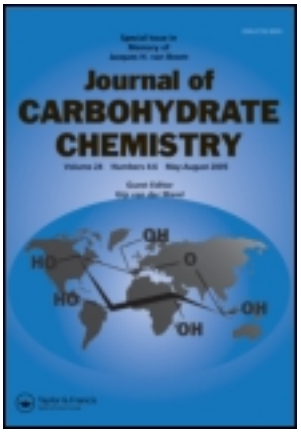

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Single-Step Multisyntheses of Glycosyl Acceptors: Benzylation of *n-1* Hydroxyl Groups of Phenylthio Glycosides of Xylose, Mannose, Glucose, Galactose, 2-Azido-2-deoxy-glucose, and 2-Azido-2-deoxy-galactose

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An array of synthons is required to access an oligosaccharide library; however, multistep and thus time-consuming synthesis is inevitable. To rapidly access such synthetic units, multiple benzylation reactions of monosaccharides under phase-transfer conditions were examined. Multiple benzyl groups were successfully incorporated in one step, especially in the cases of reactions with triol systems.

Keywords Phase transfer, Benzylation, Random reactions, Thioglycosides, Glycosyl acceptors

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INTRODUCTION

Oligosaccharides displayed on the cell surface as a part of glycoproteins or glycolipids play important roles in cellular recognition processes. Such processes include the differentiation and development of cells,[1] immune response,[2] and fertilization[3] in the animal kingdom. Other functions are the role of receptors as ligands present on the surfaces of bacteria[4] and viruses[5] that cause infectious diseases.

To access the functions and mechanisms involved in such interactions, synthetic oligosaccharides can be used as molecular probes. Satisfying the increasing need to produce such molecules relies largely on the availability of synthetic monosaccharide units. In most cases, it is relatively easy to synthesize the units, but they usually require three to five steps for just protecting group manipulations and thus are time consuming and expensive. In the course of our study to synthesize an oligosaccharide library, it was necessary to prepare a series of suitably protected monosaccharide synthetic units. Considering the large number of monosaccharides required, we came to the conclusion that the stereochemistry is not strictly controlled by neighboring group participation, but rather mildly controlled by a solvent effect[6] and anomeric effect.[7] This decision would reduce the number of required synthons.[8] Despite the potential complexity of the formation of an α- and β-anomeric mixture, it is considered that this may be advantageous for the following reasons: 1) the formation of orthoester and related byproducts can be prevented and 2) another anomer, which may be biologically important, is obtained at the same time. It should also be stressed that most of the current "stereoselective" glycosylation methods inevitably yield an unwanted anomer because the glycosylation mechanism involves SN1 character. Individual synthesis of protected monosaccharides, however, still requires multistep operations. To overcome this problem, we considered a method to introduce *n-1* protecting groups into monosaccharides having *n* hydroxyl groups in a random fashion to yield a set of monosaccharides, which can directly be used as acceptors in glycosylation reactions (Fig. 1).

Here we report the results of benzylation of phenylthio glycosides of the monosaccharides xylose, mannose, glucose, galactose, 2-azido-2-deoxy-glucose, and 2-azido-2-deoxy-galactose under phase-transfer conditions to directly access glycosyl acceptors.

RESULTS AND DISCUSSION

Prior to initiating the program, we decided to prepare monosaccharides as phenylthio glycosides because of their important roles in oligosaccharide synthesis,[9–15] especially in conjunction with the orthogonal glycosylation strategy.[16–18] In an effort to introduce *n-1* benzyl groups into

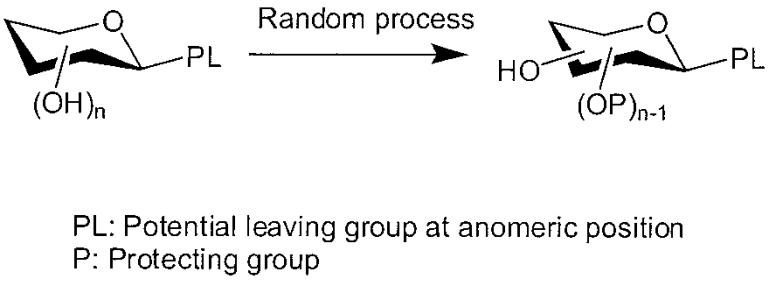
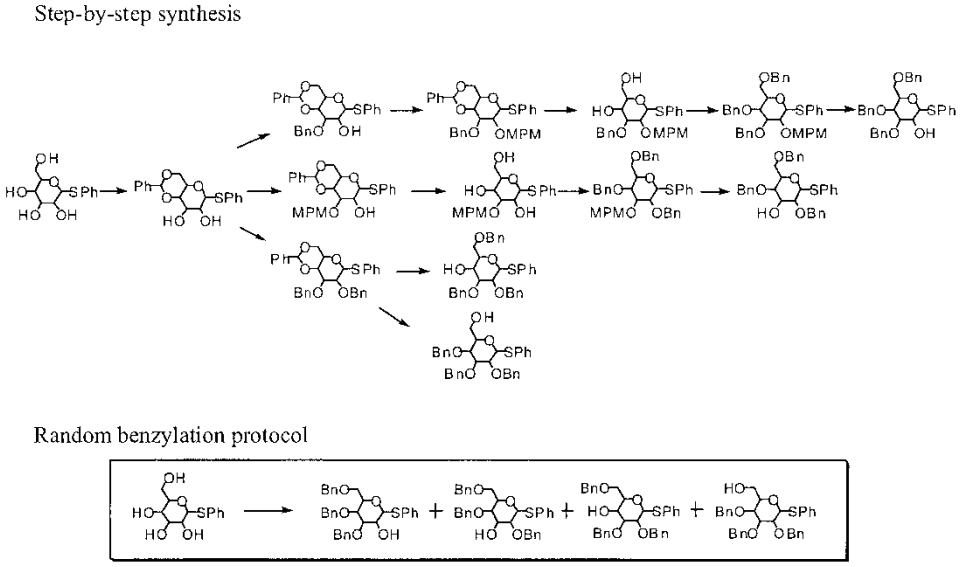


