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Glycosyltransfer mechanism of α-glucosyltransferase from *Protaminobacter rubrum*

Hiroyuki Kakinuma, Hideya Yuasa, Hironobu Hashimoto *

Department of Life Science, Faculty of Bioscience and Biotechnology, Tokyo Institute of Technology, Nagatsuta, Midoriku, Yokohama, 226-8501 Japan

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Abstract

α-Glucosyltransferase (αGT) from *Protaminobacter rubrum* catalyzes either an intramolecular transglucosylation of sucrose, giving isomaltulose, or intermolecular transglucosylations from sucrose to versatile acceptors. To obtain insights into this enzyme, especially in connection with the glycosyltransfer mechanism, kinetic studies were carried out using synthetic sucrose analogs. The k_{cat} values obtained for 1′-substituted sucrose analogs exhibited a strong correlation with Hammett substituent constants with a slope of −2, suggesting a rate-limiting glycoside cleavage with a completely protonated transition state. The α-deuterium isotope effect ($k_H/k_D = 1.20 \pm 0.08$) indicated an oxocarbenium ion-like transition state. Nojirimycin and 1′-amino-1′-deoxy and 3′-amino-3′-deoxy analogs of 6′-chlorosucrose had relatively strong inhibitory effects toward the intramolecular glucosylation, (inhibition at 10 mM being 97, 71, and 78%, respectively) in contrast to the 4′-amino-4′-deoxy and 6′-amino-6′-deoxy analogs, which showed moderate or no inhibitory activity. This tendency is presumably due to the presence of carboxylates as catalytic groups of the enzyme. These results reveal similarities between αGT and some glycosidases in the glycoside cleavage mechanism.

Keywords: *Protaminobacter rubrum*; α-Glucosyltransferase; Glucosyltransfer mechanism; 3′-Amino-6′-chloro-3′,6′-dideoxysucrose

1. Introduction

α-Glucosyltransferase (αGT) from *Protaminobacter rubrum* is an industrially important enzyme, since it produces non-cariogenic isomaltulose [6-*O*-(α-D-glucopyranosyl)-D-fructose] [1,2] from sucrose by an intramolecular transglucosylation. This enzyme also catalyzes intermolecular transglucosylation when a 6′-blocked sucrose derivative is used as a donor substrate (Scheme 1) [3,4], allowing α-D-glucosylation of the hydroxymethyl group attached to a five-membered ring structure, such as benzyl arabinofuranoside (**2**) or 2,3-*O*-isopropylideneglycerol. Depending on substrate and conditions, αGT hydrolyzes the α-glucopyranoside moiety [4,5]. Thus this enzyme can be classified as an α-glucosidase that works with retention of the anomeric configuration.

* Corresponding author. Fax: +81-45-922-2432.

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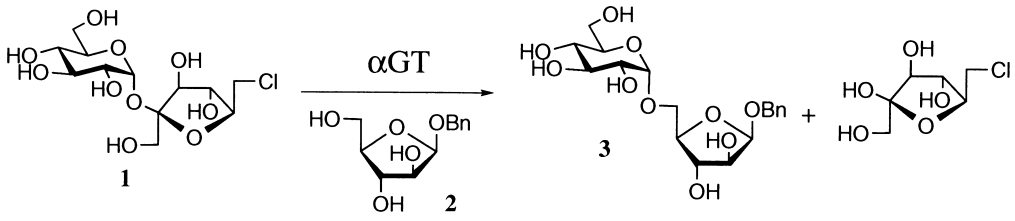


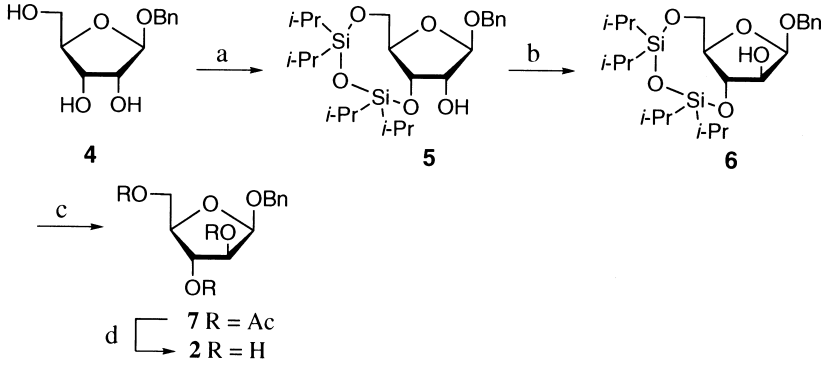
Scheme 1.

The glycosyl transfer reaction catalyzed by glycosidases has been extensively investigated [6], partly because understanding of it is important to improve the glycosyltransfer activity. Indeed, site-directed mutagenesis based on mechanistic considerations has allowed a developement of the glycosidase with high glycosyltransfer acitivity [7]. Since αGT is apparently similar to glycosidases and its glycosyltransfer activity is potentially improvable, we feel compelled to investigate its mechanism. Among a number of experimental methods to probe the mechanism of enzyme, kinetic studies with versatile substrates are considered relatively easy to obtain the information on the catalytic groups and the structure of transition states [8]. Thus we examined a Hammett analysis for the reaction rates (k_{cat}) with respect to 1′-substituents of the sucrose analogs, α-deuterium isotope effect on k_{cat}, and inhibitory effects of some aminodeoxy derivatives of sucrose. Syntheses of the 1′-deuterio-sucrose derivative and the aminodeoxy sucrose analogs are also reported.

2. Results and discussion

Syntheses of substrates and inhibitors.—To our knowledge, the synthesis of benzyl β-D-arabinofuranoside (**2**), the glycosyl acceptor, has not been reported. Indeed, all attempts to obtain the compound **2** by treatment of D-arabinose with acidic benzyl alcohol ended in a very poor yield. We thus synthesized compound **2** by inversion of the configuration at C-2 of benzyl β-D-ribofuranoside (**4**) [9]. Protection of the hydroxyl groups at the 3,5-position of β-D-ribofuranoside **4** was achieved by disiloxanylidenation to give compound **5** (Scheme 2). Swern oxidation, followed by reduction with $NaBH_4$, afforded inversion of the configuration at C-2 to give the arabinofuranoside derivative **6**. The coupling constants for the ring protons of the both compounds **5** ($J_{1,2}=0$, $J_{2,3}=J_{3,4}=5.3$ Hz) and **6** ($J_{1,2}=4.6$, $J_{2,3}=7.3$, $J_{3,4}=5.6$ Hz) suggests a twist conformation ($^3T_2$). Deprotection of the disiloxanylidene group of the compound **6** gave the desired compound **2**.

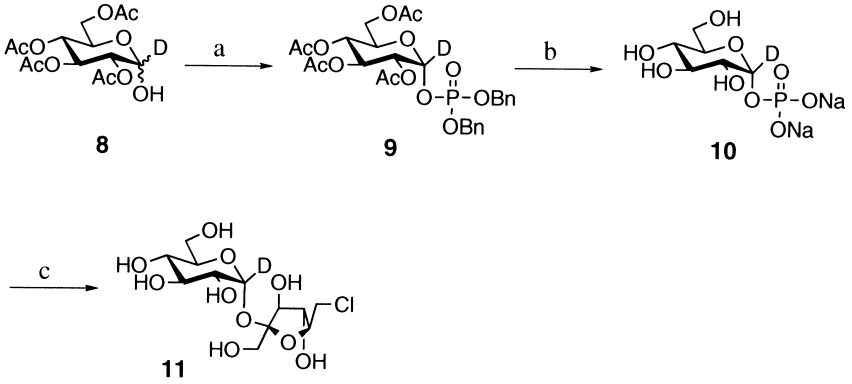
First attempt at the synthesis of [1-^{2}H]-sucrose derivative **11** was made by a glucosyl transfer from uridine 5′-(α-D-[1-^{2}H]-glucopyranosyl diphosphate) to 6-chloro-6-deoxy-D-fructose using sucrose synthetase. Though this enzyme is known to catalyze the glucosylation of 1-deoxy-1-fluoro-D-fructose to give 1′-deoxy-1′-fluorosucrose [10], our substrates gave no coupling products. Thus we turned our attention to the reverse reaction catalyzed by phosphorylase, which has been used for the chemoenzymatic synthesis of sucrose [11,12]. The substrate phosphate **10** was synthesized by dibenzyl



