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Synthesis of 1′,6′-disubstituted sucroses and their behavior as glucosyl donors for a microbial α-glucosyltransferase

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Abstract

Versatile 6′-chloro-6′-deoxy-1′-substituted sucrose derivatives were synthesized in search of an optimum donor substrate for the intermolecular transglucosylation with the α-glucosyltransferase from *Protaminobacter rubrum*. Two substituents at the C-1′ and C-6′ positions of sucrose were introduced utilizing the distinct reactivity of the corresponding sulfonates. Methyl β-D-arabinofuranoside was most efficiently glucosylated with the 1′-deoxy derivative **5**. Hydroxyl and fluoro groups at C-1′ show a tendency to enhance the intramolecular transglucosylations, giving 3-*O*-(α-D-glucopyranosyl)-D-fructose derivatives.

Keywords: Sucrose, 1′,6′-disubstituted; α-Glucosyltransferase; *Protaminobacter rubrum*

1. Introduction

Since the finding that a microorganism, *Protaminobacter rubrum*, transforms sucrose into isomaltulose [1], several other microorganisms, such as *Serratia plymuthica* [2] and *Erwinia rhapontici* [3], have been reported to catalyze the same conversion (Scheme 1).

The α-glucosyltransferase from *P. rubrum* is also known to catalyze intermolecular transglucosylation. For example, it is reported that a small amount of 5-*O*-(α-D-glucopyranosyl)-D-arabinose was obtained together with large excess of isomaltulose when a mixture of sucrose and D-arabinose was treated with *P. rubrum* [4]. In order to prevent the formation of isomaltulose and thereby improve the efficiency of intermolecular

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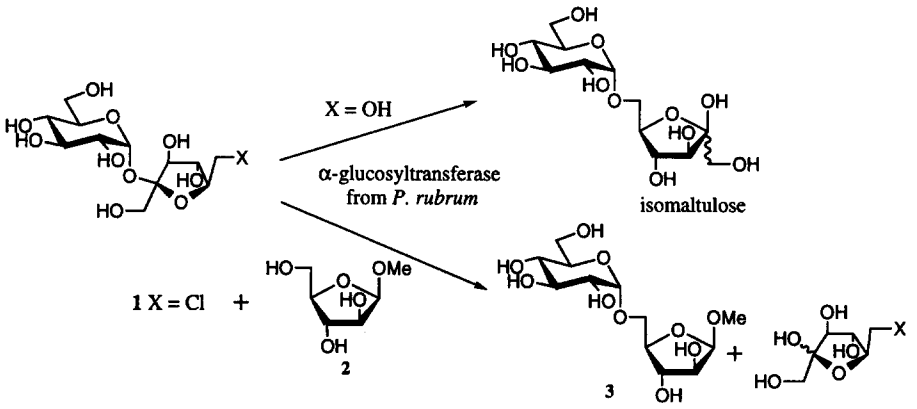
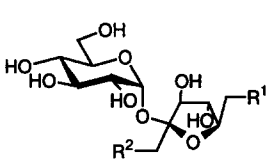


Scheme 1.

transglucosylation, a donor blocked at the 6′-position, i.e., 6′-chloro-6′-deoxysucrose (**1**) has been developed. Methyl β-D-arabinofuranoside was glucosylated to give the 5-*O*-α-D-glucopyranosyl derivative **3** in good yield [5].

The above methodology was later found to be useful for the diastereoselective as well as enantioselective α-D-glucosylation of 2,3-*O*-isopropylideneglycerol derivatives, but yields were poor to moderate, occasionally accompanied with the hydrolyzed product [6]. To create effective donors, a systematic modification at the 1′-position of **1** was planed, because the 1′-substituent is close to the glycosidic linkage and may have a significant effect on the transglucosylation. We describe herein the facile synthesis of 1′,6′-disubstituted sucroses **4–9** and their properties as glucosyl donors for the intermolecular transglucosylation.



4 R^1 = Cl, R^2 = Cl
5 R^1 = H, R^2 = H
6 R^1 = Cl, R^2 = SH
7 R^1 = Cl, R^2 = NH_2
8 R^1 = Cl, R^2 = F
9 R^1 = Cl, R^2 = OMe

2. Results and discussion

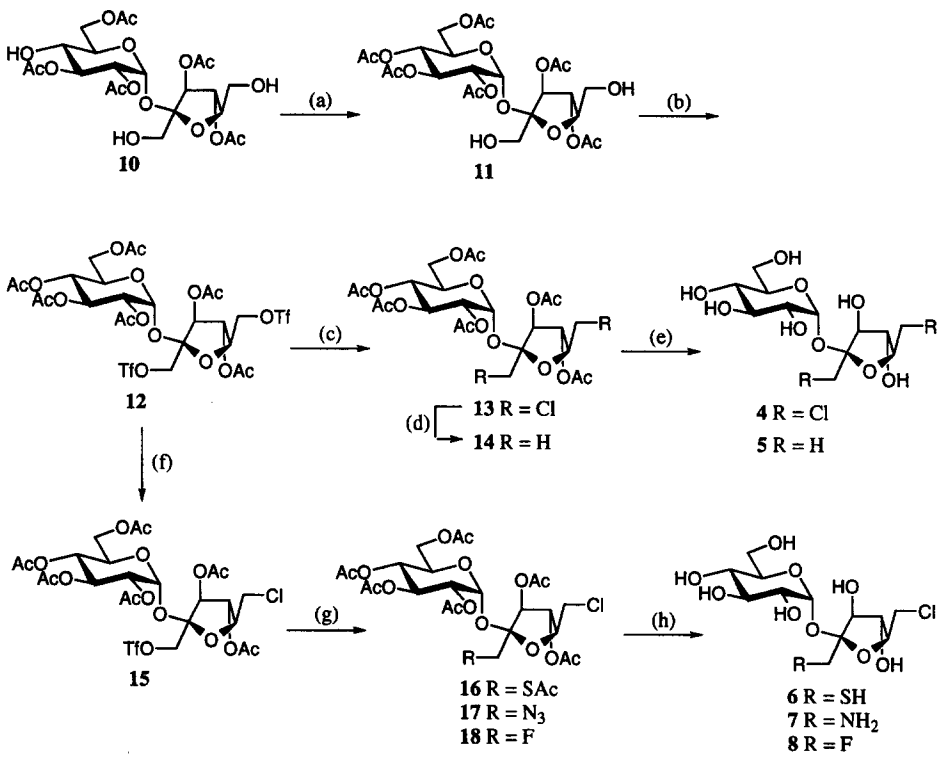
It is well documented that the 1′-hydroxyl group can be easily discriminated from the other primary hydroxyls. Indeed, the 1′-substituted sucrose derivatives have been synthesized from 6,1,6′-tri-*O*-(2,4,6-trimethybenzylsulfonyl)sucrose via the regioselective substitution at C-6 and C-6′ with benzoate, followed by a substitution reaction at C-1′ [7]. The 1′-substituted-6′-chlorosucrose would be likewise obtainable by substitution

reactions of the 1′,6′-disulfonate consecutively with chloride ion and another nucleophile.

