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SYNTHESIS OF 1-THIOSUCROSE AND ANOMERS, AND THE BEHAVIOR OF LEVANSUCRASE AND INVERTASE WITH THIS SUBSTRATE ANALOG*,†

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ABSTRACT

A Lewis acid-catalyzed condensation between 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-glucopyranose and 1,3,4,6-tetra-*O*-benzyl-D-fructofuranose in dichloromethane, followed by a l.c. separation of the deprotected resulting disaccharides, led to 1-thiosucrose (β-D-fructofuranosyl 1-thio-α-D-glucopyranoside), and α-D-fructofuranosyl 1-thio-α-D-glucopyranoside, in respective 15 and 22% overall yields. Similarly, 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose reacted with 1,3,4,6-tetra-*O*-benzyl-D-fructofuranose to give, in a 1:6 ratio, α-D-fructofuranosyl 1-thio-β-D-glucopyranoside (1-thioisosucrose) and its β,β-anomer, which were separated as their *O*-acetyl derivatives. 1-Thiosucrose is an inducer of the biosynthesis of levansucrase in *Bacillus subtilis*. It is a competitive inhibitor for this enzyme with a K_i of 10mM and likewise for yeast invertase (K_i 20 mM).

INTRODUCTION

Sucrose (β-D-fructofuranosyl α-D-glucopyranoside), a major transportable metabolite for photosynthesis in eukaryotes, and a widespread plant-reserve disaccharide, is likewise an important carbon-source for heterotrophic organisms. Numerous enzymic systems are thereby involved in its metabolism and, among them, invertase, dextran- and levan-sucrases. The latter enzyme, which catalyzes

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transfructosylation reactions from a sucrose donor to various acceptors, is one of the few glycosyltransferases for which some basic knowledge has evolved in recent years. A D-fructosyl–enzyme intermediate in the transglycosylation process has been characterized[4] for levansucrase of *Bacillus subtilis,* and the tertiary structure of the enzyme has been obtained from X-ray diffraction studies[5]. A suitable inhibitor would allow the use of the latter approach for the direct visualization of the active site of the enzyme.

Induction of levansucrase is another attractive problem, since so far sucrose is the only known inducer[6,7]. Some intriguing features, such as the relatively slow response of the bacteria, which contrasts with the rapid response to the inducer for most of the inducible systems in *E. coli*, suggest that this disaccharide does not combine directly with the repressor.

Nonmetabolizable inducers and, on the other hand, competitive inhibitors, in the line previously developed by one of our group for glycanases[1], would fill the gap for such enzymic studies. These compounds might furthermore find applications, as nonmetabolizable sweeteners, of interest in human nutrition. We now report the synthesis of the 1-thioglycosyl analog of sucrose and its anomers, and a preliminary evaluation of the interaction of this substrate analog with invertase, as well as with levansucrase and its induction system.

RESULTS AND DISCUSSION

Lemieux and Huber[8] pioneering synthesis of sucrose involved an alcoholysis of Brigls anhydride with 1,3,4,6-tetra-*O*-acetyl-D-fructose. Several more recent approaches to the synthesis of this nonreducing disaccharide, and its anomers and derivatives, were based on a Koenigs–Knorr-type reaction, *i.e.,* a nucleophilic displacement of an aryl- or alkyl-protected glucopyranosyl halide with a similarly protected D-fructose derivative[9–11], or simpler, an acid-catalyzed condensation of anomerically free, alkyl-protected glucopyranoses and fructofuranoses[9,12]. This latter reaction likely involves alcoholysis of the anomeric hydroxyl group of the D-fructofuranose reactant, in view of the expected propensity of this species to develop a positive charge at the anomeric carbon atom. In all the synthetic schemes just mentioned, problems related to the control of the anomeric configuration at both sites arose, and the yields of sucrose derivatives were usually poor. The concept of enhancement of sulfur nucleophilicity, previously successfully applied in our hands to the stereoselective synthesis of nonreducing 1-thio-disaccharides[13], suggested similar approaches for the synthesis of 1-thiosucrose.

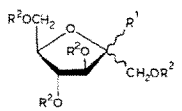
2-*S*-Acetyl-1,3,4,6-tetra-*O*-benzyl-2-thio-D-fructofuranose (**3**) was readily obtained from 1,3,4,6-tetra-*O*-benzyl-D-fructofuranose[14] (**1**), through anomeric *O*-acetylation (**2**) using either acetic anhydride–pyridine with *N,N*-dimethyl-4-pyridinamine (DAP) as catalyst or, although with a lower yield, with the acetic anhydride–sodium acetate reagent. Thiolysis of the resulting 2-*O*-acetyl derivative **2** with thioacetic acid in dichloromethane in the presence of zirconium chloride[15]

led to **3** in good yield. Attempts to deprotect the anomeric sulfur atom in the thioacetate **3** using either stoichiometric amounts of sodium methoxide in methanol[13], or phenylmercuric acetate in ethanol[16], unexpectedly failed, thus cancelling the approach involving a fructosyl 2-thiolate nucleophile for the synthesis of 1-thiosucrose. In the latter deprotection experiment, an anomeric mixture of ethyl fructosides **4** was obtained in 59% yield, showing the enhanced lability of the C–S anomeric bond in thiofructofuranose derivatives.

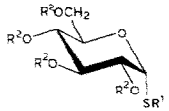
The reverse condensation-scheme involving reaction of the thiolate of 1-thio-α-D-glucopyranose[13] with a D-fructofuranosyl halide in hexamethylphosphoramide was no longer successful, owing presumably to the short life-time of fructofuranosyl halides, even when 1,3,4,6-tetra-*O*-benzoyl-D-fructofuranosyl chloride (**6**), smoothly obtained by the action of chlorotrimethylsilane on 2-*O*-acetyl-1,3,4,6-tetra-*O*-benzoyl-D-fructofuranose[17] (**5**) in dichloromethane, was used as electrophilic reagent.

In view of these disappointing results, an acid-catalyzed condensation, *i.e.*, thiolysis, involving a 1-thio-glucopyranose derivative and a suitably protected precursor of a fructofuranosyl cation was developed and successfully achieved. 2,3,4,6-Tetra-*O*-acetyl-1-thio-α-D-glucopyranose (**10**), a key intermediate in this synthesis, was prepared from the corresponding fully acetylated derivative[13] **8** by conversion[16] into a phenylmercury(II) 1-thioglycose **9**. On subsequent treatment with hydrogen sulfide at 50–60° in ethanol, **9** underwent mercury–sulfur bond fission with production, in a 49% yield, of the *O*-acetylated 1-thio-α-D-glucopyranose derivative **10**. As compared to the β-D series[16], the phenylmercury(II) derivative **9** and the thiol **10** were found to be less stable, as they decomposed both readily, in polar solvents, into untractable mixtures.

