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Transglucosylation with 6′-chloro-6′-deoxysucrose and immobilized isomaltulose-producing microorganisms using 2,2-dimethyl-1,3-dioxolane-4-methanol and its related compounds as acceptors. Steric and chemical requirement of the glucosyl acceptor

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Abstract

Enantioselective and diastereoselective α-D-glucosylation of 2,3-*O*-isopropylidene-erythritol was observed in transglucosylation with a synthetic donor using three kinds of immobilized isomaltulose-producing microorganisms. Several related compounds, including an 2,3-*O*-isopropylidenated aldotetrose dimethyl dithioacetal and an aldotetronic acid ester were also glucosylated in moderate or good yield, depending on the microorganism utilized. Steric as well as functional group factors are discussed in relation to the substrate specificity of the glucosyl acceptor.

1. Introduction

With the progress of glycobiology, studies on the substrate-recognizing mechanism of such glycoenzymes as glycosidases and glycosyltransferases have attracted attention, especially in order to design inhibitors, which are useful not only for perturbation [1] of the biosynthesis of oligosaccharide chains but also for direct elucidation of such carbohydrate-mediating recognition phenomena as fertilization [2] on the cell surface. In the field of synthetic organic chemistry, enzymes are

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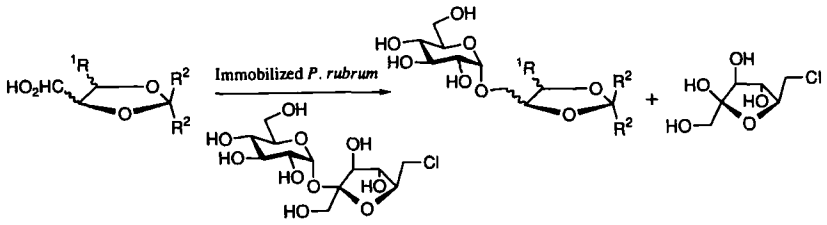
widely used as biochemical reagents [3] for enantioselective and diastereoselective conversions, and the former conversions provide a potential method of optical resolution. Here we examine the substrate specificity of an industrially used α-glucosyltransferase, using immobilized cells and various 2,2-dimethyl-1,3-dioxolane derivatives for the purpose of creating potential building blocks of glycoglycerol homologues.

Since the transformation of sucrose into 6-*O*-(α-D-glucopyranosyl)-D-fructose (isomaltulose) by *Protaminobacter rubrum* was discovered [4], several microorganisms such as *Serratia plymuthica* [5], and *Erwinia rhapontici* [6] have been reported to catalyze the same conversion. Isomaltulose is now industrially manufactured by immobilized cells [7,8] and used as a non-cariogenic agent [9] or, after hydrogenation [7], as low-caloric sweetner.

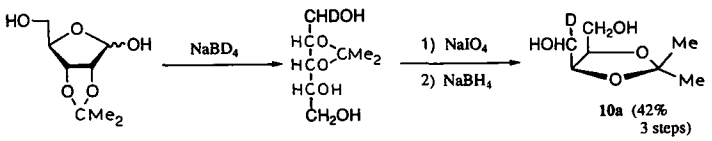
Transglucosylation was observed when a mixture of sucrose and D-arabinose was treated with *P. rubrum*, giving 5-*O*-(α-D-glucopyranosyl)-D-arabinose in 6% yield together with a large excess of isomaltulose [10]. In order to improve the efficiency of glucosyl transfer by preventing the formation of isomaltulose, 6′-chloro-6′-deoxysucrose (**1**), which has no hydroxyl group to be transglucosylated, was designed as an artificial donor and proved to be effective in conversion of several pentofuranosides to the corresponding 5-*O*-(α-D-glucopyranosyl)pentofuranosides by transglucosylation with **1** and immobilized *P. rubrum* [11a]. Among the furanosides tested, methyl β-D-arabinofuranoside (**3**) was the best acceptor, giving the corresponding disaccharide in 70% yield. Further, the partially purified enzyme obtained from *P. rubrum* transferred the α-D-glucopyranosyl moiety of *p*-nitrophenyl α-D-glucopyranoside to such ketoses as D-fructose and L-sorbose in 8–15% yield, but not to such aldoses as D-glucose, D-galactose, and D-arabinose [12]. The substrate specificity for the glucosyl donor was further examined using various 6′-substituted sucrose to reveal that 6′-deoxysucrose **2** as well as **1** have the highest efficiency [11b]. In this paper, the substrate specificity for the acceptor was elucidated using 2,2-dimethyl-1,3-dioxolane-4-methanol and related compounds.

2. Results and discussion

First of all, preliminary screening of several five-membered ring analogues of pentofuranosides demonstrated that a relatively wide structural allowance may be expected for the glucosyl acceptor of this transglucosylation. The glucosyltransfer from the donor **1** to the analogues was examined with immobilized *P. rubrum* (Scheme 1). In addition to the previously reported [11a] tetrahydrofurfuryl alcohol (**4**), 4-hydroxymethyl-1,3-dioxolane (**5a**), and 2,2-dimethyl-4-hydroxymethyl-1,3-dioxolane (1,2-*O*-isopropylideneglycerol, **6a**), were glucosylated to give the corresponding *O*-(α-D-glucopyranosyl)derivatives in 57 and 38% yields, respectively (Table 1, entries 1 and 2). Whereas no enantioselectivity was observed in the case of **4**, the 1,3-dioxolanes **5a** and **6a** were glycosylated enantioselectively with the enantiomeric ratios of 3:1 and 1:0, respectively. Although in the former case the absolute configuration of the predominant isomer of **5b** was not determined, the



Scheme 1.



Scheme 2.

configuration of glucosylated **6a** was elucidated to be *S*. The configuration was determined by the specific rotation of **6a** reformed by the action of α-glucosidase from yeast. It is noteworthy that both the enantiomers of **6a**: (*S*)-**6a** (**6aS**) and (*R*)-**6a**(**6aR**) were glucosylated (entries 3 and 4), although only the former was glucosylated from a racemic mixture as just described [2].

The finding that 1,2-*O*-isopropylideneglycerol was enantioselectively glucosylated by this α-glucosyltransferase of *P. rubrum*, prompted us to study its substrate specificity, using 2,3-*O*-isopropylidene-tetritols and related compounds as glucosyl acceptors. The glucosyl donor **1** was used in the presence of the immobilized cells just described.

Three examples of 2,3-*O*-isopropylidene-tetritols, having the L-*threo* (**7a**), D-*threo* (**8a**), and *erythro* [meso] (**9a**) [13] configurations were examined in order to elucidate the steric requirement for this α-D-glucosylation. The 1-deuterio analogue of **9a** (**10a**) was also prepared (Scheme 2) for determination of the position of glucosylation. Reduction of the readily available 2,3-*O*-isopropylidene-D-ribose with sodium borodeuteride, followed by periodate oxidation and sodium borohydride reduction gave **10a** in 42% yield (3 steps). Next, the more-polar or functionalized analogues **11a**–**18a**, in which one of the hydroxymethyl groups is substituted with such functional groups as carbamoyl and methoxycarbonyl, were also prepared as the glucosyl acceptors.

