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Sucrose analogues modified at position 3: chemoenzymatic synthesis and inhibition studies of dextransucrases

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Abstract

Conditions for the large-scale (molar) oxidation of sucrose by *Agrobacterium tumefaciens* were improved, thus leading to homogeneous solutions of 3-ketosucrose in 40% yield. Treatment of this solution with hydroxylamine or methoxylamine afforded the corresponding oximes **3a** and **3b** (isolated as acetates) in excellent yield. Dissolving-metal reduction of these oximes gave mixtures of amino disaccharides in which the *gluco* epimer (3-amino-3-deoxysucrose) was predominant. A more efficient approach to this amino sucrose was provided by the highly stereoselective hydrogenation of 3-ketosucrose peracetate (**7**), which gave exclusively the *allo* isomer **8** (2,4,6-tri-*O*-acetyl-α-D-allopyranosyl 1,3,4,6-tetra-*O*-acetyl-β-D-fructofuranoside). Upon reaction with lithium azide, the triflate derived from **8**, compound **9**, afforded 3-azido-3-deoxysucrose peracetate (**10**) which was converted into 3-amino-3-deoxysucrose (**12**). The reaction of triflate **9** with potassium ethylxanthate led to a mixture of products (the expected 3-*S*-ethoxythiocarbonyl-3-thiosucrose derivative and the peracetates of 3-thiosucrose and of 3-thiosucrose disulfide), which could be all converted into 3-thiosucrose (**17**). Sucrose analogues **12** and **17** were not substrates of dextransucrases from various strains of *L. mesenteroides*, nor did they participate in glycosyl transfer reactions to an acceptor (maltose). Compounds **3a** and **12** were found to be strong competitive inhibitors of the dextran synthesis process (dextransucrase from strain B-1397). These results indicate that **3a** and **12** compete effectively with sucrose for the sucrose binding site but are unable to participate as glycosyl donors in the polymerization or glycosyl-transfer processes.

Keywords: Sucrose analogues; 3-Amino-3-deoxysucrose; Dextransucrase; Chemoenzymatic synthesis; *Agrobacterium tumefaciens*

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1. Introduction

In the context of our studies on the utilization of enzymes for the syntheses of modified oligosaccharides, we have reported the successful conversion of cyclodextrin derivatives into modified maltooligosaccharides and the synthesis of regioselectively modified cyclodextrins [1,2]. In this paper, we describe an improved large-scale preparation of 3-ketosucrose (β-D-fructofuranosyl α-D-*ribo*-hexopyranosid-3-ulose) by microbiological oxidation of sucrose and the subsequent chemical transformations of this compound into sucrose analogues in which the 3-OH group is replaced by an amino or mercapto group. This approach is much more efficient and versatile than the selective protection–deprotection at C-3 by chemical methods which has been used for the preparation of 3-deoxy-, 3-deoxy-3-fluoro-, and *allo*-sucrose [3] [2].

These new sucrose analogues are of particular interest as potential substrates or inhibitors of enzymes involved in the biotransformation or biodegradation of sucrose such as dextransucrases and sucrose phosphorylase. Dextransucrases utilize sucrose, α-D-glucopyranosyl α-L-sorbofuranoside [5], lactulosucrose [6], *p*-nitrophenyl α-D-glucopyranoside [7], or α-D-glucopyranosyl fluoride [8] as glucosyl donors in the *de novo* synthesis of soluble or insoluble dextrans. These glucosyltransferases have extremely high glucosyl donor specificity since only 6-deoxy-6-thiosucrose has been found to be a substrate for an enzyme produced by *Streptococcus mutans* [9]. None of the other analogues of sucrose modified at C-6 [9–12], C-3 [11,13], C-4 [11], nor α-D-glucopyranosyl fluorides modified at position 2, 3, or 6 [14,15] were substrates for dextransucrases of various origins, although some of the substrate analogues were able to undergo glycosyl transfer to acceptors such as maltose. From these results, it was inferred that a potential substrate for polysaccharide synthesis should be a close analogue of sucrose with substituents in its glucosyl unit which are able to maintain hydrogen-bond donor interactions. Sucrose phosphorylase, an enzyme that catalyzes the reversible conversion of sucrose and inorganic phosphate to α-D-glucose 1-phosphate (Glc 1-P) and D-fructose, has also a strict substrate specificity: only sucrose, Glc 1-P and α-D-glucopyranosyl fluoride were accepted into the donor subsite [16].

In this paper, we also report the results of our studies of these sucrose analogues as substrates or inhibitors of dextransucrases of various strains of *L. mesenteroides*.

2. Results and discussion

The specific oxidation of sucrose (**1**) at C-3 by cells of *Agrobacterium tumefaciens* was originally described more than 30 years ago [17]. This reaction is catalyzed by a D-glucoside dehydrogenase (EC 1.1.99.13) which is inducible [18] and strongly repressed by manganese [19]. As this element is essential for growth of the organism, we determined the minimal manganese concentration to obtain a reasonable biomass yield with the highest

[2] Pietsch et al. [4] have very recently reported the preparation of a series of sucrose derivatives modified at C-3 by way of 3-ketosucrose.

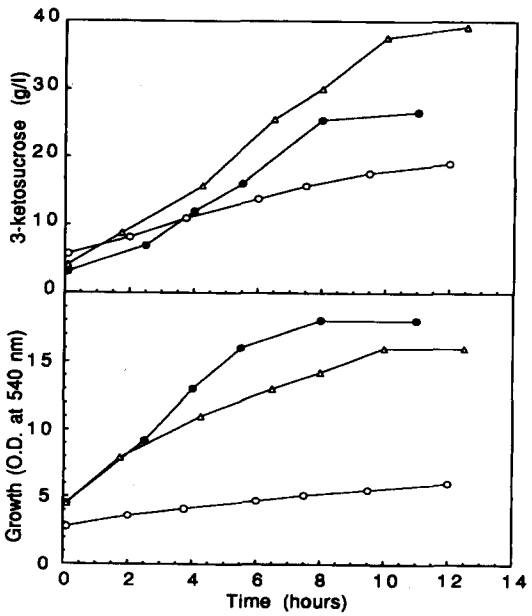
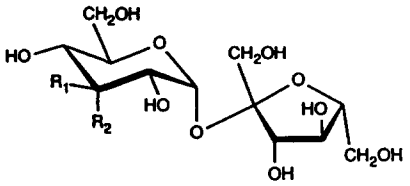


Fig. 1. Effect of initial manganese concentration on culture growth and production of 3-ketosucrose: (○) 0 mM; (Δ) 0.4 mM; (●) 2 mM.

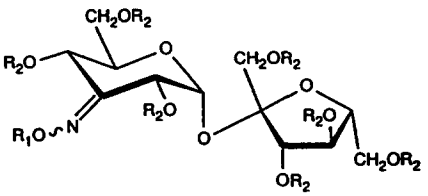
concentration of pure 3-ketosucrose (Fig. 1). With high initial sucrose concentration (100 g/L), the maximum 3-ketosucrose yield (40%) was reached with an initial manganese concentration of 0.4 mM. Lowering this concentration resulted in an insufficient growth and an incomplete sucrose consumption. In contrast, a growth improvement and a decrease in the 3-ketosucrose yield were observed at higher initial manganese concentration. The maximum 3-ketosucrose yield and concentration obtained here in manganese-limited batch growth are similar to those observed recently by using cells of *Agrobacterium tumefaciens* in a two-stage procedure (separate fermentation and disaccharide oxidation) [20]. However, in the latter procedure, additional sucrose is required for the production of an active cell mass, and the overall yield is therefore diminished. Under the conditions selected (Fig. 1), solutions of 3-ketosucrose in concentrations of up to 50 g/L could be obtained. The ^{13}C NMR spectrum of the crude mixture showed the signal of a carbonyl carbon atom at 208.8 ppm and 11 major peaks in agreement with the structure of compound **2** (see ref. [4]). Small intensity signals (~5–15%) could also be observed (71.2–65.4 ppm) and identified as a trace of fructose.

