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α-Selective Glycosylation with 5-Thioglucopyranosyl Donors; Synthesis of an IsoMaltotetraoside Mimic Composed of 5-Thioglucopyranose Units

Hiroko Matsuda,[b] Keiichiro Ohara,[a] Yasuharu Morii,[a] Masaru Hashimoto,[a,b,*] Kazuo Miyairi[a,b] and Toshikatsu Okuno[a,b]

[a]*Faculty of Agriculture and Life Science, Hirosaki University, Bunkyo-Cho 3, Hirosaki 036-8561, Japan*
[b]*The United Graduate School of Agricultural Sciences, Iwate University, Bunkyo-Cho 3, Hirosaki 036-8561, Japan*

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Abstract—A general method for α-selective glycosylation with 5-thioglucopyranosyl donors followed by efficient deprotection of the resulting products was developed. This methodology was utilized in the synthesis of an isomaltotetraoside analogue.

Carbomimetics, analogues which closely mimic carbohydrate structure, are predicted to be excellent probes not only for the investigation of the details of enzymatic reaction mechanisms that involve specific protein–sugar interactions, but also as novel drugs for some digestive or infective diseases.[1,2] Many saccharide analogues have been reported[3–5] and most of these have focused on *exo*-type glycosidases that hydrolyze the terminal glycosidic bond through recognition of the terminal carbohydrate subsite.[6] However, in the case of *endo*-type glycosidases that cleave internal glycosidic linkages, the development of enzyme inhibitors requires that oligomeric structures be prepared since *endo*-glycosidases utilize more widely dispersed subsites for recognition. Due to the increased complexity of substrate binding for *endo*-glycosidases, an inhibitor of these enzymes generally must incorporate additional functionality beyond that present at the cleavage site. Thus, mechanistic studies of *endo*-glycosidases are relatively less advanced than those on *exo*-glycosidases due to an absence of effective inhibitors of the *endo*-type enzymes.[7] Recently, oligosaccharide mimics containing *C*-glycoside linkages have been developed as *endo*-glycosidase inhibitors.[8] Since the interaction between amino acid residues and glycosidic oxygens in the enzyme–substrate complex must be considered, we anticipated that sulfur substituted saccharides would provide promising inhibitors of *endo*-glycosidases. However, the increased length of the carbon–sulfur bond compared to that of a carbon–oxygen bond could lead to a significantly altered molecular display of the atoms directly involved in enzyme binding. The oligomerization of a series of sulfur-substituted monosaccharides might result in substantial alteration in molecular size when the acetalic oxygen is replaced with sulfur. For example, as shown in Figure 1, molecular

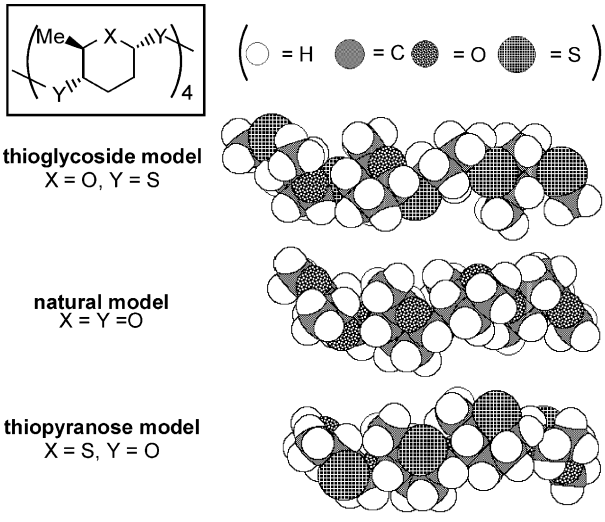

Figure 1. Comparison of a 'natural' oligosaccharide model with models of analogues containing thioglycosides and thiopyranoses.

