Characterization of Recombinant Sucrose Synthase 1 from Potato for the Synthesis of Sucrose Analogues

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Abstract: The characteristics and the application of recombinant sucrose synthase 1 (SuSy1) from potato for the synthesis of sucrose analogues are described. With UDP-Glc as donor substrate SuSy1 accepts a variety of ketoses, e.g., 1-deoxy-1-fluoro-D-fructose (**6**; 100%), L-sorbose (**7**, 55%), and D-xylulose (**8;** 42%), as well as aldoses, e.g., D-talose (**15**; 36%), D-idose (**16;** 24%), D-lyxose (**12;** 48%), L-arabinose (**13**; 36%), and D-ribose (**14**; 7%). Kinetic analyses revealed that the non-natural acceptors **6** (k_{cat}/K_m = 3.5 s^{-1} mM^{-1}), **7** (k_{cat}/K_m = 1·10^{-2} s^{-1} mM^{-1}), 8 (k_{cat}/K_m = 2·10^{-2} s^{-1} mM^{-1}), and **12** (k_{cat}/K_m = 2·10^{-2} s^{-1} mM^{-1}) were relatively poor substrates when compared to D-fructose (**5;** k_{cat}/K_m = 34.1 s^{-1} mM^{-1}). It is concluded that the configuration and/or presence of the hydroxymethyl group at C5 determine the affinity of the ketoses for SuSy1. The acceptance of aldoses can be explained by their flexible chair conformations, which lead to isosteric hydroxy groups recognized by SuSy1. The preparative synthesis of sucrose analogues yielded 1′-deoxy-1′-fluoro-β-D-fructofuranosyl-α-D-glucopyranoside (**1**), [$^{13}C_1$]-β-D-fructofuranosyl-α-D-glucopyranoside (**2**), α-D-glucopyranosyl-α-L-sorbofuranoside (**3**), and α-D-glucopyranosyl-α-D-lyxopyranoside (**4**), in a 0.1 – 1.0 g scale. The sucrose analogues **1**, **3**, and **4** were not hydrolyzed by invertase, which makes them valuable tools for studies on signal transduction pathways and sugar transport in plants.

Keywords: Enzyme catalysis; glycosides; glycoconjugates; sucrose synthase; sucrose analogues

Introduction

The plant glycosyltransferase sucrose synthase (SuSy, EC 2.4.1.13) plays an important role in the metabolism of sucrose and its conversion to polysaccharides. SuSy catalyzes the cleavage of sucrose with nucleoside diphosphates and provides the plant cell with activated sugar precursors for sucrose-starch transformation[1] and for cell wall synthesis.[2] SuSy represents a unique case among the Leloir glycosyltransferases by catalyzing *in vitro* both the cleavage of sucrose with nucleoside diphosphate yielding D-fructose and nucleotide-activated D-glucose, and the readily reversible reaction. It has been shown for developing potato tubers and other plant tissues that the reaction is also reversible *in vivo*.[3] In contrast to other enzymes of the sucrose metabolism, SuSy shows a wide specificity for nucleoside diphosphates in the sucrose cleavage direction.[4] In direction of sucrose synthesis the enzyme shows also a wide specificity.[5] This ability was utilized in the synthesis of the sucrose analogues 2-*O*-6-deoxy-α-L-sorbofuranosyl-D-glucose,[6] α-D-glucopyranosyl-β-D-*xylulo*-furanoside, and α-D-glucopyranosyl-α-D-*lyxo*-pyranoside[7] with SuSy purified from rice grains. Card et al.[8] used SuSy for the synthesis of fluoro and azido analogues of sucrose to study the binding specificity of a putative sucrose carrier protein.

The work presented in this paper comprises a detailed analysis of the substrate specificity spectrum of recombinant sucrose synthase 1 from potato and kinetic data of different acceptor substrates for the synthesis direction (Scheme 1). The sucrose analogues, 1'-deoxy-1'-fluoro-β-D-fructofuranosyl-α-D-glucopyranoside (**1**), [$^{13}C_1$]-β-D-fructofuranosyl-α-D-glucopyranoside (**2**), α-D-glucopyranosyl-α-L-sorbofuranoside (**3**), and α-D-glucopyranosyl-α-D-lyxopyranoside (**4**) were synthesized. All products were characterized by ^{1}H and ^{13}C NMR. Work is in progress to utilize the sucrose analogues for studies on sugar sensing in plants[9] and on sucrose transport in plants by *in vivo* NMR techniques (Köckenberger, in preparation), respectively.