The reductive amination of **2** should provide the most direct approach to 3-amino-3-deoxysucrose. This reaction has been reported recently by Asano et al. [21]. Both epimers at C-3 of the glucose unit were obtained in low yield (~20%) with predominance of the D-*allo* isomer [3]. We therefore considered alternative approaches to the amino sucrose analogue and investigated first the oximation of **2**, followed by the reduction of the oxime. Treatment of **2** with hydroxylamine or methoxylamine hydrochloride at constant pH (pH 4.5) afforded the oximes **3a** and **3b**, respectively, in high yield; these oximes were isolated

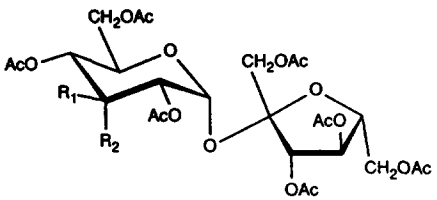
[3] We were not able to reproduce Asano's results (no amino sugar obtained under the conditions described).



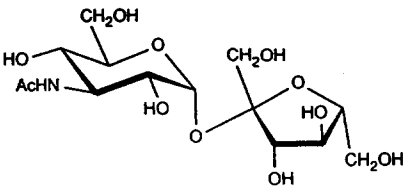
1 $R_1 = OH, R_2 = H$
2 $R_1, R_2 = O$



3a $R_1 = R_2 = H$
3b $R_1 = CH_3, R_2 = H$
4a $R_1 = R_2 = Ac$
4b $R_1 = CH_3, R_2 = Ac$



5a $R_1 = NHAc, R_2 = H$
5b $R_1 = H, R_2 = NHAc$

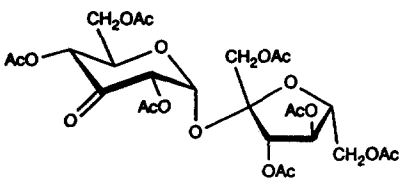


6

as their peracetates **4a** and **4b** (70 and 90%, respectively, from **2**) (see also ref. [4]). As anticipated, splitting of most of the signals in the NMR spectra of **4a** and **4b** was observed as a result of the presence of two isomers in the ratio of ~3:1. Interestingly, the minor isomer of **4b** slowly isomerized on prolonged storage, thus leading to a homogeneous product containing a single isomer. The reduction of 3-*keto*-D-*ribo*-hexopyranosides [3,22,23], as well as of the corresponding oximes [24,25] using metal hydrides or by catalytic hydrogenation, is known to produce preponderantly the corresponding axial *allo* epimer; hydrogenation of the oxime **3a** in the presence of hydrazine, conditions described by Lemieux [26], gave, indeed, after acetylation, the 3-amino-3-deoxy-*allo*-sucrose derivative **5b** [4] almost exclusively. Using dissolving-metal reduction techniques, however, oximes have led to a greater proportion of equatorial isomer [27]. Lindberg and Theander [24] have shown, for example, that methyl 3-amino-3-deoxy-β-D-glucopyranoside was the major product (isolated in 45% yield) resulting from the reduction of the corresponding oxime with sodium amalgam. The treatment of **3a** and **3b** with this reagent, as well as with lithium in ammonia, led, after acetylation, to the (unseparable) *gluco* and *allo* epimers **5a** and **5b** in ratios of ~3:1 and 5:1 in moderate yields (~30–50%). Although *gluco* epimer **6** could be isolated in pure form by HPLC after *O*-deacetylation, a different approach to 3-amino-3-deoxysucrose was sought.

Appropriate conditions (Ac_2O, DMAP–DMF) were found to prepare the peracetate of **2** (compound **7**) in high yield; standard acetylation conditions led to several degradation products. The availability of **7** provided ready access to the key intermediate **8**, an *allo*-sucrose derivative in which the 3-OH group is unprotected. Catalytic hydrogenation of **7**

over Adams' catalyst [23] gave compound **8** exclusively and in good yield. Because of its tendency to undergo acyl migration (4-OAc → 3-OAc), compound **8** was rapidly isolated (no chromatography) and converted into triflate **9**. Alternatively, compound **8** could be obtained from **2** by reduction with $NaBH_4$ (which leads preponderantly to *allo*-sucrose [28]), followed by the acetylation of the resulting mixture with a limited amount of acetic anhydride (yield, 30–40% from **2**; compound **8** was easily separated from *allo*-sucrose and sucrose octaacetates by flash chromatography). Triflate **9** reacted smoothly with lithium azide in DMF to give the C-3-inverted 3-azido-3-deoxysucrose derivative **10**. Deacetylation of **10**, followed by hydrogenation of the resulting compound **11**, gave 3-amino-3-deoxysucrose **12** in high yield.



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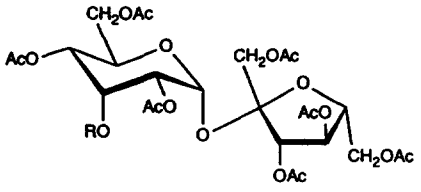
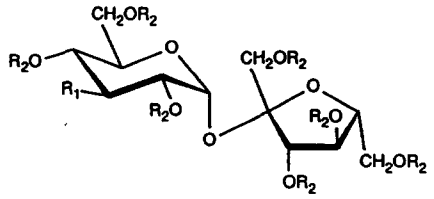


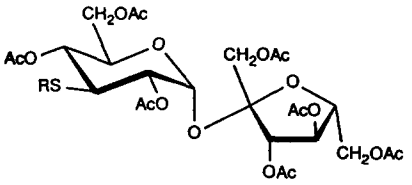
8 R = H
9 R = SO_2CF_3



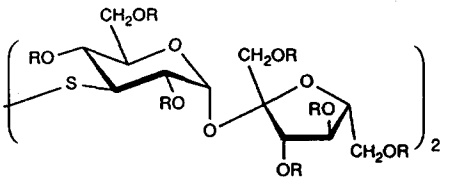
10 $R_1 = N_3$, R_2 = Ac
11 $R_1 = N_3$, R_2 = H
12 $R_1 = NH_2$, R_2 = H

Triflate **9** also constituted the ideal precursor for the preparation of 3-thiosucrose. S_N2 displacements of sugar-derived secondary sulfonates by thioacetate, thiobenzoate, or thiocyanate ions provide the most efficient approach to thio sugars [29]. For example, the first synthesis of 3-thio-D-glucose, reported by Goodman and co-workers [30], involved the displacement of a triflate at C-3 of an allofuranose derivative with a thiocyanate ion. However, on reaction with potassium thioacetate or thiobenzoate, compound **9** was found to undergo concurrent elimination, thus leading to an unseparable mixture of the desired 3-thiosucrose derivative and of an elimination product (probably C-2–C-3-unsaturated). The desired product, compound **13**, could be obtained in moderate yield (51%) using potassium ethylxanthate as the nucleophile. The reaction led also, unexpectedly, to the 3-*S*-acetyl-3-thiosucrose derivative **14** (15%), as well as to the peracetylated disulfide **15** (30%). The formation of these two products remains to be explained. Since all three products could be eventually converted into 3-thiosucrose **17**, the overall yield of the displacement was thus greater than 90%. Compound **13** could not be deacylated with sodium methoxide; however, treatment with lithium aluminum hydride [31] afforded directly 3-thiosucrose **17**, which was isolated as its peracetate **14** (80%). Conventional *O*- and *S*-deacetylation of **14** and **15**

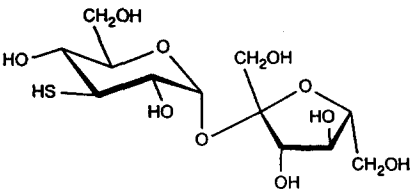
and treatment with dithioerythritol under slightly basic conditions gave 3-thiosucrose **17** in quantitative yield.