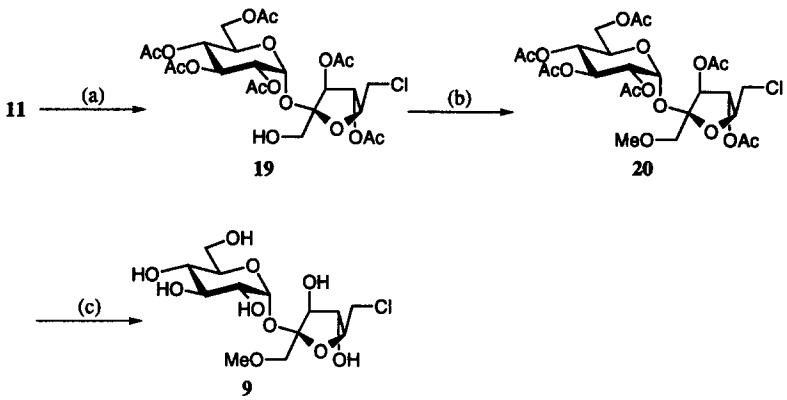
We thus selected 2,3,4,6,3′,4′-hexa-*O*-acetylsucrose (**11**) as a starting material, which was obtained in 63% from the known penta-acetate **10** [8] by regioselective silylation of the primary hydroxyls with *tert*-butylchlorodimethylsilane, acetylation of the remaining secondary hydroxyl, and then removal of the silyl groups with 70% acetic acid.

Trifluoromethanesulfonylation of **11** in the usual manner gave the 1′,6′-disulfonate **12**, which was subjected to chlorination with lithium chloride at room temperature to give the 1′,6′-dichloro compound **13** in 73% yield. The 1′,6′-dideoxy derivative **14** was obtained by reduction of **13** with tributylstannane (92% yield).

Selective chlorination at C-6′ was easily realized just by lowering the temperature to 0 °C to give the 6′-chloro-1′-sulfonate **15**. Subsequent substitution reactions at C-1′ were generally performed without isolation of **15**. Thus treatment of **15** with sodium thioacetate, sodium azide, and tris(dimethylamino)sulfur(trimethylsilyl)difluoride (TASF) at room temperature, respectively, gave the 1′-acetylthio **16** (56%), the 1′-azido **17** (76%), the 1′-fluoro **18** (49%) derivatives (Scheme 2).



Scheme 2. (a) 1) TBDMSCl, Et_3N, DMAP, 2) Py, Ac_2O, 3) 70% AcOH, 63%; (b) Tf_2O, Py; (c) LiCl, r.t., 73% (from **11**); (d) *n*-Bu_3SnH, AIBN, 92%; (e) NaOMe, MeOH, **4**: 92%, **5**: 99%; (f) LiCl, 0 °C; (g) **16**: NaSAc, r.t., 56%; **17**: NaN_3, r.t., 76%; **18**: TASF, r.t., 49% (from **11**); (h) **6**: NaOMe, MeOH, dithiothreitol, 98%; **7**: 1) Pd-C, 2) NaOMe, MeOH, 55%; **8**: NaOMe, MeOH, 99%.



Scheme 3. (a) PPh_3, CCl_4, 74%; (b) CH_2N_2, $BF_3 \cdot OEt_2$, 99%; (c) NaOMe, MeOH, 93%.

Since attempted substitution of the 6′-chloro-1′-sulfonate **15** with sodium methoxide ended in the formation of the 2,1′-anhydro derivative, the 1′-methoxy derivative **20** was synthesized by treatment of the 6′-chloro derivative **19** [9] with diazomethane and borontrifluoride etherate [10] (Scheme 3). The 1′,6′-disubstituted sucrose hexa-acetates thus obtained, except for **16** and **17**, were subjected to Zemplén deacetylation to give a series of the 1′,6′-disubstituted sucroses (**4**, **5**, **8** and **9**). The 1′-thioacetate **16** was deacetylated with sodium methoxide in the presence of dithiothreitol to give **6**. The 1′-amino derivative **7** was obtained from **17** via hydrogenation with palladium-on-charcoal, followed by Zemplén deacetylation.

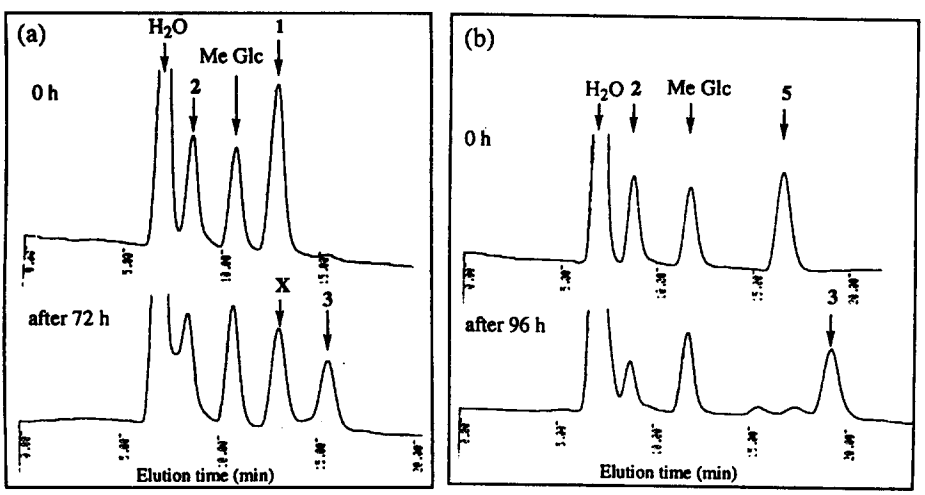


Fig. 1. HPLC chromatograms of the transglucosylation products of **1** (a) and **5** (b) in the presence of **2** by *P. rubrum*. Methyl α-D-glucopyranoside (Me Glc) was used as an internal standard. Compounds were detected by refractive index. The eluents used for (a) and (b) were 80% and 87% aq CH_3CN, respectively.