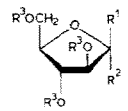
In analogy with previous results in thioglycose synthesis[15], the thiolysis-condensation reaction of 1,3,4,6-tetra-*O*-benzyl-D-fructofuranose (**1**) with thiol **10** was initially conducted with zirconium chloride, a moderately hygroscopic Lewis acid,



1 R^1 = OH, R^2 = Bzl
2 R^1 = OAc, R^2 = Bzl
3 R^1 = SAc, R^2 = Bzl
4 R^1 = OEt, R^2 = Bzl
5 R^1 = OAc, R^2 = Bz
6 R^1 = Cl, R^2 = Bz
7 R^1 = OH, R^2 = Bz

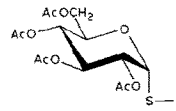


8 R^1 = R^2 = Ac
9 R^1 = HgPh, R^2 = Ac
10 R^1 = H, R^2 = Ac

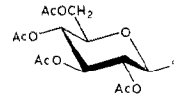


11 R^1 = X, R^2 = CH_2OBzl, R^3 = Bzl
12 R^1 = CH_2OBzl, R^2 = X, R^3 = Bzl
13 R^1 = Y, R^2 = CH_2OBzl, R^3 = Bzl
14 R^1 = CH_2OBzl, R^2 = Y, R^3 = Bzl
15 R^1 = Y, R^2 = CH_2OBz, R^3 = Bz
16 R^1 = CH_2OBz, R^2 = Y, R^3 = Bz

X =



Y =



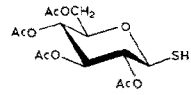


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TABLE I

TOTAL YIELDS AND RESPECTIVE RATIO OF ANOMERS OF PROTECTED 1-THIOSUCROSES **11–14** OBTAINED BY 2,3,4,6-TETRA-*O*-ACETYL-1-THIO-α- (**10**) AND -β-D-GLUCOPYRANOSE (**17**) CONDENSATION WITH 1,3,4,6-TETRA-*O*-BENZYL-D-FRUCTOFURANOSE (**1**)[a]

1-Thio-D-*glucopyranose derivative*	*Catalyst*	D-*Fruf-(2→1)-1-thio*-D-*Glcp disaccharide formed*		
		Compounds	*Ratio*	*Total yield (%)*
10	$ZrCl_4$	**11–12**	2:3	70
10	$SnCl_4$	**11–12**	1:3	32
17	$ZrCl_4$	**13–14**	6:1	73
17	$SnCl_4$	**13–14**	1:1	57

[a]In dichloromethane. Molar ratios of **10** or **17** to **1** to Lewis acid, 4:4:3.

in dichloromethane. In further systematic studies, tin chloride was found to be a convenient catalyst, but leading to some variations in the proportion of anomers (**11, 12**) of the resulting disaccharide (Table I). The respective ratio of acid catalyst to the D-fructose and D-glucose reactants was another parameter of importance in the final yield. In a typical experiment, equimolar amounts of 1,3,4,6-tetra-*O*-benzyl-D-fructofuranose (**1**) and 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-glucopyranose (**10**) were found to react smoothly, over a 30-min period, in dichloromethane in the presence of a 0.75 molar proportion of zirconium chloride, leading to a 70% yield of sirupy, t.l.c. homogeneous, condensation product **11, 12**. Debenzylation of **11, 12** with sodium in liquid ammonia was, however, cumbersome and resulted in a comparatively low yield, after subsequent conventional reacetylation, in the mixture of anomers **18, 20** (43% from **1**). All attempts to resolve these diastereoisomers at this step with either conventional preparative column chromatography or reverse-phase l.c. failed. This separation was, however, achieved on the *O*-deacylated compounds by use of a semi-preparative l.c. on an amino-bond-phase column leading to α-D-fructofuranosyl 1-thio-α-D-glucopyranoside (**19**) and its D-fructosyl anomer, β-D-fructofuranosyl 1-thio-α-D-glucopyranoside (1-thiosucrose **21**) in a 3:2 ratio.

The anomeric configuration of both disaccharides was assigned by a comparison of their optical rotations assuming that, according to Hudson's rules, an α,α anomer would be the most dextrorotatory compound (Table II). The anomeric configuration of the 1-thio-D-glucopyranosyl residues was further confirmed through an examination of the ^{1}H-n.m.r. spectra of the corresponding octa-*O*-acetyl derivatives (**18, 20**; Table III), where respective coupling constants of 6.0 and 5.5 Hz were measured for H-1–H-2 of the D-glucopyranosyl residue of both compounds in the $^4C_1$(D) conformation. Comparison of the relative chemical displacements for H-4 of the D-fructosyl residues in the 1-thiosucrose acetate **20** and its α,α anomer **18** provided another argument in favor of the proposed anomeric configuration for the D-fructofuranosyl residue in both disaccharides, as this resonance was deshielded by 0.5 p.p.m. in **20**, a value close to that found for

TABLE II

COMPARISON OF OPTICAL ROTATIONS FOR SUCROSE, ITS ANOMERS, THEIR OCTA-*O*-ACETYLATED DERIVATIVES, AND THEIR 1-THIO ANALOGS **18–25**

O-Glycosyl disaccharide	$[\alpha]_D$ *(deg.)*	*Solv.*	*Ref.*	*1-Thioglycosyl analog*	$[\alpha]_D$ *(deg.)*	*Solv.*
α-D-Fru*f*-(2→1)-α-D-Glc*p*	+109	H_2O	9	α-D-Fru*f*-(2→1)-1-thio-α-D-Glc*p* **19**	+280	MeOH
β-D-Fru*f*-(2→1)-α-D-Glc*p*	+ 66	H_2O	8	β-D-Fru*f*-(2→1)-1-thio-α-D-Glc*p* **21**	+ 26	MeOH
β-D-Fru*f*-(2→1)-β-D-Glc*p*				β-D-Fru*f*-(2→1)-1-thio-β-D-Glc*p* **23**	−146	MeOH
α-D-Fru*f*-(2→1)-β-D-Glc*p*	+ 34	H_2O	18	α-D-Fru*f*-(2→1)-1-thio-β-D-Glc*p* **25**	+ 26	H_2O
Octa-*O*-acetyl				Octa-*O*-acetyl		
α-D-Fru*f*-(2→1)-α-D-Glc*p*	+ 83	$CHCl_3$	9	α-D-Fru*f*-(2→1)-1-thio-α-D-Glc*p* **18**	+210	$CHCl_3$
β-D-Fru*f*-(2→1)-α-D-Glc*p*	+ 60	$CHCl_3$	8	β-D-Fru*f*-(2→1)-1-thio-α-D-Glc*p* **20**	+ 44	$CHCl_3$
β-D-Fru*f*-(2→1)-β-D-Glc*p*				β-D-Fru*f*-(2→1)-1-thio-β-D-Glc*p* **22**	− 38	$CHCl_3$
α-D-Fru*f*-(2→1)-β-D-Glc*p*	+ 20	$CHCl_3$	18	α-D-Fru*f*-(2→1)-1-thio-β-D-Glc*p* **24**	+ 65	$CHCl_3$