13 R = EtOCS
14 R = Ac



15 R = Ac
16 R = H



17

Whether sucrose analogues **12** and **17** could act as substrates of dextransucrases was investigated using enzymes from five different strains of *L. mesenteroides* (NRRL B-523, -1298, -1397, -1399, and -512F). No fructose was released in any of these reactions, indicating that no polymerization had occurred. Also, the transfer of the modified glucosyl residue to an acceptor (maltose) was not observed when **12** and **17** were incubated with the dextransucrase of the B-512F strain in the presence of maltose. These results constitute further evidence for the extreme specificity of the dextransucrases for the glucosyl residue and indicate that the 3-OH group must be a key polar function in the binding of the substrate to the enzyme in the polymerization process, since it can be replaced neither by a fluorine atom (*S. mutans* enzymes) [13] nor by an amino or a thiol group. It should be noted, however, that 3-deoxy-3-fluorosucrose and even 3-deoxysucrose do participate in glycosyl-transfer reactions to acceptors such as maltose (*S. mutans* enzyme) [13], in spite of a much weaker binding to the enzyme than the normal substrate, sucrose [11,13].

An examination of the inhibition of the dextran synthesis process by **3a** and **12** revealed that these analogues bind relatively tightly to the enzyme active site, in fact much more tightly than 3-deoxy- and 3-deoxy-3-fluorosucrose. Compounds **3a** and **12** competitively inhibited the polymerization process mediated by the *L. mesenteroides* B-1397 enzyme with K_i values of 1.4 and 1.0 mM, respectively, using sucrose as the substrate (K_m = 13.3 mM; similar Michaelis constants have been reported for other dextransucrases, e.g., K_m = 9–12 mM for the B-512F strain [10,11,15]). The 3-thiosucrose analogue **17** led to nonreproducible results and was not further investigated; qualitative assays have shown, however, that **17** is a much weaker inhibitor than **12**. That **3a** and **12** exhibit a high affinity for the enzyme but do not undergo glycosyl transfer (polymerization or reaction with acceptors) is quite remarkable. A similar behavior was already observed, for example, for sucrose analogues modified at C-6 (6-deoxy- and 6-deoxy-6-fluorosucrose) [10,11].

Further investigations on modified sucroses as potential substrates of dextransucrases are in progress and will be reported in due course.

3. Experimental

General methods.—NMR spectra were recorded on a Bruker AC 300 spectrometer at 300 MHz for ^1H and 75 MHz for ^{13}C. ^1H NMR data of the new compounds are given in Table 1. Mass spectra were recorded on a Nermag R-1010C spectrometer. For spectra acquired in the FAB mode, a 1:4 aq 0.1 M HCl–glycerol matrix was used. Optical rotations were measured at 20°C on a Perkin–Elmer 241 polarimeter. All solvents were evaporated under reduced pressure (40°C). When CH_2Cl_2 solutions were extracted with water, the aqueous phases were re-extracted with CH_2Cl_2, and the collected organic phases were combined and dried on Na_2SO_4. For flash chromatography, Merck Silica Gel 60 (230–400 mesh) was used.

Organism and culture conditions.—*Agrobacterium tumefaciens* (strain NCPPB 396) was obtained from the British national collection of plant pathogenic bacteria. The growth medium of Fenson et al. [32] was modified and contained: sucrose, 100 g/L; NH_4Cl, 2 g/L; $MgSO_4 \cdot 7H_2O$, 0.15 g/L; $FeSO_4 \cdot 7H_2O$, 20 mg/L; $CaCl_2 \cdot 2H_2O$, 15 mg/L; citric acid, 0.17 g/L; KH_2PO_4, 1 g/L; K_2HPO_4 2 g/L.

Batch cultures were performed at 30°C in a 2-L fermentor (Sétric Génie Industriel, Toulouse, France) containing 1.5 L of medium. The pH was kept constant at pH 7.2 by automatic addition of dil aq NH_4OH (50% v/v). Foaming was suppressed by manual addition of silicon oil (Rhodorsyl 426). The agitation was 1200 rpm, and the air flow was 1.5 L/min.

Bacterial growth was determined by turbidity at 540 nm. 3-Ketosucrose (**2**) was evaluated by the method of Fukui and Hayano [33]: an aliquot (5 μL) was diluted in NaOH (0.1 N, 3 mL) and the absorbance was measured at 340 nm. The concentration of **2** was calculated for $\epsilon = 6.5\ mM^{-1}$. The supernatant obtained by centrifugation of the broth at 10 000*g* for 20 min was used as the crude solution of **2** (typical concentrations: ~40 g/L, ~40% yield). For ^{13}C NMR data, see ref. [4] [4].

β-D-Fructofuranosyl 3-oximino-α-D-ribo-hexopyranoside (**3a**).—A solution of crude 3-ketosucrose (**2**; ~1.4 g, 4.1 mmol) was diluted with water to 100 mL. Hydroxylamine hydrochloride (0.67 g, 9.71 mmol) was then added, and the mixture was stirred at 60°C for 2 h. During this time the pH of the solution was kept at pH 4.5 by the addition of N NaOH. The solution was then freeze-dried to give crude **3a**, which was immediately acetylated in 2:1 pyridine–Ac_2O (40 mL, 15 h). The solvents were evaporated, the residue diluted in CH_2Cl_2, and the resulting mixture washed with ice-cold aq $KHSO_4$ (10%), ice-cold satd aq $NaHCO_3$, and cold water. Column chromatography of the residue (1:3 then 1:1 EtOAc–light petroleum) afforded the peracetate **4a** as mixture of *E*/*Z* isomers (1.99 g, 70%); mp 90–120°C (from ether); $[\alpha]_D^{25}$ +15° (*c* 0.5, $CHCl_3$); ^{13}C NMR ($CDCl_3$): see ref. [4]. Anal. Calcd for $C_{28}H_{37}NO_{19}$: C, 48.62; H, 5.39; N, 2.02. Found: C, 48.74; H, 5.52; N, 2.04.

[4] The chemical shift reported for C-4 in ref. [4] appears to be in error; the value should be: $\delta_{C\text{-}4}$ 72.15 (D_2O). See also ref. [21].

