TMSOTf, 75%. (h) TIPSCl, imidazole, DMF, 95%. (i) NaH, BnBr, or Bn-d_7Br. (j) TBAF, 97% in two steps (**2H**), 86% in two steps (**2D**).

gave lower selectivity and aromatic solvents behaved poorly (entries 2–6). At higher temperature (50 °C), the α-selectivity was drastically increased in CPME or $CHCl_3$/CPME, reaching as high as 17.6:1 or 19.5:1, although the yield was only modest. On the other hand, significantly deteriorated selectivity was observed in $CHCl_3$ and $(CH_2Cl)_2$ (entries 18–21). These results suggest that the origins of α-selectivity are different

Table 1
Effect of the solvent on 1,2-*cis*-glycosylations for Glc1→2Glc (Linkage 1)

Entry	Solvent	Yield (%)	α/β
1	$CHCl_3$	73	5.28:1
2	CH_2Cl_2	75	5.17:1
3	CPME	80	4.61:1
4	Et_2O	75	3.93:1
5	$(CH_2Cl)_2$	92	5.47:1
6	CCl_4	85	1.99:1
7	Benzene	100	2.45:1
8	Toluene	86	1.93:1
9	Dioxane	25	5.00:1
10	DME	45	3.52:1
11	EtCN	25	1:1.27
12	*t*-BuCN	72	1.25:1
13	$CHCl_3$/CPME	70	8.68:1
14	$CHCl_3/Et_2O$ (9:1)	91	4.33:1
15	$CHCl_3/Et_2O$ (4:1)	97	5.31:1
16	$CHCl_3/Et_2O$ (1:1)	96	9.27:1
17	$CHCl_3/Et_2O$ (1:4)	98	4.67:1
18	$CHCl_3/Et_2O$ (1:9)	93	4.35:1
19	$CHCl_3$/dioxane	72	5.73:1
20	$(CH_2Cl)_2/Et_2O$	100	6.10:1
21	$(CH_2Cl)_2$/CPME	84	8.17:1
22	$(CH_2Cl)_2$/dioxane	55	3.84:1
23	CH_2Cl_2/Et_2O	93	8.19:1
24	CH_2Cl_2/dioxane	69	4.42:1

between halogenated and ethereal solvents. In the latter case, activation of the donor likely to generate ether-coordinated intermediates **E(α)** and **E(β)**. It is plausible that glycosylation proceeds through more abundant β-oriented **E(β)**, giving α-glycoside predominantly.

The formation of Glcα1→3Man (Linkage 3) uniformly proceeded in a highly selective manner in $CHCl_3$, Et_2O, or CPME, among which Et_2O was optimum. Again, a binary system consisting of Et_2O and $CHCl_3$ gave the best result, in terms of both yield and selectivity (Table 3).

From these results, a 1:1 mixture of $CHCl_3/Et_2O$ (solvent-A) or $CHCl_3$/CPME (solvent-B) was judged to be suitable for all linkages 1–3. Therefore, these solvent systems were employed in the linear synthesis of the tetrasaccaharide **1** (Scheme 4A). The mannose derivative **2** was subjected to sequential glycosylations with **3**[20] (glycosylation-1), **4**[20] (glycosylation-2), and **5** (glycosylation-3). To begin with, coupling with **3** gave **34** [α/β=21.6:1 (solvent-A) or 17.4:1 (solvent-B)], which was converted to the disaccharide acceptor **6**.[26] The latter was coupled with **4** [α/β=18.7:1 (solvent-A) or 18.5:1 (solvent-B)] to provide **35**. Subsequent detachment of the PMB group gave **7**, which was subjected to further coupling with **5D** [α/β=21.6:1 (solvent-A) or 19.9:1 (solvent-B)] to give **8**. The overall selectivity from **2D** was 86.7% or 85.5% in solvent-A and -B, respectively.

High overall selectivity allowed us to conduct the whole series of reactions without recourse of isomer separation after each step. Thus, compound **2D** was subjected to steps 1–5, with all glycosylations performed in $CHCl_3/Et_2O$ (solvent-A) as shown in Scheme 4B to afford the desired all α-isomer of Glc_3Man_1 derivative **8** in good overall yield in high purity (47% from **2D**). Deprotection of **8** gave **1**[27] without incident.

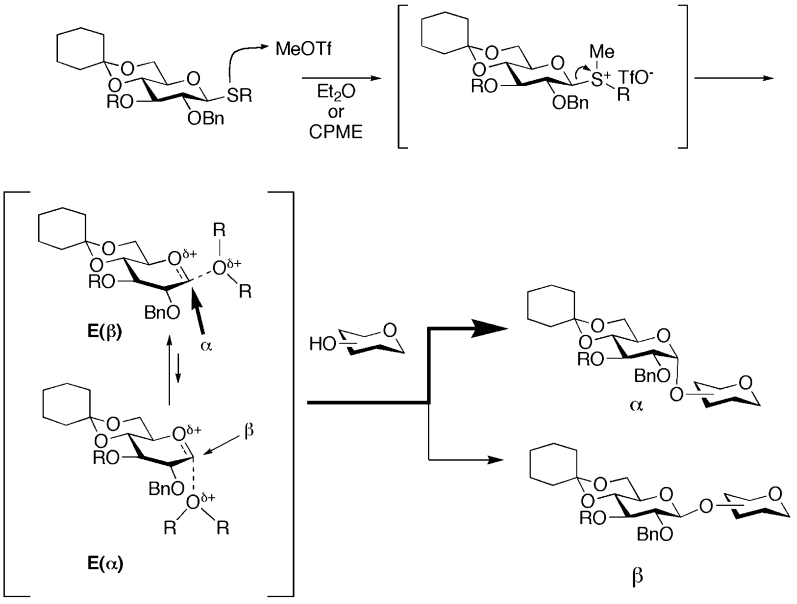


Figure 2. Plausible mechanism of α-selective glucosylation in the mixed solvent.

Table 2
Effect of the solvent on 1,2-*cis*-glycosylations for Glc1→3Glc (Linkage 2)

Entry	Solvent	Temp (°C)	Yield (%)	α/β
1	$CHCl_3$	25	60	10.9:1
2	CH_2Cl_2	25	61	5.89:1
3	$(CH_2Cl)_2$	25	96	3.68:1
4	$CHBr_3$	25	47	5.34:1
5	Benzene	25	65	1.86:1
6	Toluene	25	66	1.46:1
7	CPME	25	78	6.91:1
8	Et_2O	25	91	3.79:1
9	Dioxane	25	87	4.11:1
10	DME	25	43	3.78:1
11	$CHCl_3$/CPME	25	100	11.4:1
12	$CHCl_3/Et_2O$	25	98	11.4:1
13	Toluene/CPME	25	78	3.64:1
14	$CHCl_3$/dioxane	25	55	7.28:1
15	Toluene/dioxane	25	76	8.72:1
16	$CHCl_3$/DME	25	100	3.23:1
17	Toluene/DME	25	51	4.09:1
18	$CHCl_3$	50	100	4.95:1
19	$(CH_2Cl)_2$	50	97	4.18:1
20	CPME	50	42	17.6:1
21	$CHCl_3$/CPME	50	50	19.5:1

Table 3
Effect of the solvent on 1,2-*cis*-glycosylations for Glc1→3Man (Linkage 3)

Entry	Solvent	Yield (%)	α/β
1	$CHCl_3$	94	22.1:1
2	CPME	90	22.1:1
3	Et_2O	83	35.3:1
4	$CHCl_3/Et_2O$	92	39.9:1
5	$CHCl_3$/CPME	74	22.2:1