*Corresponding author. Fax: +81-172-39-3782; e-mail: hmasaru@cc.hirosaki-u.ac.jp

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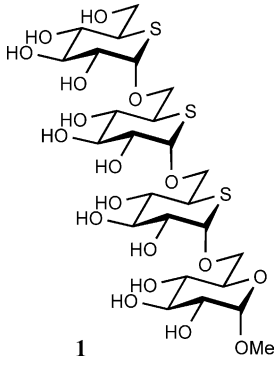
modeling calculations indicate that a tetramer of an α-(1→4)-glycan with thioglycosidic linkages ('thioglycoside' model) results in a more extended molecular structure as compared with that of a 'natural' tetrasaccharide.[9] *Endo*-glycosidases may no longer be capable of binding this type of analogue. However, as supported by similar molecular modeling studies, a tetramer of 1,4-linked thiopyranoses ('thiopyranose' model, Fig. 1) may better absorb the deviations of bond lengths and bond angles between ethers and thioethers, thereby minimizing structural change. Accordingly, oligothiosaccharides may be potential inhibitors of *endo*-glycosidases. In this report, we describe a general method for the α-selective glycosylation with 5-thioglucopyranosyl donors and an efficient deprotection protocol of the polysaccharide products. Utilizing this methodology, the synthesis of sulfur-substituted isomaltotetraoside **1** is also described.



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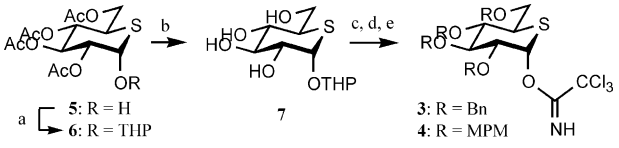
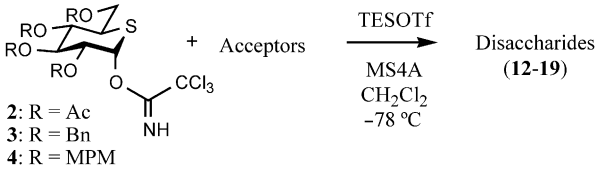
Several groups have reported extensive efforts directed at glycosylation reactions with 5-thiopyranosyl donors. Those reports typically employed *O*-acetyl-protected imidate **2** as the glycosyl donor with various promoters. However, in these examples, both the yields and the stereoselectivity of the reactions were dependent on the glycosyl acceptor.[10–13] For example, Mehta et al. disclosed the glycosylation in 80% yield but with low selectivity (α/β = 1.5:1),[11] while Izumi et al. obtained the α-disaccharide exclusively in only 22% yield.[12] In our preliminary experiments, we also examined the glycosylation using donor **2**, but the yields were quite low under many different reaction conditions (e.g., Run 1 and 2, Table 1). A general and highly stereoselective glycosylation reaction was required for the synthesis of oligothiosaccharides. Thus, we first investigated a general glycosylation reaction employing thioglucopyranosyl donors **3** and **4**.

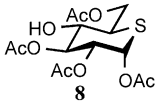
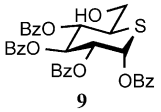
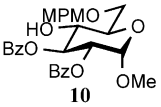
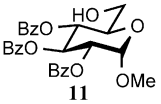


Scheme 1. (a) DHP, *p*-TsOH, CH_2Cl_2 (95%); (b) NaOMe, MeOH (87%); (c) BnBr (88% for **3**) or MPMBr (95% for **4**), NaH, DMF; (d) H^+, MeOH (90% for **3**, 81% for **4**); (e) CCl_3CN, DBU, CH_2Cl_2, 0 °C (57% for **3**, 74% for **4**).