Table 1
^{1}H NMR data (δ in ppm, J in Hz)[a]

Compound	H-1 ($J_{1,2}$)	H-2 ($J_{2,3}$)	H-3 ($J_{3,4}$)	H-4 ($J_{4,5}$)	H-5 ($J_{5,6A}$; $J_{5,6B}$)	H-6A,6B ($J_{6A,6B}$)	H-1′A,1′B ($J_{1'A,1'B}$)	H-3′ ($J_{3',4'}$)	H-4′ ($J_{4',5'}$)	H-5′ ($J_{5',6'A}$; $J_{5',6'B}$)	H-6′A,6′B ($J_{6'A,6'B}$)	Other signals or data[b]
5a	5.50 d	4.76 dd	4.57 q	4.81 t	—4.13–4.34—		4.12 AB	5.44 d	5.35 t	—4.13–4.34—		NH 5.55 (d)
	(3.5)	(11.0)	(10.5)	(9.8)			(12)	(6.0)	(6.0)			$J_{3,NH}$ 9
5b	5.57 d	4.92 t	4.85 dt	4.82 dd	—4.15–4.28—		4.14 AB	5.43 d	5.31 t	—4.15–4.28—		NH 6.68 (d)
	(3.6)	(4.6)	(4)	(12)			(12.2)	(6.0)	(6.1)			$J_{3,NH}$ 8.2
7	5.89 d	5.41 d		5.28 dd	4.51 ddd	—4.10–4.40—		5.42 d	5.32 t	—4.10–4.40—		$J_{2,4}$ 1.0
	(4.3)			(10.4)	(2.4; 4.1)			(6.0)	(6)			
8	5.60 br d	4.83 t	~4.3	4.78 dd	4.45 ddd	4.18–4.38	4.13 AB	5.47 d	5.37 t	—4.18–4.38—		$J_{1,3}$ ~0.5
	(3.7)	(3.3)	(2.9)	(10.6)	(2.6; 4.4)		(12.0)	(6.6)	(6.5)			
9	5.59 d	5.03 dd	5.29 br t	4.98 dd	4.57 ddd	4.26–4.36	4.00 s	5.53 d	5.42 t	4.19 dt	4.26–4.36	
	(4.0)	(3.3)	(2.9)	(10.6)	(2.6; 4.2)			(8.0)	(7.7)	(5.5; 5.5)		
10	5.62 d	4.71 dd	3.86 t	4.89 t	4.04–4.28			5.43 d	5.33 t	—4.04–4.28—		
	(3.7)	(11.0)	(9.5)	(10.2)				(6.2)	(6.2)			
12[c]	5.27 d	3.41 dd	3.02 t	3.30 t	—3.69–3.78—		3.55 s	4.10 d	3.94 t	—3.69–3.78—		
	(3.8)	(10.5)	(9.5)	(9.5)				(8.7)	(8.1)			
13	5.59 d	5.01 br dd	~4.1	5.14 br t	4.27 ddd	—4.03–4.18—		5.48 d	5.39 t	4.20 dt	4.27–4.38	OCH_2CH_3
	(3.3)	(~12)	(~10)	(10.2)	(2.2; 4.7)			(6.6)	(6.5)	(4.7; 6.2)	(11.7)	4.64 dq, 1.42 t
15	5.65 d	4.84 dd	3.15 br t	5.04 br t	—4.05–4.20—			5.44 d	5.39 t	—4.05–4.20—		
	(3.7)	(11.3)	(11.3)	(9.2)				(6.6)	(6.5)			
16[d]	5.41 d	occl.	3.07 t	3.56 t	3.85 ddd	3.57–3.84	3.63 AB	4.12 d	4.03 m	—3.57–3.84—		
	(3.3)	(11.0)	(10.2)	(9.5)	(1.8, 4.4)		(12)	(8.0)				
17[d,e,f]	5.36 d	3.39 dd	3.18 t	3.28 dd	—3.67–3.81—		3.61 AB	4.10 d	4.02 m	—3.67–3.81—		DTE: 2.63 m
	(3.7)	(10.6)	(10.2)	(9.2)			(12)	(8.7)				2.83 m, 3.55 m

[a] For solutions in $CDCl_3$ (δ_{CHCl_3} 7.24 ppm) unless stated otherwise. [b] Signals of $OCOCH_3$: δ 2.0–2.2 ppm. [c] Solution in D_2O at 30°C (δ_{DOH} 4.60 ppm). [d] Solution in CD_3OD (δ_{CD_2HOD} 3.30 ppm). [e] In the presence of dithioerythritol (DTE); in the absence of DTE, the spectrum is essentially identical. [f] In D_2O, the only significant difference is δ_{H-3} 3.05 ppm.

To a solution of **4a** (1.99 g, 2.8 mmol) in dry MeOH (50 mL) was added methanolic M NaOMe (2 mL), and the mixture was stirred for 7 h at room temperature. The solution was then neutralized with Amberlite IRN 77 (H^+) resin, the solvent was evaporated, and the residue was crystallized in water (0.82 g, 80%); mp 99–102°C; $[\alpha]_D^{25}$ +25° (*c* 0.5, H_2O); FABMS: *m/z* 356 $[M+H]^+$. Anal. Calcd for $C_{12}H_{21}NO_{11}\cdot 0.5H_2O$: C, 39.56; H, 6.08; N, 3.84. Found: C, 39.54; H, 6.17; N, 3.90.

β-D-Fructofuranosyl 3-methoximino-α-D-ribo-hexopyranoside (**3b**).—3-Ketosucrose (**2**; estimated amount 1.1 g, 3.3 mmol) was reacted with methoxyamine hydrochloride (1.06 g, 12.7 mmol) in water (total volume 80 mL) under the conditions described above (preparation of **3a**). Crude **3b** was acetylated and the resulting product purified by flash chromatography (1:2 EtOAc–light petroleum) to give **4b** (2.0 g, 90%) as a mixture of *E*/*Z* isomers (~3:1 ratio, not assigned). Although they crystallize well from an ether solution, these isomers could not be separated, even after repeated recrystallizations; mp 65–95°C. Analysis of the crystalline material, after a period of storage at 4°C (6–9 months), revealed that isomerization had occurred, leading to a single isomer (the major isomer present in the initial mixture); mp 92–96°C; $[\alpha]_D^{25}$ +7° (*c* 0.5, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 170.6, 170.4, 169.8, 169.6, 169.0, 168.6 (CH_3CO), 147.1 (C-3), 103.3 (C-2′), 90.2 (C-1), 78.3, 75.9, 74.6, 70.2, 68.2, 64.0, 63.6, 63.0, 62.5, 61.2,(C-1′,2,3′,4,4′,5,5′,6,6′,OCH_3), 20.7, 20.6, 20.4 (CH_3CO); CIMS (NH_3 + isobutane): *m/z* 681 $[M+NH_4]^+$. Anal. Calcd for $C_{27}H_{37}O_{18}N$: C, 48.87; H, 5.62; N, 2.11. Found: C, 48.64; H, 5.60; N 2.24

A solution of **4b** was deacetylated as described for **4a**. Compound **3b** was obtained in 88% yield; syrup; $[\alpha]_D^{25}$ +2° (*c* 0.6, MeOH); ^{13}C NMR (D_2O): δ 156.3 (C-3), 105.5 (C-2′), 92.7 (C-1), 82.9, 78.0, 75.9, 75.2, 70.1, 64.2, 63.8, 63.4, 63.0, 62.0; FABMS (+): *m/z* 392 $[M+Na]^+$, 370 $[M+H]^+$; FABMS (−): *m/z* 368 $[M-H]^-$.