Figure 1: Concept of direct conversion of nonprotected monosaccharide into a glycosyl acceptor.

monosaccharides, we decided to use phase-transfer conditions after examining various possible conditions. Unlike usual methods,[19,20] our objective was to obtain multiple products in the least number of steps possible (Sch. 1).[21] For this, we have examined the conditions to "optimize" the randomness of the introduction of *n-1* benzyl groups. The criteria for this are that (1) *n-1* hydroxyl groups are succsessfully benzylated and (2) the expected abundance of individual products is equal. 1.25–1.5 Equivalents of benzyl bromide (BnBr) for each hydroxyl group, tetra-*n*-butyl ammonium hydrogensulfate (Bu_4NHSO_4) as a catalyst, and sodium hydroxide were used in a dichloromethane water-solvent system under gentle reflux conditions at a bath temperature of 50°C. It was found that the desired compounds, which have one



Scheme 1: Comparison of the step-by-step preparation of the glycosyl accepters and the random alkylation protocol.

hydroxyl group and other hydroxyl groups that were benzylated, were produced in relatively scattered ratios.

Randomized Dibenzylation Reactions of Phenylthio-Glycosides of Monosaccharides Carrying Three Hydroxyl Groups (2/3 Benzylation Reactions)

Under the above-mentioned phase-transfer conditions, the reaction of phenyl 2-azido-2-deoxy-1-thio-β-D-galactopyranoside (GalN) yielded **GalN3**, **GalN4**, and **GalN6** in yields of 17%, 36%, and 13%, respectively (Fig. 2, Table 1). A starting material having three hydroxyl groups has a greater partition coefficient to the aqueous phase, and therefore, a greater chance exists for an alkoxide to be involved in the reaction. The dominant factor in this step should be basically the basisity of each alkoxide. In this case, the anomeric position and *C*-2 are substituted by electron withdrawing groups (e.g. phenylthio- and azido-groups). Thus, the most basic and nucleophilic alkoxide will be *O*-4. This was consistent with the analysis of the isolated mono-benzyl compound that was found to be exclusively *O*-4 benzyl compound. However, this evidence does not mean that the first generation reaction went through the benzylation at 4-OH because the major product of the dibenzylation was a 4-OH compound. This inconsistent result suggests

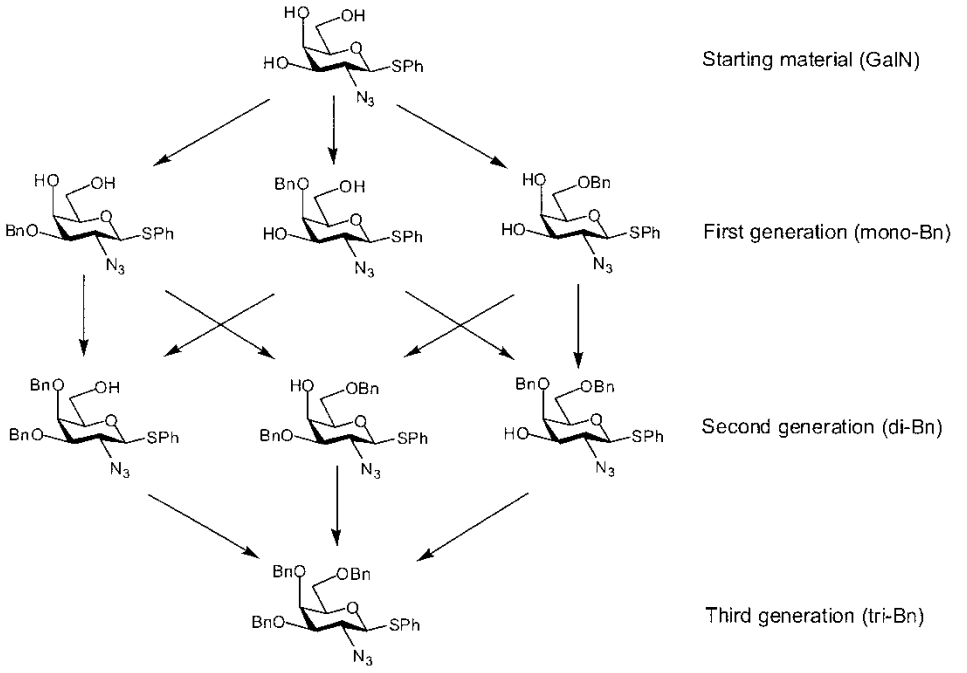


Figure 2: Generations of benzylation reaction.

Table 1: Isolated yields of dibenzylated monosaccharides with three OHs.