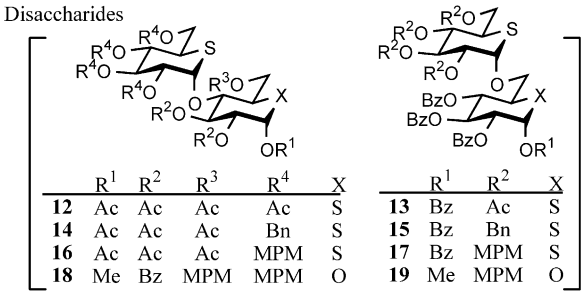
Donors **3** and **4** were prepared by standard methodology as shown in Scheme 1. It was found that *O*-benzylprotected donor **3** gave glycosides **14** and **15** in good yields by treating **3** with acceptors **8** and **9**, respectively, along with a catalytic amount of TESOTf (0.05–0.1 equiv) at −78 °C. The ^1H NMR spectra of **14** and **15** revealed that both glycosylations proceeded in high α-stereoselectivity (>98:2). Similarly, *O*-(4-methoxyphenyl)methyl (MPM) protected donor **4** provided adducts **16–19** in a highly α-stereoselective manner even though the yields were slightly lower than that of the reactions with donor **3**. In these last examples, a small amount of relatively polar material was observed by TLC analysis, suggesting that some MPM groups were cleaved during the reactions.

Table 1. Glycosylation with 5-thioglucopyranosyl donors



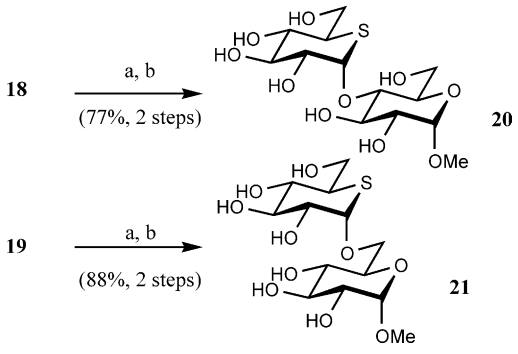
Run	Donors	Acceptors	Disaccharides (%)	α/β[a]
1	**2**	 **8**	**12** (5%)	>95:5[b]
2	**2**	 **9**	**13** (10%)	>95:5[b]
3	**3**	**8**	**14** (94%)	>98:2[b]
4	**3**	**9**	**15** (81%)	>98:2[b]
5	**4**	**8**	**16** (60%)	94:6
6	**4**	**9**	**17** (94%)	>98:2[b]
7	**4**	 **10**	**18** (87%)	>98:2[b]
8	**4**	 **11**	**19** (82%)	88:12

[a]The ratios were estimated by 400 MHz ^1H NMR spectra.
[b]The β-isomers were not detected by 400 MHz ^1H NMR spectra.



	R^1	R^2	R^3	R^4	X
12	Ac	Ac	Ac	Ac	S
14	Ac	Ac	Ac	Bn	S
16	Ac	Ac	Ac	MPM	S
18	Me	Bz	MPM	MPM	O

	R^1	R^2	X
13	Bz	Ac	S
15	Bz	Bn	S
17	Bz	MPM	S
19	Me	MPM	O



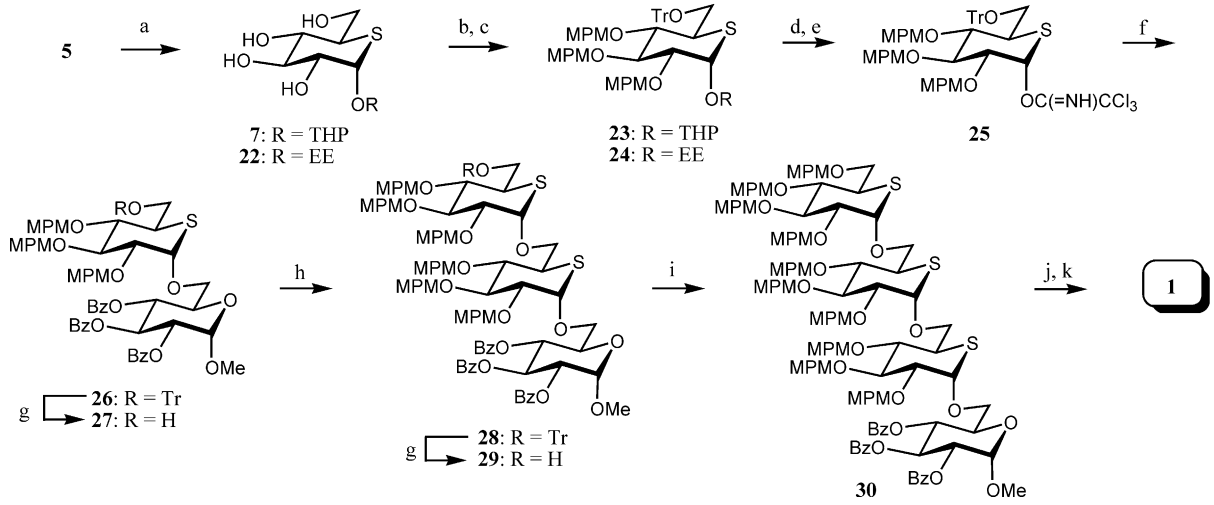
Scheme 2. (a) NaOMe, MeOH; (b) DDQ, aq CH_2Cl_2.