1,3,4,6-Tetra-O-acetyl-β-D-fructofuranosyl 3-acetamido-2,4,6-tri-O-acetyl-3-deoxy-α-D-glucopyranoside (**5a**) *and 3-acetamido-2,4,6-tri-O-acetyl-3-deoxy-α-D-allopyranosyl 1,3,4,6-tetra-O-acetyl-β-D-fructofuranoside* (**5b**).—*Method 1.* To a solution of **3a** or **3b** (300 mg, 0.84 or 0.81 mmol) in water (3 mL) was added portionwise 2.5% sodium amalgam (20 g) [34], while the pH was maintained at pH 5.5–6.5 by adding AcOH. The reaction was monitored by TLC (4:1:1 2-PrOH–AcOH–H_2O). Mercury was decanted and the clear solution was freeze-dried. The residue was acetylated in 2:1 pyridine–Ac_2O (12 mL) in the presence of *N,N*-dimethylaminopyridine (12 h). Processing of the mixture, as described above for the preparation of **4a**, afforded a residue which was purified by flash chromatography (1:1, then 3:1 EtOAc–light petroleum, then pure EtOAc). Compounds **5a** and **5b** were obtained as a 3:1 mixture in 44 and 50% yield starting from **3a** and **3b**, respectively. These epimers could not be separated by flash chromatography. A pure sample of **5a** was obtained by reacetylation of **6** (see below), and a pure sample of **5b** was provided by Method 3.

gluco Isomer **5a**: $[\alpha]_D^{25}$ +36° (*c* 0.7, $CHCl_3$); ^1H NMR: see Table 1; ^{13}C NMR ($CDCl_3$): δ 170.6–169.1 (CH_3CO), 103.7 (C-2′), 89.7 (C-1), 78.9, 75.4, 74.9, 70.0, 69.0, 68.4, (C-2,3′,4,4′,5,5′), 63.8, 63.0, 61.9 (C-1′,6,6′), 49.8 (C-3), 23.1, 20.6–20.5 (CH_3CO); CIMS (NH_3 + isobutane): *m/z* 695 $[M+NH_4]^+$, 678 $[M+H]^+$. Anal. Calcd for $C_{28}H_{39}NO_{18}$: C, 49.63; H, 5.80; N, 2.06. Found: C, 49.75; H, 6.05; N. 2.01.

allo Isomer **5b** [4]: $[\alpha]_D^{25}$ +46° (*c* 0.7, $CHCl_3$); ^1H NMR: see Table 1; Anal. Found: C, 49.64; H, 5.90; N, 2.25.

Method 2. Into a solution of **3a** (348 mg, 0.97 mmol) in a minimum amount of EtOH (3 mL) was condensed NH_3 (~ 30 mL). Small pieces of Li were added until the blue color persisted for ca. 1 min. EtOH was then added (10 mL), and most of the NH_3 was flushed by an air stream. The solvent was evaporated, and the residue was co-evaporated with toluene (3×). The product mixture was acetylated and purified as described under Method 1. Compounds **5a** and **5b** were thus obtained as a 5:1 mixture in 30% yield.

Method 3. To a solution of **3a** (300 mg, 0.84 mmol) in EtOH (5 mL) and water (5 mL) was added hydrazine monohydrate (300 μL). The solution was kept at room temperature for 2 h and then hydrogenated at 7 atm over 10% Pd–C (250 mg) for 24 h. The catalyst was removed by filtration (Celite), the solvent was evaporated, and the residue acetylated and purified by chromatography as described above, to afford only the *allo* compound **5b** (260 mg, 46%).

β-D-Fructofuranosyl 3-acetamido-3-deoxy-α-D-glucopyranoside (**6**).—To a solution of the 5:1 mixture of **5a** and **5b** (153 mg, 0.22 mmol) in MeOH (1.5 mL) was added methanolic M NaOMe (100 μL). After 3 h at room temperature, the mixture was neutralized with Amberlite IRN 77 (H^+) resin, the resin was removed by filtration, and the filtrate was concentrated. The residue was submitted to HPLC on a C_{18} reversed-phase column (μ-Bondapak, 10 μm, 19×300 mm, Waters Associates) with water as the eluent, which afforded pure **6** (53 mg, 70%); $[\alpha]_D^{25}$ +59° (*c* 0.5, H_2O); ^{13}C NMR (D_2O): δ 176.7 (CH_3CO), 105.2 (C-2′), 93.1 (C-1), 82.9, 78.2, 75.6, 74.2, 70.9, 69.0 (C-2,3′,4,4′,5,5′), 63.9, 62.9, 61.6 (C-1′,6,6′), 55.3 (C-3), 23.7 (CH_3CO); FABMS (+): *m/z* 384 $[M+H]^+$, FABMS (−): *m/z* 382 $[M-H]^-$. Anal. Calcd for $C_{14}H_{25}NO_{11} \cdot 0.5\ H_2O$: C, 42.86; H, 6.68; N, 3.57. Found: C, 43.07; H, 6.97; N, 3.54.

1,3,4,6-Tetra-O-acetyl-β-D-fructofuranosyl 2,4,6-tri-O-acetyl-α-D-ribo-hexopyranosid-3-ulose (**7**).—An aqueous solution of crude 3-ketosucrose **2** (~ 1g) was freeze-dried, and the light yellow powder (2.76 g) was treated at room temperature for 30 min in DMF (21 mL) with Ac_2O (8.5 mL) in the presence of *N,N*-dimethylaminopyridine (catalytic amount). CH_2Cl_2 (100 mL) was then added, and the mixture was washed with water (3×30 mL). Flash chromatography (1:3 then 1:2 EtOAc–light petroleum) gave pure **7** (1.22 g, 65%) as a syrup; $[\alpha]_D^{25}$ +49° (*c* 0.5, $CHCl_3$); ^1H NMR: see Table 1; ^{13}C NMR ($CDCl_3$): δ 192.5 (C-3), 170.3–169.0 (CH_3CO), 103.7 (C-2′), 92.7 (C-1), 79.0, 75.4, 74.6, 73.7, 71.9, 70.8 (C-2,3′,4,4′,5,5′), 63.4, 62.8, 62.0 (C-1′,6,6′), 20.6–19.9 (CH_3CO); FABMS: *m/z* 635 $[M+H]^+$, 657 $[M+Na]^+$. Anal. Calcd for $C_{26}H_{34}O_{18}$: C, 49.21; H, 5.40. Found: C, 48.96; H, 5.36.

2,4,6-Tri-O-acetyl-α-D-allopyranosyl 1,3,4,6-tetra-O-acetyl-β-D-fructofuranoside (**8**). —*Method 1.* To a solution of acetylated 3-ketosucrose **7** (567 mg, 0.89 mmol) in aq 95% EtOH (60 mL) was added platinum oxide (150 mg), and the mixture was hydrogenated at room temperature for 2 h (7 atm H_2). Removal of the catalyst by filtration and concentration of the filtrate led to crude **8**, which could be used without purification in the next step. Chromatography (2:3 then 1:1 EtOAc–light petroleum) of an aliquot led to pure **8** (overall yield, 57%); $[\alpha]_D^{25}$ +50° (*c* 1.3, $CHCl_3$); ^1H NMR: see Table 1; ^{13}C NMR ($CDCl_3$): δ 170.6–169.5 (CH_3CO), 103.6 (C-2′), 91.2 (C-1), 78.7, 75.3, 74.6, 68.0, 67.8, 67.7, 64.4 (C-2,3,3′,4,4′,5,5′), 63.7, 62.8, 62.3 (C-1′,6,6′), 20.8–20.5 (CH_3CO); CIMS (NH_3 + isobutane): *m/z* 654 $[M+NH_4]^+$. Anal. Calcd for $C_{26}H_{36}O_{18}$: C, 49.06; H, 5.70. Found C, 49.03; H, 5.81.