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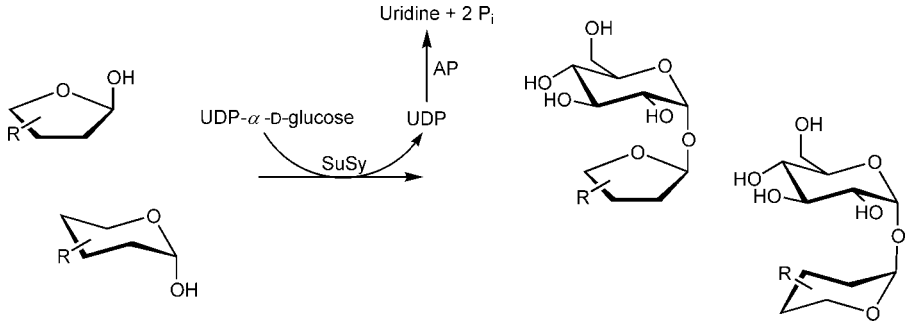

Scheme 1. General scheme for the synthesis of sucrose analogues with recombinant sucrose synthase 1 from potato.

Results and Discussion

Ketoses as Acceptor Substrates

Different ketoses were tested to explore the acceptor substrate spectrum of SuSy1. Figure 1 illustrates that the primary alcohol function at C1 of β-D-fructose (**5**) can be replaced by a fluoro group in **6**. Further variation of the ketoses reveals a crucial role for the configuration at C3 and C4. The 3*S*,4*R* configuration in L-sorbose (**7**) and D-xylulose (**8**) is important for the ketose substrate to be recognized by SuSy1, whereas D-tagatose (**9**), D-psicose (**10**), and D-sorbose (**11**) are not accepted. However, the configuration and/or presence of the hydroxymethyl group at C5 appear not to be crucial. The acceptance of **7** and **8** was also described for sucrose synthase from green peas[10] and sugar beet roots.[11] The kinetic constants for the accepted ketoses were determined to optimize the preparative syntheses of sucrose analogues. They are summarized in Table 1. Both natural substrates **5** and UDP-Glc show an inhibition at high substrate concentrations. The relatively low K_{iS} value of UDP-Glc implies that the donor substrate concentration limits the reaction rate of SuSy1. This is illustrated for **5** as a variable substrate, when already 2 mM UDP-Glc (4-fold K_m value) causes a lower V_{max} value. In comparison to enzyme preparations from potato, recombinant SuSy1 shows a 3.5-fold to 13.5-fold higher affinity for UDP-Glc.[12] The K_m value for **5** is comparable to those determined by Pressey[12b] and Murata[12c] for the potato enzyme; however, a substrate inhibition for the donor and acceptor substrate was not found. In contrast, a substrate inhibition above 20 mM of **5** was described for SuSy from maize.[13] Nakai et al.[14] found K_m values of 7.72 mM and 0.4 mM for **5** and UDP-Glc, respectively, for a recombinant sucrose synthase from mung bean seedlings expressed in *E. coli*. The kinetic analysis for **7** and **8** reveals, however, that these ketoses are poor substrates with an approx. 1000-fold decreased catalytic efficiency (k_{cat}/K_m) for SuSy1 (Table 1). We conclude that the configuration at C5 determines the affinity of the ketose substrate.

Aldoses as Acceptor Substrates

The idea to test also aldoses as acceptor substrates was derived from the fact that in aqueous solution the equilibrium form of β-D-fructose appears to be 70% pyranose and 23% furanose. The $^1C_4$ chair conformation is thermodynamically favored by the β-D-