With disaccharides **14**–**19** in hand, deprotection reactions were next attempted. Although glycosylation employing donor **3** was effective, hydrogenolysis of the *O*-benzyl ethers in either **14** or **15** was unsuccessful, providing a number of partially deprotected alcohols. The sluggishness of this reaction was most likely due to poisoning of the palladium catalyst by the sulfide functionality.[11,14]

Next, alternative deprotection methods were examined using MPM-masked disaccharides **16**–**19**. Treatment with excess 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in aqueous CH_2Cl_2[15] appeared to smoothly deprotect the MPM group based on TLC analysis. However, the resulting alcohols could not be separated by silica gel chromotography from the corresponding hydroquinone that was generated in large quantity during the reaction. Acetylation of the crude reaction products (Ac_2O in pyridine) resulted in a complex mixture. Deprotection of disaccharides **18** and **19** could be achieved by changing the order of the deprotection reactions (Scheme 2). After the benzoyl groups were removed by treatment with sodium methoxide in methanol, the resulting products were further deprotected with DDQ. Efficient cleavage of all of the MPM groups provided methyl 5′-thio-α-maltoside (**20**) and methyl 5′-thio-α-isomaltoside (**21**) in 77 and 88% yield, respectively, over two steps.[16] In each of these cases, the hydroquinone derivative could be separated easily by column chromatography because of the increased polarity of the products. Oxidation of the thioacetal moiety with DDQ was not observed. This was confirmed by observing the molecular ion peaks of both **20** and **21** at $m/z = 371$ ($[M-H]^-$) by FAB-MS (negative mode). In contrast, the same operations for **16** and **17** resulted only in decomposition of the substrates and/or products, suggesting that the C1 position of the thioglucose moiety must be protected for DDQ treatment.

With effective glycosylation and deprotection protocols in hand, a synthesis of isomaltotetraose mimic **1** was performed (Scheme 3). Glycosyl donor **25** was prepared from **5**[12] by sequential (1) C1–OH protection as the tetrahydropyranyl (THP) (→**7**) or ethoxyethyl (EE) ethers (→**22**), (2) removal of all acetyl groups, (3) selective trityl ether formation of the primary alcohol, (4) protection of the remaining hydroxyl groups as MPM ethers (→**23**, **24**), (5) regeneration of the C1–OH, and (6) formation of the trichloroacetimidate[17] (→**25**). Acidic removal the THP group of **23** gave the alcohol in low yield (30%) due to simultaneous cleavage of the trityl ether. It was found that the more labile EE group of **24** was deprotected selectively by treatment with pyridinium *p*-toluenesulfonate (PPTS) in 92% yield.[18]

As expected, the glycosylation reaction of acceptor **11** with donor **25** proceeded smoothly, providing, α-disaccharide **26** in 67% yield along with a small amount of the β-isomer (7%), after chromatographic purification. After selective cleavage of the trityl ether under acidic conditions, the resulting alcohol **27** was subjected to a second glycosylation to afford the trisaccharide **28**. This material was further converted to tetrasaccharide **30** by similar treatment. Interestingly, formation of β-glycosides was not observed in the latter two glycosylation steps based on ^1H NMR spectroscopy. Finally,