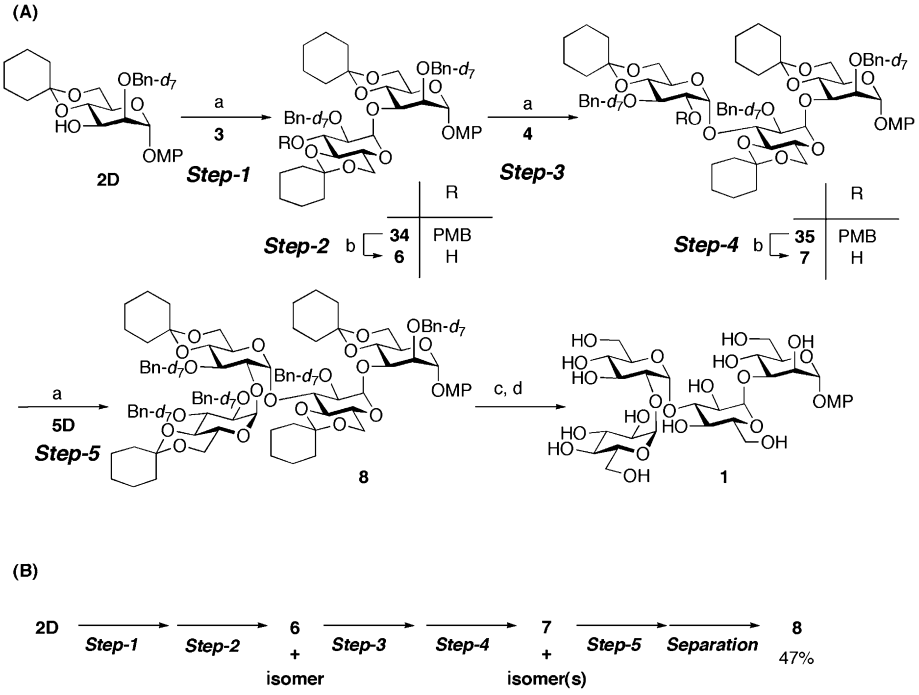
3. Conclusion

In conclusion, a stereoselective synthesis of the tetrasaccharide [Glcα1→2Glcα1→3Glcα1→3Man], which contains three continuous α-glucosidic linkages, was conducted using solvent systems identified by HTP screening. Our study revealed the synergistic effect of combined ethereal and halogenic solvents. Further effort toward facilitating and scaling down the optimization through HTP system is in progress.

4. Experimental

4.1. General procedures

Reactions sensitive to air and/or moisture were carried out under nitrogen or argon atmosphere with anhydrous solvents. Column chromatography was performed on silica gel 60N, 100–210 mesh (Kanto Kagaku Co., Ltd.). Preparative TLC was performed on silica gel 60 F_{254}, 0.5 mm thickness (E. Merck). Gel filtration chromatography was performed on Sephadex LH-20 (Pharmacia). All other reagents were purchased from Wako Pure Chemical Industries Ltd., Kanto Chemicals Co., Inc., Tokyo Kasei Kogyo Co., or Aldrich Chemical Company. Melting points were determined with Büchi 510 melting point apparatus. Optical rotations were measured with a JASCO DIP 370 polarimeter. ^{1}H NMR spectra were recorded at 400 MHz on a JEOL JNM-AL 400 spectrometer and chemical shifts are referred to internal tetramethylsilane (0 ppm) or residual solvent peaks; $CDCl_3$ (7.24 ppm), D_2O (4.65 ppm), or CD_3OD (3.30 ppm). ^{13}C NMR spectra were recorded at 100 MHz on the same instrument and chemical shifts are referred to internal $CDCl_3$ (77.00 ppm), C_6D_6 (128.00 ppm), or CD_3OD (49.0 ppm). MALDI-TOF mass spectra were recorded on a SHIMADZU Kompact MALDI AXIMA-CFR spectrometer with 2,5-dihydroxybenzoic acid as the matrix. ESI-TOF mass spectra were recorded on a JEOL AccuTOF JMS-T700LCK with CF_3CO_2Na as an internal standard. FAB mass spectra were recorded on a JEOL JMS HX110 with



Scheme 4. Synthesis of Glc_3Man_1. Reagents and conditions: (a) MeOTf (4.2 equiv), DTBMP (1.5 equiv), 4 Å MS, $CHCl_3/Et_2O$ (1:1) (solvent-A) or $CHCl_3$/CPME (1:1) (solvent-B), rt, 24 h; 87% (21.6:1) (solvent-A), 76% (17.4:1) (solvent-B) (**34**); 81% (18.7:1) (solvent-A), 84% (18.5:1) (solvent-B) (**35**); 83% (21.6:1) (solvent-A), 86% (19.9:1) (solvent-B) (**8**). (b) DDQ, CH_2Cl_2/H_2O (10:1), rt, 18 h, 97% (**6**), 86% (**7**). (c) TFA, CH_2Cl_2, rt. (d) H_2, $Pd(OH)_2$, MeOH/H_2O (2:1), 99% in two steps. (e) MeOTf (4.2 equiv), DTBMP (1.5 equiv), 4 Å MS, $Et_2O/CHCl_3$ (1:1), rt, 48 h, 47% (**8**) from **2** (**8**/other isomers=9.0:1). (f) DDQ, CH_2Cl_2/H_2O (10:1), rt, 36 h.

NBA as the matrix and PEG as an internal standard. Elemental analyses were performed with a Fisons EA1108 instrument.

4.2. General procedure for the small scale screening (Tables 1–3)

Each 100 μL of solution of acceptor **3D** (96.6 mg, 208 μmol), donor **4D** (121.2 mg, 250 μmol), and DTBMP (76.8 mg, 374 μmol) in 6.0 mL of CH_2Cl_2 were pipetted into multiplicate tubes, and the mixtures were evaporated by flashing with N_2 gas. In each tube, 3.47 μmol of acceptor, 4.17 μmol of donor, and 6.23 μmol of DTBMP were prepared for the reaction. After addition of 4 Å MS (25 mg) and each solvent (200 μL) to the mixture, methyl trifluoromethanesulfonate (2.0 μL, 18 μmol) was added to each tube. The mixtures were magnetically stirred at room temperature for 24 h, and the reactions quenched by triethylamine. The mixtures were filtered through Celite, washed with saturated aqueous $NaHCO_3$ solution, dried over Na_2SO_4, and evaporated by flashing with N_2 to give crude mixtures. Results of glycosylation are listed in Tables 1–3, which were obtained by following two analytical methods.

4.2.1. Determination of the stereoselectivity by ^1H NMR analysis

The anomeric ratios of products were estimated from the relative intensities of H-1 signals (in C_6D_6) of α- and β-isomer by analyzing the crude mixture.

4.2.2. Determination of the yield by the quantitative MALDI-TOF MASS analysis

The crude mixtures were diluted with 700 μL of CH_3CN. A 4.0 μL measure of 1.0 mM standard solution of each of the three non-labeled compounds was pre-mixed with 2.0 μL of the crude solutions for MS analysis. The resulting solutions were measured by MALDI-TOF MASS using the RASTER function. The molar ratio of labeled to non-labeled compound was obtained from the ratio of each value (mV) at the apex of the ion peak of $[M+Na]^+$.

4.3. Synthesis

4.3.1. Methoxyphenyl 4,6-O-cyclohexylidene-2,3-di-O-benzyl-β-D-glucopyranosyl-(1→3)-2-O-benzyl-4,6-cyclohexylidene-β-D-glucopyranoside (12H)

After azeotropic removal with toluene, a mixture of **10H** (16.1 mg, 35.3 μmol) and **5H** (20.0 mg, 42.5 μmol) was dissolved in $(CH_2Cl)_2$ (2 mL) containing 4 Å MS (250 mg, freshly dried) and 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP, 10.7 mg, 51.1 μmol) and the mixture was stirred for 10 min at room temperature. Solution of methyl trifluoromethanesulfonate (1 M, 51 μL, 51 μmol) in $(CH_2Cl)_2$ was added to the mixture at the same temperature. After stirring for 3d, the reaction was quenched by triethylamine and filtered through Celite pad followed by washing with ethyl acetate. The combined filtrate and washings were washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by gel