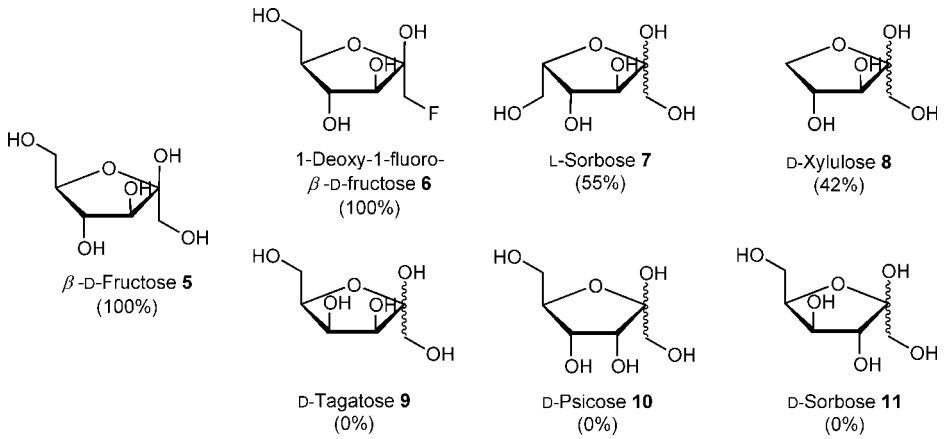

Figure 1. Ketoses as acceptor substrates for recombinant SuSy1 from potato. The monosaccharides (10 mM) were tested with the donor UDP-Glc (2 mM) in 50 mM HEPES buffer, pH 8.0, for 16 h at 30 °C. The formation of disaccharides and the conversion of UDP-Glc was monitored by HPLC. The relative yields refer to the donor substrate UDP-Glc.

Table 1. Kinetic constants of recombinant SuSy1 from potato for the sucrose synthesis direction.

Substrate	K_m [mM]	K_{iS} [mM]	V_{max} [U mg^{-1}]	k_{cat} [s^{-1}]	k_{cat}/K_m [s^{-1} mM^{-1}]
UDP-Glc[a]	0.46	2.30	21.76	142.8	310.4
5[a]	2.05	35.88	10.63	69.8	34.1
6[a]	6.28	–	3.29	21.7	3.5
7[b]	226.5	521.2	0.35	2.3	0.01
8[b]	244.3	–	0.61	4.0	0.02
12[b]	125.7	–	0.35	2.3	0.02

[a] The activity assay was performed without addition of alkaline phosphatase.
[b] The activity assay was performed with addition of alkaline phosphatase.

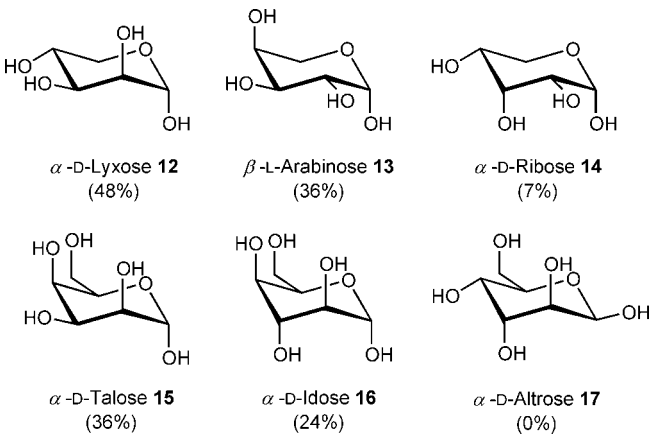

Figure 2. Aldoses as acceptor substrates for recombinant SuSy1 from potato. The monosaccharides (10 mM) were tested with the donor UDP-Glc (2 mM) in 50 mM HEPES buffer, pH 8.0, for 16 h at 30 °C. The formation of disaccharides and the conversion of UDP-Glc was monitored by HPLC. The relative yields refer to the donor substrate UDP-Glc.

fructopyranose. We hypothesized that SuSy1 may recognize the β-D-fructopyranose conformation rather than removing the β-D-fructofuranose from the equilibrium during the synthesis reaction. These led us to test more than 20 different aldoses as acceptor substrates. However, we found that only a few were readily accepted. A common feature of the accepted aldoses is that the defined anomers, as depicted in Figure 2, have the flexible $^4C_1/^1C_4$ chair conformations. Whether this feature represents an exclusive criterion for SuSy1 cannot be established completely by these investigations. The kinetic analysis (Table 1) reveals **12** to be a poor substrate. However, in comparison to the ketoses **7** and **8** the lower K_m value indicates a better affinity of SuSy1. The comparison of the accepted aldoses with the $^1C_4$ chair conformation of β-D-fructopyranose suggests that their conformational flexibility could contribute to isosteric configurations of their hydroxy groups, which are recognized by SuSy1. Figure 3 illustrates our hypothesis for **12** and **13**, which predicts also the stereoselective product formation. First evidence for our hypothesis is reported below for the preparative synthesis of a sucrose analogue, where **12** is converted by SuSy1 to yield the non-reducing disaccharide **4** (Figure 4).

