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Chemoenzymatic synthesis of the branched oligosaccharides which correspond to the core structures of N-linked sugar chains

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Abstract

Synthetic routes are described to a partial structure common to all high mannose-type sugar chains and complex-type sugar chains based on a chemoenzymatic strategy which incorporates, (a) enzymatic synthesis of oligosaccharide blocks using glycosidases, and (b) chemical synthesis of the branching oligosaccharides via regioselective coupling. All reaction products correspond to key intermediates necessary for the construction of N-linked oligosaccharides and we have synthesized the branched tetra-*manno*-oligosaccharide high mannose-type sugar chain and the branched hexa-oligosaccharide complex-type sugar chain using this simple and direct method. © 1998 Elsevier Science Ltd.

Keywords: *N*-linked oligosaccharides; Chemoenzymatic synthesis; Regioselective glycosylation; Glycosidases

1. Introduction

N-linked sugar chains have been the subject of special attention due to their role in cell surface recognition phenomena, which are of critical importance to multicellular organisms [1]. These oligosaccharides are generally divided into three categories: high mannose-type, complex-type, and hybrid-type sugar chains (Fig. 1) [2]. We are trying to develop a versatile method for the synthesis of N-linked oligosaccharides requiring as few synthetic steps as possible. For that purpose, we are employing two key synthetic strategies: (a) enzymatic synthesis of oligosaccharide blocks using glycosidases, and (b) chemical synthesis of the branching oligosaccharides via regioselective coupling. Enzymatic oligosaccharide synthesis can decrease the number of synthetic steps by eliminating the needed for protection and deprotection steps [3–6]. For the enzymatic synthesis of oligosaccharides, two different classes of reactions are known: glycosidases and glycosyltransferases. In this study, we used glycosidases, since many are commercially available and relatively inexpensive. Glycosidases can catalyze two types of reactions: reverse hydrolysis and transglycosylations. The reverse hydrolysis reaction is an equilibrium controlled

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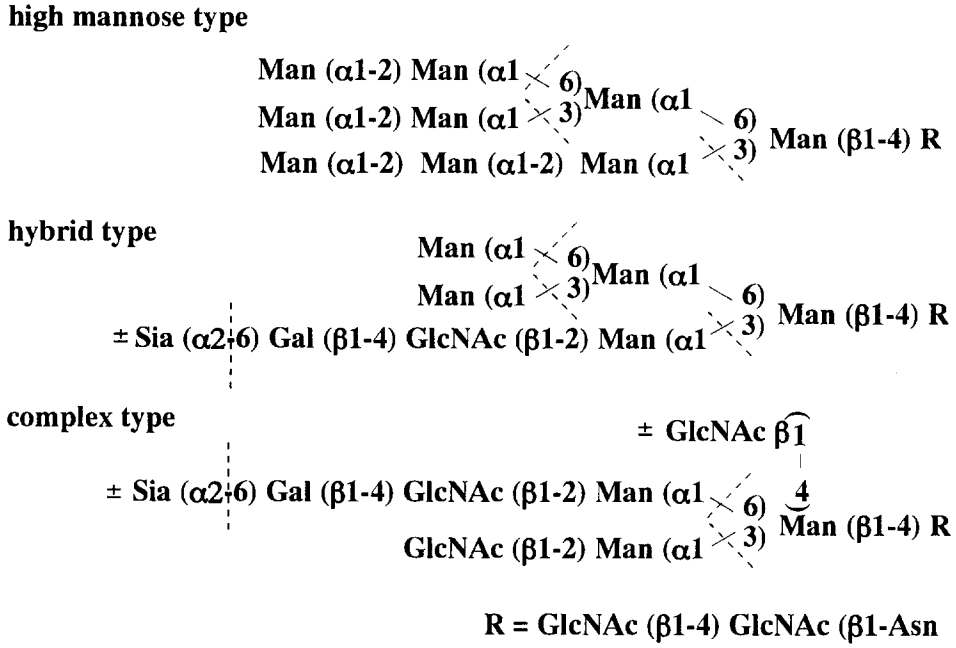


Fig. 1. Three subgroups of N-linked sugar chains.

reaction, in which a disaccharide is formed as a result of an equilibrium between monosaccharide(s) and the disaccharide. The transglycosylation reaction generally proceeds with greater regioselectivity, assuming the enzyme has been properly selected (Scheme 1).

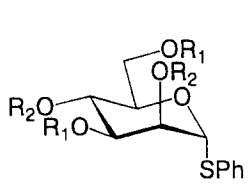
Recently, we have demonstrated the preparation of an important component of N-linked sugar chains by reverse hydrolysis and transglycosylation using glycosidases [7–11]. By combining these two types of glycosidase reaction, most of the necessary oligosaccharides were prepared. However, the construction of branched oligosaccharides observed in N-linked sugar chains cannot be done solely by glycosidase assisted reactions. Therefore, we also use traditional chemical methods [12] for the construction of the branching segments. Although chemical synthesis of branched sugar chains has been reported by many researchers [13–15], the process is complicated by the numerous protection and deprotection steps required. In contrast, regioselective glycosylation of a partially protected acceptor can eliminate many of the protection and deprotection steps [16]. For this reason, we believe that an approach employing both the enzymatic preparation of oligosaccharides and regioselective glycosylation should be extremely useful and efficient in the construction of branched oligosaccharides.

In the present study, we examined regioselective glycosylation using enzymatically synthesized donors and a 3,6- unprotected mannose acceptor in order to develop an efficient synthetic route to the branched oligosaccharides of N-linked glycopeptides.

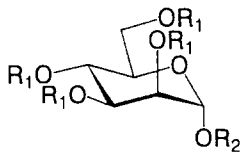
2. Results and discussion

Our target structures are shown in Fig. 1. Disconnection of the indicated bonds leads to various oligosaccharide blocks and a C-3, C-6 branched mannose. Oligosaccharide blocks such as Man(α1-2)Man, Man(α1-2)Man(α1-2)Man, GlcNAc(β1-2)Man, and Gal(β1-4)GlcNAc(β1-2)Man can be prepared enzymatically. For construction of the branched oligosaccharides, we prepared a partially protected mannose derivative **4** in which the C-3 and C-6 positions are free. Generally, the primary hydroxyl group at C-6 is more reactive than the secondary hydroxyl group at C-3. Taking this difference in reactivity into account, oligosaccharide block donors should react with C-6 regioselectively, after which the remaining hydroxyl group at C-3 can be used for the next glycosylation reaction to provide a branched oligosaccharide.

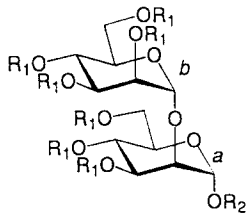
In order to synthesize the mannose derivative **4**, we first tried a selective allylation of thiophenyl mannoside **1** using the stannylation method [17], but this reaction resulted in a complicated mixture. Regioselective pivaloylation of compound **1** using pivaloyl chloride and pyridine gave the 3,6-di-*O*-pivaloylated compound **2** in 90% yield as a crystal. Subse-



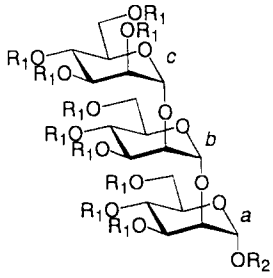
	R_1	R_2
1	H	H
2	Piv	H
3	Piv	Bn
4	H	Bn



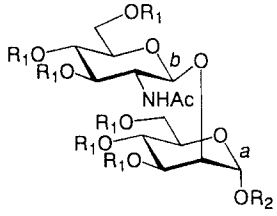
	R_1	R_2
5	H	H
6	Ac	Ac
7	Ac	H
8	Ac	$CNHCCl_3$



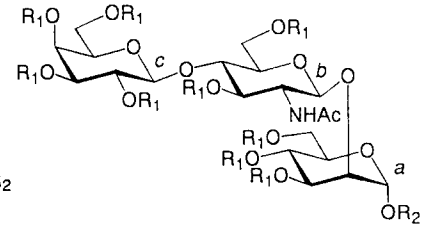
	R_1	R_2
9	H	H
10	Ac	Ac
11	Ac	H
12	Ac	$CNHCCl_3$