filtration chromatography (Bio beads SX-3, toluene/ethyl acetate=1:1) to give **12H** as a mixture of isomers (24.8 mg, 85%, α/β=5.00:1). The isomers were separated by PTLC (toluene/ethyl acetate=10:1) for characterization. Compound **12Hα**: ^{1}H NMR (C_6D_6, 400 MHz): δ 0.90–2.10 (m, cyclohexyl×2, 10H), 3.08 (td, *J*=10.0, 5.2 Hz, H-5^{Glc1}, 1H), 3.27 (s, MeO, 3H), 3.56 (t, *J*=10.8 Hz, H-6^{Glc1}, 1H), 3.68–3.74 (m, H-6^{Glc1}, H-2^{Glc2}, 2H), 3.76–3.89 (m, H-2^{Glc1}, H-4^{Glc1}, H-4^{Glc2}, H-6^{Glc2}, 4H), 4.04 (dd, *J*=10.4, 5.2 Hz, H-6^{Glc2}, 1H), 4.14 (t, *J*=9.2 Hz, H-3^{Glc1}, 1H), 4.29 (t, *J*=8.8 Hz, H-3^{Glc2}, 1H), 4.56–4.65 (m, H-5^{Glc2}, 1H), 4.86 (s, Bn, 2H), 4.88 (d, *J*=8.0 Hz, H-1^{Glc1}, 1H), 5.01 (d, *J*=11.6 Hz, Bn, 1H), 5.06 (d, *J*=10.8 Hz, Bn, 1H), 5.10 (d, *J*=10.8 Hz, Bn, 1H), 5.19 (d, *J*=11.6 Hz, Bn, 1H), 5.89 (d, *J*=3.6 Hz, H-1^{Glc2}, 1H), 6.69 (d, *J*=9.2 Hz, MP, 2H), 6.98 (d, *J*=9.2 Hz, MP, 2H), 7.10–7.75 (m, Ar, 15H); ^{13}C NMR (C_6D_6, 100 MHz): δ 22.79, 22.98, 23.34, 23.49, 26.05, 26.14, 27.85, 28.21, 38.61 (×2), 55.18, 61.51, 62.42, 63.94, 67.15, 73.04, 74.31, 75.08, 75.20, 75.44, 75.86, 78.94, 80.05, 80.13, 97.68, 99.63, 99.67, 104.05, 114.89, 119.17, 127–129.10 (over up under C_6D_6), 129.60, 138.32, 139.37, 139.91, 151.73, 156.02. MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{52}H_{62}O_{12}Na$ 901, found 901. HRMS FAB: $[M+H]^+$ calcd for $C_{52}H_{63}O_{12}$ 879.4320, found 879.4348. Compound **12Hβ**: ^{1}H NMR (C_6D_6, 400 MHz): δ 0.90–1.95 (m, cyclohexyl×2, 10H), 3.12–3.22 (m, H-5^{Glc1}, 1H), 3.20–3.28 (m, H-5^{Glc2}, 1H), 3.28 (s, MeO, 3H), 3.62–3.77 (m, H-6^{Glc1}, H-2^{Glc2}, H-3^{Glc2}, H-6^{Glc2}, 4H), 3.78–3.85 (m, H-4^{Glc1}, H-6^{Glc1}, H-4^{Glc2}, 3H), 3.89 (t, *J*=8.0 Hz, H-2^{Glc1}, 1H), 3.95 (dd, *J*=9.2, 5.2 Hz, H-6^{Glc2}, 1H), 4.17 (t, *J*=8.8 Hz, H-3^{Glc1}, 1H), 4.88 (d, *J*=11.6 Hz, Bn, 1H), 4.91 (d, *J*=7.6 Hz, H-1^{Glc1}, 1H), 4.93 (d, *J*=10.0 Hz, Bn, 1H), 4.96 (d, *J*=12.0 Hz, Bn, 1H), 5.07 (d, *J*=12.0 Hz, Bn, 1H), 5.10 (d, *J*=10.0 Hz, Bn, 1H), 5.11 (d, *J*=11.6 Hz, Bn, 1H), 5.26 (d, *J*=7.2 Hz, H-1^{Glc2}, 1H), 6.69 (d, *J*=9.2 Hz, MP, 2H), 7.00 (d, *J*=9.2 Hz, MP, 2H), 7.10–7.50 (m, Ar, 15H); ^{13}C NMR (C_6D_6, 100 MHz): δ 22.83, 22.93, 23.26, 23.42, 26.05, 26.19, 28.01, 28.06, 38.53, 38.60, 55.20, 61.91, 62.15, 67.53, 67.70, 71.83, 74.35, 74.77, 75.52, 75.58, 78.67, 79.03, 82.05, 83.11, 83.39, 99.53, 99.76, 103.45, 103.55, 114.97, 118.96, 138.88, 139.37, 139.68, 151.84, 156.02. MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{52}H_{62}O_{12}Na$ 901, found 901. HRMS FAB: $[M+H]^+$ calcd for $C_{52}H_{63}O_{12}$ 879.4320, found 879.4384.

*4.3.2. Methoxyphenyl 4,6-O-cyclohexylidene-2,3-di-O-(benzyl-d$_7$)-β-D-glucopyranosyl-(1→3)-2-O-(benzyl-d$_7$)-4,6-O-cyclohexylidene-β-D-glucopyranoside (**12D**)*

This compound was prepared from **10D** and **5D** according to the procedure described for compound **12H**. The crude mixture was subjected to MALDI-TOF Mass and ^{1}H NMR measurements, which provided the estimations of yield (70%) and selectivity (α/β=5.26:1). Finally, the isomers were separated by PTLC (SiO_2, toluene/ethyl acetate=10:1) for the sake of characterization. Compound **12Dα**: ^{1}H NMR (C_6D_6, 400 MHz): δ 0.90–2.10 (m, cyclohexyl×2, 10H), 3.08 (td, *J*=10.0, 5.2 Hz, H-5^{Glc1}, 1H), 3.27 (s, MeO, 3H), 3.55 (t, *J*=10.4 Hz, H-6^{Glc1}, 1H), 3.68–3.74 (m, H-6^{Glc1}, H-2^{Glc2}, 2H), 3.76–3.89 (m, H-2^{Glc1}, H-4^{Glc1}, H-4^{Glc2}, H-6^{Glc2}, 4H), 4.05 (dd, *J*=10.8, 5.6 Hz, H-6^{Glc2}, 1H), 4.14 (t, *J*=9.2 Hz, H-3^{Glc1}, 1H), 4.29 (t, *J*=9.2 Hz, H-3^{Glc2}, 1H), 4.57–4.65 (m, H-5^{Glc2}, 1H), 4.87 (d, *J*=8.0 Hz, H-1^{Glc1}, 1H), 5.89 (d, *J*=3.6 Hz, H-1^{Glc2}, 1H), 6.69 (d, *J*=9.2 Hz, MP, 2H), 6.98 (d, *J*=9.2 Hz, MP, 2H); ^{13}C NMR (C_6D_6, 100 MHz): δ 22.93, 23.12, 23.48, 23.63, 26.18, 26.28, 27.98, 28.35, 38.71, 38.75, 55.28, 61.60, 62.51, 64.01, 67.23, 74.38, 75.29, 75.93, 78.90, 79.99, 80.04, 97.76, 99.67, 99.71, 104.10, 114.90, 119.17, 151.69, 155.99 (+Bn-d_7×3). MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{52}H_{41}N_{21}O_{12}Na$ 923, found 923. HRMS FAB: $[M+H]^+$ calcd for $C_{52}H_{41}N_{21}O_{12}Na$ 922.5457, found 922.5397. Compound **12Dβ**: ^{1}H NMR (C_6D_6, 400 MHz): δ 0.90–1.95 (m, cyclohexyl×2, 10H), 3.10–3.20 (m, H-5^{Glc1}, 1H), 3.19–3.27 (m, H-5^{Glc2}, 1H), 3.27 (s, MeO, 3H), 3.68–3.74 (m, H-6^{Glc1}, H-2^{Glc2}, H-3^{Glc2}, H-6^{Glc2}, 4H), 3.78–3.85 (m, H-4^{Glc1}, H-6^{Glc1}, H-4^{Glc2}, 3H), 3.89 (t, *J*=8.0 Hz, H-2^{Glc1}, 1H), 3.94 (dd, *J*=9.2, 5.2 Hz, H-6^{Glc2}, 1H), 4.17 (t, *J*=8.8 Hz, H-3^{Glc1}, 1H), 4.91 (d, *J*=7.6 Hz, H-1^{Glc1}, 1H), 5.26 (d, *J*=7.2 Hz, H-1^{Glc2}, 1H), 6.69 (d, *J*=9.2 Hz, MP, 2H), 7.00 (d, *J*=9.2 Hz, MP, 2H); ^{13}C NMR (C_6D_6, 100 MHz): δ 22.97, 23.07, 23.40, 23.56, 26.18, 26.32, 28.14, 28.18, 38.65, 38.72, 55.30, 61.99, 62.24, 67.61, 67.77, 71.90, 74.44, 79.10, 81.99, 83.08, 83.37, 114.98, 118.97, 151.81, 155.97 (+Bn-d_7×3). MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{52}H_{41}N_{21}O_{12}Na$ 923, found 923. HRMS FAB: $[M+H]^+$ calcd for $C_{52}H_{41}N_{21}O_{12}Na$ 922.5457, found 922.5459.