A mixture of immobilized *P. rubrum*, methyl β-D-arabinofuranoside, and one of the synthesized 1′,6′-disubstituted sucroses was incubated at 20 °C and monitored by HPLC. All of the 1′,6′-disubstituted sucroses, except for the 1′-amino derivative **7**, were donor substrates, and product distributions barely changed after 72 h. Typical examples of the HPLC analyses are shown in Fig. 1. In most cases, except for the 1′,6′-dideoxy

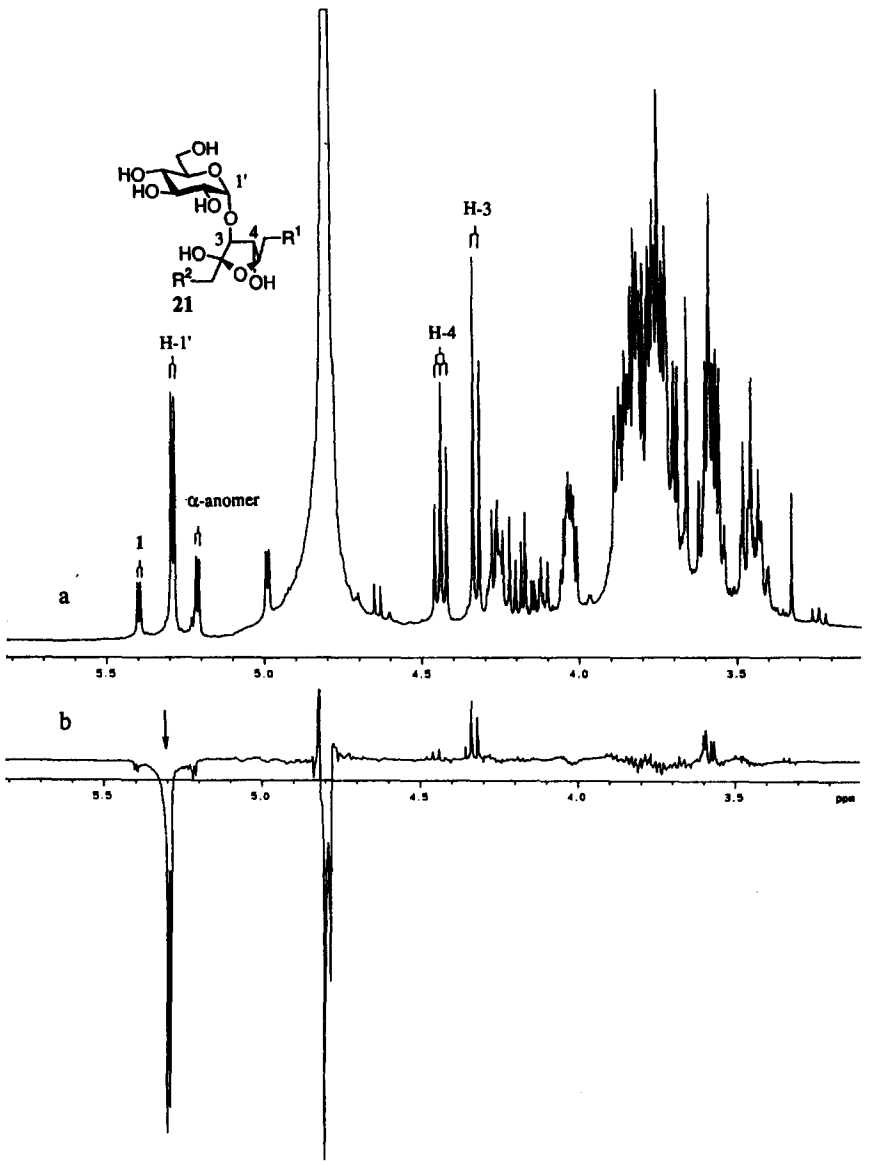


Fig. 2. ^{1}H NMR spectrum of another transglucosylation product isolated from the fraction corresponding to the peak X in Fig. 1 (a) and NOE spectrum with H-1′ irradiated (b) at 400 MHz.

Table 1
Product distribution after 96 h incubation

Donor	**3** (%)	**21** (%) [a]	Other products (%)
1 (R^1 = Cl, R^2 = OH) [b]	51	28	18 [c]
4 (R^1 = Cl, R^2 = Cl)	64	9	17 [d]
5 (R^1 = H, R^2 = H)	86	n.d. [e]	10 [f]
6 (R^1 = Cl, R^2 = SH)	42	n.d. [e]	43 [d]
8 (R^1 = Cl, R^2 = F)	31	28	19 [c]
9 (R^1 = Cl, R^2 = OMe)	62	5	24 [f]

[a] The yields were estimated from the intensity of H-1 signals in NMR spectra.
[b] The reaction was performed for 72 h.
[c] Mainly β-anomer of **21**.
[d] The starting material was mainly recovered.
[e] Not detected.
[f] Mainly D-glucose.

derivative **5** (Fig. 1b), a significant amount of the second fraction was accompanied as shown in peak X (Fig. 1a). From the ^{1}H NMR spectrum (Fig. 2a), this fraction was found to contain at least four components, the major component of which was deduced to be 6-chloro-3-*O*-(α-D-glucopyranosyl)-β-D-fructofuranose (**21**: R^1 = Cl, R^2 = OH) from the strong NOE between H-1′ and H-3 (Fig. 2b). The other three components were the α-anomer of **21**, recovered **1**, and an unidentified product.

The final yields of the intermolecularly transglucosylated product **3** and the other intramolecularly transglucosylated products are listed in Table 1. The substrates having an substituent of hydrogen acceptable, like the 1′-hydroxyl **1** and the 1′-fluoro **8** derivatives, tend to react intramolecularly, lowering the yield of **3**. As a consequence, we achieved an improvement in the intermolecular transglucosylation by using 1′,6′-deoxysucrose (**5**), which gives an 86% yield of the disaccharide **3**, compared with a yield of only 51% from 6′-chloro-6′-deoxysucrose (**1**).

Time-course studies of the intermolecular transglucosylation with various donors indicate a distinct slowdown of the rate in case of the 1′-hydroxyl derivative **1** as shown in Fig. 3. In contrast, in the case of the 1′-fluoro derivative **8**, which gave a similar amount of the intramolecularly transglucosylated product, the slowdown of the transfer rate is modest as with the other three cases. Because rather strong product inhibition of 6-chlorofructose is known [11], no inhibitory activities of other 1,6-disubstituted fructoses must be proved in order to rationalize the above results. Additional specific inhibition studies will be necessary to draw precise conclusions.