	R_1	R_2
13	H	H
14	Ac	Ac
15	Ac	H
16	Ac	$CNHCCl_3$



	R_1	R_2
17	H	H
18	Ac	Ac
19	Ac	H
20	Ac	$CNHCCl_3$

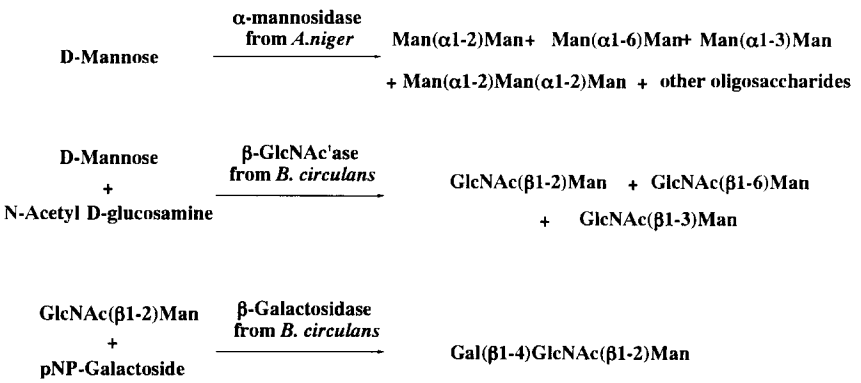


	R_1	R_2
21	H	H
22	Ac	Ac
23	Ac	H
24	Ac	$CNHCCl_3$

Scheme 1.

quent benzylation of **2** gave the 2,4-di-*O*-benzylated compound **3**, along with other side products which arose via migration of the pivaloyl groups under the basic conditions. Without further purification, this mixture was treated with lithium aluminum hydride in ether at 0 °C. Removal of the pivaloyl groups in compound **3** had not been possible using strong bases such as NaOMe, *t*-BuOK or $NaBH_4$. The reaction mixture was purified by silica gel column chromatography to obtain compound **4** in 43% yield (2 steps from **2**) (Scheme 1).

The oligosaccharide blocks were prepared enzymatically as reported previously (Scheme 2). The reverse hydrolysis of mannose in the presence of

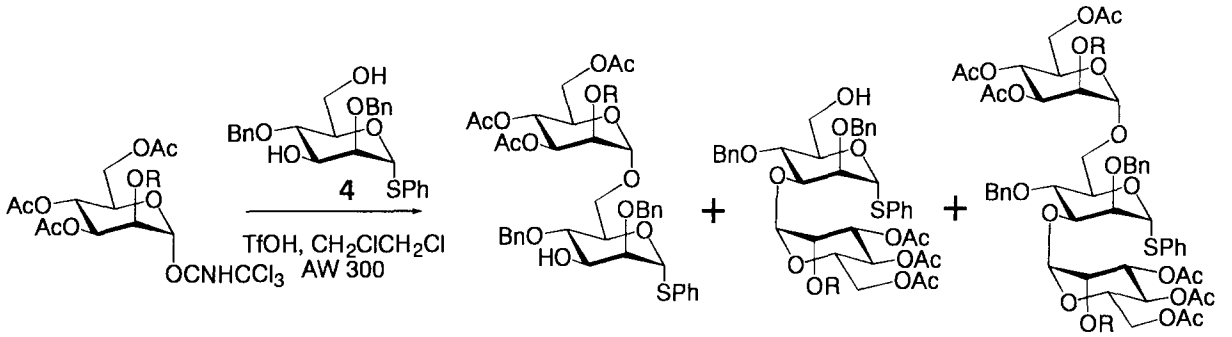


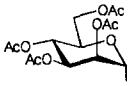
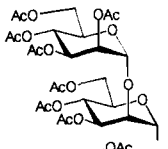
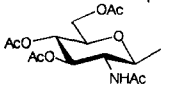
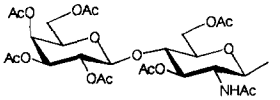
Scheme 2.

α-mannosidase from *Aspergillus niger* afforded mannooligosaccharides such as Man(α1-2)Man (**9**), Man(α1-3)Man, Man(α1-6)Man, Man(α1-2)Man(α1-2)Man (**13**) and as well as small amounts of other oligosaccharides [7]. Mannooligosaccharides **9** and **13** were isolated by successive column chromatography using an activated carbon column and an amino silica gel column in 290 mg and 14 mg from 10 g of mannose, respectively. For the synthesis of complex type oligosaccharides, GlcNAc(β1-2)Man (**17**) was also synthesized by the reverse hydrolysis of mannose and *N*-acetylglucosamine in the presence of β-*N*-acetyl glucosaminidase from *Bacillus circulans* together with GlcNAc(β1-3)Man and GlcNAc(β1-6)Man [8]. The disaccharide **17** was isolated by activated carbon column chromatography in 0.2% yield. Although the yield was low in these reverse hydrolysis reactions, the starting materials are inexpensive, (Man and GlcNAc), and were easily recovered during the activated carbon column chromatography separation. Therefore, the low yield is not a significant problem. Moreover, not only Man(α1-2)Man and GlcNAc(β1-2)Man, but also Man(α1-3)Man, Man(α1-6)Man, GlcNAc(β1-3)Man, and GlcNAc(β1-6)Man are also useful for the synthesis of high mannose-type or complex-type sugar chains.

Galactose was then regioselectively coupled to the GlcNAc residue of **17** via a β1-4 linkage by transglycosylation using β-galactosidase from *B. circulans* to give **21** in 10% yield. Usually, β-galactosidase from *B. circulans* is known to produce a small amount of the β1-6 linked disaccharide along with the β1-4 linked isomer [18], however, when a disaccharide was used as an acceptor, the β1-4 linked trisaccharide was obtained selectively.

These oligosaccharide blocks were then converted into the corresponding glycosyl donors. For the activation of the anomeric position, the glycosyl imidate [19,20] was chosen since our preliminary experiments showed that glycoside bonds of the desired α-configuration could be obtained stereoselectively when the glycosyl imidate was used as the donor. Synthesis of the mannobiose imidate **12** was performed in three



Donor	Products	C-6 glycosylated compound	C-3 glycosylated compound	C-3,6 diglycosylated compound
8	R = Ac	25	26	27
12	R = 	28	29	30
16	R = 	31	32	33
20	R = 	34	35	36
24	R = 	37		

Scheme 3.

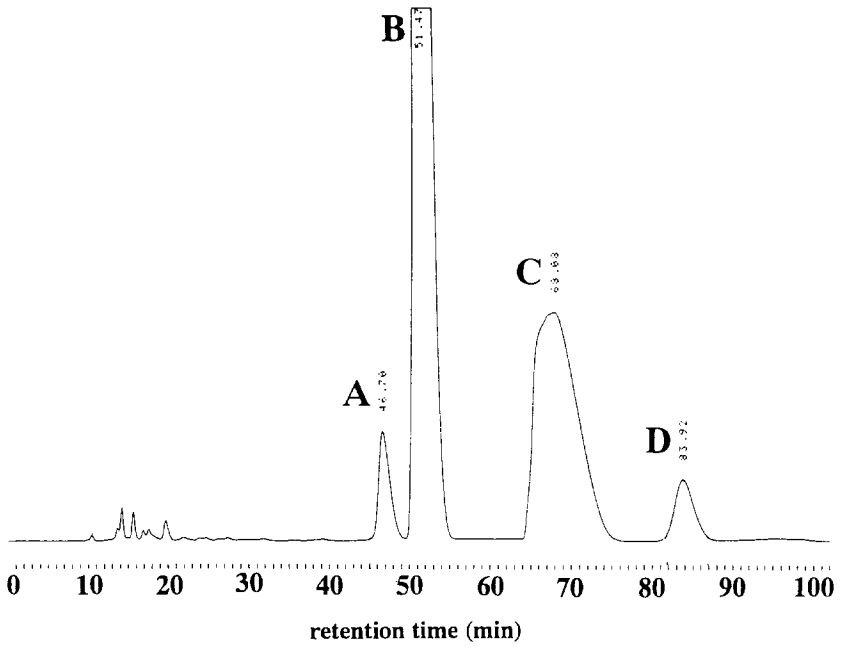


Fig. 2. HPLC of the synthetic oligosaccharides. Column: JAIGEL-ODS; eluent: acetonitrile–water, (3:2); flow rate, 5 mL/min; detection, UV monitor (254 nm); (A) **35**, (B) **4** and **36**, (C) **34**, (D) unknown compound (not sugar derivative).

steps: treatment of **9** with Ac_2O in pyridine to obtain the acetate **10**, selective deacetylation of the anomeric position using ammonium carbonate to provide **11**, and subsequent treatment with trichloroacetonitrile and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to give the mannobiose imidate **12** in 60% overall yield (3 steps). Over these three steps, only one silica gel chromatography separation was necessary. The other imidate derivatives **8**, **16**, **20**, and **24** were synthesized using a similar sequence of reactions resulting in 54%, 53%, 58%, and 52% yields, respectively.