4.4. Typical procedure for α-selective glycosylation

After azeotropic removal with toluene, to the mixture of an acceptor (5–100 μmol) and a donor (1.2 equiv) in dry $CHCl_3/Et_2O$ (1:1) or $CHCl_3$/CPME (1:1) (~15 mM) were added 4 Å MS (ca. 125 mg/mL, freshly dried) and DTBMP (1.5 equiv) and the mixture was stirred for 10 min at room temperature. MeOTf (4.2 equiv) was added to the mixture. After stirring for 24 h at the same temperature, the reaction was quenched with triethylamine followed by filtration through Celite pad and washing of pad with ethyl acetate. The combined solutions were washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by gel filtration (Bio beads SX-3, toluene/ethyl acetate=1:1) to give the mixture of the isomers.

*4.4.1. Methoxyphenyl 4,6-O-cyclohexylidene-2,3-di-O-benzyl-α-D-glucopyranosyl-(1→2)-3-O-benzyl-4,6-O-cyclohexylidene-β-D-glucopyranoside (**11H**)*

This compound was synthesized from **9H** and **5H** according to the procedure described in Section 4.4. (94%, α/β=8.06:1 in $CHCl_3$/CPME). ^{1}H NMR (C_6D_6, 400 MHz): α-isomer: δ 1.15–2.10 (m, cyclohexyl×2, 20H), 2.96 (td, *J*=9.6, 5.2 Hz, H-5^{Glc1}, 1H), 3.28 (s, OMe, 3H), 3.56 (t, *J*=10.4 Hz, H-4^{Glc1}, 1H), 3.64–3.74 (m, H-2^{Glc1}, H-3^{Glc2},

H-6^{Glc2}, 4H), 3.80 (t, J=10.0 Hz, H-6^{Glc1}, 1H), 3.83 (t, J=9.2 Hz, H-4^{Glc1}, 1H), 4.05 (dd, J=10.0, 5.2 Hz, H-6^{Glc1}, 1H), 4.18 (t, J=8.4 Hz, H-3^{Glc1}, 1H), 4.26 (t, J=9.6 Hz, H-3^{Glc1}, 1H), 4.55 (d, J=12.0 Hz, Bn, 1H), 4.67 (d, J=12.0 Hz, Bn, 1H), 4.78 (td, J=10.0, 5.6 Hz, H-5^{Glc1}, 1H), 4.91 (d, J=8.0 Hz, H-1^{Glc2}, 1H), 5.03 (d, J=12.0 Hz, Bn, 1H), 5.07 (d, J=10.8 Hz, Bn, 1H), 5.11 (d, J=10.8 Hz, Bn, 1H), 5.20 (d, J=12.0 Hz, Bn, 1H), 5.91 (d, J=4.0 Hz, H-1^{Glc1}, 1H), 6.67–7.83 (m, Ar, 19H); ^{13}C NMR (C_6D_6, 100 MHz): α-isomer: δ 22.80, 22.98, 23.39, 23.52, 25.94, 26.16, 27.91, 28.21, 38.55, 38.70, 55.25, 61.62, 62.36, 64.03, 67.05, 73.43, 74.34, 74.91, 75.08, 75.17, 76.48, 78.08, 79.04, 80.26, 97.29, 99.61 (×2), 102.65, 114.91, 118.64, 127.49–129.89 (overlapped on C_6D_6), 138.57, 139.02, 139.95, 151.05, 155.91. HRMS ESI-TOF: $[M+Na]^+$ calcd for $C_{52}H_{62}O_{12}Na$ 901.4139, found 901.4117.

4.4.2. Methoxyphenyl 4,6-O-cyclohexylidene-2,3-di-O-(benzyl-d_7)-α-D-glucopyranosyl-(1→2)-2-O-(benzyl-d_7)-4,6-O-cyclohexylidene-β-D-glucopyranoside (**11D**)

This compound was synthesized from **9D** and **5D** as described for the synthesis of **12H** except that the reaction time was 24 h (95%, α/β=3.99:1). ^{1}H NMR (C_6D_6, 400 MHz): α-isomer: δ 1.15–2.10 (m, cyclohexyl×2, 20H), 2.96 (td, J=10.0, 5.6 Hz, H-5^{Glc1}, 1H), 3.28 (s, OMe, 3H), 3.56 (t, J=10.4 Hz, H-4^{Glc1}, 1H), 3.62–3.74 (m, H-2^{Glc1}, H-3^{Glc2}, H-6^{Glc2}, 4H), 3.80 (t, J=10.0 Hz, H-6^{Glc1}, 1H), 3.83 (t, J=9.2 Hz, H-4^{Glc1}, 1H), 4.06 (dd, J=10.0, 5.2 Hz, H-6^{Glc1}, 1H), 4.17 (t, J=8.0 Hz, H-2^{Glc1}, 1H), 4.26 (t, J=9.2 Hz, H-3^{Glc1}, 1H), 4.78 (td, J=10.0, 5.2 Hz, H-5^{Glc1}, 1H), 4.91 (d, J=7.6 Hz, H-1^{Glc2}, 1H), 5.91 (d, J=3.6 Hz, H-1^{Glc1}, 1H), 6.67–6.93 (m, Ar, 4H); ^{13}C NMR (C_6D_6, 100 MHz): α-isomer: δ 22.80, 22.98, 23.38, 23.51, 25.93, 26.16, 27.91, 28.21, 38.54, 38.71, 55.25, 61.61, 62.36, 64.00, 67.03, 74.93, 75.18, 76.47, 77.88, 78.93, 80.16, 97.31, 99.61 (×2), 102.07, 114.90, 118.64, 151.04, 155.90 (+Bn-d_7×3). HRMS ESI-TOF: $[M+Na]^+$ calcd for $C_{52}H_{41}D_{21}O_{12}Na$ 922.5457, found 922.5457.

4.4.3. Methoxyphenyl 4,6-O-cyclohexylidene-2,3-di-O-benzyl-α-D-glucopyranosyl-(1→3)-2-O-benzyl-4,6-O-cyclohexylidene-α-D-mannopyranoside (**13H**)

This compound was synthesized from **2H** and **5H** according to the general procedure described in Section 4.4. (91%, α/β=22:1 in $CHCl_3$/CPME). $[\alpha]_D^{28}$ 109.8 (c 1.0, $CHCl_3$). ^{1}H NMR (C_6D_6, 400 MHz): δ 1.00–2.10 (m, cyclohexyl×2, 20H), 3.29 (s, OMe, 3H), 3.68–3.78 (m, H-2^{Glc}, H-6^{Man}, 3H), 3.80 (t, J=9.6 Hz, H-4^{Glc}, 1H), 3.84 (t, J=10.4 Hz, H-6^{Glc}, 1H), 3.99 (br t, H-2^{Man}, 1H), 4.02 (t, J=10.4, 5.2 Hz, H-6^{Glc}, 1H), 4.05–4.18 (m, H-5^{Man}, H-5^{Glc}, 2H), 4.26 (t, J=9.2 Hz, H-3^{Glc}, 1H), 4.49 (d, J=11.2 Hz, Bn, 1H), 4.59–4.72 (m, H-3^{Man}, H-4^{Man}, 2H), 4.76 (d, J=12.0 Hz, Bn, 1H), 4.84 (d, J=11.2 Hz, Bn, 1H), 4.96 (d, J=12.0 Hz, Bn, 1H), 4.99 (d, J=11.6 Hz, Bn, 1H), 5.10 (d, J=11.6 Hz, Bn, 1H), 5.53 (d, J=1.6 Hz, H-1^{Man}, 1H), 5.82 (d, J=3.6 Hz, H-1^{Glc}, 1H), 6.67–7.57 (m, Ar, 19H); ^{13}C NMR (C_6D_6, 100 MHz): δ 23.96, 23.03, 23.59, 23.67, 26.33, 28.34, 28.40, 38.94, 39.01, 55.42, 61.92, 62.66, 65.66, 66.80, 71.89, 72.43, 73.79, 74.89, 75.11, 75.31, 78.80, 79.46, 80.21, 98.82 (J_{C-H}=174.0 Hz), 98.93 (J_{C-H}=169.1 Hz), 100.13, 100.36, 115.21, 118.48, 125.89, 127.70–129.52 (overlapped on C_6D_6), 138.98, 139.61, 139.99, 150.55, 155.88. HRMS ESI-TOF: $[M+Na]^+$ calcd for $C_{52}H_{62}O_{12}Na$ 901.4139, found 901.4084.