The methyl 2,3-*O*-isopropylidene-L- (**11a**) and -D-threonate (**15a**) were prepared from 2,3-*O*-isopropylidene-L- (**7a**) and -D-threitol (**8a**), respectively, in better yields as compared with the previous preparations [14] from dimethyl 2,3-*O*-isopropyli-

[2] The structure of **6aS** is so depicted that the hydroxyl group to be glucosylated is located in a similar position to that of the best acceptor **3**, to demonstrate the structural requirement for the acceptor. This depiction applies to the other acceptors examined in this paper.

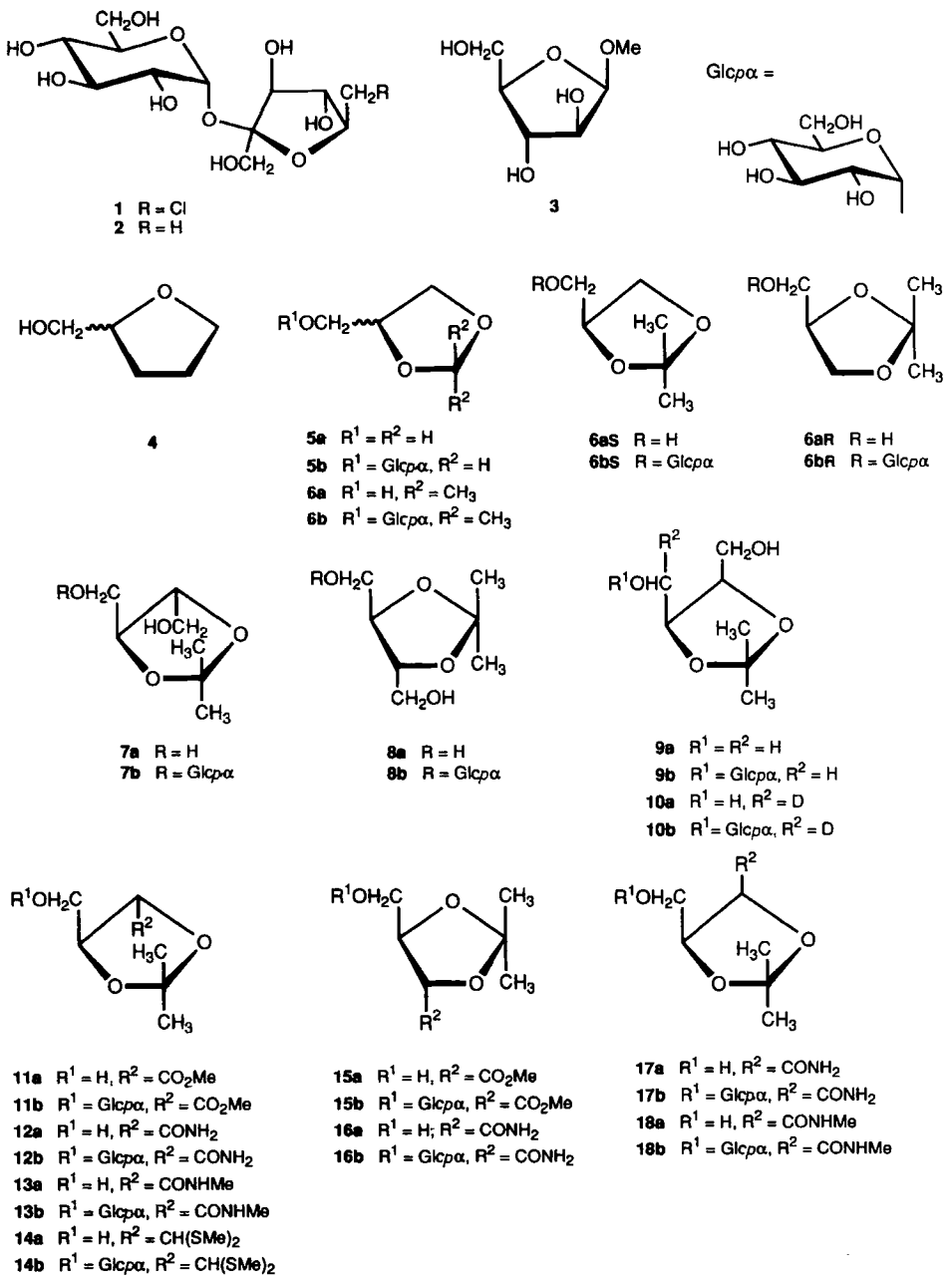
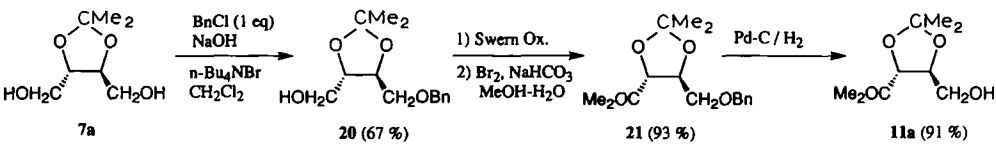


Fig. 1. Donors, acceptors, and products of transglycosylation.

dene-L- and -D-tartarate. Monobenzylation [15] of **7a**, followed by Swern and bromine [16] oxidations and *O*-debenzylation, afforded **11a** in ~55% overall yield (Scheme 3). The enantiomer **15a** was prepared from **8a** by the same methods. The



Scheme 3.

methyl threonates **11a** and **15a** were further converted into the corresponding threonamides, **12a** and **16a**, and the *N*-methylthreonamide **13a**, by aminolysis with ammonia and methylamine, respectively in excellent yields.

The 2,3-*O*-isopropylidene-D-erythronamide **17a** and its *N*-methyl analogue **18a** were also prepared by aminolysis of the readily available 2,3-*O*-isopropylidene-L-erythrono-1,4-lactone.

The 2,3-*O*-isopropylidene-tetritols of L-*threo* **7a** and D-*threo* **8a** configurations were both glucosylated in moderate yields (Table 1). In contrast to **6a**, no enantioselectivity was observed when a 1:1 mixture of **7a** and **8a** as the acceptor was subjected to transglucosylation (entry 7). The *erythro* isomer **9a** proved to be a good acceptor, showing a perfect diastereoselectivity to give the 1-*O*-glucosylated D-erythritol derivative **9b** exclusively. The glucosylated position was elucidated by the ^{13}C NMR spectrum of the 1-deuterated analogue of **9b** (**10b**), obtained by microbial glucosylation of **10a**. The deuterated hydroxymethyl carbon, readily assigned by its triplet signal, shows a downfield shift of 6.5 ppm through glucosylation (Fig. 2).

Table 1
Transglucosylation [a] of 4-hydroxymethyl-1,3-dioxolane **5a** and its homologue **6a–10a** with immobilized *P. rubrum*

Entry	Acceptor	Product	Yield (%)	Enantioselectivity or diastereoselectivity
1	**5a**	**5b**	57	3:1 [b]
2	**6a**	**6bS**	38	1:0 [b]
3	**6aS**	**6bS**	47 [d]	
4	**6aR**	**6bR**	32 [d]	
5	**7a**	**7b**	49	
6	**8a**	**8b**	43	
7	**7a**+**8a** (1:1)	**7b**+**8b**	52	1:1 [b]
8	**9a**	**9b**	54	1:0 [c]
9	**10a**	**10b**	70	1:0 [c]

[a] Transglycosylation was carried out in a 0.3 M solution of glycosyl donor **1**, and glycosyl acceptor using the immobilized *P. rubrum* in calcium propionate buffer (pH 5.5) at 37°C for 24 h (entries 1–4) or 72 h (entries 5–9).
[b] Enantioselectivity.
[c] Diastereoselectivity.
[d] Estimated by HPLC [11b].