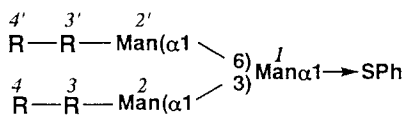
The coupling of various donors to the mannosyl acceptor was studied in order to determine the degree of regioselectivity (Scheme 3). All reactions were performed under identical conditions: a mixture of donor and acceptor was treated with a catalytic amount of trifluoromethanesulfonic acid (TfOH) in dichloroethane at −20 °C. The reaction products were isolated using HPLC attached with reverse phase column by eluting with acetonitrile–water. A representative HPLC chart is demonstrated in Fig. 2. The isolated yield was calculated based on the glycosyl donor and is summarized in Table 1. The structures of all products were confirmed by ^{1}H, ^{13}C and 2D NMR spectroscopy. A representative C–H correlation spectrum is given in Fig. 3. The chemical shifts

Table 1
Summary of the regioselectivity in the glycosylation reaction using glycosyl acceptor **4** and several donors

Donor	Ratio (donor:acceptor)	Products			Isolated yield		
		α1-6[a]	α1-3[a]	α1-3/α1-6[a]	α1-6[a]	α1-3[a]	α1-3/α1-6[a]
8	1:3	**25**	**26**	**27**	64%	7%	1%
12	1:3	**28**	**29**	**30**	49%	16%	3%
12	2.6:1	–	–	**30**	–	–	45%
16	1:3	**31**	**32**	**33**	54%	23%	9%
20	1:3	**34**	**35**	**36**	75%	10%	6%
20	2.1:1	–	–	**36**	–	–	63%
24	1:3	**37**	–	–	52%	–	–

[a]α1-6, α1-3, and α1-3/α1-6 mean the C-6 glycosylated compound, the C-3 glycosylated compound, and the C-6, C-3 diglycosylated compound, respectively.

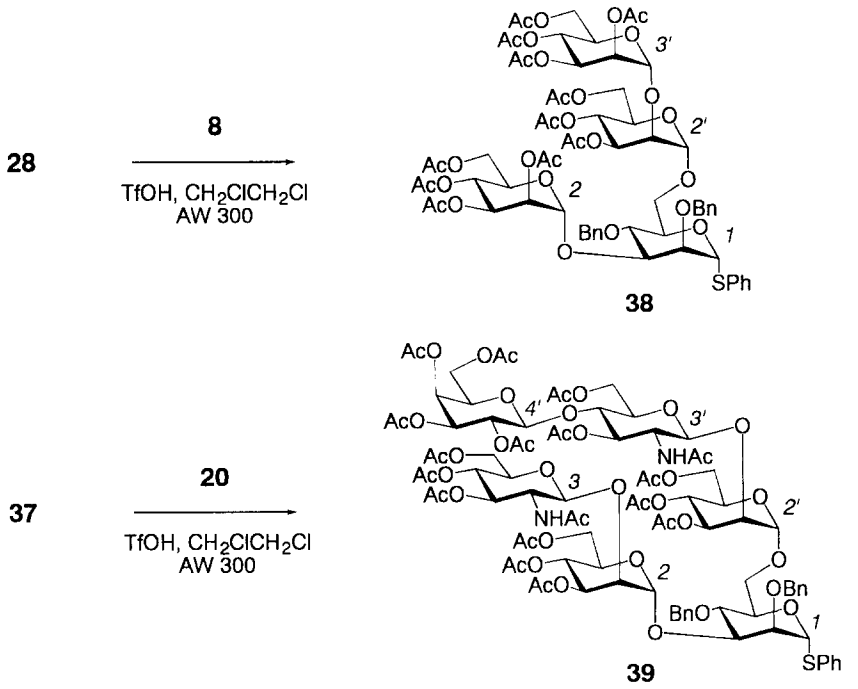
Table 2
^{13}C NMR data of the coupling products



and $^{1}J_{C,H}$ are summarized in Table 2. Even in the absence of neighboring group participation, the newly formed mannosidic linkages were found to be α-configuration from the $^{1}J_{C,H}$ values. As expected, each donor reacted preferentially with the primary hydroxyl group at C-6 position to give the 6-monoglycosylated compound (**25**, **28**, **31**, **34**, and **37**). However, the 3-monoglycosylated compound (**26**, **29**, **32**, and **35**) was also formed. Moreover, in spite of the use of a three-fold excess of acceptor, a considerable amount of the 3,6-di-*O*-glycosylated product (**27**, **30**, **33**, and **36**) was also obtained. From these results, it can be seen that the difference in reactivity between the primary and the secondary hydroxyl groups is small and that the reactivity of the primary hydroxyl group is slightly higher than the secondary group in this mannose derivative. The C-3 glycosylated product and the 3,6-di-*O*-glycosylated product is also useful for the synthesis of heterogenously branched oligosaccharide blocks or homogeneously branched oligosaccharide blocks. The pentasaccharide derivatives (**30** and **36**) were obtained as the main products when the amount of donor was increased.

The monoglycosylated compounds can be used as the glycosyl acceptors for the subsequent glycosylation reactions to synthesize the heterogeneously branched oligosaccharide blocks. Partial syntheses of some high mannose-type and complex-type sugar chains were performed using the aforementioned coupling products (Scheme 4). The coupling of compound **28** and mannose donor **8** proceeded smoothly to give the tetrasaccharide **38** in 67% yield which corresponds to a component of high mannose-type sugar chains. Similarly, oligosaccharide **39**, a component of a complex-type sugar chains, was also prepared by coupling compound **37** with glycosyl donor **20** in 56% yield. These structures were confirmed by ^{1}H, ^{13}C and 2D NMR spectroscopy.

In summary, we have successfully coupled enzymatically prepared di- and trisaccharide donors with a partially protected mannose acceptor **4** to prepare oligosaccharides which correspond to key intermediates necessary for the construction of N-linked oligosaccharides. The branched oligosaccharides **38** and **39** were synthesized using this simple and direct method. By employing synthetic strategy, many synthetic steps can be eliminated and a large variety of branched oligosaccharides can be synthesized on a preparative scale.



Scheme 4.

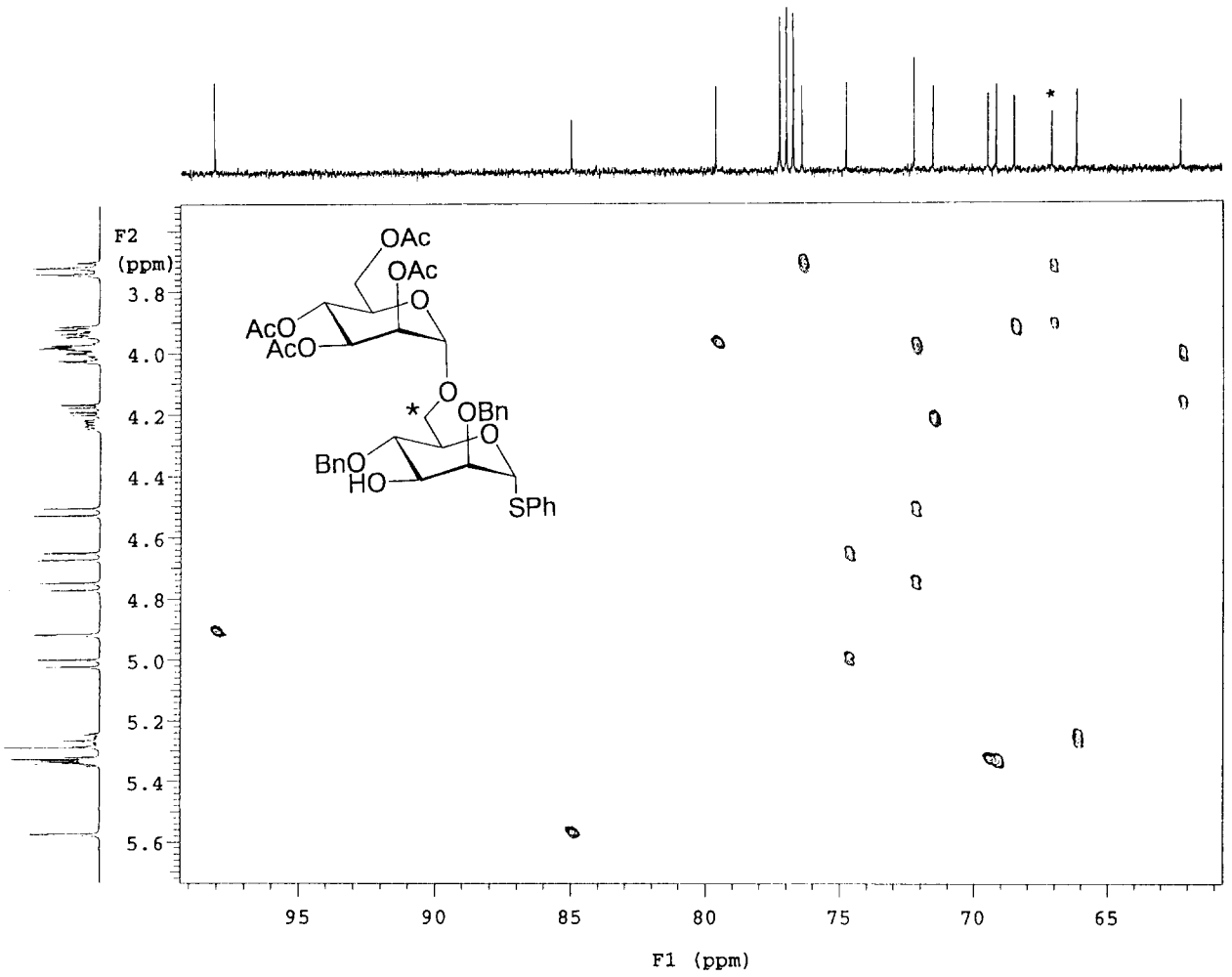


Fig. 3. GHSQC spectrum of compound **25**. * denotes glycosylated position (C-6).