4.4.4. Methoxyphenyl 4,6-O-cyclohexylidene-2,3-di-O-(benzyl-d_7)-α-D-glucopyranosyl-(1→3)-2-O-(benzyl-d_7)-4,6-O-cyclohexylidene-α-D-mannopyranoside (**13D**)

This compound was prepared from **5D** and **2D** and isolated by PTLC (hexane/ethyl acetate=5:1). $[\alpha]_D^{27}$ 101.59 (c 1.0, $CHCl_3$). ^{1}H NMR (C_6D_6, 400 MHz): δ 1.00–2.10 (m, cyclohexyl×2, 20H), 3.30 (s, OMe, 3H), 3.68–3.78 (m, H-2^{Glc}, H-6^{Man}, 3H), 3.80 (t, J=9.6 Hz, H-4^{Glc}, 1H), 3.84 (t, J=10.4 Hz, H-6^{Glc}, 1H), 3.99 (br t, H-2^{Man}, 1H), 4.02 (t, J=10.4, 5.2 Hz, H-6^{Glc}, 1H), 4.05–4.18 (m, H-5^{Man}, H-5^{Glc}, 2H), 4.26 (t, J=9.2 Hz, H-3^{Glc}, 1H), 4.59–4.72 (m, H-3^{Man}, H-4^{Man}, 2H), 5.53 (d, J=1.6 Hz, H-1^{Man}, 1H), 5.82 (d, J=4.0 Hz, H-1^{Glc}, 1H), 6.67–7.57 (m, Ar, 19H); ^{13}C NMR (C_6D_6, 100 MHz): δ 23.93, 23.06, 23.48, 23.57, 26.20, 26.21, 28.24, 28.29, 38.82, 38.90, 55.28, 61.78, 62.50, 64.90, 66.65, 71.73, 73.62, 74.96, 78.51, 79.17, 79.94, 98.65, 98.77, 99.92, 100.15, 114.98, 118.26, 127.70–129.52 (overlapped with C_6D_6), 138.42, 139.04, 139.43, 150.29, 155.60. ESI-TOF MS: $[M+Na]^+$ calcd for $C_{52}H_{41}D_{21}O_{12}Na$ 922.55, found 922.54. HRMS ESI-TOF: $[M+Na]^+$ calcd for $C_{52}H_{41}D_{21}O_{12}Na$ 922.5457, found 922.5496.

4.4.5. Methoxyphenyl 2-O-(benzyl-d_7)-3-O-(4-methoxybenzyl)-4,6-O-cyclohexylidene-α-D-glucopyranosyl-(1→3)-2-O-(benzyl-d_7)-4,6-O-cyclohexylidene-α-D-mannopyranoside (**34**)

This compound was synthesized from **2D** and **3** according to the procedure described in Section 4.4. (87%, α/β=21.6:1 in $CHCl_3$/Et_2O; 76%, α/β=17.4:1 in $CHCl_3$/CPME). $[\alpha]_D^{29}$ 88.1 (c 0.54, $CHCl_3$). ^{1}H NMR (C_6D_6, 400 MHz): δ 1.00–2.10 (m, cyclohexyl×2, 20H), 3.26 (s, OMe, 3H), 3.29 (s, OMe, 3H), 3.68–3.78 (m, H-2^{Glc}, H-6^{Man}, 3H), 3.81 (t, J=9.6 Hz, H-4^{Glc}, 1H), 3.84 (t, J=10.8 Hz, H-6^{Glc}, 1H), 3.99 (br s, H-2^{Man}, 1H), 4.03 (t, J=10.8, 5.6 Hz, H-6^{Glc}, 1H), 4.05–4.14 (m, H-5^{Man}, 1H), 4.15 (td, J=10.4, 4.8 Hz, H-5^{Glc}, 1H), 4.27 (t, J=9.2 Hz, H-3^{Glc}, 1H), 4.62–4.72 (m, H-3^{Man}, H-4^{Man}, 2H), 4.97 (d, J=11.2 Hz, PMB, 1H), 5.08 (d, J=11.2 Hz, PMB, 1H), 5.53 (d, J=2.0 Hz, H-1^{Man}, 1H), 5.82 (d, J=4.0 Hz, H-1^{Glc}, 1H), 6.66–7.57 (m, Ar, 18H); ^{13}C NMR (C_6D_6, 100 MHz): δ 22.94, 23.10, 23.49, 23.61, 26.20, 26.27, 28.24, 28.32, 38.86, 54.85, 55.27, 61.78, 62.53, 64.94, 66.66, 71.77, 73.55, 74.68, 74.85, 75.05, 78.18, 79.14, 79.96, 98.67, 98.83, 99.92, 100.15, 113.96, 114.98, 118.26, 127.88–128.78 (overlapped on C_6D_6), 129.78, 131.74, 138.46, 139.14, 150.28, 155.59, 159.53. ESI-TOF MS: $[M+Na]^+$ calcd for $C_{53}H_{50}D_{14}O_{13}Na$ 945.51, found 945.5119. HRMS ESI-TOF: $[M+Na]^+$ calcd for $C_{53}H_{50}D_{14}O_{13}Na$ 945.5123, found 945.5119.

4.4.6. Methoxyphenyl 2-O-(benzyl-d_7)-4,6-O-cyclohexylidene-α-D-glucopyranosyl-(1→3)-2-O-(benzyl-d_7)-4,6-O-cyclohexylidene-α-D-mannopyranoside (**6**)

To a solution of compound **34** (17.0 mg, 18.4 μmol) in CH_2Cl_2/H_2O (10:1, 3.3 mL) was added DDQ (5.8 mg, 28.0 μmol) and stirred at room temperature for 18 h. The reaction was quenched with ascorbic acid/citric acid buffer, which was prepared from L-ascorbic acid (0.7 g), citric acid monohydrate (1.2 g), and NaOH (0.92 g) in H_2O (100 mL), and extracted with $CHCl_3$. The combined solutions were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The resulting residue was purified by PTLC (hexane/ethyl acetate=10:1×2) to give the title compound (14.4 mg, 97%). $[\alpha]_D^{28}$ −125.2 (*c* 1.00, $CHCl_3$). ^{1}H NMR (C_6D_6, 400 MHz): δ 1.00−2.10 (m, cyclohexyl×2, 20H), 2.48 (br s, OH, 1H), 3.29 (s, OMe, 3H), 3.59 (dd, *J*=9.2, 4.0 Hz, C2-H^{Glc}, 1H), 3.69 (t, *J*=9.2 Hz, C4-H^{Glc}, 1H), 3.73−3.79 (m, C6-H^{Man}, 2H), 3.80 (t, *J*=10.4 Hz, C6-H^{Glc}, 1H), 3.98 (t, *J*=1.2 Hz, C2-H^{Man}, 1H), 4.02 (dd, *J*=10.4, 5.6 Hz, C6-H^{Glc}, 1H), 4.04−4.18 (m, C5-H^{Man}, C5-H^{Glc}, 2H), 4.39 (t, *J*=9.2 Hz, C3-H^{Glc}, 1H), 4.65−4.73 (m, C3-H^{Man}, C3-H^{Man}, 2H), 5.53 (d, *J*=1.2 Hz, C1-H^{Man}, 1H), 5.82 (d, *J*=4.0 Hz, C1-H^{Glc}, 1H), 6.68−6.90 (m, Ar, 2H); ^{13}C (C_6D_6, 100 MHz): δ 22.38, 22.54, 22.85, 22.92, 25.64, 25.71, 27.73, 38.28, 38.40, 54.83, 61.31, 61.89, 64.26, 66.24, 70.81, 71.31, 73.27, 73.48, 78.77, 79.49, 97.85, 98.49, 99.72, 99.83, 114.69, 117.97, 126.85−128.65 (overlapped on C_6D_6), 138.15, 138.47, 150.07, 155.42. MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{45}H_{42}D_{14}NaO_{12}$ 825.45, found 825.50. HRMS ESI-TOF: $[M+Na]^+$ calcd for $C_{45}H_{42}D_{14}NaO_{12}$ 825.4548, found 825.4565.