Method 2. An aqueous solution of crude 3-ketosucrose (**2**; ~1.2 g, 3.5 mmol) was diluted to 100 mL with water and treated with excess $NaBH_4$ as described by Hough and O'Brien [28]. The solid residue obtained after repeated co-evaporations with MeOH was dried and dissolved in pyridine (40 mL). The solution was cooled to 0°C and Ac_2O (5 mL, ~1.9 equiv/OH) was added. After 15 h at room temperature, the mixture was diluted with water (80 mL), and the solution was extracted with ether (100 mL, 20 mL), then with CH_2Cl_2 (3×30 mL). The combined organic phases were washed with water (10 mL), dried (Na_2SO_4), and concentrated; residual pyridine was removed by repeated co-evaporation with toluene. The residue was submitted to flash chromatography [1:1 then 2:1 (after elution of the peracetates) EtOAc–light petroleum] which afforded a mixture of *allo*-sucrose octaacetate (α-D-allopyranosyl β-D-fructofuranoside octaacetate) and of sucrose octaacetate (~2:1 ratio, 695 mg, 29%), then *allo*-sucrose heptaacetate **8** (815 mg, 37%) containing a small amount (≤5%) of an isomeric heptaacetate [2,3,6-tri-*O*-acetyl-α-D-allopyranosyl 1,3,4,6-tetra-*O*-acetyl-β-D-fructofuranoside; characteristic ^1H NMR signals ($CDCl_3$): δ 5.43 (d, 1 H, $J_{1,2}$ 4.4 Hz, H-1), 4.87 (dd, 1 H, $J_{2,3}$ 3.7 Hz, H-2), 5.57 (t, 1 H, $J_{3,4}$ 3.3 Hz, H-3), 3.67 (dd, 1 H, $J_{4,5}$ 10.3 Hz, H-4)]. Repetition of this procedure led to samples of **8** free of this byproduct.

2,4,6-Tri-O-*acetyl-3*-O-*trifluoromethanesulfonyl*-α-D-*allopyranosyl 1,3,4,6-tetra*-O-*acetyl*-β-D-*fructofuranoside* (**9**).—To an ice-cold solution of crude **8** (391 mg, 0.61 mmol) in 15:1 CH_2Cl_2–pyridine (20 mL) was added trifluoromethanesulfonic anhydride (0.5 mL). The mixture was stirred for 30 min at 0°C and then for 1 h at room temperature. The mixture was diluted with CH_2Cl_2 (100 mL), and the organic solution was washed successively with ice-cold aq 10% $KHSO_4$, ice-cold satd aq $NaHCO_3$, and water. Flash chromatography (2:3 acetone–hexane) of the residual yellow oil gave **9**, isolated as a colourless syrup (378 mg, 80% from **7**). Crystallization from ether–EtOAc gave an analytical sample; mp 126°C; $[\alpha]_D^{25}$ +49° (*c* 1.1, $CHCl_3$); ^1H NMR: see Table 1; ^{13}C NMR ($CDCl_3$): δ 170.5–168.8 (CH_3CO), 124.5, 120.2, 116.0, 111.8 (CF_3), 102.4 (C-2′), 88.0 (C-1), 82.0 (C-3), 77.8, 74.4, 73.6, 65.1, 64.4, 64.0 (C-2,3′,4,4′,5,5′), 63.7, 63.4, 61.5 (C-1′,6,6′) 20.5–19.8 (CH_3CO); DCIMS (NH_3 + isobutane): *m/z* 786 $[M+NH_4]^+$. Anal. Calcd for $C_{27}H_{35}F_3O_{20}S$: C, 42.19; H, 4.59; F, 7.42; S, 4.17. Found: C, 42.13; H, 4.35; F, 7.58; S, 4.13.

1,3,4,6-Tetra-O-*acetyl*-β-D-*fructofuranosyl 2,4,6-tri*-O-*acetyl-3-azido-3-deoxy*-α-D-*glucopyranoside* (**10**).—To a stirred solution of triflate **9** (600 mg, 0.78 mmol) in DMF (8 mL) was added lithium azide (200 mg, 4 mmol). After 12 h at room temperature, the mixture was diluted with ether, and the organic phase was washed with water. Flash chromatography (2:3 EtOAc–light petroleum) afforded **10** (360 mg, 70%) as a syrup; $[\alpha]_D^{25}$ +60° (*c* 0.6, $CHCl_3$); ^1H NMR: see Table 1; ^{13}C NMR ($CDCl_3$): δ 170.2–168.8 (CH_3CO), 103.5 (C-2′), 89.2 (C-1), 78.3, 75.3, 74.3, 70.6, 68.3, 67.8 (C-2,3′,4,4′,5,5′), 63.2, 62.8, 61.5, 60.5 (C-1′,3,6,6′), 20.2 (CH_3CO); DCIMS (NH_3 + isobutane): *m/z* 679 $[M+NH_4]^+$; IR 2112 cm^{-1} (N_3). Anal. Calcd for $C_{26}H_{35}N_3O_{17}$: C, 47.20; H, 5.33; N, 6.35. Found: C, 47.62; H, 5.48; N, 6.03.

β-D-*Fructofuranosyl 3-azido-3-deoxy*-α-D-*glucopyranoside* (**11**).—To a solution of acetylated azide **10** (300 mg, 0.45 mmol) in MeOH (6 mL) was added methanolic M NaOMe (400 μL). The mixture was stirred for 3 h, then neutralized with Amberlite IRN 77 (H^+) resin and concentrated. HPLC on a μ-Bondapak NH_2-column (10 μm, 19×50

mm, Waters Associates) using 85:15 MeCN–H_2O as the eluent afforded pure **11** (225 mg, 75%); $[\alpha]_D^{25}$ +73° (*c* 0.49, H_2O); ^1H NMR: see Table 1; ^{13}C NMR (D_2O): δ 105.2 (C-2′), 93.0 (C-1), 82.9, 77.9, 75.5, 73.7, 71.5, 69.5 (C-2,3′,4,4′,5,5′), 67.1, 63.9, 62.9, 61.5 (C-1′,3,6,6′); FABMS (+): *m/z* 368 $[M+H]^+$; FABMS (−): *m/z* 366 $[M-H]^-$. Anal. Calcd for $C_{12}H_{21}N_3O_{10}\cdot H_2O$: C, 37.40; H, 6.02; N, 10.90. Found: C, 37.23; H, 6.01; N, 10.77.