3. Experimental

General methods.—Optical rotations were measured at 25 °C with HORIBA polarimeter SEPA-300. ^{1}H and ^{13}C NMR spectra were measured on a UNITY 500 spectrometer in $CDCl_3$ and were referenced to Me_4Si. Silica gel column chromatography was performed using BW300 (Fuji Silisia Aichi, Japan). Analytical TLC was performed on aluminum plates coated with Silica Gel $60F_{254}$ (Merck). Gel for size exclusion chromatography (Bio-Beads) was a product of Bio-Rad. Reverse phase column (JAIGEL-ODS) is a product of Japan Analytical Industry (Tokyo, Japan). Acid washed molecular sieves (AW300) was purchased from Aldrich and activated at 180 °C under vacuum immediately prior to use. All glycosylation reactions were performed in anhydrous solvents under an atmosphere of dry argon.

Phenyl 2,4-di-O-benzyl-1-thio-α-D-mannopyranoside (**4**).—To a stirred solution of **1** (27.8 g, 0.10 mol) in pyridine (95 mL) was added pivaloyl chloride (27.6 mL, 0.23 mol). The mixture was stirred at 0 °C for 1.5 h, then added ethanol. The solvent was evaporated in vacuo. The residue was diluted with $CHCl_3$ and washed with aqueous copper(II) sulfate, brine, saturated aqueous $NaHCO_3$, brine, dried ($MgSO_4$) and evaporated in vacuo. The residue was washed with hexane to afford 40.8 g (0.09 mol) of **2**. A mixture of **2** (30.1 g, 0.07 mol) and benzyl bromide (32.5 mL, 0.27 mol) in Me_2NCHO (200 mL) was stirred at 0 °C. Then 50% NaH (6.6 g, 0.15 mol) was added portionwise to above mixture. The mixture was stirred for 2 h, quenched with MeOH, diluted with EtOAc, washed with brine, dried ($MgSO_4$) and evaporated in vacuo. The residue was passed by short silica gel column chromatography (hexane:EtOAc, 15:1) to afford 28.8 g of benzylated compounds. These mixture was treated with lithium aluminum hydride (3.66 g) in dry Et_2O at 0 °C. The mixture was stirred at 0 °C for 2 h, diluted with EtOAc,

washed with 1 N HCl, brine, saturated aqueous $NaHCO_3$, brine, dried ($MgSO_4$) and evaporated in vacuo. The residue was purified by silica gel column chromatography (hexane:EtOAc, 15:1). The eluent was crystallized from isopropylether to afford **4** (13.2 g, 0.03 mol, overall yield is 39% in three steps from **1**); mp 91.0–92.0 °C; R_f 0.3 (toluene:EtOAc, 3:1); $[\alpha]_D = +126°$ (c 1.00, $CHCl_3$); ^{1}H NMR; (500 MHz, $CDCl_3$) δ 7.45–7.24 (m, 15 H, arom.), 5.57 (s, 1 H, H-1), 4.92–4.56, (4 H, CH_2Ph) 4.12 (m, 1 H, H-5); ^{13}C NMR; (125 MHz, $CDCl_3$) δ 85.26 (C-1), 79.79 (C-2), 76.57 (C-4), 72.58 (C-5), 72.23 (C-3), 62.20 (C-6). Anal. Calcd. for $C_{26}H_{28}O_5S$: C, 69.00; H, 6.24; S, 7.08. Found: C, 68.82; H, 6.02; S, 6.89.

2, 3, 4, 6 - Tetra - O - acetyl - α - D - mannopyranosyl trichloroacetimidate (**8**).—To a stirred solution of mannose **5** (23.0 g, 0.13 mol) in pyridine (100 mL) was added acetic anhydride (50 mL). The mixture was stirred at room temperature for 12 h. The reaction mixture was added ethanol. The solvent was evaporated in vacuo. The residue was diluted with EtOAc and washed with aqueous copper(II) sulfate, brine, saturated aqueous $NaHCO_3$, brine, dried ($MgSO_4$) and evaporated in vacuo to give **6**. The acetate **6** was treated with 116 g of ammonium carbonate in Me_2NCHO (100 mL) at room temperature for 1 day. The reaction mixture was diluted with EtOAc and washed with water, brine, dried ($MgSO_4$) and evaporated in vacuo to give the hemiacetal derivative **7**. A mixture of **7** and trichloroacetonitrile (46 mL) in CH_2Cl_2 (40 mL) was added DBU (1.4 mL) at 0 °C. The mixture was stirred for 2 h. The reaction mixture was purified by silica gel column chromatography (hexane:EtOAc, 2:1) to afford the compound **8** (35.1 g, 0.07 mol); R_f 0.52 (toluene:EtOAc, 2:1); $[\alpha]_D = +53°$ (c 1.01, $CHCl_3$); ^{1}H NMR; (500 MHz, $CDCl_3$) δ 8.77 (s, 1 H, NH), 6.22 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-1), 5.40 (m, 1 H, H-2), 4.21 (dd, 1 H, $J_{5,6'}$ 5.0, J_{gem} 12.0 Hz, H-6e); ^{13}C NMR; (125 MHz, $CDCl_3$) δ 159.54 (C=NH) 94.41 (C-1), 71.10 (C-5), 68.66 (C-3), 67.73 (C-2), 65.31 (C-4), 61.91 (C-6). Anal. Calcd. for $C_{16}H_{20}O_{10}NCl_3$: C, 39.01; H, 4.09; N, 2.84. Found: C, 38.81; H, 3.97; N, 2.63.

O-2,3,4,6-Tetra-O-acetyl-α-D-mannopyranosyl-(1 → 2)-3,4,6-tri-O-acetyl-α-D-mannopyranosyl trichloroacetimidate (**12**).—To a stirred solution of mannobiose **9** (8.4 g, 0.025 mol) in pyridine (50 mL) was added acetic anhydride (25 mL). The mixture was stirred at room temperature for 12 h. The reaction mixture was added ethanol. The solvent was evaporated in vacuo. The residue was diluted with EtOAc and washed with aqueous copper(II) sulfate, brine, saturated aqueous $NaHCO_3$, brine, dried ($MgSO_4$) and evaporated in vacuo to give **10**. The acetate **10** was treated with 19.0 g of ammonium carbonate in Me_2NCHO (20 mL) at room temperature for 3 days. The reaction mixture was diluted with EtOAc and washed with water, brine, dried ($MgSO_4$) and evaporated in vacuo to give **11**. A mixture of **11** and trichloroacetonitrile (10 eq.) in CH_2Cl_2 (20 mL) was added DBU (0.1 eq.) at 0 °C. The mixture was stirred at 0 °C for 4 h. The reaction mixture was purified by silica gel column chromatography (hexane:EtOAc, 2:1–1:1) to afford the compound **12** (12.1 g, 0.015 mol); R_f 0.29 (toluene:EtOAc, 1:1); $[\alpha]_D = +37°$ (c 1.03, $CHCl_3$); ^{1}H NMR; (500 MHz, $CDCl_3$) δ 8.72 (s, 1 H, NH), 6.41 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1*a*), 5.47 (dd, 1 H, $J_{3,4}$ 9.5, $J_{4,5}$ 10.0 Hz, H-4*b*), 5.40 (dd, 1 H, $J_{2,3}$ 3.5, $J_{3,4}$ 10.5 Hz, H-3*b*), 5.33 (dd, 1 H, $J_{2,3'}$ 3.5, $J_{3,4}$ 9.5 Hz, H-3*a*), 4.98 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-1*b*); ^{13}C NMR; (125 MHz, $CDCl_3$) δ 160.03 (C=NH) 99.15 (C-1*b*), 95.48 (C-1*a*) 75.00 (C-2*a*), 69.70 (C-2*b*). Anal. Calcd. for $C_{28}H_{36}O_{18}NCl_3$: C, 43.06; H, 4.65; N, 1.79. Found: C, 42.84; H, 4.45; N, 2.03.