4.4.7. Methoxyphenyl 3-O-(benzyl-d_7)-4,6-O-cyclohexylidene-2-O-(4-methoxybenzyl)-α-D-glucopyranosyl-(1→3)-2-O-(benzyl-d_7)-4,6-O-cyclohexylidene-α-D-glucopyranosyl-(1→3)-2-O-(benzyl-d_7)-4,6-O-cyclohexylidene-α-D-mannopyranoside (**35**)

This compound was synthesized from **6** and **4** according to the procedure described in Section 4.4. (81%, α/β=18.8:1 in $CHCl_3/Et_2O$; 84%, α/β=18.5:1 in $CHCl_3$/CPME). $[\alpha]_D^{29}$ 90.8 (*c* 1.0, $CHCl_3$). ^{1}H NMR (C_6D_6, 400 MHz): δ 1.22−2.05 (m, cyclohexyl, 30H), 3.30 (s, MeO, 3H), 3.32 (s, MeO, 3H), 3.66−3.78 (m, H-2^{Glc1}, H-6^{Glc1}, H-2^{Glc2}, H-6^{Glc2}, H-6^{Man}, 6H), 3.79 (t, *J*=10.0 Hz, H-4^{Glc1}, 1H), 3.94 (t, *J*=9.6 Hz, H-4^{Glc2}, 1H), 3.95−4.03 (m, H-5^{Glc1}, H-6^{Glc1}, H-6^{Glc2}, H-2^{Man}, 3H), 4.05−4.13 (m, H-5^{Glc2}, H-5^{Man}, 2H), 4.31 (t, *J*=9.2 Hz, H-3^{Glc1}, 1H), 4.65 (t, *J*=9.2 Hz, H-3^{Glc2}, 1H), 4.68−4.75 (m, H-3^{Man}, H-4^{Man}, 2H), 4.84 (d, *J*=11.6 Hz, PMB, 1H), 4.88 (d, *J*=11.6 Hz, PMB, 1H), 5.54 (d, *J*=1.2 Hz, H-1^{Man}, 1H), 5.90 (d, *J*=3.6 Hz, H-1^{Glc1}/H-1^{Glc2}, 1H), 5.92 (d, *J*=3.6 Hz, H-1^{Glc1}/H-1^{Glc2}, 1H), 6.66−7.42 (m, Ar, 8H); ^{13}C NMR (C_6D_6, 100 MHz): δ 22.57 (×3), 22.90, 22.99, 23.13, 25.57, 25.78 (×2), 27.66 (×2), 27.89, 32.26, 32.45 (×2), 54.49, 54.83, 61.28, 61.67, 63.55, 63.97, 66.29, 71.42, 72.47, 72.85 (×2), 74.93 (×2), 76.86, 78.51, 78.64, 79.52, 97.29, 97.58, 98.48, 99.19, 99.62, 99.87, 113.75, 114.68, 118.00, 150.09, 155.41, 159.41 (+Bn-d_7×3). ESI-TOF MS: $[M+Na]^+$ calcd for $C_{72}H_{67}D_{21}O_{18}Na$ 1284.72, found 1284.74. HRMS ESI-TOF: $[M+Na]^+$ calcd for $C_{72}H_{67}D_{21}O_{18}Na$ 1284.7187, found 1284.7138.

4.4.8. Methoxyphenyl 3-O-(benzyl-d_7)-4,6-O-cyclohexylidene-α-D-glucopyranosyl-(1→3)-2-O-(benzyl-d_7)-4,6-O-cyclohexylidene-α-D-glucopyranosyl-(1→3)-2-O-(benzyl-d_7)-4,6-O-cyclohexylidene-α-D-mannopyranoside (**7**)

This compound was synthesized from **35** according to the procedure described for the synthesis of **6** (86%). $[\alpha]_D^{29}$ 130.9 (*c* 0.77, $CHCl_3$). ^{1}H NMR (C_6D_6, 400 MHz): δ 0.90−2.00 (m, cyclohexyl×3, 30H), 2.71 (d, *J*=8.4 Hz, OH, 1H), 3.30 (s, OMe, 3H), 3.54 (dd, *J*=9.2, 3.6 Hz, H-2^{Glc1}, 1H), 3.62 (t, *J*=9.6 Hz, H-4^{Glc1}, 1H), 3.65−3.80 (m, H-4^{Glc2}, H-6^{Glc1}, H-6^{Glc2}, H-6^{Man}, 5H), 3.85−4.00 (m, H-2^{Glc2}, H-6^{Glc1}, H-6^{Glc2}, H-2^{Man}, 4H), 4.02−4.16 (m, H-5^{Glc1}, H-5^{Glc2}, H-5^{Man1}, 3H), 4.42−4.52 (m, H-3^{Glc1}, H-3^{Glc2}, 2H), 4.62−4.65 (m, H-3^{Man}, H-4^{Man}, 4H), 3.92 (dd, *J*=2.8, 1.6 Hz, ManH-2, 1H), 4.05−4.14 (m, Glc^{1}H-3, Glc^{2}H-3, 1H), 5.54−5.57 (m, H-1^{Glc2}, H-1^{Man}, 2H), 5.86 (d, *J*=3.6 Hz, H-1^{Glc1}, 1H), 6.68−6.92 (m, Ar, 4H); ^{13}C NMR (C_6D_6, 100 MHz): δ 22.38 (×2), 22.54, 22.80, 22.89, 23.12, 25.57 (×2), 25.76, 27.67, 27.77, 38.09, 38.37, 38.47, 54.83, 61.26, 61.70, 61.91 (×2), 64.00, 64.25, 66.27, 71.25, 73.29, 73.69, 73.86, 74.11 (×2), 74.04, 77.13, 78.66, 79.91, 97.54, 98.27, 99.28, 99.86, 100.07, 100.61, 114.71, 117.99, 137.99, 150.07, 155.46. ESI-TOF MS: $[M+Na]^+$ calcd for $C_{64}H_{59}D_{21}O_{17}Na$ 1164.66, found 1164.66. HRMS ESI-TOF: $[M+Na]^+$ calcd for $C_{64}H_{59}D_{21}O_{17}Na$ 1164.6611, found 1164.6618.

4.4.9. Methoxyphenyl 2,3-di-O-(benzyl-d_7)-4,6-cyclohexylidene-α-D-glucopyranosyl-(1→2)-3-O-(benzyl-d_7)-4,6-O-cyclohexylidene-α-D-glucopyranosyl-(1→3)-2-O-(benzyl-d_7)-4,6-O-cyclohexylidene-α-D-glucopyranosyl-(1→3)-2-O-(benzyl-d_7)-4,6-O-cyclohexylidene-α-D-mannopyranoside (**8**)

This compound was synthesized from **7** and **5D** according to the procedure described in Section 4.4. (83%, α/β=21.6:1 in $CHCl_3/Et_2O$; 86%, α/β=19.9:1 in $CHCl_3$/CPME). $[\alpha]_D^{26}$ 136.1 (*c* 0.87, $CHCl_3$). ^{1}H NMR (C_6D_6, 400 MHz): δ 0.90−2.15 (m, cyclohexyl×4, 40H), 3.29 (s, OMe, 3H), 3.52−3.73 (m, H-6^{Glc1}, H-2^{Glc2}, H-6^{Glc2}, H-6^{Glc3}, H-6^{Man}, 5H), 3.82 (t, *J*=10.4 Hz, H-4^{Glc1}, 1H), 3.88 (dd, *J*=9.6, 3.6 Hz, H-2^{Glc3}, 1H), 3.90−4.00 (m, H-6^{Glc1}, H-4^{Glc2}, H-6^{Glc2}, H-4^{Glc3}, H-6^{Glc3}, H-2^{Man}, H-6^{Man}, 7H), 4.02−4.13 (m, H-5^{Glc2}, H-5^{Man}, 2H), 4.16 (dd, *J*=9.2, 4.0 Hz, H-2^{Glc1}, 1H), 4.27 (t, *J*=9.2 Hz, H-3^{Glc1}, 1H), 4.42 (t, *J*=9.2 Hz, H-3^{Glc3}, 1H), 4.45 (td, *J*=10.4, 5.2 Hz, H-5^{Glc3}, 1H), 4.59 (td, *J*=10.4, 5.6 Hz, H-5^{Glc1}, 1H), 4.66−4.75 (m, H-3^{Glc2}, H-3^{Man}, H-4^{Man}, 3H), 5.54 (d, *J*=1.6 Hz, H-1^{Man}, 1H), 5.68 (d, *J*=4.0 Hz, H-1^{Glc3}, 1H), 5.85 (d, *J*=3.6 Hz, H-1^{Glc2}, 1H), 6.20 (d, *J*=4.0 Hz, H-1^{Glc1}, 1H), 6.66−6.90 (m, Ar, 4H); ^{13}C NMR (C_6D_6, 100 MHz): δ 22.59, 22.82 (×2), 22.94, 23.10, 23.20, 23.32, 23.34, 25.87, 25.95 (×3), 27.89, 28.06 (×2), 28.51, 38.42 (×3), 38.67, 55.08, 61.56, 61.71, 62.12, 62.17, 63.52, 64.04, 64.23, 66.56, 71.61, 71.90, 73.14, 74.62, 74.94, 75.33, 75.39, 77.43, 77.47, 78.60, 78.80, 79.85, 95.08, 95.45, 97.79, 98.62, 99.46, 99.66, 99.75, 100.12,