Number of OHs	Position of OH	GalN Yields (%)[a]			GalN Ratio[b]	GlcN Yields (%)[a]			GlcN Ratio[b]	Xyl Yields (%)[a]			Xyl Ratio[b]
1 (di Bn)	2	—	—		—	—	—		—	11			1.0
	3	17			1.4	13			1.0	34	70		3.0
	4	36	66	93	3.6	17	67	98	1.5	25		88	2.0
	6	13			1.0	37			2.8	—	—		—
0 (tri Bn)	—	—	13		—	—	17		—	—	9.2		—
2 (mono Bn)	—	—	14		—	—	14		—	—	8.3		—

[a]Isolated yields after silica gel chromatography.
[b]Ratio obtained by HPLC analysis (see experimental section for retention time for each compound).

that the first-generation reaction would preferentially occur at 3- and 6-OH. The second generation would be largely affected by steric factors; thus, the 3-OBn compound preferentially produces a 3,6-di-Bn compound (**GalN4**), for example. The situation would be the same in the case of 6-OBn compound. The 4-OBn compound, however, would resist the second reaction due to the steric inpediment of the first benzyl group. Yields of dibenzylation indicate that the order of observed nucleophilicity (reactivity) is O-6 $\approx$ O-3 $>$ O-4 (Table 2). This result was further confirmed by additional experiments where the initial reaction rate was examined using one equivalent of benzyl bromide, and the reaction course was followed by ^{1}H NMR (data not shown). Furthermore, the reaction rate constant should be reduced for the second-generation reaction because of the lower partition coefficient in the aqueous phase, and the reaction is influenced by steric factor as well.

In the case of the glucosamine precursor GlcN, however, the order of the product ratio obtained was different from that of GalN (Table 1). An important observation was that the orders of reactivity of 3- and 4-OH were the same (Table 2). The observed different reactivity for 6-OHs in GalN and GlcN might be affected by the orientation of 6-OH (rotamers). Neverthless, the observed reactivity was found to be O-3 $>$ O-4 $>$ O-6. Isolation of each compound could not be accomplished by silica gel column chromatography in this case, which yielded **GlcN3** and a mixture of **GlcN4** and **GlcN6**. Thus, the latter mixture was subjected to silylation conditions using *t*-butyldimethylsilyl chloride (TBDMSCl), triethylamine (TEA), and 4-*N*,*N*-dimethylaminopyridine (DMAP) in *N*,*N*-dimethylformamide (DMF). As a result, only compound **GlcN6** having a primary hydroxyl group was silylated, and thus **GlcN6**-TBDMS and **GlcN4** were separated by silica gel chromatography. Finally, the TBDMS group was removed by means of tetra-*n*-butylammonium fluoride (TBAF).

As for phenylthio-xyloside, the hydroxyl groups are all in a transequatorial arrangement, and thus comparison of the reactivities must be more simple. The result indicated that the order of basisity of alcholate is O-2 $>$ O-4 $>$ O-3 (Table 2). Isolation of each compound was done by silica gel column chromatography.

Table 2: Ratio of benzylation.[a]

Position of OH	GalN	GlcN	Xyl	Gal	Glc	Man
2	—	—	1.8	1.7	1.7	1.4
3	1.9	1.7	1.0	1.0	1.0	1.1
4	1.0	1.5	1.5	1.2	1.6	1.0
6	2.1	1.0	—	1.7	1.2	1.1

[a]Ratio of sum of yields where target OH groups were benzylated.

Overall, total yields for the dibenzylation reactions were within 66% and 70% yields. Individual compounds could be isolated by simple column chromatography. Therefore, the procedure may be effectively used to prepare a set of glycosyl acceptors.

Randomized Tribenzylation Reactions of Phenylthioglycosides of Monosaccharides Carrying Four Hydroxyl Groups (3/4 Benzylation Reactions)

The tribenzylation reactions of monosaccharides carrying four hydroxyl groups such as phenylthiogalactoside (Gal), phenylthioglucoside (Glc), and phenylthiomannoside (Man) were found to be less efficient compared to the dibenzylation of triols (Table 3). The distributions of mono-, di-, tri-, and tetrabenzylated compounds were 4%, 20%, 35%, and 16%, respectively, for Gal. Attempts to push the reaction further resulted in the accumulation of perbenzylation product, which suggested that the partition coefficients for compounds having more than two benzyl groups were similar. The same tendency was observed for Glc as well. Attempts to isolate individual tribenzylated compounds by chromatography yielded a mixture of **Gal2**, **Gal3**, and **Gla4** (30%) and isolated **Gal6** (4.5%), and the mixture could not be resolved. HPLC, however, could resolve all four regioisomers. Although it was not practical to isolate tribenzylated phenylthio-glucoside, four isomers could be isolated by means of HPLC. In the case of Man, tribenzylated compounds, namely **Man2**, **Man3**, **Man4**, and **Man6**, were isolated on silica gel column chromatography. The reactivity of each hydroxyl group under the condition is in the order of O-2 $\approx$ O-6 $>$ O-4 $\approx$ O-3 (Gal), O-2 $\approx$ O-4 $>$ O-6 $>$ O-3 (Glc), and O-2 $>$ O-3 $\approx$ O-6 $\approx$ O-4 (Man) (Table 2). In the case of Gal, it was found that the reactivities of alcholates at O-3 and O-4 were similar, which was different from those of alcohols for the glycosylation reactions.[22]

Conclusion

Through this investigation, it was revealed that there is a tendency for the reactivity of alcoxide at O-2 to be greater than others in general. Comparison of Gal versus GalN and Glc versus GlcN suggest that azide functionality enhances the reactivity of O-3 alcholate despite its electron withdrawing character (Table 2). There exists a review article describing the reactivities of hydroxyl goups of various carbohydrates, but the results were inconsistent with ours.[23] Reasons for this are considered to be different reaction conditions and the anomeric protecting groups utilized.