O-(2,3,4,6-Tetra-O-acetyl-α-D-mannopyranosyl)-(1 → 2)-O-(3,4,6-tri-O-acetyl-α-D-mannopyranosyl)-(1 → 2)-3,4,6-tri-O-acetyl-α-D-mannopyranosyl trichloroacetimidate (**16**).—The mixture of mannotriose **13** (435 mg, 0.862 mmol) in pyridine (10 mL) was added acetic anhydride (5 mL). The mixture was stirred at room temperature for 12 h. The reaction mixture was added ethanol. The solvent was evaporated in vacuo. The residue was diluted with EtOAc and washed with aqueous copper(II) sulfate, brine, saturated aqueous $NaHCO_3$, brine, dried ($MgSO_4$) and evaporated in vacuo to give **14**. The acetate **14** was treated with 830 mg of ammonium carbonate in Me_2NCHO (5 mL) at room temperature for 13 h. The reaction mixture was diluted with Et_2O and washed with water, brine, dried ($MgSO_4$) and evaporated in vacuo to give **15**. A mixture of **15** and trichloroacetonitrile (10 eq.) in CH_2Cl_2 (2.5 mL) was added DBU (0.1 eq.) at 0 °C. The mixture was stirred at 0 °C for 1 h. The reaction mixture was purified by silica gel column chromatography (hexane:EtOAc, 1:1–1:2) to afford the compound **16** (491 mg, 0.459 mmol); R_f 0.42 (toluene:EtOAc, 2:3); $[\alpha]_D = +42°$ (c 1.01, $CHCl_3$); ^{1}H NMR; (500 MHz, $CDCl_3$) δ 8.75 (s, 1 H, NH), 6.43 (d, 1 H, $J_{1,2}$ 2.2 Hz, H-1*a*), 5.19 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1*b*), 4.96 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1*c*); ^{13}C NMR; (125 MHz, $CDCl_3$) δ 160.13 (C=NH), 99.64 (C2-1*b*), 99.34 (C-1*c*), 95.64

(C-1*a*) 77.11 (C-2*b*), 74.37 (C-2*a*). Anal. Calcd. for $C_{40}H_{52}O_{26}NCl_3$: C, 44.93; H, 4.90; N, 1.31. Found: C, 45.09; H, 4.71; N, 1.54.

O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1 → 2)-3,4,6-tri-O-acetyl-α-D-mannopyranosyl trichloroacetimidate (**20**).—A starting material **17** (461 mg, 1.20 mmol) was dissolved in pyridine (4 mL) and acetic anhydride (2 mL) was added dropwise at 0 °C. The mixture was stirred at room temperature for 12 h. The reaction was quenched with EtOH. The solvent was evaporated in vacuo. The residue was diluted with EtOAc and washed with aqueous copper(II) sulfate, brine, saturated aqueous $NaHCO_3$, dried ($MgSO_4$) and evaporated in vacuo to give the acetylated compound **18** (720 mg, 1.06 mmol). A mixture of **18** (101 mg, 0.15 mmol) and 143 mg of ammonium carbonate was stirred in Me_2NCHO (1 mL) at room temperature for 1 day. The reaction mixture was diluted with EtOAc and washed with water, brine, dried ($MgSO_4$) and evaporated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$:MeOH, 50:1) to give 19 (71 mg, 0.11 mmol). A mixture of **19** (64 mg, 0.101 mmol) and trichloroacetonitrile (5 eq.) in CH_2Cl_2 (2 mL) was added DBU (0.1 eq.) at 0 °C. The mixture was stirred at 0 °C for 2 h. The reaction mixture was purified by silica gel column chromatography (hexane:EtOAc, 1:2–1:3) to afford the compound **20** (71 mg, 0.091 mmol); R_f 0.30 ($CHCl_3$:MeOH, 20:1); $[\alpha]_D = +5$ (c 1.04, $CHCl_3$); ^{1}H NMR; (500 MHz, $CDCl_3$) δ 8.68 (s, 1 H, NH), 6.16 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1*a*), 5.40 (dd, 1 H, $J_{2,3}$ 9.3, $J_{3,4}$ 10.5 Hz, H-3*b*), 5.36 (dd, 1 H, $J_{4,5}$ 10.1 Hz, H-4*a*), 5.09 (dd, 1 H, $J_{2,3'}$ 3.7, $J_{3,4}$ 10.3 Hz, H-3*a*), 5.03 (t, 1 H, $J_{4,5}$ 9.8 Hz, H-4*b*), 4.93 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1*b*), 4.40 (m, 1 H, H-2*b*), 3.79 (m, 1 H, H-2*b*), 3.72 (m, 1 H, H-5*b*); ^{13}C NMR; (125 MHz, $CDCl_3$) δ 160.08 (C=NH), 99.52 (C2-1*b*), 94.92 (C-1*a*), 72.62 (C-2*a*), 54.76 (C-2*b*); Anal. Calcd. for $C_{28}H_{37}O_{17}N_2Cl_3$: C, 43.12; H, 4.78; N, 3.59. Found: C, 43.20; H, 4.68; N, 3.95.

O-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-O-(2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1 → 2)-3,4,6-tri-O-acetyl-α-D-mannopyranosyl trichloroacetimidate (**24**).—The mixture of **21** (112 mg, 0.205 mmol) in pyridine (10 mL) was added acetic anhydride (5 mL). The mixture was stirred at room temperature for 12 h. The reaction mixture was added ethanol. The solvent was evaporated in vacuo. The residue was diluted with EtOAc and washed with aqueous copper(II) sulfate, brine, saturated aqueous $NaHCO_3$, brine, dried ($MgSO_4$) and evaporated in vacuo to give **22**. The acetate **22** was treated with 200 mg of ammonium carbonate in Me_2NCHO (0.2 mL) at room temperature for 13 h, The reaction mixture was diluted with EtOAc and washed with water, brine, dried ($MgSO_4$) and evaporated in vacuo to give **23**. A mixture of **23** and trichloroacetonitrile (10 eq.) in CH_2Cl_2 (2 mL) was added DBU (0.1 eq.) at 0 °C. The mixture was stirred at 0 °C for 1 h. The reaction mixture was purified by silica gel column chromatography (hexane:EtOAc, 1:2–1:3) to afford the compound **24** (116 mg, 0.109 mmol); R_f 0.26 ($CHCl_3$:MeOH, 20:1); $[\alpha]_D = +11$ (c 0.92, $CHCl_3$); ^{1}H NMR; (500 MHz, $CDCl_3$) δ 8.71 (s, 1 H, NH), 6.20 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1*a*), 4.97 (dd, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 3.4 Hz, H-3*c*), 4.62 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1*b*), 4.50 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1*c*), 4.36 (m, 1 H, H-2*a*), 4.02 (m, 1 H, H-2*b*), 3.89 (t, 1 H, $J_{4,5}$ 6.8, $J_{5,6}$ 6.8 Hz, H-5*c*), 3.79 (t, 1 H, $J_{3,4}$ 9.0 Hz, H-4*b*), 3.62 (m, 1 H, H-5*b*); ^{13}C NMR; (125 MHz, $CDCl_3$) δ 160.12 (C=NH) 100.91 (C-1*c*), 100.23 (C-1*b*), 95.11 (C-1*a*), 75.79 (C-4*b*), 72.76 (C-2*a*), 62.20 (C-6*b*), 61.97 (C-6*c*), 60.87 (C-6*a*), 53.48 (C-2*b*). Anal. Calcd. for $C_{40}H_{53}O_{25}N_2Cl_3$: C, 44.98; H, 5.00; N, 2.62. Found: C, 44.77; H, 5.12; N, 3.09.

Standard coupling procedure.—A mixture of glycosyl acceptor (0.21 mmol), TfOH (5 μL) and molecular sieves (1 g, AW 300, Aldrich) in dry 1,2-dichloroethane (1 mL) was stirred at 0 °C for 30 min, then cooled at −20 °C. The glycosyl donor (0.07 mmol) was dissolved in dry 1,2-dichloroethane (4 mL) which was added dropwise to the glycosyl acceptor solution for 1 h. The reaction was quenched with saturated aqueous $NaHCO_3$, diluted with EtOAc and filtered through Celite. The filtrate was washed with brine, dried ($MgSO_4$) and evaporated in vacuo. The residue was purified by size exclusion chromatography (25 mm $\phi \times 650$ mm, Bio-Beads S-X3, Bio-Rad) or HPLC fitted with JAIGEL-ODS column (20 mm $\phi \times 250$ mm, Japan Analytical Industry).