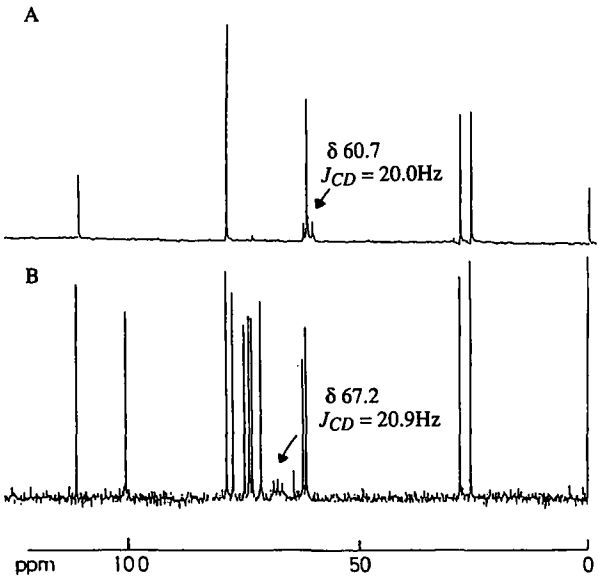


Fig. 2. ^{13}C NMR spectra of **10a** (A) and **10b** (B).

Transglucosylations using 2,3-*O*-isopropylidene-tetronates and related compounds as acceptors are summarized in Table 2. As shown in glucosylation with *P. rubrum* (entries 1, 7, 12, and 13), these 1,3-dioxolanes having methoxycarbonyl, carbamoyl, and dimethylthiomethyl substituent groups were found to be poorer

Table 2
Transglucosylation [a] of 2,3-*O*-isopropylidene-tetronates **11a,15a**, -tetronamides **12a,13a,16a–18a**, and dimethyl dithioacetal derivative **14a**

Entry	Glucosyl acceptor	Immobilized cell	Glucosylated product (%)	Glucose [c] (%)
1	**11a**	*P. rubrum*	**11h** 15	trace
2	**11a**	*S. plymuthica*	**11b** 7	0
3	**11a**	*E. rhapontici*	**11b** 39	14
4 [b]	**11a**	*E. rhapontici*	**11b** 69	
5	**12a**	*E. rhapontici*	**12b** 11	37
6	**13a**	*E. rhapontici*	**13b** 7	33
7 [b]	**14a**	*P. rubrum*	**14b** 27	
8 [b]	**14a**	*S. plymuthica*	**14b** 14	0
9 [b]	**14a**	*E. rhapontici*	**14b** 60	37
10	**15a**	*E. rhapontici*	**15b** 2	32
11	**16a**	*E. rhapontici*	**16b** 0	
12	**17a**	*P. rubrum*	**17b** 7	50
13	**18a**	*P. rubrum*	**18b** 0	57

[a] Under the same conditions as described in Table 1.
[b] The ratio of the donor **1** and the acceptor was 3.
[c] Produced by hydrolysis of **1**.

acceptors. The α-glucosyltransferase of another microorganism, *S. plymuthica* gave similar or lower yields (entries 2 and 8). However, *E. rhapontici* showed a remarkable difference and gave the glucosylated products in moderate to good yields (entries 3 and 9). The difference in steric tolerance of the acceptor-binding site of the α-glucosyltransferases of the three immobilized cells is demonstrated by transglucosylation using **11a** and **14a** as acceptors (entries 1, 2, and 3). The immobilized *E. rhapontici* gave the highest yields for both the acceptors.

In contrast to the corresponding tetritols, a distinct difference in substrate specificity between the two *threo* tetronate enantiomers was observed. Although the L-*threo* isomer **11a** was glucosylated to give **11b** in a moderate yield, the glucosylated D-*threo* isomer **15b** was produced in only very low yield. The corresponding erythronate could not be prepared because of spontaneous formation of the lactone.

From preparative and practical points of view, it is noteworthy that the yield of glucosylated product could be increased considerably by using an excess of the donor. As shown in entry 4, the yield was increased from 39 up to 69% by using 3 molar equivalents of **1**. The 2,3-*O*-isopropylidenetetronamide derivatives **12a**, **13a**, **16a**, **17a**, and **18a** proved to be poor acceptors, indicating that the basic functionality may retard the transglucosylation. The bulkiness of the carbamoyl groups does not seems to be crucial, because the more bulky dithioacetal **14a** [17] was glucosylated.

In the case of poor acceptors, the major byproduct is glucose produced by hydrolysis of **1** or 6-chloro-6-deoxy-3-*O*-(α-D-glucopyranosyl)-D-fructose. While the α-glucosyltransferase produced by *P. rubrum* converts sucrose mainly into isomaltulose (~ 80%), 1-*O*-(α-D-glucopyranosyl)-D-fructose (trehalulose) is also formed in ~ 10% yield. Nevertheless, 6-chloro-6-deoxytrehalulose was not obtained in the foregoing transglucosylation. A slight increase in the ratio of isomaltulose to trehalulose due to immobilization [12] is not well understood.

To sum up, the following common features are indicated for the substrate specificity of the acceptor. When the structures of acceptors are shown by fixing the hydroxymethyl group to be glucosylated as depicted in Fig. 3, according to the example of the best pentofuranoside acceptor **3**, two possible orientations of a 1,3-dioxolane derivative may be represented by **6aS [A]** and **6aR [B]**. The former is preferred, although the latter is also a moderate acceptor. The ring oxygen atom of

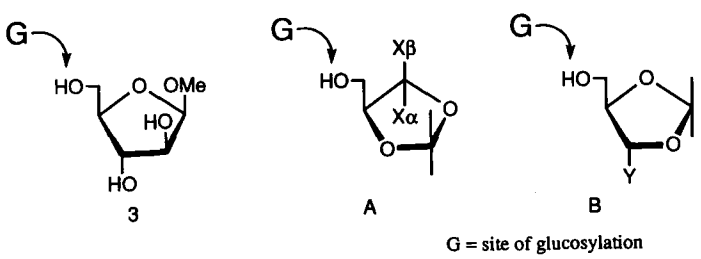


Fig. 3. Recognition of 1,3-dioxolane derivatives.

3 can be substituted with a β-face-oriented hydroxymethyl group. On the other hand, a carbamoyl group in the same position (Xβ) retards the transglucosylation. Further, for the space around Xα: the α-face of the ring oxygen in **3** seems to have electronic and steric tolerance for the glucosyltransferaseas, especially in the case of *E. rhapontici* as indicated by the results of **11a**, **12a**, and **14a**. The importance of the 3-hydroxyl group of **3** and Y of structure **B** are also suggested by the remarkable difference between **8a** and **15a** as well as **16a**. These results are expected to be closely related to the recognition of such glucosyl donors as **1**. Further elucidation of the substrate recognition is in progress.