In summary, a random benzylation method of phenylthioglycosides of a series of monosaccharides was examined in order to rapidly access a set of

Table 3: Isolated yields of tribenzylated monosaccharides with four OHs.

Number of OHs	Position of OH	Gal		Glc		Man			
		Yields (%)[a]	Ratio[b]	Yields (%)[a]	Ratio[b]	Yields (%)[a]			Ratio[c]
1 (tri Bn)	2	35	1.2		1.0	1.1			0.3
	3		6.2	48	4.8	20	65		1.0
	4		5.1		1.1	23			1.3
	6	75	1.0	87	3.6	21		93	1.1
0 (tetra Bn)	—	16	—	20	—	—	8.6		—
2 (di Bn)	—	20	—	19	—	—	9.6		—
3 (mono Bn)	—	4.0	—	—	—	—	10		—

[a]Yields after silica gel chromatography.
[b]Ratio obtained by HPLC analysis (see experimental section for retention time for each compound).
[c]Ratio estimated by ^{1}H NMR.

glycosyl acceptors. It was found that all phenylthio-glycosides can be converted into glycosyl acceptors having one hydroxyl group in single phase-transfer reactions. GalN, Xyl, and Man derivatives in particular could easily be isolated by silica gel column chromatography. HPLC can be used when simple purification is not possible. Despite some drawbacks in the purification process, the method drastically reduces the synthetic steps and thus the overall yields for most of the individual compounds are superior compared to traditional one-by-one and step-by-step methods.

EXPERIMENTAL

General Methods

Analytical thin layer chromatography (TLC) was performed on Merck Art 5715, Kieselgel 60 F_{254}/0.25 mm thickness plates. Visualization was accomplished with UV light and phosphomolybdic acid and/or sulfuric acid solution followed by heating. Column chromatography was performed with Merck Art 7734 Silicagel 60 70–230 mesh. ^{1}H NMR (500 MHz) spectra were recorded with an AVANCE 500 spectrometer (Bruker Biospin Inc.) in deuterated solvents using tetramethylsilane as an internal standard. ^{13}C NMR chemical shifts were obtained and assigned by HSQC experiments. Optical rotations were measured in a 1.0 dm tube with a Horiba SEPA-200 polarimeter at 26 $\pm$ 1°C.

^{1}H NMR Assignments

To obtain unambiguous results regarding the structures of individual compounds, all compounds were synthesized prior to this investigation. Assigned chemical shifts and coupling constants of compounds are partially listed in Tables 4 and 5, respectively.

Estimation of Compounds' Ratio

Estimation of the ratio of each compound formed in a phase-transfer alkylation reaction was carried out based on ^{1}H NMR integrals.

GalN: Although those of one of the H-6 protons of **GalN3** and **GalN4** were isolated in a spectrum of a mixture, there is no isolated signal for **GalN6**. Therefore, the ratio of each compound was estimated as follows: [**GalN6**] = {[**GalN3**] + [**GalN4**] + [**GalN6**]} − {[**GalN3**] + [**GalN4**]}, where [**GalN3**] = integral at δ 3.70 (H-6a of **GalN3**) = 0.39 and [**GalN4**] = integral at δ 3.77 (H-6a of **GalN4**) = 1.00. {[**GalN3**] + [**GalN4**] + [**GalN6**]} =

Table 4: Chemical shifts of *n-1* benzylated compounds.

Sugar	Position of OH	H-1	H-2	H-3	H-4	H-5	H-6		Benzyl methylenes
GalN	3	4.42	3.55	3.67	3.90	3.52	3.70	3.70	4.72, 4.67, 4.54, 4.49
	4	4.37	3.64	3.38	4.05	3.55	3.77	3.77	4.69, 4.65, 4.58, 4.55
	6	4.40	3.85	3.42	3.84–3.80	3.41	3.48–3.80	3.54	4.89, 4.74, 4.71, 4.55
GlcN	3	4.44	3.28	3.59	3.51	3.45	3.80	3.76	4.70, 4.65[a], 4.57
	4	4.43	3.31	3.36	3.62	3.45	3.78	3.74	4.89, 4.81, 4.59, 4.55
	6	4.46	3.33	3.54	3.52	3.38	3.87	3.71	4.88, 4.85, 4.83, 4.64
Xyl	2	4.92	3.72	3.63	3.57	4.29 3.52	—		4.83, 4.74, 4.64[b]
	3	4.63	3.33	3.73	3.51	4.04 3.21	—		4.92, 4.75, 4.70, 4.63
	4	5.20	3.73	3.64	3.69	4.42 3.53	—		4.78, 4.76, 4.61, 4.61
Gal	2	4.53	4.01	3.48	3.98	3.66	3.66	3.66	4.89, 4.74, 4.67, 4.57, 4.50,
	3	4.62	3.70–3.66	3.70–3.66	3.91	3.70–3.66	3.70–3.66 3.70–3.66		4.89, 4.74, 464, 4.65, 4.52,
	4	4.64	3.75	3.57	4.10	3.60	3.81	3.77	4.83, 4.75, 4.72, 4.68, 4.57[b]
	6	4.65	3.95	3.60	3.85	3.43	3.83	3.51	4.97, 4.82, 4.75[a], 4.63
Glc	2	4.50	3.49	3.59	3.59	3.53	3.79	3.74	4.90, 4.84, 4.82, 4.61, 4.57,
	3	4.65	3.38	3.76	3.54	3.49	3.80	3.73	4.96, 4.78, 4.67, 4.62, 4.61,
	4	4.69	3.48	3.53	3.65	3.47	3.79	3.75	4.91[a], 4.78, 4.74, 4.58, 4.55
	6	4.72	3.49	3.73	3.58	3.39	3.88	3.70	4.92, 4.91, 4.87, 4.86, 4.77,
Man	2	5.61	4.26	3.88	3.94	4.30	3.8	3.68	4.84, 4.72[b], 4.62, 4.53, 4.46
	3	5.69	4.00	3.98	3.80	4.29	3.85	3.75	4.88, 4.78, 4.66, 4.55, 4.52,
	4	5.62	4.01	3.68	4.13	4.29	3.84	3.80	4.70, 4.62, 4.58, 4.54, 4.54,
	6	5.51	3.99	3.89	4.03	4.12	3.82	3.80	4.96, 4.69[b], 4.66, 4.65, 4.61