Phenyl O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-(1 → 6)-2,4-di-O-benzyl-1-thio-α-D-mannopyranoside (**25**), Phenyl O-(2,3,4,6-tetra-O-acetyl-α-D-*mannopyranosyl)-(1 → 3)-2,4-di-O-benzyl-1-thio-α-D-mannopyranoside* (**26**), Phenyl O-(2,3,4,6-tetra-O-acetyl-α-D-*mannopyranosyl)-(1 → 3)-O-[(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-(1 → 6)]-2,4-di-O-benzyl-1-thio-α-D-mannopyranoside* (**27**).—The glycosyl acceptor **4** (92 mg, 0.203 mmol) was coupled to the glycosyl donor **8** (34 mg, 0.069 mmol) by standard coupling procedures. The reaction mixture was purified by HPLC with JAIGEL-ODS column

(acetonitrile:water, 3:2) to give the disaccharide **25**; (35 mg, 0.044 mmol), **26**; (4 mg, 0.005 mmol), and the trisaccharide **27**; (1 mg, 0.001 mmol), respectively, **25**; R_f 0.44 (toluene:EtOAc, 2:1); $[\alpha]_D$ = +77° (c 1.00, $CHCl_3$); ^{1}H NMR; (500 MHz, $CDCl_3$) δ 7.44–7.24 (m, 15 H, arom.), 5.58 (bs, 1 H, H-1 *1*), 4.92 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1 *2'*), 4.23 (m, 1 H, H-5 *1*); ^{13}C NMR; (125 MHz, $CDCl_3$) δ 98.00 (C-1 *2'*), 84.90 (C-1 *1*). Anal. Calcd. for $C_{40}H_{46}O_{14}S$: C, 61.37; H, 5.92. Found: C, 61.68; H, 5.94. **26**; R_f 0.43 (toluene:EtOAc, 2:1); $[\alpha]_D$ = +96° (c 1.02, $CHCl_3$); ^{1}H NMR; (500 MHz, $CDCl_3$) δ 7.44–7.22 (m, 15 H, arom.), 5.60 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1 *1*), 5.19 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1 *2*), 4.04 (m, 1 H, H-2 *1*), 3.89 (m, 1 H, H-5 *2*); ^{13}C NMR; (125 MHz, $CDCl_3$) δ 99.50 (C-1 *2*), 85.20 (C-1 *1*). Anal. Calcd. for $C_{40}H_{46}O_{14}S$: C, 61.37; H, 5.92. Found: C, 61.77; H, 5.90. **27**; R_f 0.31 (toluene:EtOAc, 2:1); $[\alpha]_D$ = +94° (c 1.04, $CHCl_3$); ^{1}H NMR; (500 MHz, $CDCl_3$) δ 7.44–7.25 (m, 15 H, arom.), 5.59 (d, 1 H, $J_{1,2}$ 1.0 Hz, H-1 *1*), 5.16 (d, 1 H, $J_{1,2}$ 1.0 Hz, H-1 *2*), 4.89 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-1 *2'*); ^{13}C NMR; (125 MHz, $CDCl_3$) δ 99.56 (C-1 *2*), 97.94 (C-1 *2'*), 84.92 (C-1 *1*). Anal. Calcd. for $C_{54}H_{64}O_{23}S$: C, 58.27; H, 5.79. Found: C, 58.57; H, 5.80.

Phenyl O-(2, 3, 4, 6-tetra-O-acetyl-α-D-mannopyranosyl)-(1 → 2)-O-(3,4,6-tri-O-acetyl-α-D-manno-pyranosyl)-(1 → 6)-2,4-di-O-benzyl-1-thio-α-D-mannopyranoside (**28**), Phenyl O-(2,3,4,6-tetra-O-acetyl-α-D-*mannopyranosyl)-(1 → 2)-O-(3,4,6-tri-O-acetyl-α-D-mannopyranosyl)-(1 → 3)-2,4-di-O-benzyl-1-thio-α-D-mannopyranoside* (**29**), Phenyl O-(2,3,4,6-tetra-O-acetyl-α-d-mannopyranosyl)-(1 → 2)-O-(3,4,6-tri-O-acetyl-α-D-*mannopyranosyl)-(1 → 3)-O-[(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-(1 → 2)-O-(3,4,6-tri-O-acetyl-α-D-mannopyranosyl)-(1 → 6)]-2,4-di-O-benzyl-1-thio-α-D-mannopyranoside* (**30**).—The glycosyl acceptor **4** (91 mg, 0.201 mmol) was coupled to the glycosyl donor **12** (53 mg, 0.068 mmol) by standard coupling procedures. The reaction mixture was applied to the S-X3 column (25 mm $\phi \times$ 650 mm, Bio-Rad) and eluted with toluene to give the pentasaccharide **30** (4 mg, 0.002 mmol) and the mixture of trisaccharides. The mixture was separated by HPLC with JAIGEL-ODS column (acetonitrile:water, 7:3) to give the trisaccharide **28**; (35 mg, 0.033 mmol) and **29**; (12 mg, 0.011 mmol), respectively. **28**; R_f 0.49 (toluene:EtOAc, 1:1); $[\alpha]_D$ = +80° (c 1.00, $CHCl_3$); ^{1}H NMR; (500 MHz, $CDCl_3$) δ 7.44–7.24 (m, 15 H, arom.), 5.60 (bs, 1 H, H-1 *1*), 5.38 (dd, 1 H, $J_{2,3}$ 3.5, $J_{3,4}$ 10.0 Hz, H-3 *2'*), 5.33 (t, 1 H, $J_{3,4}$ 10.0, $J_{4,5}$ 10.0 Hz, H-4 *2'*), 5.03 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1 *2'*), 4.79 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-1 *3'*), 4.23 (m, 1 H, H-5 *1*), 3.87 (m, 1 H, H-5 *2*), 3.75 (t, 1 H, $J_{3,4}$ 9.0, $J_{4,5}$ 9.0 Hz, H-4 *1*); ^{13}C NMR; (125 MHz, $CDCl_3$) δ 99.19 (C-1 *3'*), 98.84 (C-1 *2'*), 85.27 (C-1 *1*). Anal. Calcd. for $C_{52}H_{62}O_{22}S$: C, 58.31; H, 58.3; S, 2.99. Found: C, 58.31; H, 5.84; S, 2.78. **29**; R_f 45 (toluene:EtOAc, 1:1); $[\alpha]_D$ = +65° (c 1.00, $CHCl_3$); ^{1}H NMR; (500 MHz, $CDCl_3$) δ 7.43–7.25 (m, 15 H, arom.), 5.61 (d, 1 H, $J_{1,2}$ 1.5, H1-1 *1*), 5.29 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-1 *2*), 4.68 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1 *3*); ^{13}C NMR; (125 MHz, $CDCl_3$) δ 100.59 (C-1 *2*), 99.22 (C-1 *3*), 84.99 (C-1 *1*). Anal. Calcd. for $C_{52}H_{62}O_{22}S$: C, 58.31; H, 58.3; S, 2.99. Found: C, 58.08; H, 5.92; S, 2.81. **30**; R_f 0.49 (toluene:EtOAc, 1:2); $[\alpha]_D$ = +49° (c 1.02, $CHCl_3$); ^{1}H NMR; (500 MHz, $CDCl_3$) δ 7.44–7.30 (m, 15 H, arom.), 5.66 (bs, 1 H, H-1 *1*), 5.29 (bs, 1 H, H-1 *2*), 5.03 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-1 *2'*), 4.70 (bs, 1 H, H-1 *3*), 4.69 (bs, 1 H, H-1 *3'*); ^{13}C NMR; (125 MHz, $CDCl_3$) δ 100.52 (C-1 *2*), 99.25 (C-1 *3'*), 99.22 (C-1 *3*), 98.80 (C-1 *2'*) 84.98 (C-1 *1*). Anal. Calcd. for $C_{78}H_{96}O_{39}S$: C, 55.45; H, 5.73; S, 1.90. Found: C, 55.01; H, 5.40.

A mixture of glycosyl acceptor **4** (10 mg, 0.022 mmol), glycosyl donor (39 mg, 0.050 mmol), and AW 300 (0.5 g) in dry 1,2-dichloroethane (2.5 mL) was stirred at 0 °C for 30 min, then cooled at −25 °C, added TfOH (5 μL). The reaction was stirred at −25 °C for 2 h, then quenched with saturated aqueous $NaHCO_3$, diluted with EtOAc and filtered through Celite. The filtrate was washed with brine, dried ($MgSO_4$) and evaporated in vacuo. The reaction mixture was purified by gel filtration (S-X3, toluene) to give the pentasaccharide **30** (20 mg, 0.012 mmol).