Scheme 3. (a) For **22**; ethyl vinyl ether, PPTS, CH_2Cl_2, 0 °C (96%), then NaOMe, MeOH (86%); (b) TrCl, Py, DMAP (31% for **23**, 81% for **24**); (c) MPMBr, NaH, DMF (69% for **23**, 62% for **24**); (d) PPTS, MeOH (30% from **23**), or PPTS, EtOH–PrOH (92% from **24**); (e) CCl_3CN, DBU, CH_2Cl_2, 0 °C (81%); (f) **11**, TESOTf, MS4A, CH_2Cl_2, −78 °C (74%, α/β = 10:1); (g) *p*-TsOH, MeOH–THF (71% for **27**, 67% for **29**); (h) **25**, TESOTf, MS4A, CH_2Cl_2, −78 °C (47%); (i) **4**, TESOTf, MS4A, CH_2Cl_2, −78 °C (67%); (j) NaOMe, MeOH; (k) DDQ, aq CH_2Cl_2 (65%, two steps).

all protective groups of tetramer **30** were successfully removed by employing the methodology described above (NaOMe in methanol, followed by DDQ in aqueous CH_2Cl_2), giving free sulfur substituted methyl isomaltotetraoside **1** in 65% yield (two steps).[19] The tetrameric structure of **1** was confirmed by the observation of four signals of equatorial oriented anomeric protons at δ = 4.79, 4.82, 4.83, and 4.88 ppm (J = 2.9, 2.9, 2.9, and 3.9 Hz, respectively) in the ^{1}H NMR spectrum as well as the molecular ion peak at m/z = 727 ($[M-H]^-$) by FAB-MS (negative mode). Further, the ^{13}C NMR spectrum (25 carbons) fully supported the structure of **1**.

As described in this report, we have developed a general α-stereoselective glycosylation employing 5-thioglucopyranoses as well as a deprotection protocol that is applicable to the resulting oligothiosaccharides. Studies on the enzyme binding properties of **1** and the synthesis of additional oligothiosaccharides are under investigation in our laboratory.