[a]Signal ovarlapped with other methylene protons.
[b]Signal appeared as a singlet.

Table 5: Chemical shifts and coupling constants of *n-1* benzylated compounds.

Sugar	Position of OH	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,5}$	$J_{5,6}$	$J_{6,6}$	Benzyl methylenes (J_{gem})
GalN	3	9.6	9.6	2.2	—[a]		2.1 3	7.5	11.7, 11.7
	4	10.2	9.8	3.1	2.7		5.7 5.7	10.0	11.5, 12.3
	6	10.1	9.9	2.8	—[a]		4.2 8.7	11.4	11.6, 11.7
GlcN	3	10.1	9.6	8.9	9.6		2.0 4.0	11.0	11.3, 11.9
	4	9.7	9.3	8.5	9.6		4.4 4.9	10.4	11.1, 11.8
	6	10.2	9.1	9.0	9.2		2.6 6.1	6.1[b]	10.5, 11.1
Xyl	2	5.9	6.0	6.2	6.5 3.1	11.8			11.6, —[c]
	3	9.5	9.0	8.8	10.0 5.1	11.5			10.9, 11.8
	4	4.3	4.7	5.2	5.0 2.8	11.9			11.6, 12.1
Gal	2	9.6	9.4	2.7	—[a]		—[a] —[a]	—[a]	11.5, 11.7, 11.9
	3	9.5	—[a]	—[a]	—[a]		—[a] —[a]	—[a]	10.8, 11.6, 11.7
	4	9.8	9.3	3.2	—[a]		5.8 5.8	10.0	10.3, 11.8, —[c]
	6	9.7	9.4	2.7	—[a]		5.1 7.1	11.3	10.2, 11.7, —[a]
Glc	2	9.6	7.7	8.1	8.5		1.7 4.4	10.9	11.2, 10.6, 12.0
	3	9.9	9.0	8.9	9.6		4.4 5.3	10.9	11.0, 11.2, 11.9
	4	9.5	8.7	8.7	9.2		4.0 5.2	10.4	11.4, 10.3, 11.9
	6	9.8	9.1	9.0	9.5		2.7 4.8	11.7	10.2, 10.9, 10.9
Man	2	1.0	3.1	9.1	9.5		1.8 4.6	10.9	10.8, 12.0, —[c]
	3	—[a]	3.6	9.2	9.4		1.7 4.9	10.9	11.0, 11.6, 11.9
	4	0.7	2.7	9.5	9.5		3.4 5.5	10.7	12.3, 11.9, 11.7
	6	1.2	2.8	9.2	10.0		2.9 4.5	5.9[b]	10.9, 11.6, —[c]

[a]Coupling constants were not determined due to the signal ovarlap.
[b]Coupling constants were affected by broadening of the signal due to hydroxyl proton.
[c]Signal appeared as a singlet.

integral at δ 4.42–4.40 (**GalN3** + **GalN4** + **GalN6**, H-1s) = 1.66. Thus, [**GalN6**] = 1.66–1.39 = 0.27.

GlcN: In a spectrum of a mixture, one of benzylidene methylene proton of **GlcN3** and H-6a of **GlcN6** were separated, but none of the protons belonging to **GlcN4** was separated. The ratio of each compound was estimated as follows: [**GlcN4**] = {[**GlcN3**] + [**GlcN4**] + [**GlcN6**]} − {[**GlcN3**] + [**GlcN6**]}, where [**GlcN3**] = integral at δ 4.70 (one of the benzylmethylene protons, **GlcN3**) = 0.37 and [**GlcN6**] = integral at δ 3.87 (H-6a, **GlcN6**) = 1.00. {[**GlcN3**] + [**GlcN4**] + [**GlcN6**]} = integral at 4.46–4.43 (H-1s of three compounds) = 1.89. Thus, [**GlcN4**] = 1.89–1.37 = 0.52.

Xyl: Integrals of H-5 signals were used to obtain [**Xyl2**]:[**Xyl3**]:[**Xyl4**] = 1:3:2. Man: Integrals of H-1 signals were used to obtain [**Man2**]:[**Man3**]:[**Man4**]:[**Man6**] = 0.3:1:1.3:1.1.