Thus the more electronegative the substituents, the smaller the initial rate. If we assume the interglycosidic linkage of 1′,6′-disubstituted sucrose derivatives is cleaved between the anomeric carbon of the glucose moiety and the interglycosidic oxygen, this tendency is extraordinary from the mechanistic point of view, since the aglycon moiety with an extra electron-withdrawing group must acquire further leaving ability, and hydrolysis becomes easier as displayed on the reaction with some glycosidases [12]. This speculation was supported by the fact that sucrose was hydrolyzed much faster than 1′-deoxy-1′-fluorosucrose by invertase [13], which cleaves the bond between the anomeric carbon of the fructose moiety and the interglycosidic oxygen. We have too little

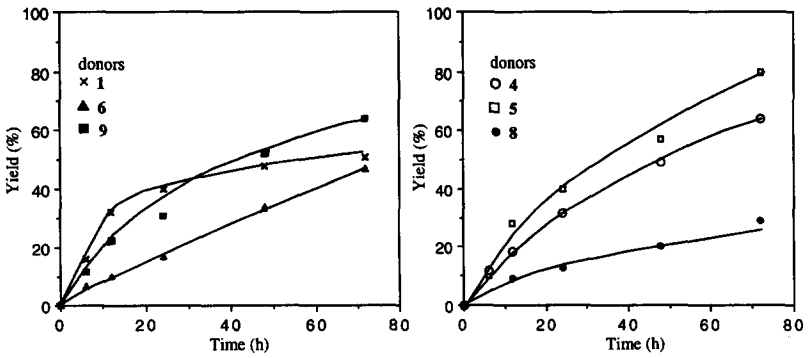


Fig. 3. Time-course of formation of transglucosylated product **3**. The mixture of one of the donor substrates, 1'-hydroxy **1**; 1'-chloro **4**; 1'-deoxy **5**; 1'-thio **6**; 1'-fluoro **8**; 1'-methoxy **9** derivatives, and the acceptor **2** were incubated with immobilized *P. rubrum* in a calcium acetate buffer at 20 °C. The reaction was monitored by HPLC.

information to explain the anomaly at this point. Investigations using purified enzyme and some other sucrose analogues are under way.

3. Experimental

General methods.—Melting points were measured with a Yanagimoto melting point apparatus and are uncorrected. Optical rotations were taken with a JASCO DIP-4 polarimeter. NMR spectra were recorded with a JEOL EX-270 or Varian Unity-plus spectrometer for solutions in $CDCl_3$ (internal Me_4Si) or D_2O (external dioxane). Flash column chromatography was performed on Wako Gel C-300 (Wako). Gel filtration was performed on Sephadex G-15 (Pharmacia). Immobilized microbials (*P. rubrum* CBS 574,77) were kindly donated by Mitsui Sugar Co. Ltd.

2,3,4,6,3',4'-Hexa-O-acetylsucrose (**11**).—To a solution of **10** (8.67 g, 15.7 mmol) in dry Me_2NCHO were added Et_3N (7.69 mL) and *tert*-butylchlorodimethylsilane (8.0 g) and 4-dimethylaminopyridine (79 mg). The mixture was stirred for 3.5 h at room temperature, then poured into cold aq $NaHCO_3$ and extracted with EtOAc (2 × 200 mL). The combined organic layer was washed with brine (2 × 100 mL), dried ($MgSO_4$), and evaporated. To a solution of the residue in dry pyridine (40 mL) was added Ac_2O (20 mL). After 1 h, the mixture was poured into aq $NaHCO_3$ and extracted with EtOAc (400 mL). The organic layer was washed with aq $NaHCO_3$ (100 mL) and brine (100 mL), dried ($MgSO_4$), and evaporated. The residue was dissolved in 70% AcOH. The mixture was stirred for 3 days at room temperature and evaporated to give a solid residue, which was purified by flash chromatography with 4:1 hexane–EtOAc to give **11** (5.89 g, 63%): $[\alpha]_D^{24}$ +40° (*c* 1.1, $CHCl_3$); mp 129–131 °C; Lit. [14] mp 132 °C, $[\alpha]_D$ +40.1° (*c* 1.1, $CHCl_3$).

2,3,4,6,3',4'-Hexa-O-acetyl-1',6'-dichloro-1',6'-dideoxysucrose (**13**).—To a solution of pyridine (0.59 mL) and Tf_2O (0.61 mL) in CH_2Cl_2 (10 mL) was added dropwise a solution of **11** (500 mg, 0.907 mmol) in CH_2Cl_2 (10 mL) at 0 °C. After 20 min, the

mixture was poured into aq $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was washed with M HCl (15 mL) and aq $NaHCO_3$ (20 mL), dried ($MgSO_4$), and then evaporated to dryness. The dry residue was dissolved in Me_2NCHO (10 mL) and LiCl (192 mg) was added to the solution. After 2.5 h, the mixture was diluted with EtOAc (30 mL) and extracted with water (20 mL). The organic layer was washed with brine, dried ($MgSO_4$), and evaporated. Flash chromatography (2:1 hexane–EtOAc) gave **13** (420 mg, 73%) as a colorless oil: $[\alpha]_D^{24}$ +47° (*c* 1.7, $CHCl_3$); Lit. [15] $[\alpha]_D$ +51.7° (*c* 1.0, $CHCl_3$). ^{1}H NMR (270 MHz, $CDCl_3$): δ 5.67 (d, 1 H, $J_{3',4'}$ 6.3 Hz, H-3'), 5.63 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 5.43 (t, 1 H, $J_{2,3}$ 9.9, $J_{3,4}$ 9.9 Hz, H-3), 5.40 (t, 1 H, $J_{4',5'}$ 6.3 Hz, H-4'), 5.07 (t, 1 H, $J_{4,5}$ 9.9 Hz, H-4), 4.91 (dd, 1 H, H-2), 4.35–4.25 (m, 2 H, H-5,5'), 4.23 (dd, 1 H, $J_{5,6a}$ 4.6, $J_{6a,6b}$ 12 Hz, H-6a), 4.15 (dd, 1 H, $J_{5,6b}$ 2.3 Hz, H-6b), 3.77 (dd, 2 H, $J_{5',6'}$ 6.3 Hz, H-6'), 3.76 (d, 1 H, $J_{1'a,1'b}$ 11.9 Hz, H-1'a), 3.59 (d, 1 H, H-1'b), 2.19, 2.11, 2.10, 2.09, 2.05, 2.02, (each s, each 3 H, OAc); ^{13}C NMR (67 MHz, $CDCl_3$) δ 170.6, 170.1, 169.8, 169.6, 169.5, 104.5, 90.2, 81.2, 76.1, 70.0, 69.6, 68.6, 68.2, 61.9, 44.7, 43.9, 20.8, 20.7, 20.6, 20.5. Anal. Calcd for $C_{24}H_{32}Cl_2O_{15}$: C, 45.65; H, 5.12. Found: C, 45.47; H, 6.12.