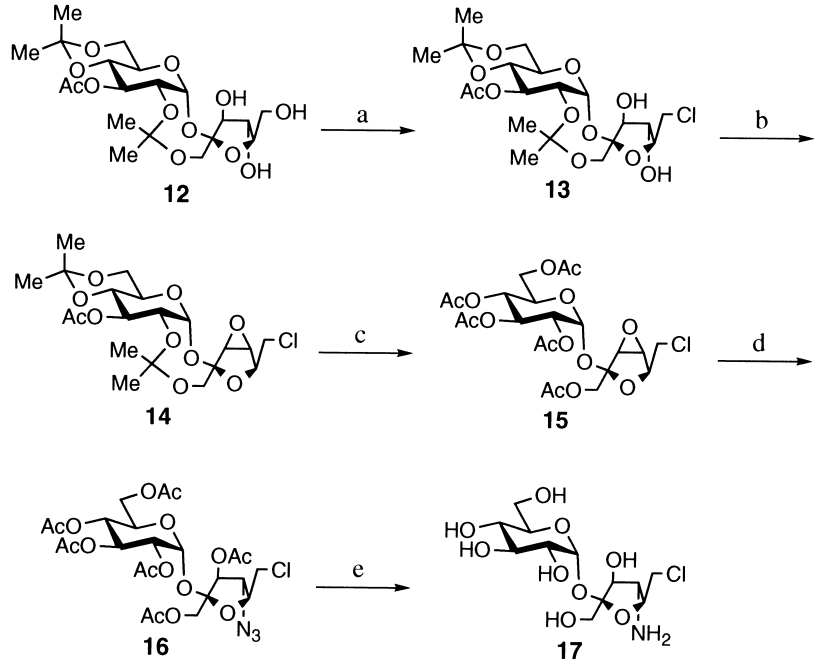
Scheme 2. (a) 1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane, imidazole, 72%; (b) 1: $(COCl)_2$–Me_2SO, Et_3N; 2: $NaBH_4$, 82%; (c) 1: Bu_4NF, 2: Py, Ac_2O, 93%; (d) NaOMe, 95%.

phosphorylation [13] of 2,3,4,6-tetra-*O*-acetyl-D-[1-^{2}H]-glucopyranose (**8**) [14], and removal of the benzyl groups by catalytic hydrogenation (Scheme 3). Transglucosylation of 6-chloro-6-deoxy-D-fructose with the phosphate **10** gave [1-^{2}H]-6′-chloro-6′-deoxysucrose (**11**), but the yield was poor.

4′-Amino-6′-chloro-4′,6′-dideoxysucrose (**17**) was synthesized (Scheme 4), according to the synthesis of 4′-acetamido-4′-deoxysucrose [15]. The 6′-chloro derivative **13**, which was derived from the suitably protected sucrose derivative **12** [16], was treated with PPh_3 and DEAD, and the *O*-isopropylidene groups were substituted with acetyl groups to give the 3′,4′-epoxide **15** with a *lyxo* configuration at the fructose moiety. The ^{1}H NMR spectrum of the compound **15**, e.g., $J_{3,4}=2.9$, $J_{4,5}=0$ Hz, was very similar to that reported for the corresponding 6′-*O*-acetate ($J_{3,4}=3.0$, $J_{4,5}=0.5$ Hz) [16], supporting the assignment. The pentaacetate **15** was treated with NaN_3 to give the 4′-azide derivative of 6′-chlorosucrose **16**. The chemical shifts for H-3′ and H-4′ (δ 5.36, 4.20) and coupling constants ($J_{3,4}=J_{4,5}=7.9$ Hz) fully support the assigned structure, as is the case for the corresponding 6′-acetate [15]. Deacetylation of **16**, followed by reduction of the azide group, afforded the desired compound **17**.

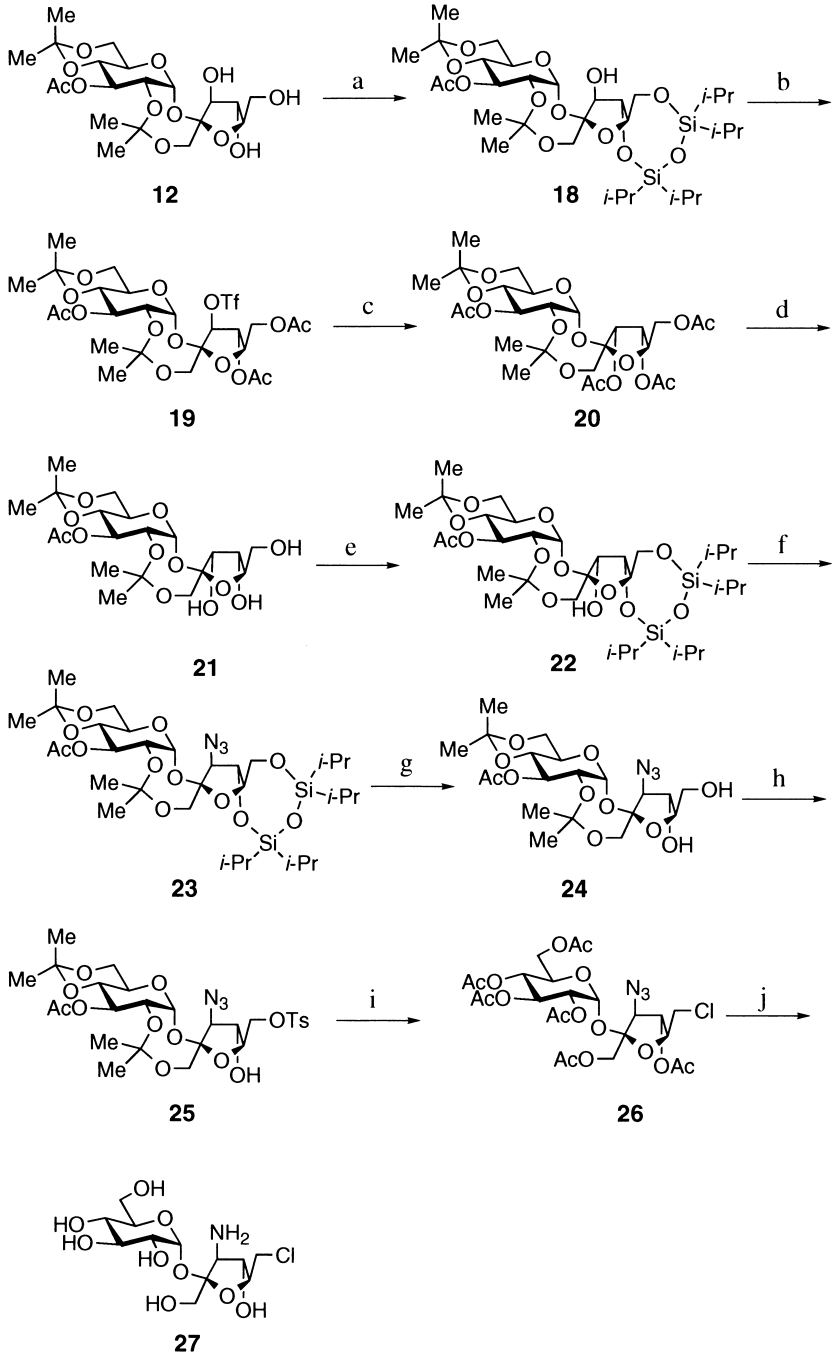


Scheme 3. (a) 1: LDA, 2: $PO(OBn)_2Cl$, 53% (α), 36% (β); (b) 1: 10% Pd–C/H_2, $NaHCO_3$, 2: Dowex 50W×8 (Na^+ form), 98%; (c) sucrose phosphorylase, 6-chloro-6-deoxyfructose, 13%.



Scheme 4. (a) PPh_3, CCl_4, 86%; (b) DEAD, PPh_3; (c) 1: 70% AcOH, 2: Py, Ac_2O, 53% (from **13**); (d) 1: NaN_3, NH_4Cl, 2: Py, Ac_2O, 84%; (e) 1: NaOMe, 2: Lindlar catalyst/H_2, quant.

Introduction of 3′-amino group into the sucrose derivative was rather problematic, and both the reductive amination of the 3′-ulose derivative and the nucleophilic ring-opening with NaN_3 of the 3′,4′-epoxide having a *ribo* configuration at the fructose moiety ended in failure. Thus, inversion of the configuration at 3′-position and substitution of the resulting hydroxyl group with azide group were employed (Scheme 5). Disiloxanylidenation of the di-*O*-isopropylidene derivative **12** gave compound **18**, in which only the 3′-OH is unprotected. Nucleophilic substitution reaction at C-3′ was first attempted for the 3′-*O*-trifluoromethanesulfonate (3′-*O*-Tf) of compound **18**. However, the reaction



Scheme 5. (a) 1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane, imidazole, 82%; (b) 1: Py, Tf_2O, 2: HF–Py, 3: Py, Ac_2O, 86%; (c) AcOCs, 18-crown-6, 99%; (d) NH_3, 0 °C, 71%; (e) 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane, imidazole, 82%; (f) 1: Py, Tf_2O, 2: NaN_3, 47%; (g) Bu_4NF, 56%; (h) Py, TsCl, 88%; (i) 1: LiCl, 2: 70% AcOH, 3: Py, Ac_2O, 82%; (j) 1: NaOMe, 2: Lindlar catalyst/H_2, 52%.

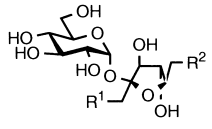
did not proceed at all, probably because of bulkiness of the disiloxanylidene group. Thus the disiloxanylidene group was removed, and acetates were introduced to the resulting hydroxyl groups to give compound **19**. The series of the 3′-*O*-Tf derivatives were unexpectedly stable, and compound **19** could be stored at 4 °C over more than one month. Compound **19** was treated with CsOAc in the presence of 18-crown-6 [17] affording the compound **20**. Selective deprotection of the acetyl groups at the 3′,4′,6′-positions of **20** with ammonia, followed by reconstruction of the disiloxanylidene group at the 4′,6′-positions, afforded compound **22**. Nucleophilic substitution reaction at the resulting 3′-OH with NaN_3, introduction of 6′-chloro group in a usual manner, and deprotection gave the desired product **27**. Stereochemistry of the fructose moiety of **27** was confirmed by the large coupling constants of the ring protons ($J_{3',4'} = J_{4',5'} = 9.2$ Hz).