3. Experimental

General methods.—Melting points were measured with a Yanagimoto MP apparatus and are uncorrected. Optical rotations were taken with JASCO DIP-4 polarimeter at 20 ± 5°C. ^{1}H NMR spectra were recorded with a JEOL PS-100 or JNM-GX-500 spectrometer. ^{13}C NMR spectra were recorded with a JEOL FX-90Q spectrometer for solutions in $CDCl_3$ (internal Me_4Si) or D_2O (external Me_4Si). Conventional and flash column chromatography were performed on Kieselgel 60 (Merck) and Wako gel C-300 (Wako) respectively. Preparative HPLC were performed on Lichroprep NH_2 (Merck) $\phi 21 \times 300$ mm. Chitopearl BCW 3000 was purchased from Fuji bouseki Co. Ltd. α-Glucosidase (EC 3.2.1.20) was purchased from Sigma. Glycerol formal (mixture of 1,3-dioxan-5-ol and 1,3-dioxolane-4-methanol) (**5a**), (±)-2,2-dimethyl-1,3-dioxolane-4-methanol (**6a**), (*S*)-(+)-2,2-dimethyl-1,3-dioxolane-4-methanol (**6aS**), (*R*)-(−)-2,2-dimethyl-1,3-dioxolane-4-methanol (**6aR**), 2,3-*O*-isopropylidene-L-threitol (**7a**), and 2,3-*O*-isopropylidene-D-threitol (**8a**) were purchased from Aldrich. Immobilized microbials were kindly donated by Mitsui Sugar Co. Ltd.

General procedure for enzymic glucosylation.—Method A. The reaction was performed on 0.833–2.78 mmol scale. To a solution of 6′-chloro-6′-deoxysucrose **1** (0.3 M) and an acceptor (0.3 M) in 20 mM calcium propionate buffer (pH 5.5) was added immobilized whole cells of *P. ruburum* (1% w/v), and the mixture was incubated with shaking for 72 h at 37°C. The mixture was filtered to remove the immobilized cells, and the filtrate was concentrated in vacuo. The residue was chromatographed on silica gel with 12:2:1 AcOEt–EtOH–H_2O to give crude glucosylated 1,3-dioxolane derivative, which had R_f 0.15 on TLC (12:2:1 AcOEt–EtOH–H_2O). The crude residue was further separated by HPLC (Lichroprep NH_2) with 17:3 CH_3CN–H_2O (10 mL/min) to afford a pure glucosylated product.

Method B. To a solution of the donor **1** (0.3 M or 0.9 M) and acceptor (0.1 or 0.3 M) in 20 mM calcium propionate buffer (pH 5.5) containing 5% DMF was added immobilized whole cells of *P. ruburum* (1% w/v), *E. rhapontici* (2% w/v), or *S. plymuthica* (4% w/v), and the mixture was incubated for 72 h at 37°C. The mixture was filtered to remove the immobilized cells, and the filtrate was concentrated in vacuo. The residue was purified by column chromatography with an appropriate solvent system to give the glucosylated 1,3-dioxolane derivative.

4-(α-D-Glucopyranosyl)oxymethyl-1,3-dioxolane (**5b**).—To a solution of **1** (1.00 g, 2.77 mmol) and **5a** (1.15 g, 11.1 mmol) in buffer solution (9.25 mL) was added immobilized *P. rubrum* (92.5 mg), and the mixture was incubated for 24 h at 37°C. The product was separated by column chromatography with 8:2:1 AcOEt–EtOH–H_2O to give **5b** (420 mg, 57%). The diastereomeric ratio of **5b** was estimated from the intensity of ^{13}C NMR signals. ^{13}C NMR (90 MHz, D_2O): δ 99.8, 99.6 (C-1), 95.7 (C-2), 75.6, 75.2 (C-4′), 74.2 (C-3), 73.2 (C-5), 72.6 (C-2), 70.8 (C-4), 69.2, 68.6 (C-6′), 67.3 (C-5′), 61.8 (C-6). Anal. Calcd for $C_{10}H_{18}O_8$: C, 45.11; H, 6.81. Found: C, 44.87; H, 6.72.

2,2-Dimethyl-4-(α-D-glucopyranosyl)oxymethyl-1,3-dioxolane (**6b**).—Compound **6b** (284 mg, 34% from **6a**: 366 mg, 2.77 mmol) was obtained as described for preparation **5b**. ^{13}C NMR (90 MHz, D_2O): δ 111.6 (C-2′), 99.7 (C-1), 75.8 (C-4′), 74.3 (C-3), 73.1 (C-5), 72.6 (C-2), 70.5 (C-4), 69.5 (C-6′), 66.7 (C-5′), 61.7 (C-6), 27.0, 25.6 (> C*Me*$_2$). Anal. Calcd for $C_{12}H_{22}O_8$: C, 48.97; H, 7.53. Found: C, 49.00; H, 7.53.

Immobilization of α-glucosidase.—To a suspension of Chitopearl BCW 3000 (5 mL, wet-resin) in H_2O (10 mL) was added α-D-glucosidase (13.4 mg). The mixture was stirred for 24 h at room temperature. The enzyme-adsorbed resin was filtered and washed with H_2O and stored at 4°C.

Determination of the configuration of compound **6b**.—The absolute configuration was determined by the specific rotation of **6a** which had been regenerated by immobilized α-glucosidase. To a solution of **6b** (89 mg, 0.30 mmol) in 50 mM sodium phosphate buffer (pH 6.8, 3.0 mL) was added a suspension of immobilized α-glucosidase (1.0 mL), and the mixture was stirred for 36 h at 37°C. The suspension was filtered to remove immobilized enzymes. The filtrate was extracted with $CHCl_3$. The extract was concentrated to give **6a** (14 mg, 35%); $[\alpha]_D$ +6.9° (*c* 4.8, MeOH); {Lit. [19] (*S*)-(+)-**6a** $[\alpha]_D$ +11.05 ± 0.5° (*c* 5.0, MeOH)}

1-O-(α-D-Glucopyranosyl)-2,3-O-isopropylidene-L-threitol (**7b**).—The glucosylation of acceptor **7b** was performed as described in method A; yield 49% (colorless syrup); $[\alpha]_D$ +80.0° (*c* 0.89, H_2O); ^{13}C NMR (90 MHz, D_2O): δ 111.4 (> CMe_2), 99.8 (C-1), 79.3, 77.3 (C-2′, 3′), 74.3 (C-3), 73.2 (C-5), 72.6 (C-2), 70.8 (C-4), 69.1 (C-1′), 62.2 (C-4′), 61.8 (C-6), 27.3 (> C*Me*$_2$). Anal. Calcd for $C_{13}H_{24}O_9$: C, 48.13; H, 7.46. Found: C, 48.29; H, 7.94.