114.93, 118.23, 125.50–129.50 (overlapped on C_6D_6), 138.31, 138.47, 139.48, 150.34, 155.67. ESI-TOF MS: $[M+Na]^+$ calcd for $C_{90}H_{75}D_{35}O_{22}Na$ 1600.96, found 1600.94. HRMS ESI-TOF: $[M+Na]^+$ calcd for $C_{90}H_{75}D_{35}O_{22}Na$ 1600.9583, found 1600.9610.

4.4.10. Preparation of *8* from *2D*

Step-1: after azeotropic removal with toluene, to the mixture of acceptor **2D** (50.0 mg, 0.108 mmol) and donor **3** (75.5 mg, 0.129 mmol) in dry $CHCl_3/Et_2O$ (1:1) (4.0 mL) were added 4 Å MS (500 mg, freshly dried) and DTBMP (33.3 mg, 0.162 mmol) and the mixture was stirred for 10 min at room temperature. MeOTf (51.3 μL, 0.453 mmol) was added to the mixture. After stirring for 48 h at the same temperature, the reaction was quenched with triethylamine followed by filtration through Celite pad and through washing of the filter cake with ethyl acetate. Combined filtrates were washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated in vacuo.

Step-2: to a solution of the above mixture in CH_2Cl_2/H_2O (10:1, 3.3 mL) was added DDQ (38.0 mg, 0.184 mmol) and stirred at room temperature for 18 h. The reaction was quenched with ascorbic acid/citric acid buffer and extracted with $CHCl_3$. The combined solutions were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was filtrated through Bio beads SX-3 with elution of toluene/ethyl acetate (1:1) to give the mixture of Glc_1Man_1 isomers. The mixture was coevaportated with toluene and used for the next glycosylation without further purification.

Step-3: the above mixture was mixed with donor **4** (75.5 mg, 0.129 mmol) in dry $CHCl_3/Et_2O$ (1:1) (4.0 mL), were added 4 Å MS (500 mg, freshly dried) and DTBMP (33.3 mg, 0.162 mmol), and MeOTf (51.3 μL, 0.453 mmol) was added to the mixture after 10 min. After stirring for 48 h at the same temperature, the reaction was quenched with triethylamine and processed as described in Step-1.

Step-4: to a solution of the above mixture in CH_2Cl_2/H_2O (10:1, 3.3 mL) was added DDQ (38.0 mg, 0.184 μmol) and stirred at room temperature for 36 h. The reaction was processed as described in Step-2. The residue was filtrated through Bio beads SX-3 with elution of toluene/ethyl acetate (1:1) to give the mixture of Glc_2Man_1 isomers. The mixture was used for next glycosylation without further purification.

Step-5: after azeotropic removal with toluene, to the solution of the above mixture and donor **5D** (62.8 mg, 0.129 mmol) in dry $CHCl_3/Et_2O$ (1:1) (4.0 mL) were added 4 Å MS (500 mg, freshly dried) and DTBMP (33.3 mg, 0.162 mmol) and the mixture was stirred for 10 min at room temperature. MeOTf (51.3 μL, 0.453 mmol) was added to the mixture. After stirring for 48 h at the same temperature, the reaction was quenched with triethylamine followed by filtration through Celite pad and washing of pad with ethyl acetate. The combined solutions were washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was filtrated through Bio beads SX-3 with elution of toluene/ethyl acetate (1:1) to give the mixture of the isomers. The resulting residue was purified by PTLC (toluene/ethyl acetate=5:1) to give the title compound as the major isomer (82.3 mg, 47%, **8**/other isomers=9.0:1).

4.4.11. Methoxyphenyl α-D-glucopyranosyl-(1→2)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→3)-α-D-mannopyranoside (*1*)

To a solution of compound **8** (8.7 mg, 5.50 μmol) in $CHCl_3$ (2 mL) was added trifluoroacetic acid (TFA, 0.2 mL) and stirred at room temperature for 2 h. The reaction mixture was concentrated in vacuo followed by azeotropic removal with toluene. Hydrogenolysis of resulting residue was carried out in the presence of $Pd(OH)_2$ (10 mg) in $MeOH/H_2O$ (2:1, 3 mL) for 4 h at room temperature. The mixture was filtered through Celite and filtrate was concentrated in vacuo. The residue was purified by gel filtration (Sephadex LH-20, MeOH/H_2O, 1:1) to give the title compound (4.2 mg, 99%). $[\alpha]_D^{28}$ 76.3 (*c* 0.48, H_2O). ^1H NMR (CD_3OD/D_2O=1:1, 400 MHz): δ 3.43 (t, J=10.0 Hz, H-4^{Glc3}, 1H), 3.43 (t, J=9.6 Hz, H-4^{Glc1}, 1H), 3.58 (dd, J=10.0, 3.6 Hz, H-2^{Glc3}, 1H), 3.58 (dd, J=10.0, 4.0 Hz, H-2^{Glc2}, 1H), 3.63 (dd, J=10.0, 4.0 Hz, H-2^{Glc1}, 1H), 3.64–3.80 (m, H-3^{Glc1}, H-6^{Glc1}, H-3^{Glc2}, H-4^{Glc2}, H-5^{Glc2}, H-6^{Glc2}, H-3^{Glc3}, H-6^{Glc3}, H-5^{Man}, H-6^{Man}, 14H), 3.76 (s, OMe, 3H), 3.88–4.50 (m, H-5^{Glc1}, H-5^{Glc3}, H-3^{Man}, H-4^{Man}, 3H), 4.26 (t, J=1.2 Hz, H-2^{Man}, 1H), 5.09 (d, J=4.0 Hz, H-1^{Glc3}, 1H), 5.23 (d, J=4.0 Hz, H-1^{Glc2}, 1H), 5.36 (d, J=1.2 Hz, H-1^{Man}, 1H), 5.46 (d, J=3.6 Hz, H-1^{Glc1}, 1H), 6.87 (d, J=8.8 Hz, MP, 2H), 7.06 (d, J=8.8 Hz, MP, 2H); ^{13}C NMR (D_2O, 100 MHz): δ 56.46, 60.78, 60.94 (×2), 61.21, 66.45, 67.43, 69.84, 69.89, 70.37, 70.50, 70.96, 71.81, 71.90, 72.06, 72.39, 72.59, 73.44, 74.06, 79.08, 80.40, 96.43, 97.13, 99.64, 101.34, 115.56, 119.23, 149.99, 155.05. HRMS ESI-TOF: $[M+Na]^+$ calcd for $C_{31}H_{48}O_{22}Na$ 795.2535, found 795.2626. HRMS FAB: $[M+Na]^+$ calcd for $C_{31}H_{48}O_{22}Na$ 795.2535, found 795.2507.

Acknowledgements

This work was partly supported by 'Ecomolecular Science' and 'Chemical Biology' programs in RIKEN, and a Grant-in-Aid for Encouragement of Young Scientist from the Ministry of Education, Culture, Sports, Science, and Technology (No. 18710196). We thank Ms. A. Takahashi for her technical assistance.

Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2007.10.087.

References and notes

1. *Essentials in Glyocbiology*; Varki, A., Cummings, R., Esko, J., Freeze, H., Hart, G., Marth, J., Eds.; Cold Spring Harbor Laboratory: New York, NY, 1999.