Enzymatic study.—Michaelis–Menten parameters were obtained by Lineweaver–Burk plot for the intermolecular transglucosylation by αGT with respect to the donor substrates (**1, 28, 29, 30, 31, 32, 33**) [5] at a constant concentration of the acceptor substrate **2** (Table 1). Variation of the 1′-substituent significantly affects the k_{cat} value (Table 1), indicating that the formation of the glucosyl–enzyme intermediate (step 1 in Fig. 1) is rate limiting, because the structures of the liberated fructose derivatives cannot affect the cleavage of the intermediate (step 2 in Fig. 1). Hammett analysis was performed for the k_{cat} values with respect to the inductive effect of the 1′-substituents (Fig. 2). The 6′-substituents of the donor were determined to be unaffected to the kinetic constants from comparisons of the donor **1** and **28** (Table 1). When omitting the SH group, a significant correlation was obtained with r and ρ values of 0.961 and −2.0, respectively. This strongly negative ρ value suggests a large degree of positive charge accumulation on the leaving oxygen at the transition state, and thus a completely protonated transition state structure (Fig. 3) [19] is indicated. This suggestion is supported from the negative ρ values reported for the acid-catalyzed hydrolyses of aryl glycopyranosides [20–22]. Deviation of the SH substituent from the regression line is presumably due to an inhibition of protonation of the leaving oxygen by a hydrogen bonding. Though the K_m values also vary depending on the donor structure, no clear explanations for this structure–activity relationship were obtained from available substituent constants.

The secondary deuterium kinetic isotope effect measured for the 6′-chlorosucrose derivatives **1** and **11** ($k_H/k_D = 1.20 \pm 0.08$) indicates a glycosyl cation-like transition state, since the values more than 1.05 reflect substantial sp^3 to sp^2 rehybridization [23]. By the same token, transition states with oxocarbenium ion characters have been suggested for an acid-catalyzed hydrolysis of methyl α-D-glucopyranoside ($k_H/k_D = 1.14$) [24], and the hydrolyses catalyzed by lysozyme ($k_H/k_D = 1.11$) [25], exo-α-glucanase ($k_H/k_D = 1.20$) [26], sucrase ($k_H/k_D = 1.14$) [27], and isomaltase ($k_H/k_D = 1.20$) [27].

The characteristics of αGT evaluated above are similar to those of α-glucosidases [27–29], whose activities are inhibited by some azasugars [29,30]. A proposed inhibition mechanism of the azasugars is that the conjugated acids of the amines bind tightly to the deprotonated catalytic group. Therefore, if αGT has an acid catalytic group, nojirimycin is considered to inhibit its activity. In practice, nojirimycin inhibited the activity by 97% at 10 mM concentration (Table 2). Interestingly, while 1′-amino-1′-deoxy (**34**) [5] and 3′-amino-3′-deoxy **27** derivatives of 6′-chlorosucrose also inhibited the activity to a considerable extent, the 4′-amino-4′-deoxy **17** and 6′-amino-6′-deoxy (**35**) [31] derivatives scarcely inhibited the activity (Table 2). This is considered due to the presence of acid catalytic groups, probably carboxylates, near the reaction center, as proposed to those in most glycosidases [30]. We thus propose a structure of the transition state of the transglucosylation catalyzed by αGT as depicted in Fig. 3, where the two

Table 1
Michaelis–Menten parameters for the transglucosylation by αGT

Donor	σ_I [a] of R^1	K_m (mM)	k_{cat} (s^{-1})	k_{cat}/K_m (s^{-1} mM^{-1})
				
1 (R^1 = OH, R^2 = Cl)	0.24	46	16	0.35
28 (R^1 = OH, R^2 = H)	0.24	43	15	0.35
29 (R^1 = Cl, R^2 = Cl)	0.47	5.9	3.0	0.51
30 (R^1 = H, R^2 = H)	0.0	66	22	0.33
31 (R^1 = SH, R^2 = Cl)	0.27	6.1	1.8	0.30
32 (R^1 = F, R^2 = Cl)	0.54	8.7	2.0	0.23
33 (R^1 = OMe, R^2 = Cl)	0.30	53	7.3	0.14

[a] The σ_I values were taken from Ref. [18].

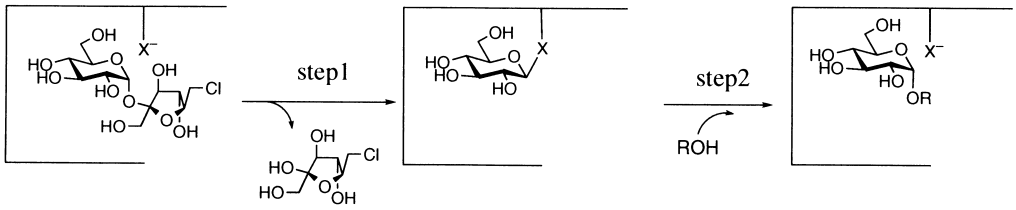


Fig. 1. A ping-pong mechanism in the transglucosylation reaction catalyzed by αGT.

carboxylates behave as catalytic groups and one of them is used for the protonation of the leaving oxygen.

3. Experimental

Melting points were measured with a Yanagimoto melting point apparatus and are uncorrected. Optical rotations were determined with a JASCO DIP-4 polarimeter. NMR spectra were obtained with a JEOL EX-270 spectrometer. In ^{1}H NMR, chemical shifts were recorded in ppm using Me_4Si (0 ppm in $CDCl_3$) or HDO (4.8 ppm in D_2O) as an internal standard. $CDCl_3$ (77.0 ppm) or dioxane (64.0 ppm in D_2O) was used as an internal standard in ^{13}C NMR. In ^{31}P NMR, the external standard H_3PO_4 was adjusted to 0 ppm. Flash column chromatography was carried out on Wako gel C-300 (Wako). Gel filtration was performed on Sephadex G-15 (Pharmacia). Sucrose phosphorylase (EC 2.4.1.7) from *Leuconostoc mesenteroides* was purchased from Sigma. α-GT from *P. rubrum* CBS 574,77 was kindly donated by Mitsui Sugar Co. Ltd.

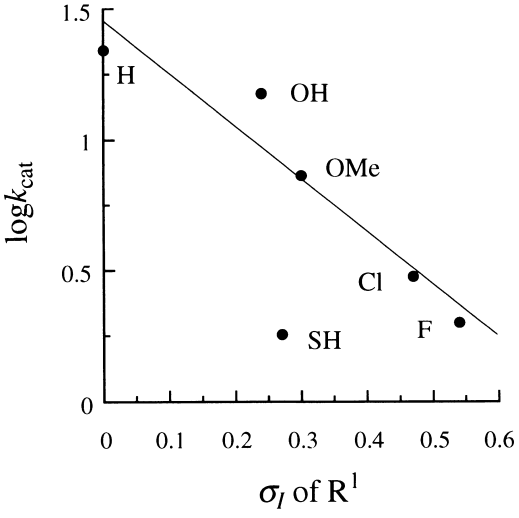


Fig. 2. Hammett plot for the transglucosylation reaction of the 1′-substituted sucrose analogs catalyzed by αGT.

Benzyl 3,5-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranoside (**5**).—To a solution of benzyl β-D-ribofuranoside (**4**) [9] (2.54 g, 10.6 mmol) in Me_2NCHO (25 mL) were added imidazole (2.88 g, 42.3 mmol) and 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (3.4 mL, 10.6 mmol) under Ar atmosphere at 0 °C. After 15 h, the mixture was diluted with aq $NaHCO_3$ and extracted with EtOAc (2×100 mL). The combined organic layer was washed with brine, dried ($MgSO_4$), and evaporated. Flash chromatography (8:1 hexane–EtOAc) gave **5** (3.69 g, 72%): $[\alpha]_D^{22}$ −84° (*c* 1.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.40–7.25 (m, 5 H, Ph), 5.03 (s, 1 H, H-1), 4.68 (d, 1 H, *J* 11.6 Hz, Ph*CH*$_2$), 4.57 (t, 1 H, $J_{2,3}$ 5.3, $J_{3,4}$ 5.3 Hz, H-3), 4.44 (d, 1 H, Ph*CH*$_2$), 4.10–4.02 (m, 2 H, H-5a, 5b), 4.09 (d, 1 H, H-2), 3.84–3.76 (m, 1 H, H-4), 2.99 (brs, 1 H), 1.15–0.98 (m, 28 H, CH(CH$_3$)$_2$). Anal. Calcd for $C_{24}H_{42}O_6Si_2$: C, 59.71; H, 8.77. Found: C, 59.63; H, 8.57.

Benzyl 3,5-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranoside (6).—To a solution of $(COCl)_2$ (0.82 mL, 9.6 mmol) in CH_2Cl_2 (15 mL) was added dropwise a solution of Me_2SO (1.33 mL, 18.7 mmol) in CH_2Cl_2 (5 mL) at −78 °C. After stirring for 15 min, a solution of **5** (1.52 g, 3.15 mmol) in CH_2Cl_2 (18 mL) was added. After stirring at −78 °C for 40 min, Et_3N (2.6 mL, 19 mmol) was added dropwise so that the pH of the solution became 6.5–7. After 5 min, the solution was warmed up to the room temperature, mixed

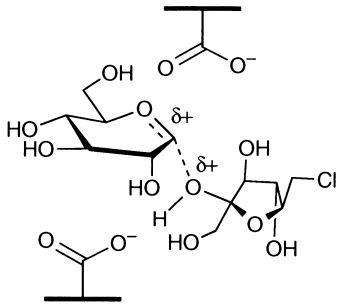
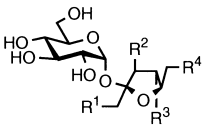


Fig. 3. A proposed structure of the transition state.