Phenyl O-(2, 3, 4, 6-tetra-O-acetyl-α-D-mannopyranosyl)-(1 → 2)-O-(3,4,6-tri-O-acetyl-α-D-mannopyranosyl)-(1 → 2)-O-(3,4,6-tri-O-acetyl-α-D-mannopyranosyl)-(1 → 6)-2,4-di-O-benzyl-1-thio-α-D-mannopyranoside (**31**), Phenyl O-(2,3,4,6-tetra-O-acetyl-α-D-*mannopyranosyl)-(1 → 2)-O-(3,4,6-tri-O-acetyl-α-D-mannopyranosyl)-(1 → 2)-O-(3,4,6-tri-O-acetyl-α-D-mannopyranosyl)-(1 → 3)-2,4-di-O-benzyl-1-thio-α-D-mannopyranoside* (**32**), Phenyl O-(2,3,4,6-tetra-O-acetyl-α-D-*mannopyranosyl)-(1 → 2)-O-(3,4,6-tri-O-acetyl-α-D-mannopyranosyl)-(1 → 2)-O-(3,4,6-tri-O-acetyl-α-D-mannopyra-nosyl)-(1 → 3)-O-[(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-(1 → 2)-O-(3,4,6-tri-O-acetyl-α-D-mannopyranosyl)-(1 → 2)-O-(3,4,6-tri-O-acetyl-α-D-mannopyranosyl)-(1 → 6)]-2,4-di-O-benzyl-1-thio-α-D-mannopyranoside* (**33**).—The glycosyl acceptor **4** (95 mg, 0.210 mmol) was coupled to the glycosyl donor **16** (75 mg, 0.070 mmol)

by standard coupling procedures. The reaction mixture was purified by gel filtration (S-X3, toluene) to give the heptasaccharide **33** (8 mg, 0.006 mmol) and mixture of the tetrasaccharides. The mixture was separated by HPLC with JAIGEL-ODS column (acetonitrile:water, 2:1) to give the tetrasaccharide **31**; (51 mg, 0.038 mmol) and **32**; (22 mg, 0.016 mmol). **31**; R_f 0.41 (toluene:EtOAc, 2:3); $[\alpha]_D$ = +69° (c 1.04, $CHCl_3$); ^{1}H NMR; (500 MHz, $CDCl_3$) δ 7.45–7.24 (m, 15 H, arom.), 5.57 (bs, 1 H, H-1 *1*), 5.38 (dd, 1 H, $J_{2,3}$ 3.5, $J_{3,4}$ 10.0 Hz, H-3 *4'*), 5.06 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-1 *3'*), 5.00 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-1 *2'*), 4.92 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1 *4'*), 4.27 (m, 1 H, H-5 *1*); ^{13}C NMR; (125 MHz, $CDCl_3$) δ 99.84 (C-1 *3'*), 99.30 (C-1 *4*), 98.63 (C-1 *2'*), 85.36 (C-1 *1*). Anal. Calcd. for $C_{64}H_{78}O_{30}S$: C, 56.55; H, 5.78; S, 2.36. Found: C, 56.32; H, 6.01; S, 2.33. **32**; R_f 0.38 (toluene:EtOAc, 2:3); $[\alpha]_D$ = +62° (c 1.05, $CHCl_3$); ^{1}H NMR; (500 MHz, $CDCl_3$) δ 7.44–7.20 (m, 15 H, arom.), 5.61 (bs, 1 H, H-1 *1*), 5.34 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1 *2*), 5.02 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-1 *3*), 4.94 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-1 *4*); ^{13}C NMR; (125 MHz, $CDCl_3$) δ 100.45 (C-1 *2*), 100.06 (C-1 *3*), 99.34 (C-1 *4*), 85.07 (C-1 *1*). Anal. Calcd. for $C_{64}H_{78}O_{30}S$: C, 56.55; H, 5.78. Found: C, 56.75; H, 5.77. **33**; R_f 0.11 (toluene:EtOAc, 2:3); $[\alpha]_D$ = +60° (c 1.03, $CHCl_3$); ^{1}H NMR; (500 MHz, $CDCl_3$) δ 7.45–7.28 (m, 15 H, arom.), 5.64 (bs, 1 H, H-1 *1*), 5.01 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-1), 5.00 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 4.99 (bs, 1 H, H-1), 4.93 (bs, 2 H, H-1); ^{13}C NMR; (125 MHz, $CDCl_3$) δ 100.53 (C-1 *2*), 100.03 (C-1 × 2 *3*, *3'*), 99.26 (C-1 *4*), 99.22 (C-1 *4'*), 98.76 (C-1 *2'*), 84.98 (C-1 *1*). Anal. Calcd. for $C_{102}H_{128}O_{55}S$: C, 54.06; H, 5.69; S, 1.41. Found: C, 54.31; H, 5.43.

Phenyl O-*(2-acetamido-3,4,6-tri*-O-*acetyl-2-deoxy-β*-D-*glucopyranosyl)-(1 → 2)*-O-*(3,4,6-tri*-O-*acetyl-α*-D-*mannopyranosyl)-(1 → 6)-2,4-di*-O-*benzyl-1-thio-α*-D-*mannopyranoside* (**34**), Phenyl O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-*glucopyranosyl)-(1 → 2)*-O-*(3,4,6-tri*-O-*acetyl-α*-D-*mannopyranosyl)-(1 → 3)-2,4-di*-O-*benzyl-1-thio-α*-D-*mannopyranoside* (**35**), Phenyl O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-*glucopyranosyl)-(1 → 2)*-O-*(3,4,6-tri*-O-*acetyl-α*-D-*mannopyranosyl)-(1 → 3)*-O-*[(2-acetamido,4,6-tri*-O-*acetyl-2-deoxy-β*-D-*glucopyranosyl)-(1 → 2)*-O-*(3,4,6-tri*-O-*acetyl-α*-D-*mannopyranosyl)-(1 → 6)]-2,4-di*-O-*benzyl-1-thio-α*-D-*mannopyranoside* (**36**).—The glycosyl acceptor **4** (94 mg, 0.208 mmol) was coupled to the glycosyl donor **20** (55 mg, 0.071 mmol) by standard coupling procedures. The reaction mixture was purified by HPLC using JAIGEL-ODS column (acetonitrile:water, 3:2) to give the trisaccharide **34**; (57 mg, 0.053 mmol), **35**; (7 mg, 0.007 mmol), and mixture of the acceptor **4** and the pentasaccharide **36**. The mixture was separated by silica gel column chromatography ($CHCl_3$:MeOH, 20:1) to give the pentasaccharide **36**; (6 mg, 0.004 mmol). **34**; R_f 0.36 ($CHCl_3$:MeOH, 20:1); $[\alpha]_D$ = +72° (c 1.41, $CHCl_3$); ^{1}H NMR; (500 MHz, $CDCl_3$) δ 7.51–7.27 (m, 15 H, arom.), 5.83 (bs, 1 H, H-1 1), 5.73 (d, 1 H, $J_{2,NH}$ 8.5 Hz, NH), 5.31 (dd, 1 H, $J_{2,3}$ 9.5, $J_{2,3}$ 10.3 Hz, H-3 *3'*), 4.81 (d, 1 H, $J_{1,2}$ 3.2 Hz, H-1 *2'*), 4.77 (d, 1 H, $J_{1,2}$ 8.6 Hz, H-1 *3'*), 4.31 (m, 1 H, H^1-5), 3.83 (t, $J_{3,4}$ 9.3 Hz, H-4 *1*), 3.32 (m, 1 H, H-5 *2'*); ^{13}C NMR; (125 MHz, $CDCl_3$) δ 99.71 (C-1 *3'*), 97.73 (C-1 *2'*), 85.49 (C-1 *1*), 54.95 (C-2 *3'*). Anal. Calcd. for $C_{52}H_{63}O_{21}NS \cdot 3/2H_2O$: C, 56.93; H, 6.06; N, 1.28. Found: C, 56.99; H, 5.68; N, 1.56. **35**; R_f 0.36 ($CHCl_3$:MeOH, 20:1); $[\alpha]_D$ = +33° (c 0.46, $CHCl_3$); ^{1}H NMR; (500 MHz, $CDCl_3$) δ 7.47–7.30 (m, 15 H, arom.), 5.59 (bs, 1 H, H-1 *1*), 5.17 (t, 1 H, $J_{3,4}$ 10.0, $J_{4,5}$ 10.0 Hz, H-3 *3*), 5.05 (bs, 1 H, H-1 *2*), 4.00 (m, 1 H, H-2 *1*); ^{13}C NMR; (125 MHz, $CDCl_3$) δ 99.95 (C-1 *3*), 99.33 (C-1 *2*), 85.62 (C-1 *1*), 53.91 (C-2 *3*). Anal. Calcd. for $C_{52}H_{63}O_{21}NS \cdot H_2O$: C, 57.40; H, 6.02; N, 1.29. Found: C, 57.61; H, 5.90; N, 1.77. **36**; R_f 0.11 (toluene:EtOAc, 2:3); $[\alpha]_D$ = +35° (c 0.96, $CHCl_3$); ^{1}H NMR; (500 MHz, $CDCl_3$) δ 7.53–7.29 (m, 15 H, arom.), 5.90 (bs, H, H1-1 *1*), 5.79 (d, 1 H, $J_{2,NH}$ 8.6 Hz, NH), 5.05 (bs, 1 H, H-1 *2*), 4.83 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1 *2'*), 4.81 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1 *3'*), 4.38 (m, 1 H, H-5 *1*); ^{13}C NMR; (125 MHz, $CDCl_3$) δ 99.80 (C-1 *3'*), 99.72 (C-1 *3*), 99.50 (C-1 *2*), 97.85 (C-1 *2'*), 85.79 (C-1 *1*). Anal. Calcd. for $C_{78}H_{98}O_{37}N_2S$: C, 55.51; H, 5.85; N, 1.66. Found: C, 55.88; H, 5.96; N, 1.93.

