droxide (149 mg) in ^2H_2O (0.75 mL, 99% ^2H) was heated on a steam bath for 2 h. The hydrogen atoms at C-2′ and C-5′ exchange in less than 1 min under these conditions. The mixture was worked up as described above (see (iii) and (iv)), and *N*-formyl-1-naphthylamine (**16**) was isolated: MS, *m/e* 172 (100%), 171 (10%), 144 (91%), 115 (56%). Thus, the sample had ca. 92 atom % ^2H in the formyl position.

Radioactivity Measurements. Radioactivity was assayed by liquid scintillation counting (Beckman LS 9000 Liquid Scintillation System). All samples were recrystallized to constant specific activity, dissolved in water, if necessary, and dispersed in Aquasol (New England Nuclear) and counted in triplicate under comparable conditions of quenching. Confidence limits shown in the tables are standard deviations from the mean.

Acknowledgment. We are grateful to Prof. John J. Miller, Department of Biology, McMaster University, for advice on and for providing facilities for the cultivation of yeast. This investigation was supported by the Natural Sciences and Engineering Research Council of Canada.

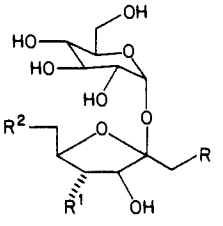
Registry No. **7**, 2908-73-8; **9**, 92844-28-5; **12**, 99310-00-6; **13**, 107-35-7; **15**, 575-36-0; **16**, 6330-51-4; **17**, 550-44-7; **18**, 2181-22-8; **19**, 98135-20-7; pyrimidine, 289-95-2; thiamin, 59-43-8; formic acid, 64-18-6; D-glucose, 50-99-7; D-fructose, 57-48-7; glycerol, 56-81-5.

Chemoenzymatic Syntheses of Fructose-Modified Sucroses via Multienzyme Systems. Some Topographical Aspects of the Binding of Sucrose to a Sucrose Carrier Protein

Peter J. Card,* William D. Hitz, and Kevin G. Ripp

Contribution No. 3806 from the Central Research & Development Department, E. I. du Pont de Nemours & Company, Inc., Experimental Stataion, Wilmington, Delaware 19898.
Received June 20, 1985

Abstract: 1′-Azido-1′-deoxysucrose (**3**) was synthesized by a sucrose synthetase mediated coupling of 1-azido-1-deoxyfructose with UDP-glucose. 6′-Deoxy- (**7**) and 6′-deoxy-6′-fluorosucrose (**8**) were prepared from the corresponding 6-substituted glucose and UDP-glucose by using an enzyme-couple consisting of glucose isomerase and sucrose synthetase. 4′-Deoxy-4′-fluorosucrose (**13**) was also prepared as above with 4-deoxy-4-fluorofructose (**14**). Fructose **14** was prepared from 4-deoxy-4-fluoroglucose

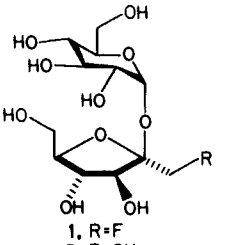


3, $R=N_3$, $R^1=R^2=OH$
7, $R^2=H$, $R=R^1=OH$
8, $R^2=F$, $R=R^1=OH$
13, $R^1=F$, $R=R^2=OH$

by conversion to 4-deoxy-4-fluorofructose 1,6-bisphosphate via a three-enzyme-couple consisting of hexokinase, phosphoglucose isomerase, and fructose 6-phosphate kinase. The bisphosphate was hydrolyzed to **14** by use of an alkaline phosphatase. The binding of these sucroses to a sucrose carrier protein is discussed in terms of the topographical surface which sucrose presents to the protein for binding.