Table 2
Inhibitory activity of the aminodeoxysucrose derivatives toward the conversion of sucrose into isomaltulose by αGT

Inhibitor	Inhibition [a] (%)
	
Nojirimycin	97
34 ($R^1 = NH_2$, $R^2 = R^3 = OH$, $R^4 = Cl$)	71
27 ($R^1 = R^3 = OH$, $R^2 = NH_2$, $R^4 = Cl$)	78
17 ($R^1 = R^2 = OH$, $R^3 = NH_2$, $R^4 = Cl$)	20
35 ($R^1 = R^2 = R^3 = OH$, $R^4 = NH_2$)	0

[a] Inhibition at inhibitor and substrate (sucrose) concentrations of 10 and 250 mM, respectively.

with H_2O, and extracted twice with $CHCl_3$. The combined organic layer was dried ($MgSO_4$) and concentrated to give a colorless oil, which was dissolved in EtOH (15 mL). To the solution was added dropwise a solution of $NaBH_4$ (234 mg, 6.19 mmol) in 2:1 EtOH–H_2O (10 mL) at 0 °C. After 15 min, the mixture was neutralized with 30% AcOH, diluted with H_2O, and then extracted with EtOAc. The organic layer was washed with brine, dried ($MgSO_4$), and evaporated. Flash chromatography (15:1 hexane–EtOAc) gave **6** (1.25 g, 82%): $[\alpha]_D^{20}$ −100° (*c* 3.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.40–7.25 (m, 5 H, Ph), 4.97 (d, 1 H, $J_{1,2}$ 4.6 Hz, H-1), 4.77 (d, 1 H, *J* 11.5 Hz, Ph*CH*$_2$), 4.50 (d, 1 H, Ph*CH*$_2$), 4.26 (dd, 1 H, $J_{2,3}$ 7.3, $J_{3,4}$ 5.6 Hz, H-3), 4.14 (ddd, 1 H, $J_{2,OH}$ 9.6 Hz, H-2), 3.98 (dd, 1 H, $J_{4,5a}$ 3.0, $J_{5a,5b}$ 9.9 Hz, H-5a), 3.89 (ddd, 1 H, $J_{4,5b}$ 8.3 Hz, H-4), 3.80 (dd, 1 H, H-5b), 2.31 (d, 1 H, OH), 1.15–0.98 (m, 28 H, CH(CH_3)$_2$). Anal. Calcd for $C_{24}H_{42}O_6Si_2$: C, 59.71; H, 8.77. Found: C, 59.58; H, 8.72.

Benzyl 2,3,4-tri-O-*acetyl*-β-D-*arabinofuranoside* (**7**).—To a solution of **6** (1.25 g, 2.59 mmol) in THF (12 mL) was added Bu_4NF in THF (1 M, 5.2 mL) at 0 °C. After 15 min, the mixture was concentrated and treated with Ac_2O (5.0 mL) and pyridine (10 mL) for 5 h. The solution was poured into aq $NaHCO_3$ and the mixture was extracted with EtOAc. The organic layer was washed with brine, dried ($MgSO_4$), and evaporated. Flash chromatography (5:1 hexane–EtOAc) gave **7** (0.88 g, 93%): $[\alpha]_D^{20}$ −132° (*c* 1.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.38–7.29 (m, 5 H, Ph), 5.40 (dd, 1 H, $J_{2,3}$ 6.6, $J_{3,4}$ 5.0 Hz, H-3), 5.33 (d, 1 H, $J_{1,2}$ 4.6 Hz, H-1), 5.06 (dd, 1 H, H-2), 4.78 (d, 1 H, *J* 11.9 Hz, Ph*CH*$_2$), 4.48 (d, 1 H, Ph*CH*$_2$), 4.43 (dd, 1 H, $J_{4,5a}$ 4.0, $J_{5a,5b}$ 11.6 Hz, H-5a), 4.22 (dd, 1 H, $J_{4,5b}$ 7.3 Hz, H-5b), 4.13 (ddd, 1 H, H-4), 2.10, 2.09, 2.05 (s, each 3 H, OAc). Anal. Calcd for $C_{18}H_{22}O_8$: C, 59.01; H, 6.05. Found: C, 58.72; H, 6.00.

Benzyl β-D-*arabinofuranoside* (**2**).—To a solution of **7** (1.15 g, 3.14 mmol) in dry MeOH (15 mL) was added NaOMe (10 mg, 0.19 mmol). The solution was stirred for 24 h and evaporated. Flash chromatography (10:1 $CHCl_3$–MeOH) gave a colorless oil, which crystallized on standing to give **2** (715 mg, 95%): mp 72–74 °C; $[\alpha]_D^{22}$ −105° (*c* 1.0, H_2O); ^{1}H NMR (D_2O): δ 7.50–7.38 (m, 5 H, Ph), 5.10 (d, 1 H, $J_{1,2}$ 4.3 Hz, H-1), 4.83 (d, 1 H, *J* 11.6 Hz, Ph*CH*$_2$), 4.62 (d, 1 H, Ph*CH*$_2$), 4.14 (dd, 1 H, $J_{2,3}$ 7.9 Hz, H-2), 4.06 (dd, 1 H, $J_{3,4}$ 6.9 Hz, H-3), 3.92 (dt, 1 H, $J_{4,5a}$ 3.3, $J_{4,5b}$ 6.9 Hz, H-4), 3.77 (dd, 1 H, $J_{5a,5b}$ 12.2 Hz, H-5a), 3.63 (dd, 1 H, H-5b); ^{13}C NMR (67 MHz, D_2O): δ 137.9, 129.5, 129.3, 129.1, 101.1, 82.9, 77.2, 75.5, 70.5, 64.1. Anal. Calcd for $C_{12}H_{16}O_5$: C, 59.99; H, 6.71. Found: C, 59.76; H, 6.75.

2,3,4,6-Tetra-O-*acetyl*-α-D-*[1-^{2}H]-glucopyranosyl dibenzylphosphate* (**9**).—To a solution of 2-propylamine (0.76 mL, 9.3 mmol) in dry THF (40 mL) was added 2.5 mL of BuLi (1.6 M in hexane) at −78 °C under Ar atmosphere. After 30 min, a solution of 2,3,4,6-tetra-*O*-acetyl-D-[1-^{2}H]-glucopyranose (**8**) [14] (698 mg, 2.00 mmol) in THF (30 mL) was added dropwise to the solution over 5 min. The mixture was stirred for 10 min, and 30 mL of dibenzylphosphorochloridate (0.45 M) in THF solution was added dropwise to the mixture over 3 min. After 10 min, the resulting mixture was warmed to room temperature, poured into aq $NaHCO_3$, and extracted with EtOAc. The organic layer was washed with brine, dried ($MgSO_4$), and evaporated. Flash chromatography (1:1 hexane–EtOAc) gave **9** (649 mg, 53%) and the β isomer (439 mg, 36%) as a colorless oil: $[\alpha]_D^{24}$ +83° (*c* 0.80, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.40–7.31 (m, 10 H, Ph), 5.49 (dd, 1 H, $J_{2,3}$ 10.2, $J_{3,4}$ 9.6 Hz, H-3), 5.11 (d, 2 H, *J* 8.6 Hz, Ph*CH*$_2$), 5.10 (dd, 1 H, $J_{4,5}$ 10.2, H-4), 5.08 (d, 2 H, *J* 8.6 Hz, Ph*CH*$_2$), 4.98 (dd, 1 H, $J_{H\text{-}P}$ 2.3 Hz, H-2), 4.17 (dd, 1 H, $J_{5,6a}$ 3.9, $J_{6a,6b}$ 12.5 Hz, H-6a), 4.08 (ddd, 1 H, $J_{5,6b}$ 2.3 Hz, H-5), 3.93 (dd, 1 H, H-6b); ^{31}P NMR (D_2O): δ −2.1. Anal. Calcd for $C_{28}H_{32}DO_{13}P$: C, 55.17; H(D), 5.62. Found: C, 55.24; H(D), 5.68.