Phenyl O-*(2-acetamido-3,4,6-tri*-O-*acetyl-2-deoxy-β*-D-*glucopyranosyl)-(1 → 2)*-O-*(3,4,6-tri*-O-*acetyl-α*-D-*mannopyranosyl)-(1 → 3)*-O-*[(2-acetamido-3,4,6-tri*-O-*acetyl-2-deoxy-β*-D-*glucopyranosyl)-(1 → 2)*-O-*(3,4,6-tri*-O-*acetyl-α*-D-*mannopyranosyl)-(1 → 6)]-2,4-di*-O-*benzyl-1-thio-α*-D-*mannopyranoside* (**36**).—A mixture of glycosyl acceptor (11 mg, 0.024 mmol), TfOH (2 μL) and AW300 (1 g) in dry 1,2-dichloroethane (1 mL) was stirred at 0 °C for 30 min, then cooled at −25 °C. The glycosyl donor (40 mg, 0.051 mmol) in dry 1,2-dichloroethane (2 mL) was added dropwise for 2 h. The reaction was quenched with saturated aqueous $NaHCO_3$, diluted with EtOAc and filtered through Celite. The filtrate was washed with brine, dried ($MgSO_4$) and evaporated in vacuo. The reaction

mixture was purified by HPLC using JAIGEL-ODS column (acetonitrile:water, 3:2) to give the trisaccharide **36**; (26 mg, 0.015 mmol).

Phenyl O-(2, 3, 4, 6-tetra-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-O-(2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1 → 2)-O-(3,4,6-tri-O-acetyl-α-D-mannopyranosyl)-(1 → 6)-2,4-di-O-benzyl-1-thio-α-D-mannopyranoside (**37**).—The glycosyl acceptor **4** (90 mg, 0.199 mmol) was coupled to the glycosyl donor **24** (75 mg, 0.070 mmol) by standard coupling procedures. The reaction mixture was purified by HPLC using JAIGEL-ODS column (acetonitrile:water, 3:2) to give the tetrasaccharide **37**; (49 mg, 0.036 mmol); R_f 0.30 ($CHCl_3$:MeOH, 20:1); $[\alpha]_D = +192°$ (c 0.70, $CHCl_3$); ^1H NMR; (500 MHz, $CDCl_3$) δ 7.52–7.31 (m, 15 H, arom.), 5.86 (bs, 1 H, H-1 *1*), 5.77 (d, 1 H, $J_{2,NH}$ 8.5 Hz, NH), 5.33 (d, 1 H, H-4 3.2 Hz *4′*), 4.79 (d, 1 H, $J_{1,2}$ 3.9 Hz, H-1 *2′*), 4.54 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1 *3′*), 4.45 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1 *4′*), 3.33 (m, 1 H, H-5 *3′*); ^{13}C NMR; (125 MHz, $CDCl_3$) δ 100.94 (C-1 *4′*), 100.15 (C-1 *3′*), 97.45 (C-1 *2′*), 85.46 (C-1 *1*), 53.56 (C-2 *3′*). Anal. Calcd. for $C_{64}H_{79}O_{29}NS$: C, 56.59; H, 5.86; N, 1.03. Found: C, 56.15; H, 5.91; N, 1.43.

Phenyl O-(2, 3, 4, 6-tetra-O-acetyl-α-D-mannopyranosyl)-(1 → 3)-O-[(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-(1 → 2)-O-(3,4,6-tri-O-acetyl-α-D-mannopyranosyl)-(1 → 6)]-2,4-di-O-benzyl-1-thio-α-D-mannopyranoside (**38**).—To a mixture of **28** (58 mg, 0.054 mmol), TfOH (5 μL) and AW300 in dry 1,2-dichloroethane (1 mL) was stirred at −20 °C under argon for 30 min. Then a solution **8** (39 mg, 0.079 mmol) in dry 1,2-dichloroethane (1.5 mL) was added dropwise to the above mixture. The mixture was stirred at −20 °C for 2 h, quenched with saturated aqueous $NaHCO_3$, diluted with EtOAc and filtered through Celite. The filtrate was washed with brine, dried ($MgSO_4$) and evaporated in vacuo. The residue was purified by size exclusion chromatography (Bio-Beads S-X3 toluene, Bio-Rad) to give **38** (51 mg, 0.036 mmol); R_f 0.62 (toluene:EtOAc, 1:2); $[\alpha]_D = +79°$ (c 1.00, $CHCl_3$); ^1H NMR; (500 MHz, $CDCl_3$) δ 7.44–7.24 (m, 15 H, arom.), 5.63 (bs, 1 H, 1^1), 5.17 (d, 1 H, $J_{1,2}$ 1.5 Hz, $1^{2'}$) 5.05 (d, 1 H, $J_{1,2}$ 1.5 Hz, 1^3), 4.73 (d, 1 H, $J_{1,2}$ 1.5 Hz, 1^4); ^{13}C NMR; (125 MHz, $CDCl_3$) δ 99.51 (1^2) 99.28 (1^3), 98.77 (1^4), 85.18 (1^1). Anal. Calcd. for $C_{66}H_{80}O_{31}S$: C, 56.57; H, 5.75; S, 2.29. Found: C, 56.33; H, 5.57; S, 2.34.

Phenyl O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1 → 2)-O-(3,4,6-tri-O-acetyl-α-D-mannopyranosyl)-(1 → 3)-O-[(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-O-(2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1 → 2)-O-(3,4,6-tri-O-acetyl-α-D-mannopyranosyl)-(1 → 6)]-2,4-di-O-benzyl-1-thio-α-D-mannopyranoside (**39**).—To a mixture of **37** (50 mg, 0.0368 mmol), TfOH (3 μL) and AW300 in dry 1,2-dichloroethane (1 mL) was stirred at −20 °C under argon for 30 min. Then a solution of **20** (34 mg, 0.0436 mmol) in dry 1,2-dichloroethane (3 mL) was added dropwise to the above mixture for 1 h. The mixture was stirred at −25 °C for 2 h, quenched with saturated aqueous $NaHCO_3$, diluted with EtOAc and filtered through Celite. The filtrate was washed with brine, dried ($MgSO_4$) and evaporated in vacuo. The residue was purified by HPLC fitted with JAIGEL-ODS column to give **31** (40 mg, 0.0203 mmol) and recovered acceptor **37** (11 mg); R_f 0.26 ($CHCl_3$:MeOH, 20:1); $[\alpha]_D = +112.3°$ (c 1.57, $CHCl_3$); ^1H NMR; (500 MHz, $CDCl_3$) δ 7.53–7.25 (m, 15 H, arom.), 5.89 (bs, 1 H, H-1 *1*), 5.79 (d, 1 H, $J_{2,NH}$ 9.0 Hz, NH), 5.31 (m, 1 H, H-4 *4′*), 4.79 (d, 1 H, $J_{1,2}$ 3.9 Hz, H-1 *2′*), 4.58 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1 *3′*), 4.43 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1 *4′*), 3.31 (m, 1 H, H-5 *3′*); ^{13}C NMR; (125 MHz, $CDCl_3$) δ 101.09 (C-1 *4′*), 100.30 (C-1 *3′*), 99.67 (C-1 *3*), 99.47 (C-1 *2*), 97.65 (C-1 *2′*), 85.70 (C-1 *1*), 54.12 (C-2 *3′*), 53.77 (C-2 *3*). Anal. Calcd. for $C_{90}H_{114}O_{45}NS$: C, 54.71; H, 5.81; N, 1.42. Found: C, 54.53; H, 5.66; N, 1.74.

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