Sucrose is the major form of transported carbon in many plant species and is actively transported across cell membranes in several tissue types.[1,2] The carrier protein responsible for this transport is quite specific for sucrose. Using monosaccharides or other disaccharides as competitive substrate inhibitors, workers using several tissue types have found only very weak competition with a very limited number of disaccharides.[3,4] This specificity suggests that either a large portion of the recognition of sucrose by the carrier protein lies with the fructose moiety or that elements of the glucose and fructose rings are involved in binding. To determine the important topographical binding regions of sucrose and the nature of that binding, we have prepared certain modified sucroses and assayed their binding to, and transport by, a sucrose carrier protein. In this paper we report on chemoenzymatic routes to sucroses in which the fructose moiety has been modified.

1′-Deoxy-1′-fluorosucrose (**1**) is an invertase hydrolysis resistant sucrose analogue that we synthesized for use in transport studies were extracellular invertase may exist.[5] While assaying the



1, R=F
2, R=OH
3, $R=N_3$

transport properties of **1**, we found that **1** was bound by the carrier protein 2 times stronger than natural sucrose (**2**) and that they were binding to the same carrier site in protoplasts derived from

(1) Giaquinta, R. T. "The Biochemistry of Plants"; Preiss, J., Ed.; Academic Press: New York, 1980.
(2) Lichtner, F. T. Spanswick, R. M. *Plant Physiol.* **1981**, *68*, 693.
(3) Giaquinta, R. T. *Plant Physiol.* **1977**, *59*, 750.
(4) Schmitt, M. R.; Hitz, W. D.; Lin, W.; Giaquinta, R. T. *Plant Physiol.* **1984**, *75*, 941.
(5) Card, P. J.; Hitz, W. D. *J. Am. Chem. Soc.* **1984**, *106*, 5348.

0002-7863/86/1508-0158$01.50/0

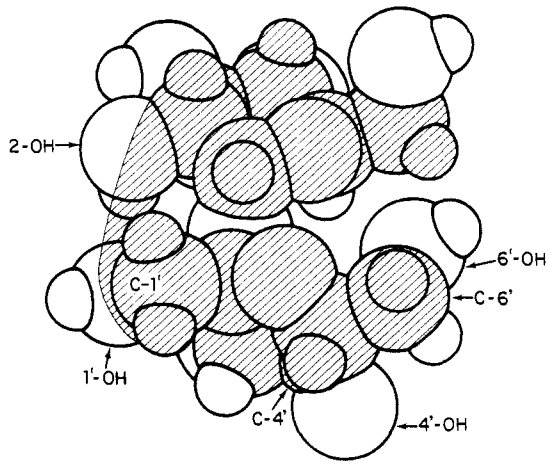


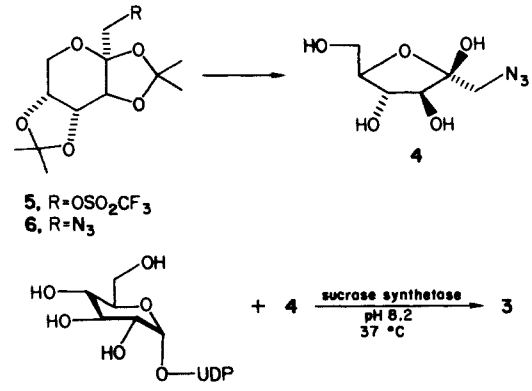
Figure 1. Topography of the hydrophobic surface which sucrose presents for binding to the carrier protein.

developing soybean cotyledons.[6] This finding indicates that the C-1′ position of sucrose (Figure 1) is within the binding domain of the carrier protein. Since replacement of the 1′-OH by fluorine doubled the binding potency, we assume that either of two situations may exist: (1) that the 1′-OH in **2** is intramolecularly hydrogen bonded to the 2-OH[7] and thus presents a hydrophobic surface for binding to the protein and that the fluorine atom just represents a more hydrophobic moiety which results in an increased binding of **1** relative to **2** or (2) that the fluorine is accepting an intramolecular hydrogen bond from the 2-OH and thus presents a hydrophobic surface to the protein. To distinguish between these two possibilities we decided to modify the 1′-position with a hydrophobic group that is not capable of accepting hydrogen bonds. We chose the azido group because it fulfills the above requirements and can be reduced to the polar amino group for additional binding studies.