α-D-*[1-^{2}H]-Glucosylphosphate, disodium salt* (**10**).—A mixture of **9** (672 mg, 1.10 mmol) and 10% Pd–C (30 mg) in 3:2 MeOH–10% $NaHCO_3$

(30 mL) was stirred under 1 atm of hydrogen gas. After 2.5 h, the suspension was filtered through a Celite pad and evaporated. The residue was dissolved in 13:6:1 MeOH–H_2O–Et_3N (10 mL), and the solution was stirred for 4.5 h and evaporated. The residue was passed through a column of Dowex 50W×8 (Na^+ form, 2×13 cm). After lyophilization, the residue was purified by gel filtration (1.7×100 cm) to give **10** (330 mg, 98%) as a white solid. ^1H, ^{13}C, and ^{31}P NMR indicated high purity of this compound: ^1H NMR (D_2O): δ 3.91 (ddd, 1 H, $J_{4,5}$ 10.2, $J_{5,6a}$ 2.3, $J_{5,6b}$ 5.3 Hz, H-5), 3.88 (dd, 1 H, $J_{6a,6b}$ 12.2 Hz, H-6a), 3.78 (dd, 1 H, $J_{2,3}$ 9.9, $J_{3,4}$ 9.2 Hz, H-3), 3.76 (dd, 1 H, H-6b), 3.51 (dd, 1 H, $J_{H\text{-}P}$ 2.0 Hz, H-2), 3.42 (dd, 1 H, H-4); ^{13}C NMR (67 MHz, D_2O): δ 73.5, 73.1, 72.3 (d, $J_{C\text{-}P}$ 7.3 Hz), 70.2, 61.2; ^{31}P NMR (D_2O): δ 1.6.

[1-^{2}H]-6′-Chloro-6′-deoxysucrose (**11**).—To a solution of 6-chloro-6-deoxyfructose (215 mg, 1.08 mmol) in 25 mM citrate buffer (pH 6.8, 4.13 mL) were added **10** (330 mg, 1.08 mmol) and sucrose phosphorylase (9.7 mg, 49 U), and the mixture was incubated at 30 °C. After 1 h, the enzyme (8.4 mg, 42 U) was further added into the mixture, and the reaction mixture was incubated for an additional 1 h. After boiling for 15 min, the mixture was filtered through a membrane and lyophilized. The residue was purified by flash chromatography (gradient from 20:1 EtOAc–EtOH to 6:2:1 EtOAc–EtOH–H_2O) to give **11** (49 mg, 13%): $[\alpha]_D^{27}$ +60° (*c* 1.0, H_2O); R_f 0.38 (6:2:1 EtOAc–EtOH–H_2O); ^1H NMR (D_2O): δ 4.24 (d, 1 H, $J_{3',4'}$ 8.3 Hz, H-3′), 4.13 (t, 1 H, $J_{4',5'}$ 8.3 Hz, H-4′), 4.04 (dt, 1 H, $J_{5',6'}$ 5.6 Hz, H-5′), 3.92–3.82 (m, 4 H, H-6,6′), 3.76 (t, 1 H, $J_{2,3}$ 9.6, $J_{3,4}$ 9.6 Hz, H-3), 3.71 (s, 2 H, H-1′), 3.56 (d, 1 H, H-2), 3.43 (t, 1 H, $J_{4,5}$ 9.6 Hz, H-4); ^{13}C NMR (67 MHz, D_2O): δ 104.6, 81.5, 77.1, 76.7, 73.3, 71.7, 70.2, 61.8, 61.1, 45.9. HRFABMS: Anal. Calcd for $C_{12}H_{20}ClDNaO_{10}$ ($M+Na^+$) 384.0784, Found 384.0808. Anal. Calcd for $C_{12}H_{20}DO_{10}$: C, 39.84; H(D), 6.13. Found: C, 39.49; H(D), 5.95.

3-O-*Acetyl-6′-chloro-6′-deoxy-1′,2:4,6-di*-O-*isopropylidenesucrose* (**13**). To a solution of **12** [16] (1.33 g, 2.87 mmol) in pyridine (15.7 mL) were added CCl_4 (3.1 mL, 32 mmol) and triphenylphosphine (1.56 g, 5.95 mmol). 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. The solution was washed with brine, dried ($MgSO_4$), and evaporated. Flash chromatography (3:1 EtOAc–hexane) gave **13** (1.19 g, 86%). The analytical sample was prepared by recrystallization from CH_2Cl_2–hexane: mp 127–132 °C; $[\alpha]_D^{15}$ +32° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 6.11 (d, 1 H, $J_{1,2}$ 4.0 Hz, H-1), 5.25 (t, 1 H, $J_{2,3}$ 9.6, $J_{3,4}$ 9.6 Hz, H-3), 4.23–4.18 (m, 1 H, H-4′), 4.14 (d, 1 H, $J_{1'a,1'b}$ 12.2 Hz, H-1′a), 4.10 (q, 1 H, $J_{4',5'}$ 6.6, $J_{5',6'}$ 6.6 Hz, H-5′), 3.90–3.70 (m, 8 H, H-2, 4, 5, 3′, 6, 6′), 3.04 (d, 1 H, H-1′b), 3.08–3.02 (m, 2 H, OH), 2.07 (s, 3 H, OAc), 1.47, 1.46, 1.40, 1.31 (s, each 3 H, CMe_2); Anal. Calcd for $C_{20}H_{31}ClO_{11}$: C, 49.74; H, 6.47. Found: C, 50.34; H 6.63.

1′,2,3,4,6-Penta-O-*acetyl-3′,4′-anhydro-6′-chloro-6′-deoxy*-tagato-*sucrose* (**15**).—To a solution of **13** (1.21 g, 2.50 mmol) in toluene (11 mL) were added DEAD (0.80 mL, 5.09 mmol) and triphenylphosphine (1.70 g, 6.49 mmol) under Ar atmosphere at 0 °C. The mixture was kept for 1.5 h at room temperature. After MeOH (5 mL) was added, the mixture was stirred for 20 min and diluted with EtOAc. The solution was washed with brine, dried ($MgSO_4$), and evaporated. Flash chromatography (2:1 EtOAc–hexane) gave a syrupy **14** (1.10 g). {^1H NMR ($CDCl_3$): δ 5.87 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 5.30 (dd, 1 H, $J_{2,3}$ 9.6, $J_{3,4}$ 9.6 Hz, H-3), 4.32 (q, 1 H, $J_{4',5'}$ 7.3, $J_{5',6'}$ 7.3 Hz, H-5′), 4.04 (d, 1 H, $J_{1'a,1'b}$ 11.9 Hz, H-1′a), 3.95–3.87 (m, 3 H), 3.79 (dd, 1 H, H-2), 3.66 (t, 1 H, $J_{4,5}$ 9.6 Hz, H-4), 3.65–3.57 (m, 4 H, H-3′, 4′, 6′), 3.42 (d, 1 H, H-1′b), 2.07 (s, 3 H, OAc), 1.46, 1.45, 1.38, 1.35 (s, each 3 H, CM_2).} Compound **14** (1.10 g) was dissolved in 70% AcOH (10 mL), and the mixture was heated at 70 °C for 20 min. After cooling to room temperature, the mixture was evaporated. To a solution of the residue in pyridine (7 mL) was added Ac_2O (3 mL). After 3 h, the mixture was poured into aq $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with aq $NaHCO_3$ and brine, dried ($MgSO_4$), and evaporated. Flash chromatography (1:1 hexane–EtOAc) gave **15** (738 mg, 53%): mp 126–127 °C; $[\alpha]_D^{15}$ +71° (*c* 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.78 (d, 1 H, $J_{1,2}$ 3,6 Hz, H-1), 5.46 (t, 1 H, $J_{2,3}$ 9.9, $J_{3,4}$ 9.9 Hz, H-3), 5.07 (t, 1 H, $J_{4,5}$ 9.9 Hz, H-4), 4.77 (dd, 1 H, H-2), 4.30 (d, 1 H, $J_{1'a,1'b}$ 11.9 Hz, H-1′a), 4.32–4.07 (m, 4 H), 4.13 (d, 1 H, H-1′b), 3.89, 3.73 (each d, each 1 H, $J_{3',4'}$ 2.9 Hz, H-3′ or 4′), 3.59 (dd, 1 H, $J_{5',6'a}$ 8.6, $J_{6'a,6'b}$ 10.9 Hz, H-6′a), 3.52 (dd, 1 H, $J_{5',6'b}$ 5.9 Hz, H-6′b), 2.13, 2.08, 2.04, 2.02 (each s, 15 H, OAc). Anal. Calcd for $C_{22}H_{29}ClO_{14}$: C, 47.79, H 5.29; Found: C, 47.55; H 5.32.

1′,2,3,3′,4,6,-Hexa-O-*acetyl-4′-azido-6′-chloro-4′,6′-dideoxysucrose* (**16**).—To a solution of **15** (384 mg, 0.695 mmol) in 8:1 EtOH–H_2O (12 mL) were added NaN_3 (348 mg, 5.35 mmol) and NH_4Cl (348 mg, 6.50 mmol), and the mixture was stirred for 73 h at 80 °C. After cooling to room temperature, the mixture was evaporated to give a residue that was acetylated with a mixture of pyridine (7 mL) and Ac_2O (3 mL). The reaction mixture was poured into aq $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with aq $NaHCO_3$ and brine, dried ($MgSO_4$), and evaporated. Flash chromatography (1:1 hexane–EtOAc) gave **16** (373 mg, 84%): mp 83–85 °C; $[\alpha]_D^{17}$ +45° (*c* 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.57 (d, 1 H, $J_{1,2}$ 3.6, H-1), 5.43 (t, 1 H, $J_{2,3}$ 9.9, $J_{3,4}$ 9.9 Hz, H-3), 5.36 (d, 1 H, $J_{3',4'}$ 7.9 Hz, H-3′), 5.07 (t, 1 H, $J_{4,5}$ 9.9 Hz, H-4), 4.90 (dd, 1 H, H-2), 4.30–4.11 (m, 4H, H-5, 5′, 6), 4.20 (t, 1 H, $J_{4',5'}$ 7.9 Hz, H-4′), 4.19 (d, 1 H, $J_{1'a,1'b}$ 11.9 Hz, H-1′a), 4.04 (d, 1 H, H-1′b), 3.79 (d, 1 H, $J_{5',6'a}$ 5.3, $J_{6'a,6'b}$ 10.9 Hz, H-6′a), 3.73 (dd, 1 H, $J_{5',6'b}$ 5.3 Hz, H-6′b), 2.24, 2.12, 2.11, 2.10, 2.06, 2.02 (each s, 18 H, OAc). ^{13}C NMR (67 MHz, $CHCl_3$): δ 170.6, 169.9, 169.7, 169.5, 102.9, 90.0, 79.8, 70.1, 69.5, 68.6, 68.5, 65.0, 63.6, 62.5, 43.3, 20.7, 20.6, 20.5. Anal. Calcd for $C_{24}H_{32}ClN_3O_{15}$: C, 45.18; H, 5.06; N 6.59. Found: C, 45.09; H, 4.97; N 6.53.