2,3,4,6,3',4'-Hexa-O-acetyl-1',6'-dideoxysucrose (**14**).—To a solution of **13** (157 mg, 0.249 mmol) in dry toluene (7.4 mL) were added tributylstannane (263 mg) and AIBN (5 mg). The solution was heated under reflux for 30 min. After cooling to room temperature, the mixture was diluted with aq KF (30 mL) and stirred for 1 h. The mixture was extracted with EtOAc. The organic layer was washed with brine, dried ($MgSO_4$), and evaporated. Flash chromatography (5:1–2:1 hexane–EtOAc) gave **14** (130 mg, 92%) as a colorless oil: $[\alpha]_D^{24}$ +34° (*c* 2.4, $CHCl_3$); Lit. [15] $[\alpha]_D$ +25.5° (*c* 1.0, $CHCl_3$). ^{1}H NMR (270 MHz, $CDCl_3$): δ 5.57 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 5.49 (t, 1 H, $J_{2,3}$ 9.9, $J_{3,4}$ 9.9 Hz, H-3), 5.23–5.17 (m, 2 H, H-3',4'), 5.08 (t, 1 H, $J_{4,5}$ 9.9 Hz, H-4), 4.86 (dd, 1 H, H-2), 4.33–4.25 (m, 2 H, H-5,6a), 4.17–4.07 (m, 2 H, H-6b,5'), 2.14, 2.10, 2.08, 2.07, 2.05, 2.02, (each s, each 3 H, OAc), 1.49 (s, 3 H, H-1'), 1.45 (d, 3 H, $J_{6',5'}$ 6.3 Hz, H-6'). Anal. Calcd for $C_{24}H_{34}O_{15}$: C, 51.24; H, 6.10. Found: C, 51.35; H, 6.17.

2,3,4,6,3',4'-Hexa-O-acetyl-6'-chloro-1',6'-dideoxy-1'-thioacetylsucrose (**16**).—Into a solution of pyridine (0.71 mL) and Tf_2O (0.74 mL) in CH_2Cl_2 (12 mL) was added dropwise a solution of **11** (600 mg, 1.09 mmol) in CH_2Cl_2 (12 mL) at 0 °C. After 40 min, the mixture was poured into aq $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was washed with M HCl (20 mL) and aq $NaHCO_3$ (20 mL), dried ($MgSO_4$), and then evaporated to dryness. The dry residue was dissolved in Me_2NCHO (12 mL) and LiCl (92 mg) was added to the solution at 0 °C. After 15 min, the mixture was diluted with EtOAc (30 mL) and extracted with water (20 mL). The organic layer was washed with brine (2 × 20 mL), dried ($MgSO_4$), and evaporated. The residue was dissolved in Me_2NCHO (12 mL) and sodium thioacetate (190 mg) was added to the solution at 0 °C. The solution was stirred for 1.5 h at room temperature, diluted with EtOAc, and extracted with water (20 mL). The organic layer was washed with brine, dried ($MgSO_4$) and evaporated. Flash chromatography (2:1 hexane–EtOAc) gave **16** (412 mg, 56%) as a colorless oil: $[\alpha]_D^{21}$ +51° (*c* 1.3, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$): δ 5.68 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 5.46 (t, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 10.5 Hz, H-3), 5.39 (d, 1 H, $J_{3',4'}$ 5.9 Hz, H-3'), 5.34 (t, 1 H, $J_{4',5'}$ 5.9 Hz, H-4'), 5.07 (dd, 1 H, $J_{4,5}$ 9.9 Hz, H-4), 4.92 (dd, 1

H, H-2), 4.30–4.08 (m, 3 H, H-5,6a,6b), 4.20 (q, 1 H, $J_{5',6'}$ 5.9 Hz, H-5'), 3.75 (d, 2 H, H-6'), 3.48 (d, 1 H, $J_{1'a,1'b}$ 14.5 Hz, H-1'a), 3.23 (d, 1 H, H-1'b), 2.37, 2.14, 2.12, 2.10, 2.08, 2.05, 2.03, (each s, each 3 H, OAc and SAc). Anal. Calcd for $C_{26}H_{35}ClO_{16}S$: C, 46.53; H, 5.27. Found: C, 46.26; H, 5.00.

2,3,4,6,3',4'-Hexa-O-*acetyl-1'-azido-6'-chloro-1',6'-dideoxysucrose* (**17**).—Into a solution of pyridine (1.77 mL) and Tf_2O (1.22 mL) in CH_2Cl_2 (20 mL) was added dropwise a solution of **11** (1.00 g, 1.81 mmol) in CH_2Cl_2 (20 mL) at 0 °C. After 20 min, the mixture was poured into aq $NaHCO_3$ and extracted with CH_2Cl_2, which was washed successively with M HCl (20 mL), water (20 mL), and aq $NaHCO_3$ (20 mL). The organic layer was dried ($MgSO_4$) and evaporated to dryness. The dry residue was dissolved in Me_2NCHO (20 mL) and LiCl (152 mg) was added to the solution at 0 °C. After 20 min, the mixture was diluted with EtOAc (50 mL) and extracted with water (20 mL). The organic layer was washed with water (20 mL) and brine (20 mL), dried ($MgSO_4$), and evaporated. The residue was dissolved in Me_2NCHO (20 mL), and NaN_3 (350 mg) was added to the solution at 0 °C. The solution was stirred for 4.5 h at room temperature, then diluted with EtOAc, and extracted with water (20 mL). The organic layer was washed with brine, dried ($MgSO_4$), and evaporated. Flash chromatography (2:1 hexane–EtOAc) gave a colorless oil. Recrystallization from hexane–diethyl ether provided **17** (873 mg, 76%) as crystals: mp 95–96 °C; $[\alpha]_D^{22}$ +59° (*c* 0.99, $CHCl_3$); ^1H NMR (270 MHz, $CDCl_3$): δ 5.61 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 5.51 (d, 1 H, $J_{3',4'}$ 6.3 Hz, H-3'), 5.44 (t, 1 H, $J_{4',5'}$ 6.3 Hz, H-4'), 5.42 (t, 1 H, $J_{2,3}$ 9.9, $J_{3,4}$ 9.9 Hz, H-3), 5.06 (t, 1 H, $J_{4,5}$ 9.9 Hz, H-4), 4.91 (dd, 1 H, H-2), 4.29–4.19 (m, 3 H, H-5,6a,6b), 4.27 (q, 1 H, $J_{5',6'}$ 6.3 Hz, H-5'), 3.78 (d, 2 H, H-6'), 3.42 (s, 2 H, H-1'), 2.18, 2.17, 2.11, 2.10 (each s, 18 H, OAc). Anal. Calcd for $C_{24}H_{32}ClN_3O_{15}$: C, 45.18; H, 5.07; N, 6.59. Found: C, 44.74; H, 5.12; N, 6.34.