1′-Azidosucrose (**3**) was prepared via a sucrose synthetase (EC 2.4.1.13) catalyzed coupling of UDP-glucose with 1-azidofructose (**4**), as previously described[5] for the preparation of **1**. Thus, triflate **5**[5] reacted with sodium azide in DMF to afford a 91% yield of azide **6**. Hydrolysis of **6** with an acidic ion-exchange resin gave **4** (92%, indicated as the β-D-fructofuranose). When **4** and UDP-glucose were incubated with the sucrose synthetase preparation at 35 °C and the pH was maintained at 8.2 via addition of 1 M KOH on a pH-stat, **3** was obtained in low yield. The structure of **3** was assigned on the basis of its mode of synthesis and its ^{13}C NMR spectrum (see Experimental Section), which is superimposable with that of **2** except for C-1′, which is shifted upfield by the azido moiety to δ 53.0. In contrast to 1-fluorofructose, which rapidly affords **1** in 60–88% yield, as judged by both the yield and rate of formation of **3**, **4** is a very poor substrate for sucrose synthetase. However, no other 1-substituted fructoses (deoxy, chloro, methoxy, or sulfhydryl) afforded sucroses using this enzyme.

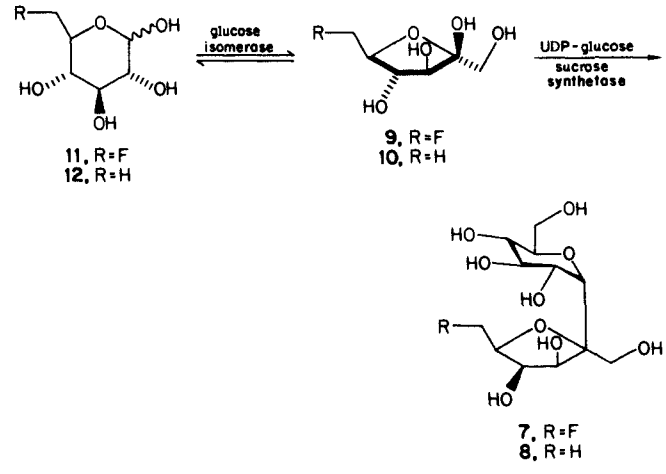
Assays[6,7] of the binding of **3** with the carrier protein revealed that substitution of the fluorine atom with the azido grouup afforded an equally potent binder. Thus it appears that the 1′-OH group in **2** is intramolecularly hydrogen bonded where it presents a nonpolar surface for interaction with the carrier protein and that the more hydrophobic fluoro and azido groups therefore result in more potent binders. In contrast to this, reduction of the azido group to the more polar amino group afforded a molecule whose binding potency was reduced when compared to **2**.

Lemieux[8,9] has provided overwhelming evidence that the main



driving force in the binding of oligosaccharides to proteins is hydrophobic in nature, where a hydrophobic cleft on the surface of the protein binds a hydrophobic portion of the carbohydrate topography. Looking at the CPK model of sucrose in Figure 1, one can envision that the β-face of the glucose moiety and the α-face of the fructose moiety define such a wedge-shaped hydrophobic surface and that C-1′ lies on this surface. It was thus of interest to us to determine whether substitution of the 6′-OH with a more hydrophobic moiety would result in a more potent binding molecule since C-6′ also lies on this surface.