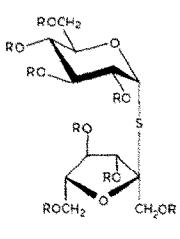
TABLE III

COMPARISON OF ^{1}H-N M R DATA FOR OCTA-*O*-ACETYL DERIVATIVES OF SUCROSE, ITS ANOMERS, AND THEIR 1-THIO ANALOGS, **18, 20, 22,** AND **24**[a]

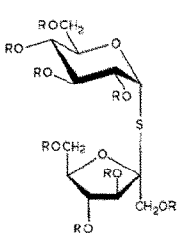
Compound[b]	*Chemical shifts (δ) and coupling constant (Hz)*[c]												
	D-Glucopyranosyl residue							*D-Fructofuranosyl residue*					
	H-1 ($J_{1,2}$)	*H-2* ($J_{2,3}$)	*H-3* ($J_{3,4}$)	*H-4* ($J_{4,5}$)	*H-5* ($J_{5,6a}$)	*H-6a* (J_{6ab})	*H-6b* ($J_{5,6b}$)	*H-1a,b* ($J_{1a,b}$)	*H-3* ($J_{3,4}$)	*H-4* ($J_{4,5}$)	*H-5* ($J_{5,6a}$)	*H-6a* ($J_{6a,b}$)	*H-6b* ($J_{5,6b}$)
α-D-Fruf-(2→1)-1-thio-α-D-Glcp (**18**)	6.04	5.01	5.25	5.05	4.36	4.35	4.05	4.4	5.36	5.05	4.15	4.45	4.18
	d	dd	dd	dd	m	m	m	m	d	dd	m	m	m
	(6.0)	(10.5)	(10.0)	(9.5)					(3.5)	(7.0)			
α-D-Fruf-(2→1)-α-D-Glcp[19]	5.51	5.03	5.53	5.13				4.05–4.57	5.50	4.82	4.4	4.38	3.90
	d	t	t '	t				d	d	t	t		
	(3.6)	(10.0)	(10.0)	(10.0)				(12.0)	(1.0)	(3.0)	(3.0)	(3.0)	(10.0)
β-D-Fruf-(2→1)-1-thio-α-D-Glcp (**20**)	5.88	5.12	5.28	5.07		4.01–4.03		4.20	5.69	5.57		4.01–4.03	
	d	dd	t	tl		m		m	d	m		m	
	(5.5)	(10.0)	(10.0)	(10.0)					(7.0)	(7.0)			
β-D-Fruf-(2→1)-α-D-Glcp[19]	5.70	4.83	5.46	5.06					5.48	5.38			
	d	t	t	t									
	(4.0)	(10.0)	(10.0)	(10.0)					(5.0)				
β-D-Fruf-(2→1)-1-thio-β-D-Glcp (**22**)	4.98	5.11	5.24	5.03	3.8	4.1–4.5		4.15	5.77	5.43	4.30	4.75	4.12
	d	dd	t	tl	o	m		m	d	m	m	d	d
	(10.5)	(10)	(10)	(10)	(3.5, 4.5)				(7.0)	(7.0)		(13.0)	
α-D-Fruf-(2→1)-1-thio-β-D-Glcp (**24**)	4.87	5.02	5.22	5.06	3.72	4.45	4.26	4.10	5.34	5.07	4.55	4.15–4.22	
	d	dd	t	t	o	dd	dd		d	dd	m	m	
	(10)	(10.5)	(10.5)	(10.5)	(3.0)	(12)	(5.0)		(2.5)	(7.0)			
α-D-Fruf-(2→1)-β-D-Glcp[19]	4.96	5.02	5.18	4.98	3.73			3.94–4.35	5.41	4.84	4.57	4.42	4.18
	d	dd	dd	dd	m			d	d	dd	o	d	d
	(10.0)	(10.0)	(9.0)	(10.0)				(12.0)	(2.0)	(5.0)	(3.0)	(12.0)	

[a]For a solution in chloroform-*d*. [b]Octa-*O*-acetyl derivative. [c]For each compound: 1st line, chemical shifts; 2nd line, multiplicity; and 3rd line, coupling constant (in parentheses).

the *O*-glycosyl series[19]. This deshielding had been previously assigned[20] to a diastereotopic effect of the D-glucopyranosyl ring oxygen atom in β- and α-D-fructofuranosyl anomers of fructosyl→glucosides.



18 R = Ac
19 R = H



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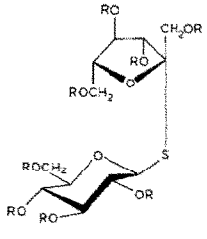
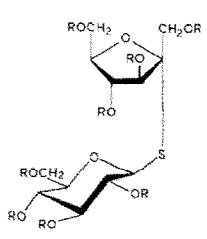
21 R = H



22 R = Ac
23 R = H



24 R = Ac
25 R = H

The ready access to 1-thiosucrose **21** and its anomer **19**, through the aforementioned synthesis, suggested a similar sequence of reactions for the preparation of 1-thioisosucrose (**25**). Thus 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose[21] (**17**) was treated with 1,3,4,6-tetra-*O*-benzyl-D-fructofuranose (**1**) under conditions similar to those used for the obtention of the mixture **11** + **12**. The product homogeneous, in t.l.c. and obtained in a 73% yield, was converted into its octa-*O*-acetyl derivative through the previously described sequence involving a Birch reduction, followed by acetylation with acetic anhydride–pyridine. The resulting syrupy mixture of two components was resolved on a silica gel column to yield cristalline 1,3,4,6-tetra-*O*-acetyl-α-D-fructofuranosyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (octa-*O*-acetyl 1-thioisosucrose, **24**) as the minor component, together with its syrupy β-D-fructofuranosyl anomer **22** (ratio 1:6). Both octa-*O*-acetyl disaccharides **22** and **24** were alternatively obtained, although in inverse ratio, from the zirconium chloride condensation of 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose (**17**) with 1,3,4,6-tetra-*O*-benzoyl-D-fructofuranose (**7**) in dichloromethane, followed by conventional successive *O*-deacylation–*O*-reacetylation of the resulting mixture **15** + **16**. The overall yield of this synthesis is comparable to that of the previous one; in fact, this approach avoided the critical debenzylation-step that lowers the yield in the former sequence of reaction. However, the thiolysis condensation reaction of **17** and **7** was less favored, in agreement with the electron-withdrawing effect of acyl groups, which raises the energy of formation of the postulated D-fructosyloxy carbenium ion-intermediate. Subsequently, this approach failed when applied to the synthesis of 1-thiosucrose from **7** and **10**, and this may be ascribed to a lower stability of the 1-thio-α-D nucleophile **10** as compared to its β-D anomer **17**.