Procedures

Phenyl 2-Azido-4,6-di-*O*-benzyl-2-deoxy-1-thio-β-D-galactopyranoside (GalN3), phenyl 2-azido-3,6-di-*O*-benzyl-2-deoxy-1-thio-β-D-galactopyranoside (GalN4), and phenyl 2-azido-3,4-di-*O*-benzyl-2-deoxy-1-thio-β-D-galactopyranoside (GalN6). To a solution of phenyl 2-azido-2-deoxy-1-thio-β-D-galactopyranoside (0.22 g, 0.75 mmol) dissolved in CH_2Cl_2 (50 mL) was added Bu_4NHSO_4 (0.051 g, 0.2 equiv.), 5% NaOH (3.0 mL, 5 equiv.), and BnBr (0.27 mL, 3 equiv.), and the resultant mixture was stirred under gentle reflux (50°C) overnight (18 hr). At that time, TLC indicated approximately 70% conversion. The organic layer was washed with water, dried with Na_2SO_4, and concentrated to dryness under vaccum. The residue was subjected to a preparative TLC (6:1 toluene-EtOAc) to obtain dibenzylated compounds, of which ^{1}H NMR and COSY analyses revealed the ratio of **GalN3**/**GalN4**/**GalN6** to be 1.4/3.6/1. Purification of the reaction mixture on silica gel column chromatography using toluene-EtOAc (30:1) afforded tribenzyl compound (50 mg, 13%), dibenzylated compounds, **GalN3** (56 mg, 17%), **GalN4** (120 mg, 36%), and **GalN6** (43 mg, 13%), and a mixture of monobenzylated compounds (37 mg, 14%).

GalN3: m.p. 54.5–55.5°C; $[\alpha]_D$+17.2°C (c = 1.02, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 138.0–127.6 (aromatic C), 86.5 (*C*-1), 77.4 (*C*-3), 75.2 (*C*-4), 75.1 (Ph*C*H$_2$), 74.2 (*C*-5), 73.6 (Ph*C*H$_2$), 68.1 (*C*-6), and 63.4 (*C*-2).

Anal. Calcd for $C_{26}H_{27}N_3O_4S$ (477.58): C, 65.39; H, 5.70; N, 8.80. Found: C, 65.42; H, 5.76; N, 8.72.

GalN4: m.p. 77–78°C; $[\alpha]_D$ −30.5°C (c = 0.96, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 137.7–127.7 (aromatic carbons), 86.2 (*C*-1), 81.0 (*C*-3), 77.0 (*C*-5), 73.6 and 71.9 (Ph*C*H$_2$ × 2), 69.3 (*C*-6), 65.5 (*C*-4), and 60.8 (*C*-2).

Anal. Calcd for $C_{26}H_{27}N_3O_4S$ (477.58): C, 65.39; H, 5.70; N, 8.80. Found: C, 65.32; H, 5.80; N, 8.56.

GalN6: m.p. 80.5–82°C; $[\alpha]_D$ −21.5°C (c = 1.03, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 138.0–127.8 (aromatic carbons), 86.4 (*C*-1), 82.6 (*C*-3), 78.9 (*C*-5), 74.1, and 72.7 (Ph*C*H_2 × 2), 71.8 (*C*-4), 62.1 (*C*-6), and 61.6 (*C*-2).

Anal. Calcd for $C_{26}H_{27}N_3O_4S$ (477.58): C, 65.39; H, 5.70; N, 8.80. Found: C, 65.53; H, 5.84; N, 8.62.

Phenyl 2-Azido-4,6-di-*O*-benzyl-2-deoxy-1-thio-β-D-glucopyranoside (GlcN3), phenyl 2-azido-3,6-di-*O*-benzyl-2-deoxy-1-thio-β-D-glucopyranoside (GlcN4), and phenyl 2-azido-3,4-di-*O*-benzyl-2-deoxy-1-thio-β-D-glucopyranoside (GlcN6). To a solution of phenyl 2-azido-2-deoxy-1-thio-β-D-glucopyranoside (1.1 g, 3.8 mmol) dissolved in CH_2Cl_2 (50 mL) was added Bu_4NHSO_4 (0.26 g, 0.2 equiv.), 5% NaOH (15 mL, 5 equiv.), and BnBr (1.3 mL, 3 equiv.), and the resultant mixture was stirred under gentle reflux (50°C) overnight (15 hr). At that time, TLC indicated approximately 70% conversion. The organic layer was washed with water, dried with Na_2SO_4, and concentrated to dryness under vaccum. The residue was subjected to a preparative TLC (6:1 toluene-EtOAc) to obtain dibenzylated compounds, of which ^1H NMR and COSY analyses revealed the ratio of **GlcN3**/**GlcN4**/**GlcN6** to be 1/1.5/2.8. Purification of the reaction mixture on silica gel column chromatography using toluene-EtOAc (30:1) afforded tribenzyl compound (17%), **GlcN3** (13%), a mixture of **GlcN4** and **GlcN6** (54%), and a mixture of monobenzylated compounds (14%). A mixture of **GlcN4** and **GlcN6** (0.98 g, 2.1 mmol) was then dissolved in DMF (20 mL). To this solution, TBDMS-Cl (464 mg, 1.5 equiv.), triethyamine (0.87 mL, 3 equiv.), and DMAP (0.12 mg, 0.5 equiv.) were added and the resultant mixture was stirred for 30 min at rt. The mixture was diluted with EtOAc and the organic layer was washed with water, dried over Na_2SO_4, and concentrated to dryness under vacuum. The resultant residue was subjected to silica gel column chromatography and eluted with toluene-EtOAc (30:1) to afford **GlcN4**[24] (17%) and TBDMS-derivative of **GlcN6**. The latter dissolved in THF (14 mL) was added to tetra-*n*-butylammonium fluoride (TBAF, 1 mL, 2.5 equiv.) and the mixture was stirred at rt for 1 hr. After evaporation of the solvent, purification of the residue on silica gel chromatography using toluene-EtOAc (20:1) afforded **GlcN6**[25] (37%).