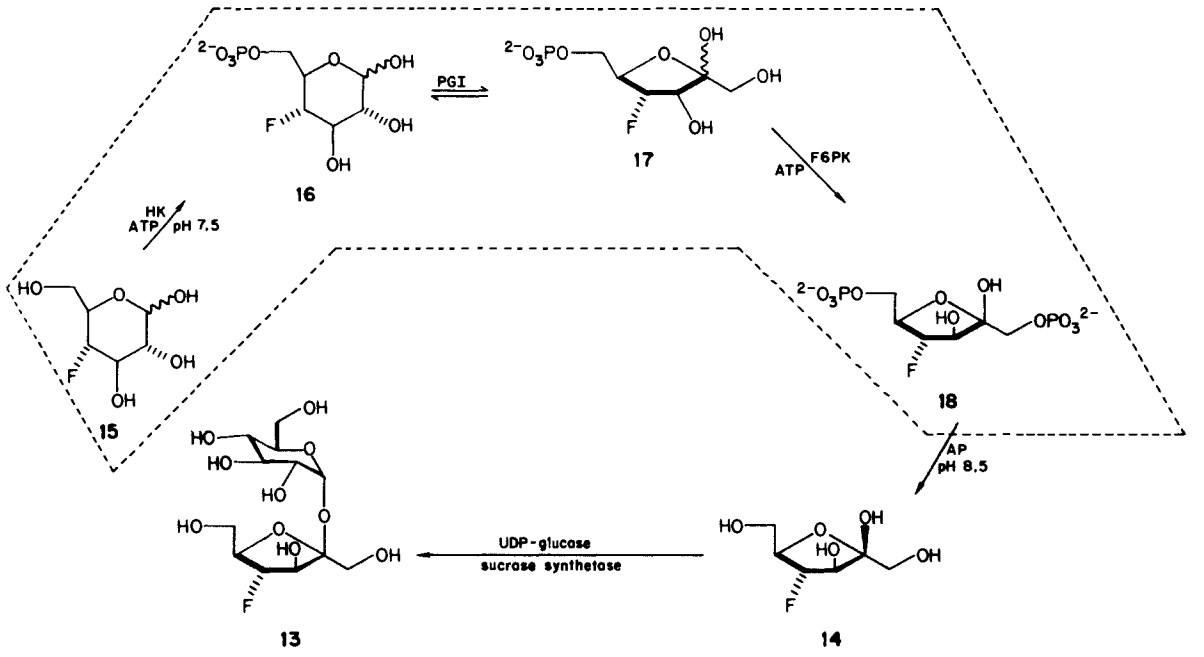
The synthesis of the 6′-substituted sucroses **7** and **8** represents an interesting challenge. The preparation of these derivatives using sucrose synthetase requires the availability of the 6-substituted fructoses **9** and **10** (indicated in the β-fructofuranose conformation for clarity). While **9** and **10** could be synthesized chemically, these methods would require multiple protection and deprotection steps which we chose to avoid. Instead, we investigated the use of the enzyme glucose isomerase to isomerize the readily available 6-substitutred glucoses **11** and **12**[10] into **9** and **10**, respectively.



6-Deoxy-6-fluoroglucose (**11**) was easily obtained (98%) via acidic ion exchange resin catalyzed hydrolysis of 6-deoxy-6-fluoro-1,2-*O*-isopropylidene-α-D-glucofuranose.[11] Using a glucose isomerase (EC. 5.3.1.5), Bock[12] reported that **12** was a substrate and that 15% of **10** was present at equilibrium. The enzyme employed in this investigation (see Experimental Section) also afforded a 10–15% equilibrium concentration of **10** (or **9**), as evidenced via analytical HPLC. However, in contrast to the reported enzyme,[12] the present enzyme did not accept 6-thio- or 6-*O*-methylglucose as substrates. The physical separation of **10** from **12** would be impractical since the yield of **10** would be extremely low and the actual separation tedious. We thus chose to solve this problem by coupling the isomerization reaction with the sucrose formation step, which is not reversible under these reaction conditions. The

(6) Hitz, W. D.; Schmitt, M. R.; Card, P. J.;;Giaquinta, R. T. Plant Physiol. **1985**.
(7) Card, P. J.; Hitz, W. D.; Ripp, K. G. *Proc. Natl. Acad. Sci. U.S.A.*, in preparation.
(8) Bock, K.; Lemieux, R. U. *Carbohydr. Res.* **1982**, *100*, 63.
(9) (a) Hindsgaul, O.; Norberg, T.; Le Pendu, J.; Lemieux, R. U. *Carbohydr. Res.* **1982**, *109*, 109. (b) Kabat, E. A.; Liao, J.; Burzynska, M. H.; Wong, T. C.; Thogersen, H.; Lemieux, R. U. *Mol. Immun.* **1981**, *18*, 893.
(10) Sigma Chemical Co.
(11) Card, P. J.; Reddy, G. S. *J. Org. Chem.* **1983**, *48*, 4734.
(12) Bock, K.; Meldal, M.; Meyer, B.; Wiebe, L. *Acta Chem. Scand., Ser. B* **1983**, *37*, 101.