1-O(α-D-Glucopyranosyl)-2,3-O-isopropylidene-D-threitol (**8b**).—The glucosylation of acceptor **8a** was performed in the same manner as described in method A; yield 43% (colorless syrup); $[\alpha]_D$ +84.6° (*c* 0.75, H_2O); ^{13}C NMR (90 MHz, D_2O): δ 111.4 (> CMe_2), 99.8 (C-1), 79.3, 77.4 (C-2′, 3′), 74.3 (C-3), 73.2 (C-5), 72.7 (C-2), 70.8 (C-4), 69.1 (C-1′), 62.3 (C-4′), 61.8 (C-6), 27.4 (> C*Me*$_2$). Anal. Calcd for $C_{13}H_{24}O_9$: C, 48.13; H, 7.46. Found: C, 48.14; H, 7.71.

1-O-(α-D-Glucopyranosyl)-2,3-O-isopropylidene-D- erythritol (**9b**).—The glucosylation of acceptor **9a** was performed in the same manner as described in method A; yield 54% (colorless syrup); $[\alpha]_D$ +92.9° (*c* 1.04, H_2O); ^{13}C NMR (90 MHz, D_2O): δ 110.6 (> CMe_2), 100.0 (C-1), 78.2, 76.8 (C-2′, 3′), 74.4 (C-3), 73.3 (C-5), 72.7 (C-2), 70.8 (C-4), 67.5 (C-1′), 61.9 (C-6), 61.1 (C-4′), 28.0, 25.7 (> C*Me*$_2$). Anal. Calcd for $C_{13}H_{24}O_9$: C, 48.13; H, 7.46. Found: C, 47.95; H, 7.45.

[$^2H_1$]-2,3-O-Isopropylidene-D-etrythritol (**10a**).—To a solution of 2,3-*O*-isopropylidene-D-ribose (1.0 g, 5.3 mmol) in a mixture of H_2O (58 mL) and EtOH (10 mL) was added dropwise a solution of $NaBD_4$ (522 mg, 12.5 mmol) in H_2O (23 mL), with cooling in an ice bath. The mixture was stirred at room temperature for 44 h, and neutralized with 1.7 M AcOH. To the mixture was added $NaIO_4$ (1.24 g, 5.79 mmol) portionwise over 5 min at 0°C. After stirring at room temperature for 3 h, the mixture was concentrated to ~7 mL. A precipitated colorless solid was removed by filtration and washed with EtOAc. The aqueous filtrate was extracted with EtOAc several times. The extracts were washed with H_2O, dried ($MgSO_4$), and concentrated to give crude 4-[$^2H_1$]-2,3-*O*-isopropylidene-L-erythrofuranose (600 mg) as a syrup. This compound was reduced with $NaBH_4$ in the same manner as just described, to give **5** as a crude syrup, which was purified by column chromatography with 60:1 EtOAc–EtOH (60:1) and further by distillation at 172°C, 2 mmHg, to give a colorless syrup (362 mg, 42%); $[\alpha]_D$ 0°(*c* 1.0, MeOH); ^{13}C NMR (90 MHz, D_2O): δ 110.3 (> CMe_2) 60.7 (t, $J_{C,D}$ 22.0 Hz, C-1), 78.2 (C-2, 3), 61.0 (C-4), 28.0, 25.7 (> CMe_2). Anal. Calcd for $C_7H_{15}O_4$: C, 51.52; H, 9.26. Found: C, 51.59; H, 8.85.

1-O-(α-D-Glucopyranosyl)-1-[$^2H_1$]-2,3-O-isopropylidene-D-erythritol (**10b**).—The glucosylation of acceptor **10a** was performed in the same manner as described in method A; yield 70% (colorless syrup); $[\alpha]_D$ +93.0° (*c* 1.00, H_2O); ^{13}C NMR (90 MHz, D_2O): δ 110.6 (> CMe_2), 100.0 (C-1), 78.3, 76.7 (C-2′, 3′) 74.3 (C-3), 73.2 (C-5), 72.7 (C-2), 70.8 (C-4),) 67.2 (t, $J_{C,D}$ 20.9 Hz, C-1′), 61.8 (C-6), 61.1 (C-4′), 27.9, 25.6 (> CMe_2). Anal. Calcd for $C_{13}H_{25}O_9$: C, 48.00; H, 7.75. Found: C, 47.64; H, 7.70.

Methyl 2,3-O-isopropylidene-L-threonate (**11a**).— A solution of **21** (3.0 g, 10.7 mmol) in MeOH (45 mL) was hydrogenolyzed in the presence of 10% Pd–C (1.6 g) at room temperature for 1 h. The filtered mixture was concentrated and the residue purified by column chromatography with 3:1 hexane–EtOAc to give **11a** (1.9 g, 91%) as a syrup; $[\alpha]_D$ −8.1° (*c* 0.85, $CHCl_3$), ^1H NMR (100 MHz, $CDCl_3$): δ 4.60 (d, 1 H, $J_{2,3}$ 7.8 Hz, H-2), 4.36 (ddd, 1 H, $J_{3,4a}$ 3.5 Hz, $J_{3,4b}$ 4.0 Hz, H-3), 4.08 (dd, $J_{4a,4b}$ 13 Hz, H-4a), 3.96 (s, 3 H, CO_2Me), 3.96–3.78 (m, 1 H, H-4b), 1.56, 1.52 (each s, each 3 H, > CMe_2); ^{13}C NMR (90 MHz, $CDCl_3$): δ 171.3 (C-1), 111.4 (> CMe_2), 79.3, 75.0 (C-2,3), 61.9 (C-4), 52.4 (CO_2Me) 26.8, 25.6 (> CMe_2). Anal. Calcd for $C_8H_{14}O_5$: C, 50.52; H, 7.42. Found: C, 50.39; H, 7.40.

Methyl 4-O-(α-D-Glucopyranosyl)-2,3-O-isopropylidene-L-threonate (**11b**).—The glucosylation of acceptor **11a** was performed as described in method B. (*i*) Donor **1** (200 mg, 0.554 mmol) and **11a** (105 mg, 0.554 mmol) were treated with immobilized *P. rubrum* (18 mg) in the buffer solution (1.85 mL). The product was separated by column chromatography with 10:1 EtOAc–EtOH to give **11b** (29 mg, 15%). (*ii*) Donor **1** (200 mg, 0.554 mmol) and **11a** (105 mg, 0.554 mmol) were treated with immobilized *E. rhapontici* (37 mg) in the buffer solution (1.85 mL). The products were separated by column chromatography with 10:1 EtOAc–EtOH to give **11b** (53 mg, 39%) and glucose (14 mg). When 3 equiv of **1** were used, donor **1** (284 mg, 0.789 mmol) and **11a** (50 mg, 0.263 mmol) were treated with immobilized *E. rhapontici* (17 mg) in the buffer solution (0.88 mL). The product was separated by

column chromatography with 10:1 EtOAc–EtOH to give **11b** (64 mg, 69%). (*iii*) Donor **1** (250 mg, 0.693 mmol) and **11a** (132 mg, 0.693 mmol) were treated with immobilized *S. plymuthica* (93 mg) in the buffer solution (2.31 mL). The product was separated by column chromatography with 10:1 EtOAc–EtOH to give **11b** (17 mg, 7%); $[\alpha]_D$ +73.9° (*c* 0.25, H_2O); ^{13}C NMR (90 MHz, D_2O): δ 173.5 (C-1), 113.3 (> CMe_2), 99.8 (C-1′), 78.6, 76.0 (C-2,3), 74.2 (C-3′), 73.2 (C-5′), 72.6 (C-2′), 70.7 (C-4′), 68.6 (C-4), 61.7 (C-6′), 27.0, 25.8 (> CMe_2). Anal. Calcd for $C_{14}H_{20}O_{10}$: C, 47.72; H, 6.87. Found: C, 47.79; H, 7.18.