2,3,4,6,3',4'-Hexa-O-*acetyl-6'-chloro-1',6'-dideoxy-1'-fluorosucrose* (**18**).—Into a solution of pyridine (1.47 mL) and Tf_2O (1.41 mL) in CH_2Cl_2 (20 mL) was added dropwise a solution of **11** (1.20 g, 2.18 mmol) in CH_2Cl_2 (24 mL) at 0 °C. After 40 min, the mixture was poured into aq $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was washed with M HCl (20 mL), water (20 mL), and aq $NaHCO_3$ (20 mL), dried ($MgSO_4$), and then evaporated to dryness. The dry residue was dissolved in Me_2NCHO (20 mL) and LiCl (184 mg) was added to the solution at 0 °C. After 10 min, the mixture was diluted with EtOAc (100 mL) and extracted with water (20 mL). The organic layer was washed with water (20 mL) and brine (20 mL), dried ($MgSO_4$), and evaporated. The residue was dissolved in CH_2Cl_2 (24 mL), and TASF (1.8 g) was added to the solution under an Ar atmosphere. The solution was stirred for 48 h at room temperature, then washed with brine and aq $NaHCO_3$, dried ($MgSO_4$), and evaporated. Flash chromatography (2:1 hexane–EtOAc) gave **18** (654 mg, 49%) as crystals: mp 118–119 °C; $[\alpha]_D^{21}$ +33° (*c* 1.2, $CHCl_3$); ^1H NMR (270 MHz, $CDCl_3$): δ 5.60 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 5.50 (d, 1 H, $J_{3',4'}$ 6.3 Hz, H-3'), 5.44 (t, 1 H, $J_{4',5'}$ 6.3 Hz, H-4'), 5.43 (dd 1 H, $J_{2,3}$ 10.6, $J_{3,4}$ 9.7 Hz, H-3), 5.06 (t, 1 H, $J_{4,5}$ 9.7 Hz, H-4), 4.90 (dd, 1 H, H-2), 4.50 (dd, 1 H, $J_{1'a,1'b}$ 10.2, $J_{1'a,F}$ 46.5 Hz, H-1'a), 4.34 (dd, 1 H, $J_{1'b,F}$ 46.5 Hz, H-1'b), 4.29 (ddd, 1 H, $J_{5,6a}$ 2.3, $J_{5,6b}$ 5.0 Hz, H-5), 4.26–4.12 (m, 3 H, H-6a,6b,5'), 3.78 (d, 2 H, $J_{5',6'}$ 6.3 Hz, H-6'), 2.19, 2.11, 2.08, 2.05, 2.02 (each s, 18 H, OAc); ^{13}C NMR (67 MHz, $CDCl_3$): 170.7, 170.1, 170.0, 169.9, 169.7, 169.5, 103.3 (d, J_{CF} 20.6 Hz), 90.3,

81.2 (d, J_{CF} 180.7 Hz), 81.0, 76.2, 75.9, 70.0, 69.5, 68.6, 68.2, 62.0, 43.9, 20.8, 20.7, 20.7, 20.6, 20.6, 20.5. Anal. Calcd for $C_{24}H_{32}ClFO_{15}$: C, 46.87; H, 5.26. Found: C, 46.67; H, 4.62.

2,3,4,6,3′,4′-Hexa-O-acetyl-6′-chloro-6′-deoxysucrose (**19**).—To a solution of **11** (1.10 g, 1.85 mmol) in pyridine (13 mL) were added CCl_4 (2.6 mL) and triphenylphosphine (1.29 g). After 30 min at 60 °C, MeOH (10 mL) was added to the mixture, and the mixture was stirred for 5 min at the same temperature. After cooling to room temperature, the mixture was diluted with EtOAc (100 mL). The organic layer was washed with brine (2 × 50 mL), dried ($MgSO_4$), and evaporated. Flash chromatography (3:1 EtOAc–hexane) gave **19** as crystals. Recrystallization from diethyl ether gave **19** (836 mg, 74%): mp 125–126 °C; $[\alpha]_D^{22}$ +29° (*c* 0.85, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$): δ 5.61 (d, 1 H, $J_{1,2}$ 3.3 Hz, H-1), 5.49–5.41 (m, 3 H, H-3,3′,4′), 5.06 (t, 1 H, $J_{3,4}$ 9.9, $J_{4,5}$ 9.9 Hz, H-4), 4.93 (dd, 1 H, $J_{2,3}$ 10.6 Hz, H-2), 4.30 (ddd, 1 H, $J_{5,6a}$ 2.6, $J_{5,6b}$ 2.0 Hz, H-5), 4.25–4.18 (m, 2 H, H-5′,6a), 4.13 (dd, 1 H, $J_{6'a,6'b}$ 11.8 Hz, H-6b), 3.78 (d, 2 H, $J_{6',5}$ 6.3 Hz, H-6′), 3.73 (dd, 1 H, $J_{1'a,OH}$ 7.6, $J_{1'a,1'b}$ 12.5 Hz, H-1′a), 3.61 (dd, 1 H, $J_{1'b,OH}$ 6.3 Hz, H-1′b), 2.55 (dd, 1 H, OH), 2.20, 2.11, 2.09, 2.05, 2.02 (each s, 18 H, OAc). Anal. Calcd for $C_{24}H_{33}ClO_{16}$: C, 47.02; H, 5.44. Found: C, 46.79; H, 5.32.