Scheme I[a]



[a] Enzymatic transformations run simultaneously in one pot are enclosed by dashed lines.

second (irreversible) reaction therefore drives the isomerization reaction to near completion. Thus incubation of **11** or **12** and UDP-glucose with both the glucose isomerase and sucrose synthetase preparations, using a pH-stat to maintain reaction conditions as described for the preparation of **3**, afforded **7** and **8** in 73% and 53% isolated yield, respectively. The structures of **7** and **8** follow from their mode of synthesis and their ^{13}C NMR spectra. For instance, the ^{13}C NMR spectrum of **7** is almost identical with that of sucrose,[5] with three exceptions. In **7** C-6′ is shifted downfield to δ 82.2 where it appears as a doublet due to splitting by the α-fluorine atom ($J_{C,F}$ = 167.5 Hz). In addition, both C-4′ and C-5′ are coupled with F-6′, $J_{C,F}$ = 8.2 and 18.3 Hz, respectively.

As might be expected from the work of Guthrie,[13] and analogous to the case of **1**,[5] incubation of **7** or **8** with invertase[10] at pH 6.5 gave no hydrolysis as measured by glucose appearance.

Assays of the binding of **7** and **8** with the carrier protein[7] revealed that substitution of the 6′-OH with a more hydrophobic moiety produced a more potent (2 times) binding molecule than **2** and suggest that indeed C-6′ also lies on the hydrophobic surface depicted in Figure 1.

To further define the contribution of the α-face of the fructose moiety to the overall binding of sucrose to the carrier protein, we desired to prepare and assay 4′-deoxy-4′-fluorosucrose (**13**). As can be seen in Figure 1, the 4′-OH group lies on the edge of the hydrophobic surface, and therefore its involvement in binding to the carrier protein is of interest in determining the boundaries of this surface.

To prepare **13** we required 4-deoxy-4-fluorofructose (**14**) to couple with UDP-glucose under sucrose synthetase catalysis. 4-Fluorofructose **14** has previously been prepared via a multistep synthesis[14] which we chose to avoid because of its excessive length. Instead, we again investigated a chemoenzymatic route to **14** (Scheme I). We previously developed[11,15] a short and efficient route to 4-deoxy-4-fluoroglucose (**15**) based upon DAST chemistry. Fluoroglucose **15** is an obvious starting material for the synthesis of **14**. We and many others[12] have found that glucose isomerase requires the 4-OH group for glucoses to be substrates for the enzyme. In this particular case the fluorine atom is not an effective substitute, and thus **15** is not isomerized to **14** via glucose isomerase. To overcome this problem we noted that Bessell[16] had reported analytical-scale experiments which showed that **15** is a substrate for hexokinase (EC 2.7.1.1) and that the resulting 4-deoxy-4-fluoroglucose 6-phosphate (**16**) was isomerized by phosphoglucose isomerase (EC 5.3.1.9) to 4-deoxy-4-fluorofructose 6-phosphate (**17**). Since fructose 6-phosphate is not a substrate for sucrose synthetase, another enzyme couple was required to drive the isomerization reaction to completion. 6-Phosphate **17** is a substrate for fructose-6-phosphate kinase[16] (EC 2.7.1.11), and therefore the irreversible formation of **18** can be used to drive the isomerization. Thus when **15** was incubated with ATP, hexokinase (HK), phosphoglucose isomerase (PGI), and fructose-6-phosphate kinase (F6PK) in one pot at 37 °C and the pH maintained at 7.5 on a pH-stat, **18** was formed in situ but not isolated. Instead, the proteins were denatured on a steam bath and removed by filtration. The solution pH was raised to 8.5, and the phosphate groups were removed by an alkaline phosphatase (AP) (EC 3.1.3.1) while the pH was maintained at 8.5. This procedure afforded **14** in 30% isolated yield. Incubation of **14** and UDP-glucose with sucrose synthetase under the normal conditions gave **13** in 16% yield. The structure of **13** follows from its ^{13}C NMR spectrum, which shows C-4′ as a doublet coupled to the α-fluorine atom $J_{C,F}$ = 183 Hz), β-fluorine couplings with both C-5′ (J = 24 Hz) and C-3′ (J = 20.8 Hz), and a γ-fluorine coupling with C-2′ (J = 9 Hz).