2,3-O-Isopropylidene-L-threonamide (**12a**).—To a solution of **11a** (210 mg, 1.10 mmol) in MeOH (0.5 mL) was added 28% aq NH_3 (0.5 mL) at 0°C. The mixture was warmed to room temperature and kept for 2 h, and concentrated. The residue was purified by column chromatography with 1:2 hexane–EtOAc, to give **12a** (273 mg, 96%) as a colorless syrup; $[\alpha]_D$ −43.5° (*c* 0.77, $CHCl_3$); ^1H NMR (100 MHz, $CDCl_3$): δ 7.04–6.64 (brd, 2 H, NH_2), 4.28 (d, 1 H, $J_{2,3}$ 7.9 Hz, H-2), 4.24–4.04 (m, 1 H, H-3), 3.95–3.81 (m, 2 H, H-4a,4b), 3.76–3.58 (m, 1 H, OH), 1.56, 1.52 (each s, each 3 H, > CMe_2); ^{13}C NMR (90 MHz, $CDCl_3$): δ 174.4 (C-1), 110.8 (> CMe_2), 79.4, 76.6 (C-2,3), 62.4 (C-4), 26.9, 25.9 (> CMe_2). Anal. Calcd for $C_7H_{13}NO_4$: C, 47.99; H, 7.48; N, 7.80. Found: C, 47.56; H, 7.42; N, 7.77.

4-O-(α-D-Glucopyranosyl)-2,3-O-isopropylidene-L-threonamide (**12b**).—The glucosylation of acceptor **12a** was performed as described in method B. Donor **1** (400 mg, 1.11 mmol) and **12a** (194 mg, 1.11 mmol) were treated with immobilized *E. rhapontici* (74 mg) in the buffer solution (3.7 mL). The products were separated as described in method A to afford glucose (75 mg) and **12b** (43 mg, 11%); $[\alpha]_D$ +95.4° (*c*0.69, H_2O); ^{13}C NMR (90 MHz, D_2O): δ 176.4 (C-1), 113.2 (> CMe_2), 99.8 (C-1′), 79.3, 76.5 (C-2,3), 74.3 (C-3′), 73.2 (C-5′), 72.7 (C-2′), 70.7 (C-4′), 69.0 (C-4), 61.7 (C-6′), 27.2, 26.1 (> CMe_2). Anal. Calcd for $C_{13}H_{23}NO_9$: C, 46.29; H, 6.87; N, 4.15. Found: C, 45.66; H, 6.97; N, 4.05.

N-Methyl-2,3-O-isopropylidene-L-threonamide (**13a**).—To 40% (w/v) aq CH_3NH_2 was added **11a** (494 mg, 260 mmol), and the mixture was kept at room temperature for 0.5 h. The mixture was concentrated in vacuo and the residue purified by column chromatography with 1:2 hexane–EtOAc, to give **13a** (432 mg, 88%) as a colorless syrup; $[\alpha]_D$ −10.6° (*c* 1.72, MeOH); ^1H NMR (100 MHz, $CDCl_3$): δ 6.70 (brd, 1 H, N*H*Me), 4.25 (d, 1 H, $J_{2,3}$ 8.5 Hz, H-2), 4.18–3.80 (m, 3 H, H-3,4a,4b), 3.52 (t, 1 H, *J* 5 Hz, OH), 2.84 (d, 3 H; $J_{Me,NH}$ 6 Hz, NH*Me*), 1.47 (s, 6 H, > CMe_2); ^{13}C NMR (90 MHz, $CDCl_3$): δ 171.3 (C-1), 110.7 (> CMe_2), 79.3, 77.4 (C-2,3), 62.7 (C-4), 26.9, 25.7 (> CMe_2). Anal. Calcd for $C_8H_{15}NO_4$: C, 50.78; H, 7.99; N, 7.40. Found: C, 50.88; H, 7.52; N, 7.37.

4-O-(α-D-Glucopyranosyl)-2,3-O-isopropylidene-N-methyl-L-threonamide (**13b**).—The glucosylation of acceptor **13a** was performed as described in method B. Donor **1** (500 mg, 1.44 mmol) and **13a** (273 mg, 1.44 mmol) were treated with immobilized *E. rhapontici* (96 mg) in the buffer solution (4.8 mL). The products were separated by column chromatography with 12:2:1 EtOAc–EtOH–H_2O to give **13b** (36 mg, 7%) and glucose (86 mg); $[\alpha]_D$ +92.1° (*c* 0.70, H_2O); ^{13}C NMR (90 MHz, D_2O): δ 173.6 (C-1), 113.2 (> CMe_2), 99.8 (C-1′), 79.3, 76.8 (C-2,3), 74.3 (C-3′), 73.2 (C-5′), 72.7 (C-2′), 70.7 (C-4′), 69.0 (C-4), 61.7 (C-6′), 27.2, 26.2 (> CMe_2), 26.7 (NMe).

Anal. Calcd for $C_{14}H_{25}NO_9$: C, 47.86; H, 7.17; N, 3.99. Found: C, 47.34; H, 7.46; N, 3.87.

4-O-(α-D-Glucopyranosyl)-2,3-O-isopropylidene-L-threose dimethyl dithioacetal (**14b**).—The glucosylation of acceptor **14a** was performed in the same manner as described in method B(*i*) Donor **1** (310 mg, 0.860 mmol) and **14a** (68 mg, 0.290 mmol) were treated with immobilized *P. rubrum* (28.7 mg) in the buffer solution (2.87 mL). The product was separated by column chromatography with 9:1 EtOAc–EtOH to give crude **14b** (31 mg, 27%). (*ii*) Donor **1** (300 mg, 0.832 mmol) and **14a** (66 mg, 0.277 mmol) were treated with immobilized *E. rhapontici* (55.4 mg) in the buffer solution (2.77 mL). The products were separated by column chromatography with 9:1 EtOAc–EtOH to give **14b** (67 mg, 60%) and glucose (55 mg). (*iii*) Donor **1** (300 mg, 0.832 mmol) and **14a** (66 mg, 0.277 mmol) were treated with immobilized *S. plymuthica* (110.8 mg) in the buffer solution (2.77 mL). The product was separated by column chromatography with 9:1 EtOAc–EtOH (9:1) to give crude **14b** (15 mg, 14%); $[\alpha]_D$ +44° (*c* 0.33, H_2O); ^{13}C NMR (90 MHz, D_2O): δ 111.8 (> CMe_2), 99.8 (C-1′), 80.2, 79.8 (C-2,3), 74.3 (C-3′), 73.2 (C-5′), 72.6 (C-2′), 70.7 (C-4′), 69.9 (C-4), 61.6 (C-6′), 56.9 (C-1), 27.4, 27.2 (> CMe_2), 14.6, 14.1 (SMe). Anal. Calcd for $C_{15}H_{28}O_8S_2$: C, 44.99; H, 7.05. Found: C, 44.70; H, 6.94.