Enzymatic Synthesis of Sucrose Analogues

Figure 4 and Table 2 summarize the synthesis of the sucrose analogues 1′-deoxy-1′-fluoro-β-D-fructofuranosyl-α-D-glucopyranoside (**1**), [$^{13}C_1$]-β-D-fructofura-

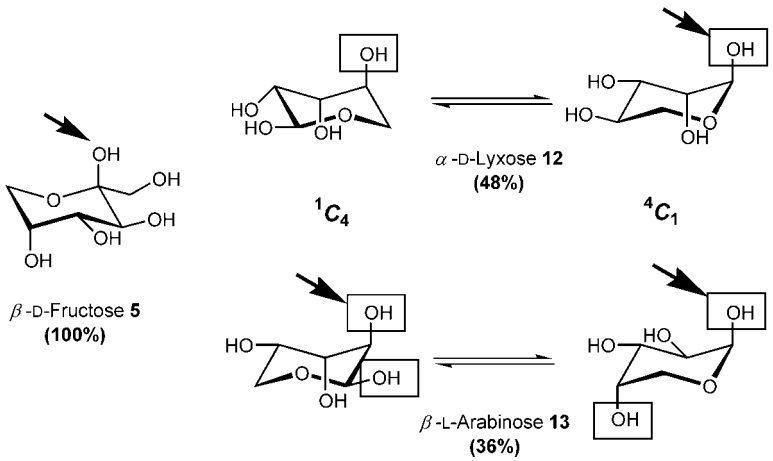

Figure 3. Hypothetical model to demonstrate the isosteric configurations of the hydroxy groups of α-D-lyxose and β-L-arabinose in comparison to the $^1C_4$ chair conformation of β-D-fructose. Isosteric hydroxy groups are highlighted. Putative sites for the transfer of the donor substrate are indicated by arrows. For α-D-lyxose the model product was confirmed by preparative synthesis and characterization by NMR analysis.

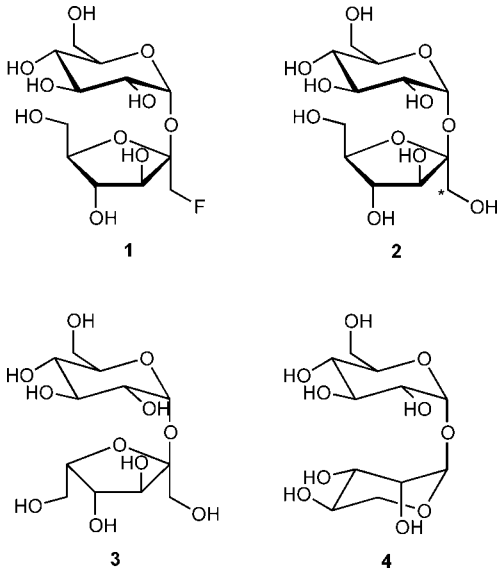

Figure 4. Products of the enzymatic synthesis with recombinant SuSy1: 1-deoxy-1-fluorofructofuranosyl-α-D-glucopyranoside (**1**), [$^{13}C_1$]-β-D-fructofuranosyl-α-D-glucopyranoside (**2**), α-D-glucopyranosyl-α-L-sorbofuranoside (**3**), and α-D-glucopyranosyl-α-D-lyxopyranoside (**4**).