Assays[6,7] of the binding of **13** with the sucrose carrier protein suggest that **13** is no more or less potent a binder than is natural sucrose (**2**) itself. Thus it would appear that C-4′ may lie at the extreme edge of the hydrophobic surface (Figure 1) which is presented to the carrier protein for binding but that the 4′-OH group is not involved in this binding interaction.

In total, the binding of these substituted sucroses suggests that a large portion of substrate binding by this carrier protein is in fact a hydrophobic interaction. Tests of all the fructoside hydroxyls excpt 3′-OH also suggest that the fructose moiety of sucrose contributes only to the hydrophobic surface, without polar interactions with the protein. While it seems likely that the glucose ring also contributes to this surface, the glucose portion of sucrose could also provide hydroxyls for a polar interaction, as suggested

(13) Guthrie, R. D.; Jenkins, I. D.; Rogers, P. J.; Wing, F. S.; Watters, J. J.; Yamasaki, R. *Carbohydr. Res.* **1979**, *75*, C1.
(14) Budesinsky, M.; Cerny, M.; Dolezalova, J.; Kulhanek, M.; Pacek, J.; Tadra, M. *Collect. Czech. Chem. Commun.* **1984**, *49*, 267.
(15) Card, P. J. *J. Org. Chem.* **1983**, *48*, 393.
(16) Bessell, E. M.; Thomas, P. *Biochem. J.* **1973**, *131*, 77.

for other protein–oligosaccharide binding.[9]

Experimental Section

General Methods. All chemical reactions were performed under a nitrogen atmosphere. Melting points were determined with a Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were determined on a Nicolet 7199 FT-IR spectrometer. Optical rotations were determined on a Perkin-Elmer 241 MC polarimeter. The 360-MHz ^{1}H NMR and 90.78-MHz ^{13}C NMR spectra were obtained on a Nicolet NT WB 360 spectrometer. The ^{19}F NMR spectra were obtained on a Varian XL-100 spectrometer and are referenced to external trichlorofluoromethane. Crude glucose isomerase was a generous gift from Dr. F. Armbruster of Corn Products Corp. Sucrose synthetase was obtained as previously described.[5] Uridine-5′-diphosphoglucose and the remaining enzymes were obtained from Sigma. HPLC was performed on an HP 1084B liquid chromatograph that was equipped with a refractive index detector. Analyses were obtained at room temperature on a Du Pont Zorbax-NH_2 column using 7:3 CH_3CN/H_2O as eluent.

1-Azido-1-deoxy-2,3:4,5-di-*O*-isopropylidene-D-fructopyranose (6). A solution of **5**[5] (11.76 g; 30 mmol) in DMF (100 mL) at 80 °C was saturated with sodium azide. After stirring at 80 °C for 18 h, the mixture was poured into water and extracted with ether. The ether layer was washed with water, dried ($MgSO_4$), and concentrated to a colorless solid. Chromatogrphy on silica gel (4:1 hexane/ether) afforded 7.75 g (91%) of **6**: mp 55.5–60 °C; 360-MHz ^{1}H NMR ($CDCl_3$) δ 1.34 (s, 3 H), 1.46 (s, 3 H), 1.48 (s, 3 H), 1.56 (s, 3 H), 3.43 (AB q, 2 H, H-1 + H-1′, $J_{1,1'}$ = 13 Hz, $\Delta\nu$ = 29 Hz), 3.77 (d, H-6_{eq}, $J_{6eq,lax}$ = 13 Hz), 3.93 (dd, H-6_{ax}, $J_{6ax,5}$ = 2 Hz), 4.23 (dd, H-5, $J_{4,5}$ = 8 Hz), 4.28 (d, H-3, $J_{3,4}$ = 3 Hz), 4.61 (dd, H-4); IR (KBr) 2110, 1070 cm^{-1}; $[\alpha]_D$ − 88.9° (*c* 1.07, $CHCl_3$).