Methyl 2,3-O-isopropylidene-D-threonate (**15a**).—Hydrogenolysis of **23** (1.0 g, 3.6 mmol) was performed in the same manner as described for preparation of **11a** to afford **15a** (560 mg, 82%) as a colorless syrup; $[\alpha]_D$ + 7.5° (*c* 0.96, $CHCl_3$); ^1H NMR (100 MHz, $CDCl_3$): δ 4.61 (d, 1 H, $J_{2,3}$ 7.8 Hz, H-2), 4.31 (ddd, 1 H, $J_{3,4a}$ 3.0, $J_{3,4b}$ 4.0 Hz, H-3), 4.01 (dd, $J_{4a,4b}$ 12.4 Hz, H-4a), 3.85 (s, 3 H, CO_2Me), 3.90–3.63 (m, 1 H, H-4b), 1.51, 1.47 (each s, each 3 H, > CMe_2). Anal. Calcd for $C_8H_{14}O_5$: C, 50.52; H, 7.42. Found: C, 50.52; H, 7.48.

Methyl 4-O-(α-D-glucopyranosyl)-2,3-O-isopropylidene-D-threonate (**15b**) *and methyl 4-O-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl)-2,3-O-isopropylidene-D-threonate* (**15b′**).—The glucosylation of acceptor **15a** was performed in the same manner as described in method B. Donor **1** (200 mg, 0.552 mmol) and **15a** (105 mg, 0.552 mmol) were treated with immobilized *E. rhapontici* (37 mg) in the buffer solution (1.85 mL). The product was separated by column chromatography with 10:1 EtOAc–EtOH to give **15b** (4.6 mg); ^{13}C NMR (90 MHz, D_2O): δ 173.5 (C-1), 113.3 (> CMe_2), 99.6 (C-1′), 78.5, 75.8 (C2,3), 74.2 (C-3′), 73.3 (C-5′), 72.6 (C-2′), 70.6 (C-4′), 67.5 (C-4), 61.7 (C-6′), 27.0, 25.9 (> CMe_2). Conventional acetylation (pyridine–Ac_2O) of **15b**, followed by purification on PLC with 3:2 hexane–EtOAc gave **15b′** (6 mg, 2% in 2 steps); ^1H NMR (500 MHz, $CDCl_3$): δ 5.49 (t, 1 H, $J_{3',2'}$ 10.2, $J_{3',4'}$ 9.5 Hz, H-3′), 5.23 (d, 1 H, $J_{1',2'}$ 3.8 Hz, H-1′), 5.08 (t, 1 H, $J_{4',5'}$ 10.3 Hz, H-4′), 4.93 (dd, 1 H, H-2′), 4.51 (d, 1 H, $J_{2,3}$ 7.6 Hz, H-2), 4.33 (ddd, 1 H, $J_{3,4a}$ 2.8, $J_{3,4b}$ 4.6 Hz, H-3), 4.28 (dd, 1 H, $J_{6a',5'}$ 4.3, $J_{6a',6b'}$ 12.2 Hz, H-6a′), 4.10 (dd, 1 H, $J_{6b',5'}$ 2.3 Hz, H-6b′), 4.06 (ddd, 1 H, H-5′), 3.89 (dd, 1 H, $J_{4a,4b}$ 11.6 Hz, H-4a), 3.89–3.82 (m, 1 H, H-4b), 3.83 (s, 1 H, CO_2Me), 2.10, 2.07, 2.04, 2.02 (each s, each 3 H, OAc), 1.45, 1,43 (each s, each 3 H, > CMe_2).

2,3-O-Isopropylidene-D-threonamide (**16a**).—Ammonolysis of compound **15a** was performed as described for preparation **12a** to afford **16a** quantitatively as a colorless syrup; $[\alpha]_D$ −29.7° (*c* 1.38, $CHCl_3$); ^1H NMR (100 MHz, $CDCl_3$): δ

7.04–6.64 (brd, 2 H, NH_2), 4.27 (d, 1 H, $J_{2,3}$ 7.9 Hz, H-2), 4.28–4.05 (m, 1 H, H-3), 4.00–3.88 (m, 2 H, H-4a,4b), 3.64–3.34 (m, 1 H, OH), 1.51 (s, 6 H, $>CMe_2$). Anal. Calcd for $C_7H_{13}NO_4$: C, 47.99; H, 7.48; N, 7.80. Found: C, 47.65; H, 7.50; N, 7.84.

2,3-O-Isopropylidene-L-erythronamide (**17a**).—To a solution of **19** (1.07 g, 6.77 mmol) in EtOH (2 mL) at 0°C was added 28% NH_3 (2 mL) and the mixture was stirred for 0.5 h at room temperature. A residue obtained by concentration of the solvent was crystallized from EtOH to give **17a** (625 mg, 53%); mp 116–117°C; $[\alpha]_D$ −74.7° (*c* 0.95, MeOH); ^{1}H NMR (100 MHz, $CDCl_3$): δ 6.91–6.23 (brd, 2 H, $CONH_2$), 4.76–4.47 (m, 2 H, H-2,3), 3.92–3.63 (m, 2 H, H-4,4′), 3.54 (dd, $J_{OH,4}$ 4.3, $J_{OH,4'}$ 9.9 Hz, OH); ^{13}C NMR (90 MHz, $CDCl_3$): δ 173.9 (C-1), 110.1 ($>CMe_2$), 77.5, 77.6 (C-2,3), 61.5 (C-4), 24.4, 26.9 ($>CMe_2$), Anal. Calcd for $C_7H_{13}NO_4$: C, 47.99; H, 7.48; N, 7.80. Found: C, 47.59; H, 7.43; N, 7.95.

4-O-(α-D-Glucopyranosyl)-2,3-O-isopropylidene-L-erythronamide (**17b**).—The glucosylation of acceptor **17a** (106 mg, 0.554 mmol) was performed as described in method A to afford **17b** (13 mg, 7.0%) as a colorless syrup and glucose (50 mg, 50%); ^{13}C NMR (90 MHz, D_2O): δ 174.7 (C-1′), 112.1 ($>CMe_2$), 100.0 (C-1), 76.9, 76.1 (C-2′,3′), 74.2 (C-3), 73.2 (C-5), 72.7 (C-2), 70.6 (C-4), 67.7 (C-4′), 61.7 (C-6), 27.0, 24.9 ($>CMe_2$).

N-Methyl-2,3-O-isopropylidene-L-erythronamide (**18a**).—To 40% aq (w/v) CH_3NH_2 was added **19** (205 mg, 1.28 mmol), and the mixture was kept for 1 h at room temperature. The mixture was concentrated to give white crystals quantitatively; mp 108°C (recrystallized from $CHCl_3$); $[\alpha]_D$ −65° (*c* 1.4, MeOH); ^{1}H NMR (100 MHz, $CDCl_3$): δ 6.87 (brs, 1 H, NH), 4.82–4.49 (m, 2 H, H-2,3), 3.97–3.57 (m, 3 H, H-4,4′,OH), 1.58, 1.45 (each s, each 3 H, $>CMe_2$); ^{13}C NMR (90 MHz, $CDCl_3$): δ 171.2 (C-1), 109.2 ($>CMe_2$), 77.8, 76.8 (C-2,3), 61.5 (C-4), 26.9, 24.4 ($>CMe_2$), 25.7 (NHMe). Anal. Calcd for $C_8H_{15}NO_4$: C, 50.78; H, 7.99; N, 7.40. Found: C, 50.60; H, 7.83; N, 7.42.