As for 1-thiosucrose octaacetate **20** and its α-D-fructofuranosyl anomer **18**, the ^{1}H-n.m.r. spectra of peracetylated disaccharides **22** and **24** (Table III) confirmed unambiguously the retention of the anomeric configuration of the 1-thio-D-glucopyranosyl residue, with an anomeric coupling-constant of 10.5 Hz, for both derivatives. In agreement with the previously reported steric effect of the D-gluco-

pyranosyl residue on the chemical displacements of the protons of the D-fructosyl residue[19], the H-4 fructosyl resonance of the β,β-1-thiodisaccharide **22** was deshielded by 0.35 p.p.m. as compared to the corresponding signal of its α,β anomer **24**. Furthermore coupling constants of 7 Hz were observed for H-3–H-4 of β-fructofuranosides **20** and **22**, as compared to respective values of 3.5 and 2.5 Hz for α-fructofuranosides **18** and **24**, in close agreement with values found for methyl 3,4,6-tri-*O*-acetyl-β-D-fructofuranoside and its α-D anomer[18]. *O*-Deacylation of acyl disaccharides **22** and **24** led to β-D-fructofuranosyl 1-thio-β-D-glucopyranoside (**23**) and its diastereoisomer α-D-fructofuranosyl 1-thio-β-D-glucopyranoside (1-thioisosucrose, **25**) having comparative, specific optical rotations in agreement with Hudson's rules (Table II).

In a preliminary examination of the enzymic reactivity of this series of substrate analogs, the effect of 1-thiosucrose (**21**) on the catalytic activity of yeast invertase and levansucrase from *Bacillus subtilis* was assessed. Furthermore, **21** was checked as a gratuitous inducer of levansucrase production by *B. subtilis*. The velocity pattern of invertase for the hydrolysis of sucrose was established in the pre-

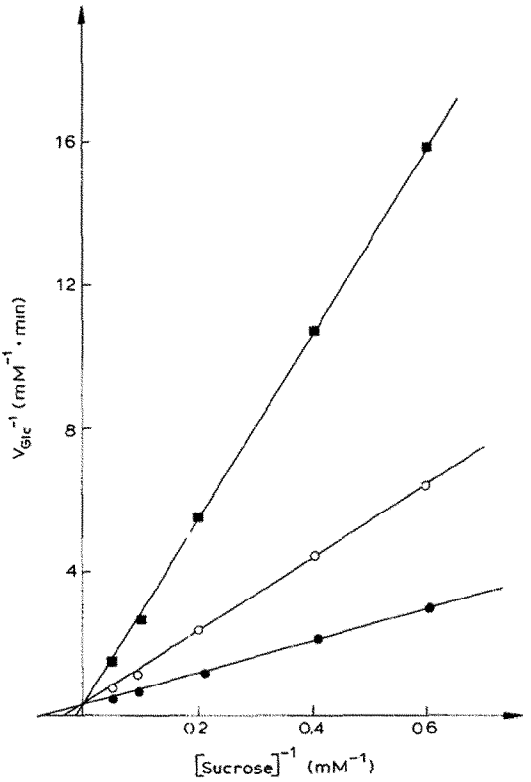


Fig. 1. Effect of various concentrations of 1-thiosucrose (**21**) on yeast-invertase catalytic activity (Lineweaver–Burk presentation): —●—●—, no addition of **21**, —○—○—, 22.5mM **21** added, and —■—■—, 110mM **21** added.

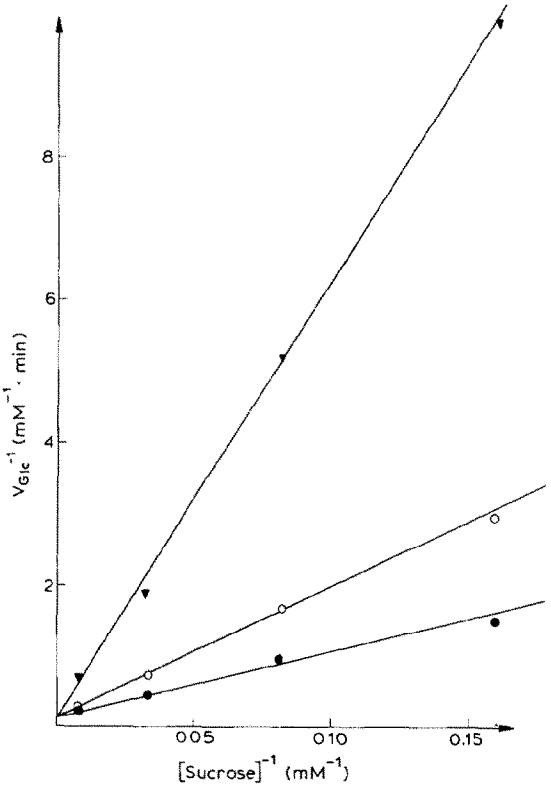


Fig. 2. Effect of various concentrations of 1-thiosucrose (**21**) on the rate of exchange of the D-glucopyranosyl residue of sucrose with [^{14}C]D-glucose, catalyzed by *B. subtilis* levansucrase (Lineweaver–Burk presentation): —●—●—, no addition of **21**, —○—○—, 17mM **21** added, and —▲—▲—, 84mM **21** added.

sence of various concentrations of [^{14}C]sucrose. From kinetic data (Fig. 1), it could be concluded that **21** is a competitive inhibitor of yeast invertase. The inhibition constants (K_i) of 20mM, as estimated from the Dixon plot, is of the same magnitude value as the Michaelis constant of yeast invertase for sucrose (16mM) under the same conditions of pH and temperature[22].

The exchange-velocity pattern of levansucrase with sucrose and D-glucose in the presence of 1-thiosucrose (**21**) was established, at fixed concentration of D-[^{14}C]glucose, with various concentrations of sucrose and **21**. Kinetic data (Fig. 2) showed an inhibition pattern in agreement with a competitive inhibition effect of **21** with respect to levansucrase. The value of K_i (10 ±1mM), as determined from Dixon plot, is nearly the value of the Michaelis constant of *B. subtilis* levansucrase for sucrose[23].

The effect of 1-thiosucrose (**21**) on levansucrase production by *B. subtilis* was evaluated taking into account the bacterial-cell growth in the presence of this sub-

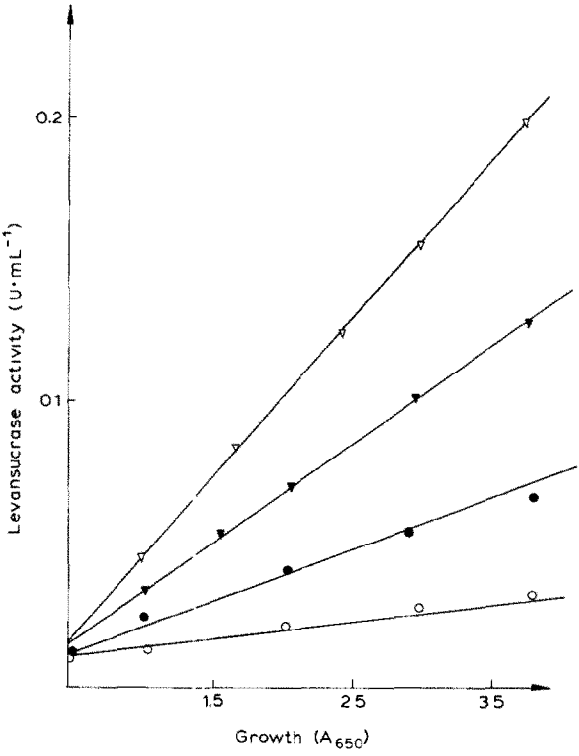


Fig. 3. Comparative productions of levansucrase by *B. subtilis* grown on mineral medium, supplemented with D-glucose (—○—○—); with addition of 7mM 1-thiosucrose (**21**) (—●—●—); with addition of 0.7mM sucrose (—▲—▲—); and with simultaneous addition of 0.7mM sucrose and 7mM **21** (—△—△—).