GlcN3: $[\alpha]_D$ −31.4°C (c = 1.01, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 138.2–127.7 (aromatic carbons), 86.1 (*C*-1), 79.1 (*C*-5), 77.3 (*C*-3), 77.3 (*C*-4), 74.8 and 73.5 (Ph*C*H_2 × 2), 68.8 (*C*-6), and 65.0 (*C*-2).

Anal. Calcd for $C_{26}H_{27}N_3O_4S$ (477.58): C, 65.39; H, 5.70; N, 8.80. Found: C, 65.33; H, 5.71; N, 8.70.

Phenyl 3,4-di-*O*-benzyl-1-thio-β-D-xylopyranoside (Xyl2), phenyl 2,4-di-*O*-benzyl-1-thio-β-D-xylopyranoside (Xyl3), and phenyl 2,3-di-*O*-benzyl-1-thio-β-D-xylopyranoside (Xyl4). To a solution of phenyl 1-thio-β-D-xylopyranoside (0.20 g, 0.83 mmol) dissolved in CH_2Cl_2 (10 mL) was added Bu_4NHSO_4 (0.056 g, 0.2 equiv.), 5% NaOH (3.0 mL, 4.5 equiv.), and BnBr

(0.23 mL, 2.3 equiv.), and the resultant mixture was stirred under gentle reflux (50°C) overnight (18 hr). At that time, TLC indicated approximately 70% conversion. The organic layer was washed with water, dried with Na_2SO_4, and concentrated to dryness under vaccum. The residue was subjected to a preparative TLC (6:1 toluene-EtOAc) to obtain dibenzylated compounds, of which ^{1}H NMR and COSY analyses revealed the ratio of **Xyl2**/**Xyl3**/**Xyl4** to be 1/3/2. Purification of the reaction mixture on silica gel column chromatography using toluene-EtOAc (30:1) afforded tribenzyl compound (9.2%), dibenzylated compounds, **Xyl2** (11%), **Xyl3** (34%), and **Xyl4** (25%), and a mixture of monobenzylated compounds (8.3%).

Xyl2: m.p. 73–74°C; $[\alpha]_D$ −127°C ($c = 0.99$, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 131.9–127.6 (aromatic carbons), 88.9 (*C*-1), 79.2 (*C*-3), 75.9 (*C*-4), 73.9 and 72.4 (Ph*C*H$_2$ × 2), 70.8 (*C*-2), and 63.4 (*C*-5).

Anal. Calcd for $C_{25}H_{26}O_4S$ (422.54): C, 71.06; H, 6.20. Found: C, 71.26; H, 6.28.

Xyl3: m.p. 80.5–81.5°C; $[\alpha]_D$ −15.3°C ($c = 1.00$, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 138.1–127.7 (aromatic carbons), 88.1 (*C*-1), 80.5 (*C*-2), 77.9 (*C*-3), 77.2 (*C*-4), 75.2 and 73.1 (Ph*C*H$_2$ × 2), and 67.5 (*C*-5).

Anal. Calcd for $C_{25}H_{26}O_4S$ (422.54): C, 71.06; H, 6.20. Found: C, 71.16; H, 6.03.

Xyl4: m.p. 81.5–82°C; $[\alpha]_D$ −76.2°C ($c = 1.02$, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 131.1–127.2 (aromatic carbons), 86.7 (*C*-1), 77.8 (*C*-3), 77.7 (*C*-2), 73.7 and 73.4 (Ph*C*H$_2$ × 2), 68.0 (*C*-4), and 64.6 (*C*-5).

Anal. Calcd for $C_{25}H_{26}O_4S$ (422.54): C, 71.06; H, 6.20. Found: C, 71.06; H, 6.26.

Phenyl 3,4,6-Tri-*O*-benzyl-1-thio-β-D-galactopyranoside (Gal2), phenyl 2,4,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (Gal3), phenyl 2,3,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (Gal4), and phenyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (Gal6). To a solution of phenyl 1-thio-β-D-galactopyranoside (0.20 g, 0.74 mmol) dissolved in CH_2Cl_2 (10 mL) was added Bu_4NHSO_4 (51 mg, 0.2 equiv.), 5% NaOH (4.1 mL, 7 equiv.), and BnBr (0.44 mL, 5 equiv.), and the resultant mixture was stirred under gentle reflux (50°C) overnight (16 hr). More BnBr (1 equiv.) was added and stirred overnight. Further addition of 5% NaOH and BnBr (each 1 equiv. × 2) every 24 hr resulted in about 40% formation of tribenzylated compounds. The organic layer was washed with water, dried with Na_2SO_4, and concentrated to dryness under vaccum. The residue was subjected to a preparative TLC (6:1 toluene-EtOAc) to obtain tribenzylated compounds, of which ^{1}H NMR and COSY analyses revealed the ratio of **Gal2**/**Gal3**/**Gal4**/**Gal6** to be 1.2/6.2/5.1/1. Purification of the reaction mixture on silica gel column chromatography using toluene-EtOAc (30:1) afforded tetrabenzyl compound (16%), a mixture of **Gal2**, **Gal3** and **Gal4** (30%), **Gal6** (4.5%), a mixture of dibenzylated