Anal. Calcd for $C_{12}H_{19}O_5N_3$: C, 50.52; H, 6.71; N, 14.73. Found: C, 50.74; H, 6.66; N, 14.84.

1-Azido-1-deoxy-D-fructose (4). A solution of **6** (7.06 g; 24.8 mmol) in EtOH (30 mL) and water (90 mL) was treated with Bio-Rad AG 50W-X8 (12.5 g, hydrogen form), and the mixture was heated at 60–70 °C for 3 h. The reaction mixture was filtered, decolorized, and then extracted with ether. The aqueous layer was partially concentrated on a rotatory evaporator to remove the EtOH and then lyophilized to afford **4** (4.91 g; 92%%) as a colorless syrup: IR (neat) 3250, 2100, 1070 cm^{-1}; $[\alpha]_D$ −30.3° (*c* 1.03, MeOH).

1′-Azidosucrose (3). 1′-Azidosucrose (**3**) was prepared from **4** and 0.25 equiv of UDP-glucose and the reaction was performed as described in ref 5. **3** was obtained in 15% yield as an amorphous solid; IR (KBr) 2110 cm^{-1}; ^{13}C NMR (D_2O) δ 53.0 (C-1′), 60.8 (C-6), 62.4 (C-6′), 69.9 (C-4), 71.4 (C-2), 72.9 (C-5), 73.1 (C-3), 74.2 (C-4′), 77.5 (C-3′), 81.5 (C-5′), 92.7 (C-1), 104.1 (C-2′); $[\alpha]_D$ +65.8° (*c* 0.95, H_2O); mass spectrum, 367 (M^+). This material was hygroscopic and we were unable to obtain satisfactory combustion analytical data.

Glucose Isomerase. A Pharmacia PD-10 Sephadex G-25 column was equilibrated with 25 mL of a buffer containing 20 mM Tris·HCl, 0.1 mM EDTA, 20 mM $MgCl_2$, and 1 mM DTT at pH 8.2. Crude glucose isomerase (2.5 mL) was run into the column, and the eluent was discarded. The above buffer (3.5 mL) was added to the column and the collected enzyme solution was kept cold until use.

6-Deoxy-6-fluoroglucose (11). A solution of 6-deoxy-6-fluoro-1,2-*O*-isopropylidene-α-D-glucofuranose[11] (18.97 g; 85 mmol) in 340 mL of 3:1 H_2O:EtOH was treated with 42 g of Bio-Rad AG 50W-X[8] (hydrogen form) at 65 °C for 3.5 h. Processing as per **4** afforded 15.1 g (98%) of **11** as a colorless solid.

6′-Deoxy-6′-fluorosucrose (7). A solution of 6-fluoroglucose **11** (728 mg; 4 mmol) and UDP-glucose (2.6 g; 4 mmol) in water (15 mL) containing 200 μL of 1 M $MgCl_2$ was treated with the glucose isomerase (2.5 mL) and sucrose synthetase (2.5 mL) preparations, and the resulting mixture was incubated at 37 °C under N_2. The pH of the reaction was maintained at 8.2 on a pH-stat via addition of IN KOH. Reaction progress was monitored by the rate of H^+ appearance and was terminated by heating to 100 °C for 10–15 min. Precipitated protein was removed from the reaction mixture by membrane filtration, and the filtrate was passed through a Bio-Rad AG1-X8 (OH^-form) column. The column was eluted with water, and lyophylization afforded 1.0 g (73%) of **7** as a colorless solid: mp 170.5-172.5 °C; ^{13}C NMR (D_2O) δ 59.2 (s, C-6), 60.0 (s, C-1′), 68.2 (s, C-4), 70.0 (s, C-2), 71.3 (s, 1 C), 71.5 (s, 1 C), 71.6 (d, C-4′), $J_{C,F}$ = 8.2 Hz), 75.1 (s, C-3′) 78.4 (d, C-5′, $J_{C,F}$ = 18.3 Hz), 82.2 (d, C-6′), $J_{C,F}$ = 167.5 Hz), 91.2 (s, C-1), 102.8 (s, C-2′); ^{19}F NMR (H_2O) δ −222 (dt, $J_{H\text{-}6',F}$ = 47, Hz, $J_{H\text{-}5',F}$ = 20 Hz); $[\alpha]_D$ +53.5° (*c* 1.04, H_2O).