strate analog. For a 30mM concentration of **21** the cells did not grow and, for a lower concentration (7mM), the growth remained exponential, but the generation time increased from 47 min, in the absence of the substrate analog, to 66 min. At the latter concentration, production of levansucrase by the cells was distinctly stimulated. However, the differential rate of synthesis (0.035 $U.A._{650}^{-1}$) was about ten-fold lower than that found for cells grown in the presence of the same concentration of sucrose, the natural inducer. When sucrose was together present at a concentration that fully induces the sucrose-transport system[7] (0.7mM, Fig. 3), the level of levansucrase biosynthesis by **21** was not significantly increased, and this raises the question of the transport system for this molecule.

In conclusion, the Lewis acid-catalyzed thiolysis of glycoses, previously adapted to the stereoselective synthesis of 1-thioglycoses in the presence of zirconium chloride[15], has been extended to the synthesis of nonreducing 1-thiodisaccharides in the sucrose series, taking advantage of the expected, enhanced rate of cationisation of fructofuranose derivatives. This approach allowed a complete

stereocontrol at the anomeric center of the reacting 1-thio-D-glucopyranose, and the yields of condensation products were considerably higher than those previously observed for the synthesis of the corresponding *O*-glycosyl disaccharides[8–12]. Levansucrase synthesis of *B. subtilis* was induced by the addition of 1-thiosucrose to the culture medium. Furthermore, this substrate analog was found to be the first disaccharide acting as competitive inhibitor for both levansucrase and invertase.

EXPERIMENTAL

General methods. — Melting points were determined with a Zeiss hot-stage equipped with a microscope and correspond to "corrected melting-points". Optical rotations were measured with a Perkin–Elmer model 241 polarimeter. ^{1}H-N.m.r. spectra were recorded at 250 MHz with a Cameca spectrometer (Thomson C.S.F. Paris) by Mr. H. Reutenauer and assignments were confirmed by the INDOR technique. ^{13}C-N.m.r. spectra were recorded at 25.18 MHz by Mrs. M. L. Dheu-Andries and Mr. C. Gey with a Bruker WP 100 instrument. Chemical shifts are reported in δ values relative to the signal of tetramethylsilane, and coupling constants in Hz (s, singlet; d, doublet; dd, doublet of doublets; m, multiplet; o, octet). Mass spectra were recorded by Mr. C. Bosso with an AEI MS-30 double-beam mass spectrometer, connected to a Finnigan-Mat SS-100 MS computer, in the chemical-ionization mode (c.i.) using 33:77 (v/v) isobutane–ammonia as reactant gas, with a source temperature of 150°, an electron energy of 110–170 eV, a filament emission of 520 νA and an ion energy of 2–4 kV; fragment intensities are given in brackets (%) with reference to base peak. Acylation reactions were followed by extraction with the solvent indicated. "Usual work-up" means successive washing of the organic layer with ice-cold solutions of 10% (w/v) $KHSO_4$, sat. $NaHCO_3$, and water. Solutions were dried (Na_2SO_4) and evaporated under reduced pressure at temperatures <45°. T.l.c. was performed on precoated glass plates (0.25 mm) coated with Silica gel 60_F-254 (E. Merck, Darmstadt, F.R.G.) in the following eluants (v/v): (*A*) 1:1 diethyl ether–hexane; (*B*) 4:1 diethyl ether–hexane; (*C*) 2:3 ethyl acetate–hexane; (*D*) diethyl ether; (*E*) 2:1 diethyl ether–hexane; (*F*) 4:1 diethyl ether–hexane; and (*G*) 65:25:4 chloroform–methanol–water. Components were detected by u.v. light and by spraying the plates with 10% H_2SO_4, with subsequent heating. Column chromatography was performed with Silica gel 60 (70–230 mesh; E. Merck, Darmstadt, F.R.G.) and the same eluants. Liquid chromatography (l.c.) was performed by Mrs. D. Dupeyre with a Waters Assoc. instrument fitted with a pumping system M 6000 A, a high pressure injector U 6K, a differential refractometer detector R 401, and a semi-preparative, Waters Micro-Bondapak-NH_2 (30 cm × 7.8 mm i.d.), stainless-steel column. The eluant was 21:4 acetonitrile–water at a pressure of 21 MPa.

Enzymic assays. — *Bacillus subtilis* 168 Marburg strain QB 112 (genotype Sac U^h 32), inducible for the synthesis of levansucrase, was used for the induction experiments. Bacteria were grown at 37°, in mineral medium supplemented with D-

glucose (10 g/L). One absorbance unit at 650 nm of cell suspension corresponds to $1.4\ 10^8$ cells · mL^{-1} and 70 μg protein · mL^{-1}.

The rate of enzyme synthesis was estimated from measurements of enzyme activity on aliquots (50 μL) of cell suspension, during exponential growth. The pH was adjusted at 6 by the addition of phosphate buffer (pH 6, 2M, 5 μL). To this sample, the following solution (5 μL) was added: lysozyme, 10 mg · mL^{-1}, DNase 0.1 mg · mL^{-1}, and magnesium chloride 2 mg · mL^{-1}. Incubation was conducted for 5 min at 37°. The activity of levansucrase was assayed on this suspension of lyzed bacteria. The differential rate of enzyme synthesis is defined as the slope of the straight line obtained when units of enzyme activity are plotted against absorbance units of the culture at 650 nm.

Levansucrase was prepared from culture supernatant of *B. subtilis* according to the usual method[23]. The activity was estimated by measuring the initial velocity of the exchange reaction catalyzed by the enzyme[24]. One unit of enzyme activity (U) corresponds to 2 μg of enzyme.

Activity of yeast invertase (Sigma Chemical Co., St. Louis, MO 63178) was estimated from the measurements of the initial-velocity value for sucrose hydrolysis. A reaction mixture (90 μL) containing [^{14}C]sucrose (90 μg) and 1-thiosucrose (**21**) at various concentrations in 0.1M acetate buffer, pH 5, was incubated at 37° in a temperature-controlled cell. The reaction was initiated by addition of an enzyme stock-solution (10 μL), and 8 aliquots of 10 μL were withdrawn at intervals of 1 min. Samples were analyzed by descending paper chromatography, with 4:1:1 (v/v) 1-butanol–acetic acid–water as developing solvent. Radioactive products were identified by autoradiography with a Kodak X-Ray film. The radioactive spots were counted with a liquid spectrometer. Initial velocities were defined as V_{Glc} expressed as the molarity of D-glucose released per min.