4′-Amino-6′-chloro-4′,6′-dideoxysucrose (**17**).—To a solution of **16** (285 mg, 0.447 mmol) in dry MeOH (6 mL) was added NaOMe (7.0 mg, 0.13 mmol), and the mixture was stirred for 2 h. To the mixture was added Lindlar catalyst (100 mg), and the mixture was stirred under 1 atm of hydrogen for 2 h. The mixture was filtered and evaporated. The residue was purified by gel filtration and lyophilized to give **17** (161 mg, 100%) as a white solid: $[\alpha]_D^{17}$ +71° (*c* 0.90, H_2O); ^1H NMR (D_2O): δ 5.43 (d, 1 H, $J_{1,2}$ 3.9 Hz, H-1), 4.52 (d, 1 H, $J_{3',4'}$ 9.6 Hz, H-3′) 4.39–4.10 (m, 1 H, H-5′), 3.97–3.72 (m, 10 H), 3.59 (dd, 1 H, $J_{2,3}$ 9.9 Hz, H-2), 3.43 (t, 1 H, $J_{3,4}$ 9.9, $J_{4,5}$ 9.9 Hz, H-4). ^{13}C NMR (67 MHz, D_2O): δ 105.1, 93.5, 78.6, 74.8, 73.6, 73.2, 71.7, 70.3, 61.3, 61.2, 57.6, 45.2. Anal. Calcd for $C_{12}H_{22}ClNO_9$: C, 40.06; H, 6.16; N, 3.89. Found: C, 39.92; H, 6.14; N, 3.85.

3-O-*Acetyl-1′,2:4,6-di*-O-*isopropylidene-4′,6′*-O-*(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)sucrose* (**18**).—To a solution of **12** (3.50 g, 7.54 mmol) in Me_2NCHO (35 mL) were added imidazole (2.2 g, 32 mmol) and 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (2.4 mL, 7.5 mmol) under Ar atmosphere at 0 °C. After 1 h, aq $NaHCO_3$ was added to the mixture, and it was extracted with EtOAc (2×50 mL). The combined organic layer was washed with brine, dried ($MgSO_4$), and evaporated. Flash chromatography (6:1 hexane–EtOAc) gave **18** (4.35 g, 82%): $[\alpha]_D^{23}$ +3.3° (*c* 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.99 (d, 1 H, $J_{1,2}$ 4.0 Hz, H-1), 5.20 (t, 1 H, $J_{2,3}$ 9.6, $J_{3,4}$ 9.6 Hz, H-3), 4.25 (dd, 1 H, $J_{3',4'}$ 7.3, $J_{4',5'}$ 5.3 Hz, H-4′), 4.10 (d, 1 H, $J_{1'a,1'b}$ 12.5 Hz, H-1′a), 3.98 (d, 1 H, H-3′), 3.93–3.60 (m, 8 H, H-2, 4, 5, 5′, 6, 6′), 3.44 (d, 1 H, H-1′b), 2.06 (s, 1 H, OAc), 1.47, 1.44, 1.41, 1.30 (each s, each 3H, CMe_2), 1.13–1.02 (m, 28 H, $CH(CH_3)_2$). ^{13}C NMR (67 MHz, $CHCl_3$): δ 102.7, 101.2, 99.6, 91.1, 81.7, 80.8, 80.3, 71.5, 70.4, 66.1, 65.7, 64.1, 62.2, 28.9, 25.3, 23.9, 20.9, 18.9, 17.5, 17.4, 17.3, 17.0, 13.4, 13.2, 13.0, 12.7, 12.5. Anal. Calcd for $C_{32}H_{58}O_{13}Si_2$: C, 54.37; H, 8.27. Found: C, 54.47; H, 8.28.

3,4′,6′-Tri-O-*acetyl-1′,2:4,6-di*-O-*isopropylidene-3′*-O-*trifluoromethanesulfonylsucrose* (**19**).—To a solution of pyridine (2.0 mL, 25 mmol) and trifluoromethanesulfonic anhydride (2.0 mL, 12 mmol) in CH_2Cl_2 (43 mL) was added dropwise a solution of **18** (4.35 g, 6.15 mmol) in CH_2Cl_2 (40 mL) at 0 °C. After 1 h, the mixture was poured into aq $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was dried ($MgSO_4$) and evaporated. The residue was dissolved in pyridine (20 mL) and the mixture was transferred into a Teflon tube. To the mixture was added HF–pyridine solution (5 mL) at 0 °C. After 15 min, the mixture was poured into aq $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with brine, dried ($MgSO_4$), and evaporated. The residue was dissolved in pyridine (10 mL) and Ac_2O (5 mL). After 3 h, the mixture was poured into aq $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with brine, dried ($MgSO_4$), and evaporated. The residue was crystallized from hexane to give **19** as a white solid (3.62 g, 86%): mp 133 °C (dec.); $[\alpha]_D^{21}$ +13° (*c* 1.2, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 6.09 (d, 1 H, $J_{1,2}$ 3,6 Hz, H-1), 5.45 (dd, 1 H, $J_{3',4'}$ 5.9, $J_{4',5'}$ 4.3 Hz, H-4′), 5.23 (t, 1 H, $J_{2,3}$ 9.6, $J_{3,4}$ 9.6 Hz, H-3), 4.94 (d, 1 H, H-3′), 4.32–4.24 (m, 2 H, H-6′), 4.16 (d, 1 H, $J_{1'a,1'b}$ 12.2 Hz, H-1′a), 3.96 (dd, 1 H, $J_{5,6a}$ 5.0, $J_{6a,6b}$ 10.2 Hz, H-6a), 3.90–3.80 (m, 1 H, H-5), 3.86 (dd, 1 H, H-2), 3.69 (dd, 1 H, $J_{5,6b}$ 3.3 Hz, H-6b), 3.67 (t, 1 H, $J_{4,5}$ 9.9 Hz, H-4), 3.49 (d, 1 H, H-1′b), 2.14, 2.05, 2.04 (each s, each 3 H, OAc), 1.47, 1.46, 1.41, 1.30 (each s, each 3 H, CMe_2). ^{13}C NMR (67 MHz, $CHCl_3$): δ 170.4, 169.6, 169.5, 102.9, 101.7, 99.7, 91.8, 85.9, 79.1,

76.5, 71.7, 71.2, 70.3, 64.9, 64.6, 64.4, 61.9, 28.9, 25.3, 23.7, 20.9, 20.7, 20.4, 18.9, 16.8, 12.5. Anal. Calcd for $C_{25}H_{35}F_3O_{16}S$: C, 44.12; H, 5.18. Found: C, 44.10; H, 5.38.

3,3′,4′,6′-Tetra-O-*Acetyl-1′,2:4,6-di*-O-*isopropylidene*-psico-*sucrose* (**20**).—To a solution of **19** (3.60 g, 5.29 mmol) in dry Me_2NCHO (36 mL) were added AcOCs (3.00 g, 15.6 mmol) and 18-crown-6 (4.20 g, 15.9 mmol), and the solution was heated at 95 °C for 3 h. After cooling to room temperature, the mixture was diluted with EtOAc (150 mL) and extracted with water. The organic layer was washed with brine, dried ($MgSO_4$), and evaporated. Flash chromatography (2:1 hexane–EtOAc) gave **20** (3.10 g, 99%) as a white solid: mp 172–174 °C; $[\alpha]_D^{23}$ +28° (*c* 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 6.04 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 5.40 (d, 1 H, $J_{3',4'}$ 4.9 Hz, H-3′), 5.40 (dd, 1 H, $J_{4',5'}$ 8.5 Hz, H-4′), 5.22 (t, 1 H, $J_{2,3}$ 9.3, $J_{3,4}$ 9.3 Hz, H-3), 4.46–4.39 (m, 1 H, H-5′), 4.32 (dd, 1 H, $J_{5',6'a}$ 4.6, $J_{6'a,6'b}$ 11.5 Hz, H-6′a), 4.20 (dd, 1 H, $J_{5',6'b}$ 7.9 Hz, H-6′b), 3.95 (d, 1 H, $J_{1'a,1'b}$ 12.2 Hz, H-1′a), 3.89 (dd, 1 H, $J_{5,6a}$ 5.0, $J_{6a,6b}$ 9.6 Hz, H-6a), 3.81 (dd, 1 H, H-2), 3.78 (dd, 1 H, $J_{5,6b}$ 4.6 Hz, H-6b), 3.65 (d, 1 H, H-1′b), 3.62 (dd, 1 H, $J_{4,5}$ 9.9 Hz, H-4), 2.13, 2.06, 2.05, 2.04 (each s, each 3 H, OAc), 1.46, 1.44, 1.38, 1.28 (each s, each 3 H, CMe_2). ^{13}C NMR (67 MHz, $CHCl_3$): δ 170.5, 170.0, 169.2, 169.0, 106.9, 101.3, 99.6, 91.4, 79.6, 75.5, 72.3, 71.6, 71.4, 70.6, 65.0, 64.0, 63.9, 62.1, 29.0, 25.4, 24.0, 20.9, 20.8, 20.4, 18.9. Anal. Calcd for $C_{26}H_{38}O_{15}$: C, 52.88; H, 6.49. Found: C, 52.68; H, 6.56.