2,3,4,6,3′,4′-Hexa-O-acetyl-6′-chloro-6′-deoxy-1′-O-methylsucrose (**20**).—To a solution of **19** (614 mg, 1.00 mmol) in CH_2Cl_2 (6.1 mL) were added $BF_3 \cdot OEt_2$ (24 μL) and a solution of CH_2N_2 in CH_2Cl_2 (21 mL) [10] at 0 °C. The solution was stirred for 1.5 h at 0 °C, then diluted with aq $NaHCO_3$, and extracted with CH_2Cl_2 (2 × 50 mL). The combined organic layers was washed with water, dried ($MgSO_4$), and evaporated. Flash chromatography (2:1 hexane–EtOAc) gave **20** (620 mg, 99%) as a colorless oil: $[\alpha]_D^{24}$ +42° (*c* 1.7, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$): δ 5.61 (d, 1 H, $J_{3',4'}$ 6.3 Hz, H-3′), 5.60 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 5.41 (t, 1 H, $J_{2,3}$ 9.9, $J_{3,4}$ 9.9 Hz, H-3), 5.41 (t, 1 H, $J_{4',5'}$ 6.3 Hz, H-4′), 5.07 (t, 1 H, $J_{4,5}$ 9.9 Hz, H-4), 4.88 (dd, 1 H, H-2), 4.32–4.11 (m, 3 H, H-5,6a,6b), 4.22 (t, 1 H, $J_{5',6'}$ 6.3 Hz, H-5′), 3.77 (d, 2 H, H-6′), 3.56 (d, 1 H, $J_{1'a,1'b}$ 10.9 Hz, H-1′a), 3.41 (d, 1 H, H-1′b), 3.40 (s, 3 H, OMe), 2.15, 2.10, 2.09, 2.05, 2.02, (each s, 18 H, OAc). Anal. Calcd for $C_{25}H_{35}ClO_{16}$: C, 47.88; H, 5.64. Found: C, 47.85; H, 5.85.

1′,6′-Dichloro-1′,6′-dideoxysucrose (**4**).—To a solution of **13** (422 mg, 0.669 mmol) in dry MeOH (7 mL) was added NaOMe (5 mg). The mixture was left at room temperature overnight and evaporated. The residue was purified by gel filtration to give **4** (233 mg, 92%), after lyophilization, as a white solid: $[\alpha]_D^{24}$ +59° (*c* 1.0, MeOH); Lit. [15] $[\alpha]_D$ +67° (*c* 1.0, MeOH). ^{1}H NMR (270 MHz, D_2O): δ 5.43 (d, 1 H, $J_{1,2}$ 4.0 Hz, H-1), 4.42 (d, 1 H, $J_{3',4'}$ 8.3 Hz, H-3′), 4.13 (t, 1 H, $J_{4',5'}$ 8.3 Hz, H-4′), 4.13–4.04 (m, 1 H, H-5′), 3.89 (d, 2 H, $J_{5',6'}$ 5.3 Hz, H-6′), 3.90–3.73 (m, 5 H, H-5,6a,6b, 1′a, 1′b), 3.76 (t, 1 H, $J_{2,3}$ 9.6, $J_{3,4}$ 9.6 Hz, H-3), 3.57 (dd, 1 H, H-2), 3.45 (t, 1 H, $J_{4,5}$ 9.6 Hz, H-4); ^{13}C NMR (67 MHz, D_2O): δ 104.2, 93.5, 81.9, 77.0, 76.2, 73.5, 73.3, 71.1, 70.2, 61.1, 45.6, 44.1. Anal. Calcd for $C_{12}H_{20}Cl_2O_9$: C, 38.00; H, 5.33. Found: C, 38.09; H, 5.15.

1′,6′-Dideoxysucrose (**5**).—To a solution of **14** (238 mg, 0.423 mmol) in dry MeOH (5 mL) was added NaOMe (6 mg). The mixture was stirred for 6 h at room temperature and evaporated. The concentrated residue was dissolved in water (10 mL), and the solution was washed with EtOAc (10 mL). The aqueous layer was decolorized with charcoal (0.5 g), evaporated, and dried in desiccator to give **5** (131 mg, 99%) as a white

solid: $[\alpha]_D^{21}$ +45° (*c* 1.2, H_2O); ^1H NMR (270 MHz, D_2O): δ 5.33 (d, 1 H, $J_{1,2}$ 4.0 Hz, H-1), 3.99–3.79 (m, 4 H, H-5,6a,6b,5′), 3.95 (d, 1 H, $J_{3',4'}$ 8.2 Hz, H-3′), 3.90 (t, 1 H, $J_{4',5'}$ 8.2 Hz, H-4′), 3.76 (t, 1 H, $J_{2,3}$ 9.6, $J_{3,4}$ 9.6 Hz, H-3), 3.57 (dd, 1 H, H-2), 3.51 (t, 1 H, $J_{4,5}$ 9.6 Hz, H-4), 1.56 (s, 3 H, H-1′), 1.35 (d, 3 H, $J_{6',5'}$ 5.9 Hz); ^{13}C NMR (67 MHz, D_2O): δ 105.1, 93.8, 81.8, 79.5, 77.2, 73.5, 73.1, 72.2, 70.1 60.8, 21.5, 19.5. Anal. Calcd for $C_{12}H_{22}O_9$: C, 46.44; H, 7.16. Found: C, 46.78; H, 7.04.

6′-Chloro-1′,6′-dideoxy-1′-thiosucrose (**6**).—To a solution of **16** (400 mg, 0.596 mmol) in dry MeOH (4.4 mL) were added NaOMe (6 mg) and dithiothreitol (46 mg) under an Ar atmosphere. The solution was stirred for 2.5 h and evaporated. The residue was purified by gel filtration to give **6** (220 mg, 98%), after lyophilization, as a white solid: $[\alpha]_D^{20}$ +27° (*c* 1.0, H_2O); ^1H NMR (270 MHz, D_2O): δ 5.41 (d, 1 H, $J_{1,2}$ 4.0 Hz, H-1), 4.51 (d, 1 H, $J_{3',4'}$ 8.3 Hz, H-3′), 4.14 (t, 1 H, $J_{4',5'}$ 8.3 Hz, H-4′), 4.06 (dt, 1 H, $J_{5',6'}$ 4.3 Hz, H-5′), 3.88 (d, 2 H, H-6′), 3.89–3.75 (m, 3 H, H-5,6a,6b), 3.74 (dd, 1 H, $J_{2,3}$ 9.9, $J_{3,4}$ 9.5 Hz, H-3), 3.57 (dd, 1 H, H-2), 3.45 (t, 1 H, $J_{4,5}$ 9.5 Hz, H-4), 3.08 (d, 1 H, $J_{1'a,1'b}$ 14.5 Hz, H-1′a), 2.88 (d, 1 H, H-1′b); ^{13}C NMR (67 MHz, D_2O): δ 105.8, 93.3, 81.8, 77.1, 76.4, 73.4, 73.3, 71.9, 70.2, 61.1, 45.8, 28.3. Anal. Calcd for $C_{12}H_{21}ClO_9S$: C, 38.24; H, 5.63. Found: C, 38.26; H, 5.45.