β-D-Fructofuranosyl 3-amino-3-deoxy-α-D-glucopyranoside (**12**).—A solution of **11** (143 mg, 0.39 mmol) in EtOH (10 mL) was hydrogenated (H_2, 7 atm) in the presence of neutralized Raney nickel (1.6 mL in H_2O) for 20 h at room temperature. Removal of the catalyst by filtration and evaporation of the solvent gave homogeneous **12**; $[\alpha]_D^{25}$ +75° (*c* 0.51, H_2O); ^1H NMR: see Table 1; ^{13}C NMR (D_2O): δ 105.1 (C-2′), 93.0 (C-1), 82.9, 77.9, 75.5, 73.9, 72.6, 70.7 (C-2,3′,4,4′,5,5′), 63.9, 62.8, 61.8 (C-1′,6,6′), 55.4 (C-3); FABMS: *m/z* 342 $[M+H]^+$, 364 $[M+Na]^+$. Anal. Calcd for $C_{12}H_{23}NO_{10}\cdot 0.5H_2O$: C, 41.14; H, 6.91; N, 3.99. Found: C, 41.79; H, 6.79; N, 3.49.

1,3,4,6-Tetra-O-acetyl-β-D-fructofuranosyl 2,4,6-tri-O-acetyl-3-S-[(ethoxy)thiocarbonyl)]-3-thio-α-D-glucopyranoside (**13**), *1,3,4,6-tetra-O-acetyl-β-D-fructofuranosyl 2,4,6-tri-O-acetyl-3-S-acetyl-3-thio-α-D-glucopyranoside* (**14**), *and 3,3′-dithiobis-[(1,3,4,6-tetra-O-acetyl-β-D-fructofuranosyl) 2,4,6-tri-O-acetyl-3-deoxy-α-D-glucopyranoside*] (**15**).—To a solution of triflate **9** (1.23 g, 1.6 mmol) in DMF (26 mL) was added a solution of potassium ethylxanthate (840 mg, 5.2 mmol) in DMF (17 mL). The mixture was stirred at room temperature overnight. Most of the solvent was then evaporated in vacuo, and the residue was dissolved in ether. The solution was washed with water, dried, and concentrated; flash chromatography (2:3 EtOAc–hexane) of the residue afforded, in this order, compound **13** (0.60 g, 51%), compound **14** (166 mg, 15%), and compound **15** (312 mg, 30%).

Compound **13**: $[\alpha]_D^{25}$ +39° (*c* 0.5, $CHCl_3$); ^1H NMR: see Table 1; ^{13}C NMR ($CDCl_3$): δ 170.5–169.1 (CH_3CO), 103.5 (C-2′), 89.4 (C-1), 78.5, 75.4, 74.6, 70.3, 69.7, 69.0, 66.8, (C-2,3′,4,4′,5,5′, OCH_2), 64.0, 63.1, 62.1 (C-1′,6,6′), 51.1 (C-3); DCIMS (NH_3 + isobutane): *m/z* 758 $[M+NH_4]^+$. Anal. Calcd for $C_{29}H_{40}O_{18}S_2$: C, 47.02; H, 5.44; S, 8.65. Found: C, 46.79; H, 5.57; S, 8.04.

Compound **14**: mp 123–124°C (ether); $[\alpha]_D^{25}$ +48° (*c* 0.5, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 192.8 (CH_3COS), 170.6–169.3 (CH_3COO), 103.8 (C-2′), 89.5 (C-1), 79.0, 75.7, 75.1, 69.7, 69.2, 67.0 (C-2,3′,4,4′,5,5′), 64.0, 63.0, 62.1 (C-1′,6,6′), 44.1 (C-3), 30.6 (CH_3COS), 20.6–20.4 (CH_3COO); DCIMS (NH_3 + isobutane): *m/z* 712 $[M+NH_4]^+$. Anal. Calcd for $C_{28}H_{38}O_{18}S$: C, 48.41; H, 5.51; S, 4.61. Found: C, 48.36; H, 5.57; S, 4.78.

Disulfide **15**: $[\alpha]_D^{25}$ +36° (*c* 0.7, $CHCl_3$); ^1H NMR: see Table 1; ^{13}C NMR ($CDCl_3$): δ 170.7–169.4 (CH_3CO), 103.6 (C-2′), 89.4 (C-1), 78.4, 75.5, 74.2, 69.6, 69.0, 67.3 (C-2,3′,4,4′,5,5′), 63.5, 63.4, 62.3 (C-1′,6,6′), 51.3 (br, C-3), 20.6 (CH_3CO); DCIMS (NH_3 + isobutane): *m/z* 1320 $[M+NH_4]^+$. Anal. Calcd for $C_{52}H_{70}O_{34}S_2$: C, 47.92; H, 5.41; S, 4.92. Found: C, 48.07; H, 5.56; S, 4.20.

Compound **14**.—*From compound* **13**. To a solution of **13** (160 mg, 0.21 mmol) in THF (9.5 mL) was added, under Ar, a solution of M $LiAlH_4$ in THF (5 mL). After 1.5 h at room temperature, the solution was cooled to 0°C, and the excess $LiAlH_4$ was decomposed with EtOAc (3.5 mL) and MeOH (19 mL). The solution was neutralized with AcOH and then freeze-dried. The resulting solid was acetylated with 3:1 pyridine–Ac_2O (12 mL) in the presence of *N,N*-dimethylaminopyridine (catalytic amount). After the usual workup,

flash chromatography of the residue (1:2 then 1:1 EtOAc–light petroleum) afforded **14** (120 mg, 79%) and the disulfide **15** (14 mg, 10%).

3,3'-Dithiobis(β-D-fructofuranosyl 3-deoxy-α-D-glucopyranoside) (**16**).—*From compound* **14**. To a solution of **14** (220 mg, 0.31 mmol) in MeOH (10 mL) was added M methanolic NaOMe (0.4 mL). The mixture was stirred for 3 h at room temperature, then neutralized with Amberlite IRN 77 (H^+) resin, and the solvent was evaporated to give compound **17** (92 mg, 81%) containing a small amount of **16**. Dimerization occurred during HPLC (μ-Bondapak NH_2-column, as described for compound **11**,with 75:25 MeCN–H_2O) which afforded **17** (9 mg, 9%) and **16** (55 mg, 47%).

Compound **16**: $[\alpha]_D^{25}$ +72° (*c* 0.5, EtOH); ^{1}H NMR: see Table ; ^{13}C NMR (D_2O): δ 105.2 (C-2'), 93.2 (C-1), 82.9, 77.8, 75.5, 74.5, 69.9, 67.4 (C-2,3',4,4',5,5'), 63.9, 62.8, 62.0 (C-1',6,6'), 58.8 (C-3); FABMS: *m/z* 737 $[M+Na]^+$. Anal. Calcd for $C_{24}H_{42}O_{20}S_2$: C, 40.36; H, 5.93; S, 8.98. Found: C, 40.65; H, 6.10; S, 8.85.

From acetylated disulfide **15**. Deacetylation of **15** as described above afforded **16** in quantitative yield.

β-D-Fructofuranosyl 3-thio-α-D-glucopyranoside (**17**).—Treatment of disulfide **16** (20 mg, 0.028 mmol) in solution in water (200 μL) with dithioerythritol (8.4 mg, 0.054 mmol) afforded **17** quantitatively; ^{1}H NMR: see Table 1; ^{13}C NMR (D_2O): δ 105.1 (C-2'), 92.9 (C-1), 82.9, 77.9, 75.6, 74.7, 73.4, 71.7 (C-2,3',4,4',5,5'), 63.9, 62.8, 62.0 (C-1',6,6'), 47.0 (C-3), 75.0, 28.8 (dithioerythritol); IR: 2567 cm^{-1} (w, SH); FABMS: *m/z* 397 $[M+K]^+$, 381 $[M+Na]^+$, 359 $[M+H]^+$.