nosyl-α-D-glucopyranoside (**2**), α-D-glucopyranosyl-α-L-sorbofuranoside (**3**), and α-D-glucopyranosyl-α-D-lyxopyranoside (**4**). The repetitive use of all enzymes during the syntheses (repetitive-batch mode) resulted in efficient enzyme productivities and space-time yields (Table 2). Moreover, these data also reflect the catalytic efficiency of SuSy1 with reference to the acceptor substrates. In comparison with the ketose **7** the affinity of SuSy1 for the aldose **12** is higher and therefore results in higher enzyme productivity. This is also evident in the case of the [$^{13}C_1$]-labeled natural substrate **5** and the derivative **6**. In comparison to previous published synthesis we could improve the overall yield for the products **1** and **2**. Card and Hitz[8a] synthesized **1** with SuSy from barley seeds with an overall yield of 59% (507 mg). Duker and Serianni[15] synthesized **2** with an overall yield of 25% (85.6 mg) using 50 U SuSy. With sucrose synthase purified from rice grains Grothus et al.[7] synthesized **3** in an analytical scale (16.5 mg) using 5 U enzyme with an overall yield of only 7.5%. This synthesis was improved by taking into account the kinetic data and by using both a fed-batch-technique and an addition of alkaline phosphatase. Peters et al.[6] synthesized 2-*O*-6-deoxy-α-L-sorbofuranosyl-α-D-glucopyranoside with an overall yield of 17% (50 mg) using 8 U SuSy from rice grains.

Table 3. Assay of sucrose analogues as substrates of invertase.

Product	Relative activity [%]
1[a]	100
2	0.04
3	0.06
4	0.12

[a] Unlabeled sucrose.

In studies on sugar signaling[9,16] and sugar transport[17] in plants it is sometimes advantageous if sucrose analogues are not hydrolyzed by invertases. Such analogues can be used to determine unambiguously the response between the signaling compound and the receptor. Table 3 demonstrates that the products **2**, **3**, and **4** are not hydrolyzed by invertase. Therefore, the very low residual activity of invertase for these sucrose analogues makes them valuable tools for further biochemical studies.

Conclusion

The presented results provide for the first time details of the structural requirements for acceptor substrates to be recognized by recombinant SuSy1 from potato. Although some compounds acted as poor substrates the synthesis yielded unique sucrose analogues for biochemical studies of signal transduction and sugar transport in plants. Further information is still required to investigate the donor substrate spectrum of this enzyme. However, already it can be concluded that SuSy1 represents a unique biocatalyst for use in carbohydrate engineering.

Table 2. Sucrose analogues synthesized with recombinant SuSy1 from potato.

Product	Synthesis yield [%]	Enzyme productivity[a] [mg U^{-1}]	Space-time yield [g L^{-1} day^{-1}]	Overall yield [%]	Amount [g]
1	100[b]	25	1.68	85[b]	0.86
2	94[b]	61	3.03	55[b]	0.71
3	81[c]	3.7	0.57	35[c]	0.079
4	81[c]	7.1	1.60	69[c]	0.160

[a] Milligrams of formed product per unit of recombinant SuSy1.
[b] With reference to the acceptor substrate.
[c] With reference to the donor substrate.

Experimental Section

Recombinant Sucrose Synthase 1 from Potato (*Solanum tuberosum*)

The gene sus1 from *Solanum tuberosum* L., which encodes SuSy1, was cloned in the expression plasmid pDR195 under the control of the proton ATPase (*PMA1*) promoter. The plasmid was transformed in the *Saccharomyces cerevisiae* strain 22574d. The recombinant yeast cells expressing SuSy1 constitutively during growth were cultivated on a 30-L scale yielding 796 g wet cells. From 200 g homogenized cells 874 U SuSy1 with a yield of 81% were obtained by a partial purification step with anion exchange chromatography. The enzyme fraction was free of contaminating invertases and phosphatases. With reference to the SuSy1 activity 0.05% of hydrolases cleaving nucleotide sugars, and 13% hexokinase as well as 0.05% phosphoglucose isomerase were present. The partially purified SuSy1 fraction was used for the synthesis of nucleotide-activated sugars and sucrose analogues. Kinetic studies required further purification by immobilized metal ion chromatography (IMAC) giving an 86% yield. The activities of contaminating enzymes could be minimized to 10% of the activities reported above.

Enzyme Assay

Enzyme activity of SuSy1 was measured in the direction of sucrose cleavage. In a total volume of 1 mL of 200 mM HEPES buffer, pH 7.6, recombinant SuSy1 from potato was incubated with 2 mM UDP (Sigma, Deisenhofen) and 500 mM sucrose for 10 min at 30 °C.[18] The reaction was stopped by heating at 95 °C for 5 min. The formation of UDP-α-D-glucose (UDP-Glc) was monitored by HPLC.[19] One unit is defined as the amount of enzyme for the formation of 1 μmol UDP-Glc per min under standard conditions.