2,3-O-Isopropylidene-L-erythrono-1,4-lactone (**19**).—A 2.0 M bromine solution in 9:1 MeOH–H_2O (3.4 mL) was added to a solution of 2,3-*O*-isopropylidene-L-erythrofuranose [18] (359 mg, 2.24 mmol) in 9:1 MeOH–H_2O (4.5 mL) buffered with $NaHCO_3$ (3.76 g). To the mixture stirred at room temperature for 3 h was added solid sodium thiosulfate to quench excess bromine, and undissolved materials were filtered off and washed with ether. After dilution with H_2O, the filtrate was extracted with ether. The combined organic layers were dried ($MgSO_4$), and concentrated to give a syrup that was purified by column chromatography with 2:1 hexane–EtOAc to give **19** (313 mg, 88%); $[\alpha]_D$ +139° (*c* 1.4, $CHCl_3$); ^{1}H NMR (100 MHz, $CDCl_3$): δ 5.50–4.88 (m, 1 H, H-3), 4.81 (d, $J_{2,3}$ 5.5 Hz, H-2), 4.60–4.47 (m, 2 H, H-4a,4b), 1.45, 1.49 (each s, each 3 H, $>CMe_2$); ^{13}C NMR (90 MHz, $CDCl_3$): δ 174.3 (C-1), 113.9 ($>CMe_2$), 74.7, 70.3 (C-2,3), 64.1 (C-4), 26.8, 25.6 ($>CMe_2$), Anal. Calcd for $C_7H_{10}O_4$: C, 53.16; H, 6.37. Found: C, 53.59; H, 6.18.

1-O-Benzyl-2,3-O-isopropylidene-L-threitol (**20**).—To a suspension of **7a** (8.3 g, 51.5 mmol) in a mixture of 4 M NaOH (37 mL) and CH_2Cl_2 (37 mL) was added benzyl chloride (6.52, 51.5 mmol) and Bu_4NBr (852 mg). The mixture was stirred for 14 h at 50°C, and after cooling, extracted with CH_2Cl_2 (80 mL × 3). The extracts were dried ($MgSO_4$), and concentrated in vacuo. The residue was purified

by flash column chromatography with 1:1 hexane–EtOAc to give **20** (8.7 g, 67%); $[\alpha]_D$ + 8.7° (*c* 1.2, $CHCl_3$); ^1H NMR (100 MHz, $CDCl_3$): δ 7.40–7.18 (m, 5 H, Ph), 4.68 (s, 2 H, CH_2Ph), 4.24–3.56 (m, 6 H, H-1a,1b,2,3,4a,4b), 1.46 (s, 6 H, > CMe_2). Anal. Calcd for $C_{14}H_{20}O_4$: C, 66.64; H, 7.51. Found: C, 66.22; H, 7.99.

*Methyl 4-O-benzyl-2,3-O-isopropylidene-*L*-threonate* (**21**).—To a solution of $(COCl)_2$ (8.76 g, 6.8 mmol) in CH_2Cl_2 (40 mL) was added dropwise a solution of Me_2SO (1.08 g, 0.138 mol) in CH_2Cl_2 (30 mL) at −78°C. After stirring for 15 min, a solution of **20** in CH_2Cl_2 (30 mL) was added. After stirring for 15 min at −78°C, Et_3N (25.1 g, 0.284 mmol) was added dropwise to the mixture. After 5 min, the resulting solution was warmed to room temperature, mixed with H_2O, and extracted with CH_2Cl_2 three times. The combined organic layer was dried ($MgSO_4$), and concentrated to give crude acyclic L-threose derivative as a yellow oil. Without further purification, a 2.0 M bromine solution in 9:1 MeOH–H_2O (86 mL) was added to a solution of aldehyde in 9:1 MeOH–H_2O (69 mL) buffered with $NaHCO_3$ (58 g). To the mixture stirred at room temperature for 15 h was added solid sodium thiosulfate to quench excess bromine, and undissolved material was filtered and washed with ether. After dilution with H_2O, the filtrate was extracted with ether three times. The combined organic layer was dried ($MgSO_4$), and concentrated in vacuo. The crude product was purified by column chromatography with 6:1 hexane–EtOAc to give **21** (8.92 g, 93%) as a colorless oil; $[\alpha]_D$ + 20.5° (*c* 0.85, $CHCl_3$); ^1H NMR (100 MHz, $CDCl_3$): δ 7.50–7.20 (m, 5 H, Ph), 4.67 (s, 2 H, CH_2Ph), 4.60–4.28 (m, 2 H, H-2,3), 4.00–3.60 (m, 2 H, H-4a,4b), 3.83 (s, 3 H, CO_2Me), 1.58, 1.48 (each s, each 3 H, > CMe_2). *Anal.* Calcd for $C_{15}H_{20}O_5$: C, 64.27; H, 7.19. Found: C, 63.65; H, 6.76.

*1-O-Benzyl-2,3-O-isopropylidene-*D*-threitol* (**22**).—Monobenzylation of **8a** (2.0 g, 12.3 mmol) was performed as described for preparation of **20** to give **22** (2.3 g, 74%) as a colorless syrup $[\alpha]_D$ − 8.2° (*c* 1.13, $CHCl_3$); ^1H NMR (100 MHz, $CDCl_3$): δ 7.40–7.18 (m, 5 H, Ph), 4.59 (S, 2 H, CH_2Ph), 4.20–3.48 (m, 6 H, H-1a,1b,2,3,4_a,4_b), 1.44 (s, 6 H, > CMe_2). Anal. Calcd for $C_{14}H_{20}O_4$: C, 66.64; H, 7.51. Found: C, 66.64; H, 7.75.

*Methyl 4-O-benzyl-2,3-O-isopropylidene-*D*-threonate* (**23**).—Swern oxidation and bromine oxidation of **22** (1.0 g, 3.69 mmol) was performed as described for preparation of **21** to give **23** (939 mg, 84%), $[\alpha]_D$ −25.9° (*c* 0.71, $CHCl_3$); ^1H NMR (100 MHz, $CDCl_3$): δ 7.49–7.23 (m, 5 H, Ph), 4.67 (s, 2 H, CH_2Ph), 4.57–4.29 (m, 2 H, H-2,3), 3.97–3.61 (m, 2 H, H-4a,4b), 3.83 (s, 3 H, CO_2Me), 1.52, 1.48 (each s, each 3 H, > CMe_2); ^{13}C NMR (90 MHz, $CDCl_3$): δ 171.0 (C-1), 137.9, 128.3, 127.6 (Ph), 111.6 (> CMe_2), 78.4, 77.1 (C-2,3), 73.6 (CH_2Ph), 69.8 (C-4), 52.3 (CO_2Me), 26.9, 25.7 (> CMe_2). Anal. Calcd for $C_{15}H_{20}O_5$: C, 64.27; H, 7.19. Found: C, 63.34; H, 7.26.

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