2. *Complehensive Natural Products Chemistry*; Barton, D., Nakanishi, K., Meth-Cohn, O., Eds.; Elsevier Science: Amsterdam, 1999; Vol. 3.
3. (a) Dwek, R. *Chem. Rev*. **1996**, *96*, 683–720; (b) *Carbohydrates in Chemistry and Biology*; Ernst, B., Hart, G. W., Sinaÿ, P., Eds.; Wiley-VCH: Weinheim, 1999; Vols. 3 and 4; (c) *Glycoscience*; Fraser-Reid, B., Tatsuta, K., Thiem, J., Eds.; Springer: Berlin, 2001; Vols. I–III.
4. Kjellén, L.; Lindahl, U. *Annu. Rev. Biochem*. **1991**, *60*, 443–475.
5. Levery, S. B.; Weiss, J. B.; Salyan, M. E.; Roberts, C. E.; Hakomori, S.; Magnani, J. L.; Strand, M. *J. Biol. Chem*. **1992**, *267*, 5542–5551.
6. Hakomori, S.; Siddiqui, B.; Li, Y.-T.; Su-Chen Li, S.-C.; Hellerqvist, C. G. *J. Biol. Chem*. **1971**, *246*, 2271–2277.
7. (a) Hakomori, S. *Annu. Rev. Biochem*. **1981**, *50*, 733–764; (b) Natori, T.; Koezuka, Y.; Higa, T. *Tetrahedron Lett*. **1993**, *34*, 5591–5592; (c) Natori, T.; Morita, M.; Akimoto, K.; Koezuka, Y. *Tetrahedron* **1994**, *50*, 2771–2784.
8. (a) Painter, T. J.; Watkins, W. M.; Morgan, W. T. J. *Nature* **1965**, *206*, 594–597; (b) Lloyd, K. O.; Kabat, E. A.; Layug, E. J.; Gruezo, F. *Biochemistry* **1966**, *5*, 1489–1501.
9. (a) Szarek, W. A.; Horton, D. *Anomeric Effect*; American Chemical Society: Washington, DC, 1979; (b) Deslongchamps, P. *Stereoelectronic Effect in Organic Chemistry*; Pergamon: Oxford, 1983; (c) Juaristi, E.; Cuevas, G. *The Anomeric Effect*; CRC: Boca Raton, 1995; (d) Kirby, A. J. *Stereoelectronic Effect*; Oxford University Press: Oxford, 1996; (e) Perrin, C. L. *Tetrahedron* **1995**, *51*, 11901–11935; (f) Randell, K. D.; Johnston, B. D.; Green, D. F.; Pinto, B. M. *J. Org. Chem*. **2000**, *65*, 220–226; (g) Vaino, A. R.; Szarek, W. A. *J. Org. Chem*. **2001**, *66*, 1097–1102; (h) Perrin, C. L.; Kuperman, J. *J. Am. Chem. Soc*. **2003**, *125*, 8846–8851.
10. For recent review for the synthesis of 1,2-*cis*-glycoside, see: Demchenko, A. V. *Synlett* **2003**, 1225–1240.
11. (a) *Carbohydrates in Chemistry and Biology*; Ernst, B., Hart, G. W., Sinaÿ, P., Eds.; Wiley-VCH: Weinheim, 1999; Vols. 1 and 2; (b) See Ref. 3c.
12. Ishiwata, A.; Ito, Y. *Tetrahedron Lett*. **2005**, *46*, 3521–3524.
13. For the synthesis of Glc_3Man_1, see: (a) Ogawa, T.; Nukada, T.; Kitajima, T. *Carbohydr. Res*. **1983**, *123*, C12–C15; (b) Ennis, S. C.; Cumpstey, I.; Fairbanks, A. J.; Butters, T. D.; Mackeen, M.; Wormald, M. R. *Tetrahedron* **2002**, *58*, 9403–9411.
14. For the synthesis of Glc_3Man_1 by intramolecular aglycon derivery, see: (a) Fairbanks, A. J. *Synlett* **2003**, 1945–1958; (b) Attolino, E.; Cumpstey, I.; Fairbanks, A. J. *Carbohydr. Res*. **2006**, *341*, 1609–1618.
15. For the synthesis of glucosylated high mennose type glycan, see: (a) Matsuo, I.; Wada, M.; Manabe, S.; Yamaguchi, Y.; Otake, K.; Kato, K.; Ito, Y. *J. Am. Chem. Soc*. **2003**, *125*, 3402–3403; (b) Matsuo, I.; Kashiwagi, T.; Totani, K.; Ito, Y. *Tetrahedron Lett*. **2005**, *46*, 4197–4200; (c) Matsuo, I.; Totani, K.; Tatami, A.; Ito, Y. *Tetrahedron* **2006**, *62*, 8262–8277.
16. Breuer, W.; Bause, E. *Eur J. Biochem*. **1995**, *228*, 689–696.
17. Meddour, A.; Courtieu, J. *Tetrahedron: Asymmetry* **2000**, *11*, 3635–3644.
18. (a) Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*, 3rd ed.; Wiley: New York, NY, 1999; (b) Kocienski, P. *Protecting Groups*; Thieme: Stuttgart, 2003.
19. (a) Roy, R.; Andersson, O.; Letellier, M. *Tetrahedron Lett*. **1992**, *33*, 6053–6056; (b) Zhang, Z.; Ollmann, I. R.; Ye, X.-S.; Wischnat, R. F.; Baasov, T.; Wong, C.-H. *J. Am. Chem. Soc*. **1999**, *121*, 734–753.
20. Details for the synthetic procedure and data for glycosyl donors and acceptors are in Supplementary data.
21. *Solvents and Solvent Effects in Organic Chemistry*, 3rd ed.; Reichardt, C., Ed.; Wiley-VCH: Weinheim, 2003.
22. (a) Wulff, G.; Rohle, G. *Angew. Chem., Int. Ed. Engl*. **1974**, *13*, 157–170; (b) Hashimoto, S.; Hayashi, M.; Noyori, R. *Tetrahedron Lett*. **1984**, *25*, 1379–1382; (c) Fukase, K.; Hasuoka, A.; Kinoshita, I.; Aoki, Y.; Kusumoto, S. *Tetrahedron* **1995**, *51*, 4923–4932; (d) Fukase, K.; Kinoshita, I.; Kanoh, T.; Nakai, Y.; Hasuoka, A.; Kusumoto, S. *Tetrahedron* **1996**, *52*, 3897–3904; (e) Uchiro, H.; Mukaiyama, T. *Chem. Lett*. **1996**, 271–272; (f) Demchenko, A.; Stauch, T.; Boons, G. J. *Synlett* **1997**, 818–820; (g) Fukase, K.; Nakai, Y.; Kanoh, T.; Kusumoto, S. *Synlett* **1998**, 84–86; (h) Manabe, S.; Ito, Y.; Ogawa, T. *Synlett* **1998**, 628–630; (i) Fraser-reid, B.; Udodong, U. E.; Wu, Z. F.; Ottosson, H.; Merritt, J. R.; Rao, C. S.; Roberts, C.; Madsen, R. *Synlett* **2003**, 1225–1240.
23. Tokimoto, H.; Fujimoto, Y.; Fukase, K.; Kusumoto, S. *Tetrahedron: Asymmetry* **2005**, *16*, 441–447.
24. The reaction of glycosyl donor with cyclic ether, reported by Dabideen and Gervay-Hague, gave also the β-adduct. See: (a) Dabideen, D. R.; Gervay-Hague, J. *Org. Lett*. **2004**, *6*, 973–975; (b) El-Badri, M. H.; Willenbring, D.; Tantillo, D. W.; Gervay-Hague, J. *J. Org. Chem*. **2007**, *72*, 4663–4672.
25. In the presence of thioether, see: Park, J.; Kawatkar, S.; Kim, J.-H.; Boons, G.-J. *Org. Lett*. **2007**, *9*, 1959–1962.
26. Oikawa, O.; Yoshioka, T.; Yonemitsu, O. *Tetrahedron Lett*. **1982**, *23*, 885–888.
27. For a conformational study of Glc_3Man_1 derivative, see: Alvarado, E.; Nukada, T.; Ogawa, T.; Ballou, C. E. *Biochemistry* **1991**, *30*, 881–886.