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16. Spectral data, **20**: ^{1}H NMR (400 MHz, D_2O, 38 °C) δ 3.05 (1H, m, C′5*H*), 3.42 (3H, s, C1OCH_3), 3.59 (1H, dd, J = 3.9, 9.7 Hz, C2*H*), 3.62–3.67 (2H, C′3*H*, C′4*H*), 3.73–3.77 (2H, C4*H*, C5*H*), 3.82–3.85 (2H, C′2*H*, C6*H*), 3.88–3.90 (3H, C′6*H* ×2, C6*H*), 3.95 (1H, t, J = 9.7 Hz, C3*H*), 4.81 (1H, d, J = 3.9 Hz, C1*H*), 5.33 (1H, d, J = 3.4 Hz, C′1*H*). ^{13}C NMR (100 MHz, D_2O, 38 °C) δ 43.9 (C′5), 55.2 (C1O*C*H_3), 60.2 (C′6), 61.0 (C6), 70.2 (C5), 71.3 (C2), 73.4 (C′4), 73.9 (C′3), 74.1 (C3), 75.5 (C2), 75.6 (C4), 82.8 (C′1), 99.2 (C1). FAB-MS (%, rel. int.) 371 ($[M-H]^-$, 100): HR-MS (FAB, negative) calcd for $C_{13}H_{23}O_{10}S$ $[M-H]^-$: 371.1012, found m/z = 371.0975. **21**: ^{1}H NMR (400 MHz, D_2O, 38 °C) δ 3.10 (1H, ddd, J = 3.4, 4.9, 10.3 Hz, C′5*H*), 3.43 (3H, s, C1OCH_3), 3.49 (1H, dd, J = 9.0, 10.0 Hz, C4*H*), 3.57 (1H, dd, J = 3.9, 9.7 Hz, C2*H*), 3.64 (1H, dd, J = 8.8, 10.2 Hz, C′4*H*), 3.67 (1H, dd, J = 9.0, 9.7 Hz, C3*H*), 3.69 (1H, dd, J = 8.8, 9.3 Hz, C′3*H*), 3.72 (1H, dd, J = 2.0, 11.0 Hz, C6*H*), 3.83 (1H, m, C5*H*), 3.85 (1H, dd, J = 2.9, 9.3 Hz, C′2*H*), 3.87 (1H, dd, J = 3.4, 11.4 Hz, C′6*H*), 3.92 (1H, dd, J = 4.9, 11.4 Hz, C′6*H*), 4.13 (1H, dd, J = 4.6, 11.2 Hz, C6*H*), 4.77 (1H, d, J = 3.0 Hz, C′1*H*), 4.82 (1H, d, J = 3.9 Hz, C1*H*). ^{13}C NMR (100 MHz, D_2O, 38 °C) δ 43.1 (C′5), 55.3 (C1O*C*H_3), 60.2 (C′6), 66.6 (C6), 69.7 (C4), 70.2 (C5), 71.3 (C2), 73.5 (C′4), 73.6 (C3), 74.2 (C′3), 75.4 (C′2), 81.9 (C′1), 99.4 (C1). FAB-MS (%, rel. int.) 371 ($[M-H]^-$, 21), HR-MS (FAB, negative) calcd for $C_{13}H_{23}O_{10}S$ $[M-H]^-$: 371.1012, found m/z 371.1000.
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18. Deprotection of EE group in **24** was performed by sequential operations of (i) stirring in EtOH in the presence of PPTS for 30 min, (ii) evaporation of the mixture, and (iii) stirring for 2 h after dilution with PrOH. Substrate **24** was insoluble in EtOH. The reactions in PrOH or in a mixed solvent system (PrOH:EtOH = 3:1) were less effective.
19. A solution of **30** (11.5 mg, 5.10 μmol) in MeOH (2.0 mL) was stirred with NaOMe (2.5 mg, 46.3 μmol) at room temperature for 1 h. The mixture was neutralized by the addition of DOWEX 50W (H^+ form), filtered, and concentrated in vacuo. Without further purification, the residue was stirred with DDQ (46.2 mg, 204 μmol) in a mixture of CH_2Cl_2 (1.0 mL) and H_2O (100 μL) at room temperature for 12 h. After concentration, the residue was purified by silica gel column chromatography (MeOH/AcOEt = 40:60) to give **1** (2.4 mg, 65% in two steps). ^{1}H NMR (400 MHz, D_2O, 43 °C) δ 3.24 (1H, brddd, J = 3.4, 4.8, 9.8 Hz), 3.32–3.37 (1H, m), 3.39–3.44 (1H, m), 3.50 (3H, s), 3.56 (1H, t, J = 9.8 Hz), 3.64 (1H, dd, J = 3.9, 9.8 Hz), 3.67–3.79 (7H), 3.87–4.00 (9H), 4.10–4.17 (2H), 4.22 (1H, dd, J = 4.9, 9.8 Hz), 4.79 (1H, d, J = 2.9 Hz), 4.82 (1H, d, J = 2.9 Hz), 4.83 (1H, d, J = 2.9 Hz), 4.88 (1H, d, J = 3.9 Hz). ^{13}C NMR (100 MHz, D_2O) δ 40.8 (d), 40.9 (d), 43.3 (d), 55.4 (q), 66.7 (t), 60.3 (t), 66.9 (t), 67.0 (t), 69.8 (d), 70.2 (d), 71.4 (d), 73.6 (d), 73.7 (d), 74.23 (d), 74.25 (d), 74.31 (d), 74.32 (d), 74.34 (d), 75.28 (d), 75.34 (d), 75.40 (d), 81.68 (d), 81.71 (d), 81.92 (d), 99.5 (d). FAB-MS (%, rel. int.) 727 ($[M-H]^-$, 19), HR-MS (FAB, negative) calcd for $C_{25}H_{43}O_{18}S_3$ $[M-H]^-$: 727.1612, found m/z 727.1591.