2-O-*Acetyl-1,3,4,6-tetra*-O-*benzyl-α,β*-D-*fructofuranose* (**2**). — (*a*) A solution of 1,3,4,6-tetra-*O*-benzyl-D-fructofuranose[14] (**1**, 203 mg, 0.38 mmol), in 3:4 (v/v) acetic anhydride–pyridine (3.5 mL) containing *N,N*-dimethyl-4-pyridinamine (DAP, 2 mg, 20 μmol), was stirred for 18 h at room temperature. Methanol (4 mL) was added, and the mixture concentrated under reduced pressure to a syrup, which was diluted with dichloromethane (150 mL) and processed as usual. The syrupy residue, which showed on t.l.c. (*A*) a major component (R_F 0.45), was purified on a column of silica gel with the same eluant to give **2** as a syrup (155 mg, 71%); ^{1}H-n.m.r. (chloroform-*d*): δ 2.01 (CH_3COO, α anomer), and 1.85 (CH_3COO, β anomer; ratio α to β, 4:1); ^{13}C-n.m.r. (chloroform-*d*): δ 110.7 (C-2, α anomer), 106.9 (C-2, β anomer), and 21.9 (CH_3COO); m.s.: *m/z* 600 [10, $(M + NH_4)^+$] 540 (100, 600-AcOH), and 523 (42, $M^+ - CH_3OCO\cdot$).

Anal. Calc. for $C_{36}H_{38}O_7$: C, 74.20; H, 6.57. Found: C, 73.97; H, 6.56.

(*b*) A solution of compound **1** (214 mg, 0.4 mmol) in acetic anhydride (2 mL) was added to a boiling solution of sodium acetate (160 mg, 1.95 mmol) in acetic anhydride (2 mL). The mixture was boiled under reflux for 10 min, then cooled in ice, and methanol (6 mL) added. The syrupy residue, resulting from evaporation *in*

vacuo, was dissolved in dichloromethane (100 mL), and the solution successively washed with cold, saturated $NaHCO_3$ and ice-cold water. A chromatographic purification, as described under (*a*), led to **2** (70 mg, 30%) as a 2:3 α and β anomer mixture (^{1}H-n.m.r.).

2-S-Acetyl-1,3,4,6-tetra-O-benzyl-2-thio-α,β-D-fructofuranose (**3**). — To a solution of **2** (obtained by method *a*; 101 mg, 0.18 mmol) in dichloromethane (2 mL) were added thioacetic acid (0.1 mL) and zirconium chloride (20 mg, 80 μmol). After a 1-h stirring at room temperature, a major component was detected in t.l.c. (*A*). Dichloromethane (100 mL) was added and the solution processed as usual. A column chromatography (silica gel, 30 g; *A*) led to the thioacetate **3** (84 mg, 80%); ^{1}H-n.m.r. (chloroform-*d*): δ 2.30 (CH_3, SAc, α anomer), and 2.17 (CH_3, SAc, β anomer; ratio α to β, 2:3); ^{13}C-n.m.r. (chloroform-*d*): δ 98.5 (C-2, α anomer), 98.1 (C-2, β anomer), 31.8 (CH_3, SAc, α anomer), and 31.4 (CH_3, SAc, β anomer); m.s.: *m/z* 616 [3, $(M + NH_4)^+$], 540 (3, 616 − AcSH), and 523 (11, $M^+ - CH_3$, COS·).

Anal. Calc. for $C_{36}H_{38}O_6S$: C, 72.22; H, 6.40; S, 5.35. Found: C, 72.22; H, 6.42; S, 5.52.

Ethyl 1,3,4,6-tetra-O-benzyl-α,β-D-fructofuranoside (**4**). — A mixture of **3** (463 mg, 0.77 mmol) and phenylmercuric acetate (259 mg, 0.77 mmol) in absolute ethanol (8 mL) was boiled under reflux for 40 min. Filtration of the resulting solution and evaporation led to an oil which showed a major component on t.l.c. (*B*), and was purified by column chromatography (260 mg, 59%); ^{1}H-n.m.r. (chloroform-*d*): δ 7.6–7.0 (m, $C_6H_5CH_2O$), 4.84–4.08 (m, $PhCH_2$), 4.35 (d, $J_{3,4}$ 7.0, H-3, β), 4.17 (t, $J_{4,5}$ 7.0, H-4, β), 4.16 (m, H-5; α,β), 4.09 (d, $J_{3,4}$ 2.5, H-3, α), 3.86 (dd, $J_{4,5}$ 6.0, H-4, α), 3.68–3.55 (m, H-1α,β, H-6a,b; α,β), and 1.22 (t, CH_3CH_2); ^{13}C-n.m.r. (chloroform-*d*): δ 108 (C-2, α anomer), and 104.2 (C-2, β anomer); m.s.: *m/z* 586 [100, $(M + NH_4)^+$], 540 (70, 586 − EtOH), 523 (41, $M^+ - OEt$·), and 447 (60, $M^+ - PhCH_2OCH_2$·).

Anal. Calc. for $C_{36}H_{40}O_6$: C, 76.03; H, 7.09; O, 16.88. Found: C, 75.77; H, 7.30; O, 17.03.

1,3,4,6-Tetra-O-benzoyl-D-fructofuranosyl chloride (**6**). — A mixture of 1,3,4,6-tetra-*O*-benzoyl-D-fructofuranose[17] (264 mg, 0.44 mmol) in 1:1 (v/v) acetic anhydride–pyridine (4 mL) containing a catalytic amount of DAP was stirred for 18 h at room temperature. Usual work-up, followed by filtration on a column of silica gel led to 2-*O*-acetyl-1,3,4,6-tetra-*O*-benzoyl-D-fructofuranose (**5**; 167 mg, 59%), which was, without further characterization, dissolved in dichloromethane (1 mL). Chlorotrimethylsilane (0.8 mL) was added, and the solution kept for 6 h at room temperature, and then evaporated to give a syrupy material (100 mg), homogeneous in t.l.c. (*C*); ^{13}C-n.m.r. (chloroform-*d*): δ 106.85 (C-2).