Kinetic Data

All kinetic measurements in the synthesis direction were performed in 200 mM HEPES buffer, pH 8.0 at 30 °C. The kinetic constants for UDP-Glc (Sigma, Deisenhofen) and the acceptors **5** and **6** were obtained at a constant concentrations of 10 mM for the natural acceptor **5** and a constant concentration of 2 mM for the donor substrate UDP-Glc. The kinetic data **7**, **8**, and **12** were determined at a constant concentration of 1 mM UDP-glucose and in the presence of 1 U mL^{-1} alkaline phosphatase (Roche Diagnostics, Mannheim). Initial rate measurements were obtained by allowing a maximum conversion of 10% of the variable substrate followed by HPLC detection[19] of formed UDP or UMP and uridine when using alkaline phosphatase. The kinetic constants were obtained by a non-linear regression analysis of the data using the Michaelis-Menten equation: $V = (V_{max} \times S)/(S + K_m)$ or the kinetic equation for substrate inhibition: $V = (V_{max} \times S)/(S + K_m + S^2/K_{iS})$.

Variation of Acceptor Substrates

Different ketoses and aldoses (10 mM) were tested as acceptor substrates in the synthesis reaction of SuSy1 with UDP-Glc (1 mM) as donor substrate. The assay mixtures containing 200 mU mL^{-1} SuSy1 and 1 U mL^{-1} alkaline phosphatase were incubated in 200 mM HEPES buffer, pH 8.0, for 16 h at 30 °C. The reaction was stopped by heating for 5 min at 95 °C. The formation of disaccharides was monitored by HPLC analysis using an Aminex HPX-87C column (300 × 7.8 mm, BioRad, Munich) and elution with distilled water at 85° C. The formation of UDP, UMP, and uridine was also observed by HPLC.[19]

Synthesis of 1-Deoxy-1-fluorofructofuranosyl-α-D-glucopyranoside (1)

1-Deoxy-1-fluoro-D-fructose (**6**) was obtained by the reaction described by Card and Hitz.[8a] In brief, the readily available 2,3:4,5-di-*O*-isopropylidene-D-fructopyranose was converted into the triflate using the procedure described by Binkley et al.[20] The triflate was fluorinated by TASF [tris(dimethylamino)sulfur(trimethylsilyl) difluoride, Aldrich Chemicals] in refluxing tetrahydrofuran. After removal of the isopropylidene protection groups, 1-deoxy-1-fluoro-D-fructose was obtained as a syrup in 75% yield. The synthesis of 1'-deoxy-1'-fluorosucrose was carried out by the repetitive-batch technique.[21] The reaction mixture (100 mL) containing 0.96 mmol 1-deoxy-1-fluorofructose (176 mg) and 1 mmol UDP-α-D-glucose in 200 mM HEPES buffer, pH 8.0, was gently stirred at 30 °C after adding of 40 U recombinant SuSy1 and 200 U alkaline phosphatase (Roche Diagnostics, Mannheim). The course of the reaction was monitored by HPLC analysis of the product with an Aminex HPX-87C column as described above. After 48 h the enzymes were recovered by ultrafiltration and used in a second and third batch, after the addition of new substrates. The total yield of the combined product solutions was 2.9 mmol (100%) for 1'-deoxy-1'-fluorosucrose. In a first purification step the product solution was adjusted to pH 8.6 and loaded onto an anion exchanger column ($HCOO^-$ form) filled with AG 1-X8 resin (100 – 200 mesh, 122 mL bed volume, BioRad, Munich), which was equilibrated with distilled water. Elution with distilled water (linear velocity: 56.5 cm h^{-1}) resulted in a product pool, which was concentrated by vacuum evaporation to a final volume of 5 mL. The disaccharide was further purified by chromatography on an AG 50 W-X8 resin column (200 – 400 mesh, Ca^{2+} form, 1532 mL bed volume, BioRad, Munich). Elution with distilled water (linear velocity: 3 cm h^{-1}) gave the fractions containing the disaccharide, which were pooled and lyophilized. The dry product was dissolved in 10 mL absolute methanol and crystallized at 25 °C. The product **1** was obtained as a white solid in an overall yield of 85% corresponding to 2.5 mmol (860.5 mg) with an HPLC purity of 89%. NMR spectroscopy (Bruker, DAX500) (11.7 T) of **1** revealed the typical couplings between ^{19}F and ^{1}H or ^{13}C. ^{1}H NMR (500 MHz, D_2O): δ = 4.39 (1'-H, m, $J_{H,F}$ = 46.6 Hz, $J_{H,H}$ = 10.4 Hz); ^{19}F NMR (470 MHz, D_2O, $CFCl_3$): δ = –229.4 (m, $J_{F,H}$ = 46.6 Hz); ^{13}C NMR (125 MHz, D_2O): δ = 80.7 (1'-C, d, $J_{C,F}$ = 174.2 Hz), 101.8 (2'-C, d, $J_{C,F}$ = 19.6 Hz).