compounds (20%), and a mixture of monobenzylated compounds (4%). A mixture of tribenzyl compounds was separated by HPLC (Column: Inert SIL C8-3, 5 μm [4.6 × 150 mm; GL Sciences Inc.]; Flow: 1.0 mL/min; Elution: Gradient from hexane-EtOH (99:1) to hexane-EtOH (50:50) during 60 min; Detection: 250 nm). Retension time for each compound was as follows: **Gal2**[26] (6.0 min), **Gal3**[17] (6.4 min), **Gal4**[27] (6.5 min), and **Gal6**[28] (10 min).

Phenyl 3,4,6-Tri-*O*-benzyl-1-thio-β-D-glucopyranoside (Glc2), phenyl 2,4,6-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (Glc3), phenyl 2,3,6-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (Glc4), and phenyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (Glc6). To a solution of phenyl 1-thio-*β*-D-glucopyranoside (0.20 g, 0.74 mmol) dissolved in CH_2Cl_2 (10 mL) was added Bu_4NHSO_4 (51 mg, 0.2 equiv.), 5% NaOH (4.1 mL, 7 equiv.), and BnBr (0.44 mL, 5 equiv.), and the resultant mixture was stirred under gentle reflux (50°C) overnight (16 hr). More BnBr (1 equiv.) was added and stirred overnight. Further addition of 5% NaOH and BnBr (each 1 equiv. × 2) every 24 hr resulted in about 40% formation of tribenzylated compounds. The organic layer was washed with water, dried with Na_2SO_4, and concentrated to dryness under vaccum. The residue was subjected to a preparative TLC (6:1 toluene-EtOAc) to obtain tribenzylated compounds, of which ^{1}H NMR and COSY analyses revealed the ratio of **Glc2**/**Glc3**/**Glc4**/**Glc6** to be 1/4.8/1.1/3.6. Purification of the reaction mixture on silica gel column chromatography using toluene-EtOAc (30:1) afforded tetrabenzyl compound (20%), a mixture of **Glc2** and **Glc3** (27%), a mixture of **Glc4** and **Glc6** (21%), and a mixture of dibenzylated compounds (19%). A mixture of tribenzyl compounds was separated by HPLC (Column: Inert SIL C8-3, 5 μm [4.6 × 150 mm; GL Sciences Inc.]; Flow: 1.0 mL/min; Elution: Hexane-EtOH (99:1); Detection: 250 nm). Retension times for each compound were as follows; **Glc2**[29] (5.0 min), **Glc3** (4.8 min), **Glc4**[30] (7.8 min), and **Glc6**[31] (6.8 min).

Glc3: m.p. 89.5–90°C; $[\alpha]_D$ −6.56°C (c = 1.02, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 138.2–127.5 (aromatic carbons), 87.1 (*C*-1), 80.6 (*C*-2), 78.8 (*C*-5), 78.7 (*C*-3), 77.4 (*C*-4), 75.2, 74.7 and 73.5 (Ph*C*H_2 × 3), and 69.1 (*C*-6).

Anal. Calcd for $C_{33}H_{34}O_5S$ (542.69): C, 73.04; H, 6.31. Found: C, 73.27; H, 6.16.

Phenyl 3,4,6-Tri-*O*-benzyl-1-thio-α-D-mannopyranoside (Man2), phenyl 2,4,6-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (Man3), phenyl 2,3,6-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (Man4), and phenyl 2,3,4-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (Man6). To a solution of phenyl 1-thio-*α*-D-mannopyranoside (0.20 g, 0.74 mmol) dissolved in CH_2Cl_2 (10 mL) was added Bu_4NHSO_4 (0.051 g, 0.2 equiv.), 5% NaOH (4.1 mL, 7 equiv.), and BnBr (0.44 mL, 5 equiv.), and the resulted mixture was stirred under gentle reflux (50°C) overnight (16 hr). More BnBr (1 equiv.) was added and stirred overnight. Further addition of 5% NaOH and BnBr (each 1 equiv. × 2) every 24 hr

resulted in about 40% formation of tribenzylated compounds. The organic layer was washed with water, dried with Na_2SO_4, and concentrated to dryness under vaccum. The residue was subjected to a preparative TLC (6:1 toluene-EtOAc) to obtain tribenzylated compounds, of which ^{1}H NMR and COSY analyses revealed the ratio of **Man2**/**Man3**/**Man4**/**Man6** to be 0.3/1/1.3/1.1. Purification of the reaction mixture on silica gel column chromatography using toluene-EtOAc (30:1) afforded tetrabenzyl compound (8.6%), **Man2**[32] (1.1%), **Man3**[33] (20%), **Man4**[34] (23%), **Man6**[35] (21%), a mixture of dibenzylated compounds (9.6%), and a mixture of monobenzylated compounds (10%).

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