3-O-*Acetyl-1′,2:4,6-di*-O-*isopropylidene*-psico-*sucrose* (**21**).—Into a solution of **20** (3.10 g, 5.25 mmol) in dry MeOH (97 mL) was bubbled NH_3 (g) for 15 min at 20 °C. After completion of the reaction (4 h) was confirmed by TLC, the mixture was evaporated and the residue was co-evaporated with MeOH. Flash chromatography (50:1 EtOAc–EtOH) gave **21** (1.73 g, 71%) as a colorless oil: $[\alpha]_D^{23}$ +31° (*c* 1.1, $CHCl_3$); R_f 0.63 (10:1 EtOAc–EtOH); ^1H NMR ($CDCl_3$): δ 6.19 (d, 1 H, $J_{1,2}$ 4.0 Hz, H-1), 5.22 (t, 1 H, $J_{2,3}$ 9.2, $J_{3,4}$ 9.2 Hz, H-3), 4.83–4.76 (m, 1 H, H-4′), 4.19–4.11 (m, 2 H, H-3′, 5′), 4.09 (d, 1 H, $J_{1'a,1'b}$ 12.8 Hz, H-1′a), 3.97 (brd, 1 H, $J_{6'a,6'b}$ 12.8 Hz, H-6′a), 3.87 (d, 1 H, H-1′b), 3.83 (dd, 1 H, H-2), 3.87–3.77 (m, 2 H, H-5, 6′b), 3.64 (dd, 1 H, $J_{4,5}$ 9.9 Hz, H-4), 3.68–3.58 (m, 2 H, H-6), 3.38, 3.13 (each brs, each 1 H, OH), 3.02 (brd, 1 H, *J* 10.6 Hz, OH), 2.08 (s, 3 H, OAc), 1.47, 1.45, 1.38, 1.34 (each s, each 3 H, CMe_2). ^{13}C NMR (67 MHz, $CHCl_3$): δ 170.5, 107.4, 101.5, 99.8, 91.3, 85.5, 77.2, 71.3, 71.2, 70.7, 68.8, 65.0, 64.0, 61.8, 61.2, 28.9, 25.3, 24.2, 21.0, 18.9. Anal. Calcd for $C_{20}H_{32}O_{12}$: C, 51.72; H, 6.94. Found: C, 51.67; H, 7.01.

3-O-*Acetyl-1′,2:4,6-di*-O-*isopropylidene-4′,6′*-O-*(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)*-psico-*sucrose* (**22**).—The compound **21** (2.02 g, 4.35 mmol) was silylated in the similar manner as described for the synthesis of **18** to give **22** (2.52 g, 82%) as amorphous: $[\alpha]_D^{23}$ +11° (*c* 1.2, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.91 (d, 1 H, $J_{1,2}$ 4.0 Hz, H-1), 5.22 (t, 1 H, $J_{2,3}$ 9.6, $J_{3,4}$ 9.6 Hz, H-3), 4.59 (dd, 1 H, $J_{3',4'}$ 5.0, $J_{4',5'}$ 5.9 Hz, H-4′), 4.15–4.03 (m, 2 H), 4.07, (d, 1 H, $J_{1'a,1'b}$ 12.4 Hz, H-1′a), 4.05 (d, 1 H, H-3′), 3.81 (d, 1 H, H-1′b), 3.81–3.64 (m, 5 H), 3.79 (dd, 1 H, H-2), 3.60 (t, 1 H, $J_{4,5}$ 9.6 Hz, H-4), 2.06 (s, 3 H, OAc), 1.46, 1.44, 1.40, 1.29 (each s, each 3 H, CMe_2), 1.16–0.91 (m, 28 H, $CH(CH_3)_2$). ^{13}C NMR (67 MHz, $CHCl_3$): δ 170.1, 107.2, 101.0, 99.6, 91.2, 83.8, 75.8, 71.6, 70.7, 66.2, 64.3, 63.9, 62.3, 29.0, 25.4, 24.0, 21.0, 18.9, 17.5, 17.3, 17.2, 17.0, 13.2, 13.0, 12.8, 12.6. Anal. Calcd for $C_{32}H_{58}O_{13}Si_2$: C, 54.37; H, 8.27. Found: C, 54.31; H, 8.67.

3-O-*Acetyl-3′-azido-3′-deoxy-1′,2:4,6-di*-O-*isopropidene-4′,6′*-O-*(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)sucrose* (**23**).—Into a solution of pyridine (1.1 mL, 14 mmol) and trifluoromethanesulfonic anhydride (1.1 mL, 6.5 mmol) in CH_2Cl_2 (25 mL) was added dropwise a solution of **22** (2.53 g, 3.58 mmol) in CH_2Cl_2 (25 mL) at 0 °C. After 15 min, the mixture was poured into aq $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was washed with brine, dried ($MgSO_4$), and evaporated to dryness. The residue was dissolved in Me_2NCHO (25 mL), NaN_3 (726 mg, 11.2 mmol) and 18-crown-6 (2.8 g, 11 mmol) were added to the solution, and the mixture was stirred at 90 °C for 40 min. After cooling to room temperature, the mixture was diluted with EtOAc, washed with water and brine, dried ($MgSO_4$), and evaporated. Flash chromatography (6:1 hexane–EtOAc) gave **23** (1.23 g, 47%) as a white solid: $[\alpha]_D^{22}$ +29° (*c* 2.3, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.94 (d, 1 H, $J_{1,2}$ 4.0 Hz, H-1), 5.28 (t, 1 H, $J_{2,3}$ 9.6, $J_{3,4}$ 9.6 Hz, H-3), 4.60 (dd, 1 H, $J_{3',4'}$ 8.3, $J_{4',5'}$ 5.3 Hz, H-4′), 4.11 (d, 1 H, $J_{1'a,1'b}$ 12.2 Hz, H-1′a), 4.03–3.91 (m, 3 H, H-5′, 6′), 3.82 (dd, 1 H, H-2), 3.72 (dd, 1 H, $J_{5,6b}$ 4.3, $J_{6a,6b}$ 10.2 Hz, H-6a), 3.85–3.78 (m, 2 H, H-5, 6b), 3.63 (t, 1 H, $J_{4,5}$ 9.6 Hz, H-4), 3.40 (d, 1 H, H-1′b), 3.29 (d, 1 H, H-3′), 2.06 (s, 3 H, OAc), 1.46, 1.45, 1.39, 1.31 (each s, each 3 H, CMe_2), 1.14–0.93 (m,

28 H, $CH(CH_3)_2$). ^{13}C NMR (67 MHz, $CHCl_3$): δ 169.8, 104.5, 101.2, 99.5, 91.4, 82.7, 71.7, 71.4, 70.6, 69.2, 66.0, 65.7, 64.4, 62.2, 28.9, 25.4, 23.9, 20.9, 19.0, 17.6, 17.5, 17.3, 17.0, 16.9, 16.8, 13.4, 13.2, 13.0, 12.8, 12.5. Anal. Calcd for $C_{32}H_{57}N_3O_{12}Si_2$: C, 52.51; H ,7.85. Found: C, 52.82; H, 8.35.

3-O-Acetyl-3′-azido-3′-deoxy-1′,2:4,6-di-O-isopropylidenesucrose (**24**).—To a solution of **23** (1.17 g, 1.60 mmol) in THF (11 mL) was added a 1 M solution of Bu_4NF in THF (3.2 mL) at 0 °C. After 10 min, the mixture was diluted with EtOAc, and the solution was washed with water, brine, dried ($MgSO_4$), and evaporated. Flash chromatography (2:1 EtOAc–hexane) gave **24** (440 mg, 56%) as a white solid: mp 193–196 °C; $[\alpha]_D^{22}$ +17° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 6.20 (d, 1 H, $J_{1,2}$ 4.0 Hz, H-1), 5.30 (dd, 1 H, $J_{2,3}$ 9.2, $J_{3,4}$ 9.6 Hz, H-3), 4.93 (dd, 1 H, $J_{3',4'}$ 9.2, $J_{4',5'}$ 7.6 Hz, H-4′), 4.14 (d, 1 H, $J_{1'a,1'b}$ 12.2 Hz, H-1′a), 4.03 (dt, 1 H, $J_{5',6'}$ 1.5 Hz, H-5′), 3.97–3.80 (m, 3 H, H-5, 6′), 3.88 (d, 1 H, H-2), 3.66 (dd, 1 H, $J_{4,5}$ 9.2 Hz, H-4), 3.65–3.58 (m, 2 H, H-6), 3.46 (d, 1 H, H-1′b), 3.26 (d, 1 H, H-3′), 3.13–3.00 (m, 1 H, OH), 2.92 (brs, 1 H, OH), 2.08 (s, 3 H, OAc), 1.47, 1.45, 1.38, 1.34 (each s, each 3 H, CMe_2). ^{13}C NMR (67 MHz, $CHCl_3$): δ 169.8, 105.1, 101.8, 99.7, 91.5, 83.9, 71.3, 71.1, 70.5, 69.6, 67.6, 66.1, 64.8, 61.8, 60.8, 28.9, 25.2, 24.0, 20.9, 18.9. Anal. Calcd for $C_{20}H_{31}N_3O_{11}$: C, 49.08; H, 6.38; N, 8.58. Found: C, 48.90; H, 6.46; N, 8.31.