Synthesis of [$^{13}C_1$]-β-D-Fructofuranosyl-α-D-glucopyranoside (2)

The synthesis was also carried out with the repetitive-batch technique. The reaction mixture (100 mL) containing

1 mmol [$^{13}C_1$]-β-D-fructofuranoside (Euriso-top, Gif-Sur-Yvette, France) and 1 mmol UDP-Glc in 200 mM HEPES buffer, pH 8.0, was gently stirred at 30 °C after the addition of 20 U recombinant SuSy1 from potato and 200 U alkaline phosphatase. The course of reaction was controlled by HPLC. After 24 h the enzymes were recovered by ultrafiltration and used in the three successive batches, together with new substrates. The yield of the combined product solutions was 3.78 mmol (94%) for **2**. Prior to isolation the product solution was adjusted to pH 8.6 and then loaded onto an anion exchanger column (Cl^- form) filled with Dowex 1x2 resin (100 – 200 mesh, 400 mL bed volume, Serva, Heidelberg), which was equilibrated with distilled water. Elution with distilled water (linear velocity: 22 cm h^{-1}) resulted in a product pool, which was concentrated by vacuum evaporation to a final volume of 16 mL. The disaccharide (aliquots of 2 mL product solution) was further purified by chromatography on a Bio-Gel P2 resin column (extra fine, 500 mL bed volume, BioRad, Munich). Elution with distilled water (linear velocity: 5 cm h^{-1}) gave the disaccharide-containing fractions, which were pooled, combined, and lyophilized. The product **2** was obtained in an overall yield of 55% corresponding to 2.07 mmol (712 mg) with an HPLC purity of 89%. NMR spectroscopy (Bruker, DPX 400 advance series) revealed the typical signals and couplings. ^{13}C NMR (100 MHz, D_2O): δ = 62.2 (s, $C_{1'}$); ^{1}H NMR (400 MHz, D_2O): δ = 3.56 (1'-H, dd, J_{HC} = 144 Hz), 5.33 (1-H, dd, J_{HC} = 169 Hz).

Synthesis of α-D-Glucopyranosyl-α-L-sorbofuranoside (3)

The synthesis was carried out in a final volume of 75 mL using the fed-batch technique. The reaction mixture containing 22.5 mmol **7** and 0.125 mmol UDP-Glc in 200 mM HEPES buffer, pH 8.0, was gently stirred at 30 °C after the addition of 50 U recombinant SuSy1 from potato and 225 U alkaline phosphatase. The course of reaction was controlled by HPLC as described above. After an incubation time of 19 h 0.4 mmol UDP-Glc was fed to the reaction mixture over the next 48 h. After the batch has been stirred for another 25 h the enzymes were separated by ultrafiltration. The synthesis yield was 0.51 mmol (81%) of **3**. The first purification step was carried out as described above for **1**. The disaccharide was further purified by chromatography on a preparative HPLC column [carbohydrate-Ca column (300 × 20 mm, CS, Langerwehe) with elution by distilled water (flow rate: 1.2 mL min^{-1}). The pooled and lyophilized fractions gave **3** in an overall yield of 35% (0.220 mmol, 79 mg). ^{1}H NMR (500 MHz, D_2O): δ = 5.31 (1-H, d), 4.40 (4'-H, t), 4.31 (5'-H, dq), 4.14 (3'-H, d), 3.61–3.82 (1'-$H_{a,b}$, 3-H, 5-H, 6-$H_{a,b}$, m), 3.50 (2-H, dd), 3.34 (4-H, t); $^3J_{1,2}$ = 4.1, $^3J_{3',4'}$ = 7.3, $^3J_{4',5'}$ = 7.6, $^4J_{2,4}$ = 10.0 Hz; ^{13}C NMR (125 MHz, D_2O): δ = 104.03 (C-2'), 92.66 (C-1), 78.59 (C-5'), 77.74 (C-3'), 75.13 (C-4'), 73.11 (C-3), 73.05 (C-5), 71.48 (C-2), 69.84 (C-4), 61.06 (C-6'), 60.97 (C-1'), 60.81 (C-6). ESI-MS (Finnigan MAT, LCQ): found: 341.1 *m/z* for $[M - H]^-$, calculated: 341.12 *m/z* for $[M - H]^-$.