Enzymatic studies.—Dextransucrases from *Leuconostoc mesenteroides* strains NRRL B-523, B-1298, B-1397, and B-1399 were provided by BioEurope (Toulouse, France). The enzyme from the B-512F strain was obtained from Sigma Chemical Co. The formation of fructose was monitored by HPLC (μ-Bondapak NH_2-column, 10 μm, 125Å, 19×50 mm, Waters Associates; eluent, 80:20 MeCN–H_2O) using an enzymatic assay (Boehringer–Mannheim kit No. 139106), or by the ferricyanide method [35]. The method described by Dische [35] was simplified as follows: an alkaline solution of potassium ferricyanide was prepared by dissolving $K_3[Fe(CN)_6]$ (300 mg) and $Na_2CO_3 \cdot 10H_2O$ (28.0 g) in water (1 L), and adding 10 drops of 10 N NaOH. To this solution (1.25 mL) was added the solution to be analyzed (75 μL); the mixture was heated in a boiling water bath for 5 min, and the absorbance at 420 nm measured. The concentration in fructose was then determined by comparison with a standard.

Polymerization process.—The sucrose analogues **12** and **17** (10 mg) were each incubated with dextransucrase (600 mU) in 20 mM NaOAc buffer (1 mL, pH 5.4) at 30°C for 20 h, and the process (formation of fructose) was monitored by HPLC.

Acceptor reactions.—Compounds **3a**, **12**, and **17** (50 mg/mL) were each incubated with the B-512F dextransucrase in the presence of maltose (20 mg/mL), and the process (formation of fructose and other new products) was monitored by HPLC.

Inhibition studies.—All assays were performed using the enzyme from the B-1397 strain (60–140 mU) in a reaction volume of 600 μL (20 mM NaOAc buffer) at 30°C. Determination of K_m: [**1**], 4.8, 9.7, 14.6, 19.4, 24.3, 29, 34, and 43.5 mM; fructose measured by the ferricyanide method. Oxime **3a** as inhibitor: [**1**], 4.8, 14.6, 29, and 44 mM; [**3a**], 0, 3.5, 7, and 14 mM; fructose determined by enzymatic assay, after treatment of the sample for 2 min at 100°C. Amino sucrose **12** as inhibitor: [**1**], 4.8, 9.7, 19.4, and 29 mM; [**12**],

0, 1.7, and 2.4 mM; fructose measured by the ferricyanide method. Lineweaver–Burk representations of the kinetic measurements revealed that **3a** and **12** were competitive inhibitors. The values of K_i (1.4 and 1.0 mM, respectively) were determined from Dixon plots.

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References

[1] C. Simiand, S. Cottaz, C. Bosso, and H. Driguez, *Biochimie*, 74 (1992) 75–80.
[2] S. Cottaz, C. Apparu, and H. Driguez, *J. Chem. Soc., Perkin Trans.* 1, (1991) 2235–2241.
[3] T.P. Binder and J.F. Robyt, *Carbohydr. Res.*, 147 (1986) 149–154.
[4] M. Pietsch, M. Walter, and K. Buchholz, *Carbohydr. Res.*, 254 (1994) 183–194.
[5] J.C. Mazza, A. Akgerman, and J.R. Edwards, *Carbohydr. Res.*, 40 (1975) 402–406.
[6] E.J. Hehre and H. Suzuki, *Arch. Biochem. Biophys.*, 113 (1966) 675–683.
[7] T.P. Binder and J.F. Robyt, *Carbohydr. Res.*, 124 (1983) 287–299.
[8] D.S. Genghof and E.J. Hehre, *Proc. Soc. Exp. Biol. Med.*, 140 (1972) 1298–1301.
[9] T.P. Binder and J.F. Robyt, *Carbohydr. Res.*, 140 (1985) 9–20.
[10] S.H. Eklund and J.F. Robyt, *Carbohydr. Res.*, 177 (1988) 253–258.
[11] A. Tanriseven and J.F. Robyt, *Carbohydr. Res.*, 186 (1989) 87–94.
[12] M.K. Battacharjee and R.M. Mayer, *Carbohydr. Res.*, 142 (1985) 277–284.
[13] T.P. Binder and J.F. Robyt, *Carbohydr. Res.*, 154(1986) 229–238.
[14] T.J. Grier and R.M. Mayer, *Arch. Biochem. Biophys.*, 212 (1981) 651–659.
[15] A.G. Michiels, A.Y. Wang, D.S. Clark, and H.W. Blanch, *Appl. Biochem. Biotechnol.*, 31 (1991) 237–245.
[16] A.M. Gold and M.P. Osher, *Biochem. Biophys. Res. Commun.*, 42 (1971) 469–474.
[17] M.J. Bernaerts and J. De Ley, *J. Gen. Microbiol.*, 22 (1960) 129–136.
[18] L.R. Nakamura and D.D. Tyler, *J. Bacteriol.*, 129 (1977) 830–835.
[19] W.M. Kurowski, A.H. Fensom, and S.U. Pirt, *J. Gen. Microbiol.*, 90 (1975) 191–202.
[20] E. Stoppok, K. Matalla, and K. Buchholz, *Appl. Microbiol. Biotechnol.*, 36 (1992) 604–610.
[21] N. Asano, K. Katayama, M. Takeuchi, T. Furumoto, Y. Kameda, and K. Matsui, *J. Antibiot.*, 42 (1989) 585–590.
[22] Y. Kondo, *Carbohydr. Res.*, 30 (1973) 386–389.
[23] E. Bar-Guilloux, J. Defaye, H. Driguez, and D. Robic, *Carbohydr. Res.*, 45 (1975) 217–236.
[24] B. Lindberg and O. Theander, *Acta Chem. Scand.*, 13 (1959) 1226–1230.
[25] Y. Tsuda, Y. Okuno, M. Iwaki, and K. Kanemitsu, *Chem. Pharm. Bull.*, 37 (1989) 2673–2678.
[26] R.U. Lemieux, K. James, T.L. Nagabhushan, and Y. Ito, *Can. J. Chem.*, 51 (1973) 33–41.
[27] L. Anderson and H.A. Lardy, *J. Am. Chem. Soc.*, 72 (1950) 3141–3147.
[28] L. Hough and E. O'Brien, *Carbohydr. Res.*,84 (1980) 95–102.
[29] D.H. Ball and F.W. Parrish, *Adv. Carbohydr. Chem. Biochem.*, 24 (1969) 139–197.
[30] P.A. Risbood, T.S. Phillips, and L. Goodman, *Carbohydr. Res.*, 94 (1981) 101–107.
[31] J.M. Heap and L.N. Owen, *J. Chem. Soc. Sect. C*, (1970) 707–712.
[32] A.H. Fenson, W.M. Kurowski, and S.J. Pirt, *J. Appl. Chem. Biotechnol.*, 24 (1974) 457–467.
[33] S. Fukui and K. Hayano, *Agric. Biol. Chem.*, 33 (1969) 1013–1017.

[34] B.S. Furniss, A.J. Hannaford, P.W.G. Smith, and A.R. Tatchell (Eds.), *Vogel's Textbook of Practical Organic Chemistry*, 5th ed., Longmans, UK, 1989, p 464.
[35] Z. Dische, *Methods Carbohydr. Chem.*, 1 (1962) 512–514.