Anal. Calcd for $C_{12}H_{21}FO_{10}$: C, 41.86; H, 6.15. Found: C, 41.61; H, 6.14.

6′-Deoxyysucrose (8). 6-Deoxyglucose (656 mg; 4 mmol) was treated as above and afforded 690 mg (53 %) of **8** as a colorless solid: mp 175–178 °C; ^{13}C NMR (D_2O) δ 17.8 (C-6′), 60.0 (C-6), 61.1 (C-1′), 69.1, 70.9, 72.0, 72.5, 76.0, 77.2, 78.5, 91.9 (C-1), 103.2 (C-2′).

Anal. Calcd for $C_{12}H_{22}O_{10}$: C, 44.17; H, 6.80. Found: C, 44.24; H, 6.68.

4′-Deoxy-4′-fluorosucrose (13). A solution of 4-fluoroglucose (776 mg; 4 mmol) and ATP (5.01 g; 8.4 mmol; neutralized to pH 7.5 via addition of 2 M $KHCO_3$) in 8 mL of water and 4.5 mL of 1 M $MgCl_2$ was treated with hexokinase (6000 units), phosphoglucose isomerase (2500 units), and fructose-6-phosphate kinase (2500 units). The mixture was incubated at 37 °C under N_2 while the pH of the reaction was maintained at 7.5 on a pH-stat via addition of 1 N KOH. Reaction progress was monitored by the rate of H^+ appearance and was terminated by heating to 100 °C for 10–15 min. Precipitated protein was removed by membrane filtration, the pH was raised to 8.5, and alkaline phosphatase (5000 μm) was added. Reaction pH was maintained at 8.5 via a pH-stat. When reaction progress ceased the pH was lowered to 7.0 by addition of 1 N HCl. The protein was denatured on a water bath and then removed via membrane filtration. The resulting solution was passed through a mixed-bed column containing AG 50W-X8 (H^+ form) and AG1-X8 (formate form). Lyophylization afforded 218 mg (30%) of **14** as a solid foam.

A solution of **14** (2 mol) and UDP-glucose (1.95 g; 3 mmol) in water (30 mL) was incubated at 37 °C with sucrose synthetase (5 mL) while the pH at 8.2 was maintained. Usual processing afforded 110 mg (16%) of **13** as an amorphous solid: ^{13}C NMR (D_2O) δ 60.5 (C-6), 61.6 (C-1′), 62.3 (C-C′), 69.5 (C-4), 71.4 (C-2), 72.8, 72.9, 75.5 (d, C-3′, $J_{C\text{-}3,F\text{-}4}$ = 20.8 Hz), 79.7 (d, C-5′, $J_{C\text{-}5',F\text{-}4}$ = 24.2 Hz), 92.8 (C-1), 96.3 (d, C-4′, $J_{C\text{-}4',F\text{-}4}$ = 183 Hz), 104.7 (d, C-2′, $J_{C\text{-}2',F\text{-}4}$ = 9 Hz); ^{19}F NMR (D_2O) 0 −203.6 (ddd, J = 59, 21.6, 16.2 Hz); $[\alpha]_D$ +61.6° (*c* 0.99, H_2O); mass spectrum, 345 (M + H), 327 (M - OH). This material was hygroscopic and we were unable to obtain satisfactory combustion analytical data.