Synthesis of α-D-Glucopyranosyl-α-D-lyxopyranoside (4)

The synthesis of **4** was performed in a final volume of 58 mL with the fed-batch technique. The reaction mixture containing 9 mmol of **12** and 0.045 mmol UDP-Glc in 200 mM HEPES buffer, pH 8.0, was gently stirred at 30 °C after addition of 26.5 U recombinant SuSy1 and 115 U alkaline phosphatase. The course of reaction was monitored by HPLC as described before. After incubation for 1.5 h 0.684 mmol UDP-Glc was added to the reaction mixture over 38 h. After the batch had been stirred for another 9 h, the reaction was terminated by separation of the enzymes with ultrafiltration. The synthesis yield was 0.59 mmol (81%) for **4**. Product isolation was carried out as described for **3**. For the sucrose analogue **4** an overall yield of 69% (0.50 mmol, 159.9 mg) was obtained. ^{1}H NMR (500 MHz, D_2O): δ = 5.09 (1-H, d); 4.99 (1'-H, d), 3.89 (2'-H, t), 3.87 (3'-H, t), 3.79–3.85 (5'-$H_{a,b}$, 3-H, 5-H, 6-$H_{a,b}$, m), 3.68 (4'-H, ddd), 3.55 (2-H, t), 3.38 (4-H, t); $^3J_{1,2}$ = 3.8 , $^3J_{1',2'}$ = 2.8, $^3J_{2',3'}$ = 3.0, $^3J_{3',4'}$ = 2.2, $^3J_{3,4}$ = 9.4, $^4J_{1',3'}$ = 12.3 Hz; ^{13}C NMR (125 MHz, D_2O): δ = 95.72 (C-1'), 94.18 (C-1), 73.00 (C-3), 72.77 (C-5), 71.25 (C-2), 70.77 (C-3'), 69.95 (C-2'), 69.93 (C-4), 67.03 (C-4'), 63.23 (C-5'), 60.87 (C-6). ESI-MS (Finnigan MAT, LCQ): found: 311.0 *m/z* for $[M - H]^-$, calculated: 311.11 *m/z* for $[M - H]^-$.

Invertase Assay

The sucrose analogues **1**, **3**, and **4** were tested as substrates of yeast invertase (Sigma, Deisenhofen) and compared to the natural substrate sucrose. Invertase catalyzes the hydrolysis of sucrose, which results in the formation of D-glucose and D-fructose. The photometric invertase assay described by Bergmeyer and Bernt[22] was used. In summary, D-glucose was measured through a conversion with glucose oxidase. The concomitant formation of hydrogen peroxide was coupled to the peroxidase-catalyzed oxidation of the dye ABTS [2,2'-azinobis(3-ethylbenzthiazoline)-6-sulfonate]. In detail, the assay was carried out by incubation of 10 U ml^{-1} invertase (Sigma, Deisenhofen), 9 U ml^{-1} glucose oxidase (Sigma, Deisenhofen), and 1.5 U ml^{-1} peroxidase (Merck, Darmstadt) in 0.2 M phosphate buffer (200 μl), pH 6.0 at 25 °C, containing 0.0125 mM sucrose or sucrose analogues, and 0.5 mg ml^{-1} ABTS. The change of absorption was observed at 405 nm giving initial rate measurements as ΔE min^{-1}. The initial rate of invertase for sucrose was set to 100%.

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