2,3,4,6-Tetra-O-acetyl-1-phenylmercury(II)-thio-α-D-glucopyranose (**9**). — A mixture of 2,3,4,6-tetra-*O*-acetyl-1-*S*-acetyl-1-thio-α-D-glucopyranose[25] (**8**, 1.22 g, 3.0 mmol) and phenylmercuric acetate (1.02 g, 3.0 mmol) in absolute ethanol (20 mL) was boiled under reflux for 2 h. The resulting black precipitate was filtered off

(Celite). The filtrate was taken to dryness and the resulting syrup was purified by column chromatography (*C*) to yield **9** (736 mg, 38%) as a solid foam, m.p. 59–61°, $[\alpha]_D^{20}$ +134° (*c* 1.0, chloroform); ^{1}H-n.m.r. (chloroform-*d*): δ 6.20 (d, $J_{1,2}$ 5.5, H-1), 5.66 (t, $J_{3,4}$ 10, H-3), 5.13 (dd, $J_{2,3}$ 10, H-2), 5.06 (dd, $J_{4,5}$ 10, H-4), 4.76 (o, $J_{5,6a}$ 5, H-5), 4.27 (dd, $J_{6a,6b}$ 12, H-6a), and 4.08 (dd, $J_{5,6b}$ 2.5, H-6b); ^{13}C-n.m.r. (chloroform-*d*): δ 79.5 (C-1), 72.2, 70.1, 68.9, 68.0, and 62.5.

Anal. Calc. for $C_{20}H_{24}HgO_9S$: C, 37.47; H, 3.77. Found: C, 37.39; H, 4.06.

*2,3,4,6-Tetra-*O*-acetyl-1-thio-α-*D*-glucopyranose* (**10**). — Hydrogen sulfide was bubbled for 10 min into a solution of the phenylmercury(II)thio derivative **9** (602 mg, 0.94 mmol) in ethanol at a temperature kept between 50 and 60°. The resulting precipitate was removed by filtration and the filtrate, which showed a major component in t.l.c. (*D*; R_F 0.6), was purified by column chromatography (*E*) to yield **10** (130 mg, 38%), m.p. 92–93°, $[\alpha]_D^{20}$ +168° (*c* 1.0, chloroform); ^{1}H-n.m.r. (chloroform-*d*): δ 5.88 (d, $J_{1,2}$ 5.5, H-1), 4.98 (dd, $J_{2,3}$ 10, H-2), 5.37 (t, $J_{3,4}$ 10, H-3), 5.04 (t, $J_{4,5}$ 10, H-4), 4.41 (m, H-5), 4.26 (dd, $J_{5,6a}$ 5, $J_{6a,6b}$ 12, H-6a), and 4.08 (dd, $J_{5,6b}$ 2.5); ^{13}C-n.m.r. (chloroform-*d*): δ 77.2 (C-1), 70.4, 70.0, 68.4, and 61.7; m.s.: *m/z* 382 [48, $(M + NH_4)^+$], and 331 (100, $M^+ - SH\cdot$).

Anal. Calc. for $C_{14}H_{20}O_9S$: C, 46.15; H, 5.53; S, 8.79. Found: C, 45.94; H, 5.55; S, 8.64.

The direct treatment of the crude phenylmercury(II)thio derivative **9** with hydrogen sulfide without the column-chromatography-purification step afforded **10** with a 49% overall yield from **8**. The resulting product was used for the next step.

*1,3,4,6-Tetra-*O*-benzyl-α,β-*D*-fructofuranosyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-*D*-glucopyranoside* (**11, 12**). — Crude syrupy **10**,prepared from **8** (3.0 g, 7.4 mmol), was treated with 1,3,4,6-tetra-*O*-benzyl-D-fructofuranose[14] (**1**, 2.0 g, 3.7 mmol) and zirconium chloride (646 mg, 2.8 mmol) in dichloromethane (55 mL) for 30 min at room temperature. Dichloromethane (100 mL) was added, and the solution washed successively with cold, saturated aqueous solution of $NaHCO_3$ (50 mL), and then water. Drying and evaporation led to a syrupy residue, which was purified by column chromatography (220 g, *E*) affording successively unreacted, starting **1** (300 mg. 15%) and syrupy **11, 12** (2.37 g, 72%).

Anal. Calc. for $C_{48}H_{54}O_{14}S$: C, 65.00; H, 6.14; S, 3.61. Found: C, 64.97; H, 6.24; S, 3.39.

*1,3,4,6-Tetra-*O*-acetyl-α,β-*D*-fructofuranosyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-*D*-glucopyranoside* (**18, 20**). — To the mixture of anomers **11** and **12** (190 mg, 0.2 mmol), in liquid ammonia (30 mL) at −78°, were slowly added, with magnetic stirring, fragments of metallic sodium until persistance of a blue coloration for more than 30 min. The excess of reagent was eliminated by addition of NH_4Cl, and the mixture left to evaporate at room temperature. The solid residue was suspended in 1:1 pyridine–acetic anhydride (v/v) (10 mL) and stirred for 16 h at room temperature. Methanol (20 mL) was added and the mixture evaporated to an oil which was diluted with chloroform. The organic layer was processed as usual. Evaporation led to a syrup that was purified by column chromatography (*D*) to give syrupy **18, 20** (87 mg, 43% from **1**; R_F 0.4, *D*). Resolution, of this mixture of anomers by classical chromatographic techniques failed.

α- (**19**) *and β-*D*-Fructofuranosyl 1-thio-α-*D*-glucopyranoside (1-thiosucrose,* **21**). — To the mixture of anomers **18, 20** (973 mg, 1.4 mmol) in methanol (20 mL), was added M sodium methoxide in methanol (20 μL), and the solution was stirred for 16 h at room temperature. Neutralization with Amberlite IRN 77 and evaporation to dryness led to a residue that was submitted to a semi-preparative, l.c. chromatography in a Micro-Bondapak-NH_2 column (30 cm × 7.8 mm i.d., stainless steel; Waters Associates, Milford, MA 01757; eluant, 21:4 acetonitrile–water). Elution gave successively **19** and **21**.

Compound **19**: hygroscopic, solid foam (266 mg, 22% from **1**), $[\alpha]_D^{20}$ +280° (*c* 1.0, methanol); ^{13}C-n.m.r. (D_2O): δ 95.4 (C-2, Fru), 85.2, 84.0, 81.6, 78.0, 75.0, 74.2, 71.5, 70.5, 64.4, and 61.5.

Anal. Calc. for $C_{12}H_{22}O_{10}S \cdot 1.5\ H_2O$: C, 37.40; H, 6.49; S, 8.31. Found: C, 37.51; H, 6.42; S, 8.08.

Compound **21**: hygroscopic, solid foam (176 mg, 15% from **1**), $[\alpha]_D^{20}$ +26° (*c* 1.33, methanol); ^{13}C-n.m.r. (D_2O): δ 95.5 (C-2, Fru), 83.5, 83.3, 77.3, 75.4, 75.1, 74.5, 71.4, 70.0, 65.3, 63.0 and 61.1.

Anal. Calc. for $C_{12}H_{22}O_{10}S \cdot 1.5\ H_2O$: C, 37.40; H, 6.49; S, 8.31. Found: C, 37.63; H, 6.50; S, 8.24.