3-O-Acetyl-3′-azido-3′-deoxy-1′,2:4,6-di-O-isopropylidene-6′-O-p-toluenesulfonylsucrose (**25**).—To a solution of **24** (330 mg, 0.674 mmol) in pyridine (3.3 mL) were added *p*-toluenesulfonyl chloride (257 mg, 1.35 mmol) and DMAP (20 mg, 0.16 mmol) at 0 °C under Ar atmosphere. After the mixture was stirred at room temperature for 12 h, it was poured into aq $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with brine, dried ($MgSO_4$), and evaporated. Flash chromatography (2:1 hexane–EtOAc) gave **25** (382 mg, 88%) as a colorless oil: $[\alpha]_D^{22}$ +25° (*c* 1.6, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.79 (d, 2 H, J_{AB} 8.3 Hz, Ph), 7.36 (d, 2 H, Ph), 5.93 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 5.27 (t, 1 H, $J_{2,3}$ 9.6, $J_{3,4}$ 9.6 Hz, H-3), 4.50 (dd, 1 H, $J_{3',4'}$ 8.6, $J_{4',5'}$ 6.3 Hz, H-4′), 4.30–4.11 (m, 3 H, H-5′, 6′), 4.08 (d, 1 H, $J_{1'a,1'b}$ 12.5 Hz, H-1′a), 3.87 (dd, 1 H, $J_{5,6a}$ 4.6, $J_{6a,6b}$ 9.9 Hz, H-6a), 3.80 (d, 1 H, H-2), 3.71 (dd, 1 H, $J_{5,6b}$ 4.9 Hz, H-6b), 3.62 (dd, 1 H, $J_{4,5}$ 9.2 Hz, H-4), 3.41 (d, 1 H, H-1′b), 3.27 (d, 1 H, H-3′), 2.46 (s, 3 H, Me), 2.07 (s, 3 H, OAc), 1.46, 1.43, 1.38, 1.30 (each s, each 3 H, CMe_2). ^{13}C NMR (67 MHz, $CHCl_3$): δ 170.0, 145.0, 132.5, 129.8, 127.9, 105.5, 101.4, 99.6, 98.7, 91.3, 79.7, 74.4, 71.5, 71.3, 70.7, 67.7, 65.4, 64.3, 62.0, 28.9, 25.2, 23.8, 21.6, 20.9, 18.9, Anal. Calcd for $C_{27}H_{37}N_3O_{13}S$: C, 50.38; H, 5.79; N, 6.53. Found: C, 50.34; H, 5.45; N, 6.30.

1′,2,3,4,4′,6-Hexa-O-acetyl-3′-azido-6′-chloro-3′,6′-dideoxysucrose (**26**).—To a solution of **25** (382 mg, 0.593 mmol) in Me_2NCHO (4.0 mL) was added LiCl (142 mg, 3.35 mmol) at 80 °C for 12 h under an Ar atmosphere. After cooling to room temperature, the mixture was diluted with EtOAc and washed with water and brine. The organic layer was dried ($MgSO_4$) and evaporated. The residue was dissolved in 70% AcOH (5 mL), and the solution was stirred at 80 °C. After 2 h, the mixture was evaporated, and the residue was treated with pyridine (2 mL) and Ac_2O (1 mL). The mixture was poured into aq $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with brine, dried ($MgSO_4$), and evaporated. Flash chromatography (2:1 hexane–EtOAc) gave **26** (309 mg, 82%) as crystals: mp 137–139 °C; $[\alpha]_D^{22}$ +39° (*c* 0.60, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.57 (d, 1 H, $J_{1,2}$ 4.0 Hz, H-1), 5.48 (dd, 1 H, $J_{2,3}$ 10.3, $J_{3,4}$ 9.9 Hz, H-3), 5.40 (dd, 1 H, $J_{3',4'}$ 8.6, $J_{4',5'}$ 7.3 Hz, H-4′), 5.04 (t, 1 H, $J_{4,5}$ 9.9 Hz, H-4), 5.00 (dd, 1 H, H-2), 4.27–4.17 (m, 2 H, H-5, 5′), 4.18–4.16 (m, 2 H, H-6′), 4.12 (d, 1 H, $J_{1'a,1'b}$ 7.6 Hz, H-1′a), 4.09 (d, 1 H, H-1′b), 3.95 (d, 1 H, H-3′), 3.85 (dd, 1 H, $J_{5,6a}$ 7.3, $J_{6a,6b}$ 11.9 Hz, H-6a), 3.79 (dd, 1 H, $J_{5,6b}$ 5.9 Hz, H-6b), 2.17, 2.13, 2.12, 2.11, 2.04, 2.01(each s, each 3 H, OAc). Anal. Calcd for $C_{24}H_{32}ClN_3O_{15}$: C, 45.18; H, 5.06; N, 6.59. Found: C, 44.84; H, 5.35; N, 6.37.

3′-Amino-6′-chloro-3′,6′-dideoxysucrose (**27**).—To a solution of **26** (34 mg, 0.053 mmol) in dry MeOH (2.0 mL) was added NaOMe (3.0 mg, 0.056 mmol) under an Ar atmosphere. After 5 h, Lindlar catalyst (20 mg) was added to the solution, and the mixture was stirred under 1 atm of hydrogen for 30 min. After insoluble material was removed off by filtration, the filtrate was neutralized with 0.1 M HCl, and it was lyophilized. The residue was purified by gel filtration and lyophilized to give **27** (10 mg, 52%) as a white solid: $[\alpha]_D^{22}$ +74° (*c* 0.72, H_2O); ^1H NMR (D_2O): δ 5.44 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 4.46 (dd, 1 H, $J_{3',4'}$ 9.2, $J_{4',5'}$ 9.2 Hz, H-4′), 4.21 (dt, 1 H, $J_{5',6'a}$ 5.3, $J_{5',6'b}$ 5.3 Hz, H-5′), 4.03 (d, 1 H, H-3′), 3.95–3.74 (m, 6 H), 3.75 (t, 1 H, $J_{2,3}$ 9.9, $J_{3,4}$ 9.9 Hz, H-3), 3.62 (dd,

1 H, H-2), 3.48 (t, 1 H, $J_{4,5}$ 9.9 Hz, H-4); ^{13}C NMR (67 MHz, D_2O): δ 105.6, 92.7, 82.8, 78.0, 73.4, 73.3, 71.7, 70.3, 61.8, 61.2, 60.8, 46.0. Anal. Calcd for $C_{12}H_{22}ClNO_9$: C, 40.06; H, 6.16; N, 3.89. Found: C, 39.73; H, 5.97; N, 3.81.

Kinetic studies.—Initial rates of transglucosylation from 1′6′-disubstituted sucrose analogs to acceptor **2** were measured for 5–6 different donor concentrations, which ranged from 5 to 50 mM, and a constant concentration of the acceptor **2** (100 mM) in 0.1 mL of calcium phosphate buffer (50 mM, pH 5.6) at 30 °C. Reaction were initiated by addition of 7.1 unit of α-GTase in the same buffer (10 μL) and stopped by addition of 1 M Na_2CO_3 (30 μL) after 45 min. It was confirmed that linearity of the initial rate maintained at least 1 h. The amounts of the products were determined by HPLC, (Erma ERC-NH-117 column; 85% CH_3CN; flow rate, 1.0 mL/min; monitor, 254 nm, Hitachi 655A UV detector). Enzyme concentration was measured by Bradford method [32]. The M_r was estimated to be 62000 by SDS-PAGE. Values of K_m and k_{cat} were determined from a Lineweaver–Burk plot.

The secondary deuterium kinetic isotope effects were determined by comparison of the k_{cat} values of the transglucosylation with protio **1** or deuterio donor substrate **11**, respectively under the same manner as described above. The experiments were carried out five times to give the k_H/k_D values of 1.33, 1.20, 1.19, 1.16, and 1.12, respectively. The means ± standard deviation is 1.20 ± 0.08.

The rate of intramolecular transglucosylation was determined by detecting the isomaltulose formation. Sucrose (300 mM) and the inhibitors (0 or 12 mM) were incubated in 250 μL of calcium phosphate buffer (50 mM, pH 5.6) at 20 °C. Reaction was initiated by addition of αGT (50 μL, 0.8 U). After 1 h, the reaction was stopped by addition of 125 μL of 0.3 M $Ba(OH)_2$ and 0.3 M $ZnSO_4$, respectively, and the mixture was centrifuged. The concentration of isomaltulose in the supernatant was measured by Somogyi–Nelson's method [33].

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