1′-Amino-6′-chloro-1′,6′-dideoxysucrose (**7**).—The mixture of **17** (400 mg, 0.627 mmol) and 5% palladium-on-charcoal (100 mg) in dry MeOH (20 mL) was stirred under 1 atm of hydrogen. After 30 min, the mixture was filtered and evaporated. The residue was dissolved in dry MeOH (8.6 mL), and NaOMe (8 mg) was added. The solution was stirred for 3 h and evaporated. The residue was purified by gel filtration to give **7** (125 mg, 55%), after lyophilization, as a white solid: $[\alpha]_D^{17}$ +66° (*c* 0.83, H_2O); ^{13}C NMR (67 MHz, D_2O): 104.6, 93.0, 81.6, 78.5, 76.7, 73.5, 73.4, 71.9, 70.3, 61.2, 45.9. Anal. Calcd for $C_{12}H_{22}ClNO_9$: C, 40.06; H, 6.18; N, 3.89. Found: C, 39.77; H, 5.61; N, 3.51.

6′-Chloro-1′,6′-dideoxy-1′-fluorosucrose (**8**).—To a solution of **18** (614 mg, 0.998 mmol) in dry MeOH (12 mL) was added NaOMe (6 mg). The solution was stirred for 24 h and evaporated. The residue was purified by gel filtration to give **8** (370 mg, 99%) as a white solid: $[\alpha]_D^{20}$ +58° (*c* 0.63, H_2O); ^1H NMR (270 MHz, D_2O): δ 5.40 (d, 1 H, $J_{1,2}$ 4.0 Hz, H-1), 4.59 (dd, 1 H, $J_{1'a,1'b}$ 10.9, $J_{1'a,F}$ 46.2 Hz, H-1′a), 4.50 (dd, 1 H, $J_{1'b,F}$ 46.2 Hz, H-1′b), 4.25 (d, 1 H, $J_{3',4'}$ 7.6 Hz, H-3′), 4.15 (t, 1 H, $J_{4',5'}$ 7.6 Hz, H-4′), 4.07 (dd, 1 H, $J_{5',6'}$ 5.6 Hz, H-5′), 3.89 (d, 2 H, H-6′), 3.92–3.77 (m, 3 H, H-5,6a,6b), 3.75 (dd, 1 H, $J_{2,3}$ 10.2, $J_{3,4}$ 9.6 Hz, H-3), 3.55 (dd, 1 H, H-2), 3.43 (t, 1 H, $J_{4,5}$ 9.6 Hz, H-4); ^{13}C NMR (67 MHz, D_2O): 102.9 (d, J_{CF} 20.7 Hz), 93.6, 81.7, 81.2 (d, J_{CF} 175.8Hz), 76.9, 76.2, 73.4, 73.2, 71.7, 70.2, 61.2, 45.8. Anal. Calcd for $C_{12}H_{20}ClFO_9$: C, 39.73; H, 5.57. Found: C, 39.76; H, 5.34.

6′-Chloro-6′-deoxy-1′-O-methylsucrose (**9**).—To a solution of **20** (770 mg, 1.23 mmol) in dry MeOH (8 mL) was added NaOMe (5 mg). The solution was stirred for 12 h and evaporated. The residue was purified by gel filtration to give a colorless oil, which crystallized on standing. Recrystallization from EtOH gave **9** (428 mg, 93%): mp 88–90 °C; $[\alpha]_D^{22}$ +70° (*c* 1.0, H_2O); ^1H NMR (270 MHz, D_2O): δ 5.38 (d, 1 H, $J_{1,2}$ 4.0 Hz, H-1), 4.20 (d, 1 H, $J_{3',4'}$ 8.6 Hz, H-3′), 4.11 (t, 1 H, $J_{4',5'}$ 8.6 Hz, H-4′), 4.02 (dt, 1 H, $J_{5',6'}$ 4.0 Hz, H-5′), 3.87–3.82 (m, 3 H, H-5,6a,6b), 3.80 (d, 1 H, $J_{1'a,1'b}$ 9.9 Hz, H-1′a), 3.75 (d, 1 H, H-1′b), 3.70 (t, 1 H, $J_{2,3}$ 9.9, $J_{3,4}$ 9.9 Hz, H-3), 3.62 (d, 2 H, H-6′), 3.53 (dd, 1 H, H-2), 3.43 (t, 1 H, $J_{4,5}$ 9.9 Hz, H-4), 3.42 (s, 3 H, OMe); ^{13}C NMR (67 MHz,

D_2O): 104.0, 93.5, 81.5, 77.4, 76.4, 73.4, 73.3, 71.9, 71.7, 70.2, 61.2, 59.7, 45.9. Anal. Calcd for $C_{13}H_{23}ClO_{10}$: C, 41.66; H, 6.20. Found: C, 41.80; H, 5.97.

General procedure of enzymatic glucosylation.—HPLC analysis. The reactions were performed on 0.095–0.11 mmol scale. To a solution of one of the donor **1**, **4–9** (0.150 M) and the acceptor **2** (0.225 M) in calcium acetate buffer containing methyl α-D-glucopyranoside (0.15 M) as an internal standard (50 mM, pH 5.6) was added the immobilized cell of *P. rubrum* (1% w/v), and the mixture was incubated at 20 °C. The 10 μL aliquots of reaction mixture was taken at 6, 12, 24, 48, 72 h, and stored at 0 °C. The 2 μL of samples were injected onto the HPLC column, and the amount of product **3** was determined by comparing the peak areas with that of the internal standard. The HPLC analysis was performed on an ERC-NH-117 column (Erma) with 80–87% CH_3CN as a solvent at the flow rate of 1.0 mL/min using refractive index detector (RI-3H, Japan Analytical Industry).

Isolation of methyl 5-O-(α-D-glucopyranosyl)-β-D-arabinofuranoside (**3**).—To a solution of **1** (36 mg, 0.10 mmol) and **2** (25 mg, 0.15 mmol) in the buffer solution (0.33 mL) was added immobilized cell (3.3 mg), and the mixture was incubated at 20 °C for 72 h. The reaction mixture was filtered to remove the immobilized cells, and the filtrate was concentrated. Flash chromatography (12:2:1 AcOEt–EtOH–H_2O) of the residue gave **3** (15 mg, 46%) and **21** (15 mg).

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