*1,3,4,6-Tetra-*O*-acetyl-α-*D*-fructofuranosyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-*D*-glucopyranoside* (**18**). — Disaccharide **19** (30 mg, 84 mmol) in 1:1 (v/v) pyridine–acetic anhydride (1 mL) was kept for 16 h at room temperature. Methanol (2 mL) was added and the resulting solution evaporated. The remaining oil was diluted with chloroform (50 mL) and the solution processed as usual. Filtration on a small silica gel column with eluant *D* afforded **18** (58 mg, 100%), m.p. 127–128° (diethyl ether), $[\alpha]_D^{20}$ +210° (*c* 1.08, chloroform).

Anal. Calc. for $C_{28}H_{38}O_{18}S$: C, 48.41; H, 5.51; S, 4.62. Found: C, 48.45; H, 5.58; S, 4.68.

*1,3,4,6-Tetra-*O*-acetyl-β-*D*-fructofuranosyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-*D*-glucopyranoside* (**20**). — Disaccharide **21** (30 mg, 84 mmol) was processed as described for **19**, leading to **20** as an hygroscopic syrup (60 mg, 100%), $[\alpha]_D^{20}$ +44° (*c* 1.12, chloroform).

Anal. Calc. for $C_{28}H_{38}O_{18}S \cdot H_2O$: C, 47.19; H, 5.66; S, 4.50. Found: C, 47.18; H, 5.63; S, 4.06.

*1,3,4,6,-Tetra-*O*-acetyl-β-* (**22**) *and -α-*D*-fructofuranosyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-*D*-glucopyranoside* (**24**). — (*a*) 2,3,4,6-Tetra-*O*-acetyl-1-thio-β-D-glucopyranose[21] (**17**, 1.5 g, 4 mmol), 1,3,4,6-tetra-*O*-benzyl-D-fructofuranose[14] (**1**, 2.2 g, 4 mmol), and zirconium chloride (683 mg, 2.93 mmol) in dichloromethane (60 mL) were stirred for 35 min at room temperature. Dichloromethane (100 mL) was added and the solution washed with a cold saturated aqueous solution of $NaHCO_3$, then with water, dried, and evaporated. The oily residue was purified by column chromatography (120 g, *E*) leading to a syrup (2.62 g, 73%), homogeneous in t.l.c. (*E*), which was, without further characterization, suspended in liquid ammonia (150 mL) at −78°. Fragments of metallic sodium were added slowly, with

magnetic stirring, until persistance of a blue coloration for more than 30 min. The excess of reagent was eliminated by addition of NH_4Cl and the mixture left to evaporate at room temperature. A suspension of the solid residue in 1:1 (v/v) pyridine–acetic anhydride (60 mL) was stirred for 16 h at room temperature. Methanol (75 mL) was added, and the mixture evaporated to an oil which was diluted with chloroform, and the solution processed as usual. Purification of the two-component (t.l.c., *D*) oily residue (4.02 g) by column chromatography (115 g, *D*) led successively to **22** and **24**.

Compound **22**: Syrup (915 mg, 33%), $[\alpha]_D^{20}$ −38° (*c* 1, chloroform); ^{13}C-n.m.r. (chloroform-*d*): δ 92.9 (C-2, Fru), 80.2, 79.5, 76.6, 76.1, 75.9, 74.2, 69.3, 68.4, 65.0, 64.7, and 62.4.

Anal. Calc. for $C_{28}H_{38}O_{18}S \cdot H_2O$: C, 47.19; H, 5.66; S, 4.50. Found: C, 46.83; H, 5.67; S, 4.51.

Compound **24**: Crystals (174 mg, 6%), m.p. 104° (ether), $[\alpha]_D^{20}$ +65° (*c* 0.83, chloroform); ^{13}C-n.m.r. (chloroform-*d*): δ 92.7 (C-2, Fru), 81.9, 80.0, 78.3, 76.1, 74.0, 69.4, 68.2, 64.9, 62.6, and 62.1.

Anal. Calc. for $C_{28}H_{38}O_{18}S$: C, 48.41; H, 5.51; S, 4.62. Found: C, 48.29; H, 5.22; S, 4.70.

(*b*) 1,3,4,6-Tetra-*O*-benzoyl-α-D-fructofuranose[26] (**7**, 596 mg, 1 mmol), 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose[21] (**17**, 546 mg, 1.5 mmol), and zirconium chloride (400 mg, 1.7 mmol) in dichloromethane (8 mL) were stirred for 60 h at room temperature. Dichloromethane (300 mL) was added and the solution was washed with a cold, saturated aqueous solution of $NaHCO_3$, then with water, dried, and evaporated. The oily residue, which showed in t.l.c. (*D*) a major component, was purified by column chromatography (*F*) leading to syrupy **15, 16** (486 mg, 51%), homogeneous in t.l.c. It was, without further characterization, dissolved in methanol and deacylated by stirring with M sodium methoxide in methanol (0.5 mL) for one night. Neutralization with Amberlite IRN 77, and evaporation to dryness in the presence of toluene led to a solid residue, homogeneous in t.l.c. (*G*) which was acetylated for one night in 1:1 (v/v) pyridine–acetic anhydride (10 mL). Addition of methanol, and processing of this mixture as usual gave a syrupy material which was purified by column chromatography (40 g, *D*), affording **24** (245 mg, 35%) after crystallization from ether. The β,β-anomer **22** (58 mg, 8%) was recovered by concentration of the mother liquors.

β-D-Fructofuranosyl 1-thio-β-D-glucopyranoside (**23**). — To a solution of the acetylated β,β-1-thiodisaccharide (**22**; 224 mg, 0.32 mmol) in methanol (5 mL), was added M sodium methoxide in methanol (0.1 mL), and the solution stirred overnight. Neutralization with Amberlite IRN 77 and evaporation led to a solid, hygroscopic foam (95 mg, 83%), $[\alpha]_D^{20}$ −146° (*c* 1, methanol); ^{13}C-n.m.r. (D_2O); δ 96.0 (C-2, Fru), 83.8, 82.1, 80.4, 78.2, 77.1, 75.7, 72.9, 70.4, 65.0, 63.3, and 61.7.

Anal. Calc. for $C_{12}H_{22}O_{10}S \cdot 2.5\ H_2O$: C, 35.73; H, 6.75; S, 7.95. Found: C, 35.80; H, 6.76; S, 7.65.

α-D-Fructofuranosyl 1-thio-β-D-glucopyranoside (1-thioisosucrose) (**25**). —

The acetylated α,β-1-thiodisaccharide **24** (224 mg, 0.32 mmol) was treated as for **22** leading, after evaporation of the methanol solution, to a microcrystalline powder (90 mg), m.p. 180–182°, $[\alpha]_D^{20}$ +26° (*c* 0.9, water); ^{13}C-n.m.r. (D_2O); δ 95.3 (C-2, Fru), 84.7, 84.2, 82.6, 80.8, 78.2, 76.9, 73.0, 70.3, 65.1, 61.7, and 61.6.

Anal. Calc. for $C_{12}H_{22}O_{10}S$: C, 40.22; H, 6.19; S, 8.95. Found: C, 40.34; H, 6.19; S, 8.69.

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