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Perspective

1,5-Anhydro-D-fructose; a versatile chiral building block: biochemistry and chemistry

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Abstract

There is a steadily increasing need to expand sustainable resources, and carbohydrates are anticipated to play an important role in this respect, both for bulk and fine chemical preparation. The enzyme α-(1→4)-glucan lyase degrades starch to 1,5-anhydro-D-fructose. This compound, which has three different functional properties, a prochiral center together with a permanent pyran ring, renders it a potential chiral building block for the synthesis of valuable and potentially biologically active compounds. 1,5-Anhydro-D-fructose is found in natural materials as a degradation product of α-(1→4)-glucans. The occurrence of lyases and the metabolism of 1,5-anhydro-D-fructose are reviewed in the biological part of this article. In the chemical part, the elucidated structure of 1,5-anhydro-D-fructose will be presented together with simple stereoselective conversions into hydroxy/amino 1,5-anhydro hexitols and a nojirimycin analogue. Synthesis of 6-*O*-acylated derivatives of 1,5-anhydro-D-fructose substituted with long fatty acid residues is carried out using commercially available enzymes. Those reactions lead to compounds with potential emulsifying properties. The use of protected derivatives of 1,5-anhydro-D-fructose for the synthesis of natural products is likewise reviewed. The potential utilization of this chemical building block is far from being exhausted. Since 1,5-anhydro-D-fructose now is accessible in larger amounts through a simple-enzyme catalyzed degradation of starch by α-(1→4)-glucan lyase, the application of 1,5-anhydro-D-fructose may be considered a valuable contribution to the utilization of carbohydrates as the most abundant resource of sustainable raw materials.

Keywords: 1,5-Anhydro-D-fructose; α-(1→4)-Glucan lyase; 1,5-Anhydro-D-glucitol; Biosynthesis; Carbohydrates; Chiral building block; Enzyme and enzyme reactions; Glycogenolysis; Antioxidants

1. Introduction

1,5-Anhydro-D-fructose (1,5AnFru, **1**), which was first synthesized in 1980,[1] is an attractive chiral building block, since it possesses different functional properties, ready for selective modifications (Fig. 1). Besides, 1,5AnFru has a permanent pyran form due to the lack of an anomeric carbon atom, and it can therefore be transformed into high-value products by selective reactions. To some extent this has been prevented by the limited access to **1** in larger amounts, since the known chemical synthesis is laborious and fairly low yielding.[1] Recently, a glucan lyase, which degrades starch and related oligosaccharides from the nonreducing end to give 1,5AnFru, was identified.[2] More than ten lyases isolated from different sources have been investigated. The production of the said carbohydrate has been optimized to such a level that **1** may now be considered a potential cheap chiral starting material for organic synthesis.[3]

This review article comprises the biochemistry and chemistry of 1,5AnFru as well as the characterization of the lyase, as a separate class of starch degrading enzymes. In the biochemical part, we will concentrate on the metabolism of 1,5AnFru in bacteria, algae, fungi and humans. The effects of **1** and its metabolite 1,5-anhydro-D-glucitol (1,5AnGlc-ol, **2**), on the general carbohydrate metabolism in cells, especially on starch and glycogen degradation to glucose and glucose-1-phosphate, catalyzed by amylase and phosphorylase, respectively, will also be discussed, together with their role in diseases such as diabetes. In the chemical part, the handling and utilization of **1** will be discussed. Despite the complex structure of 1,5AnFru (**1**), the use of the compound as a chiral starting material for preparation of valuable rare sugar derivatives, and for the synthesis of natural products and potential glycosidase inhibitors, will be presented.

This review should thus draw the attention to 1,5-anhydro-D-fructose as a viable chiral building block, as well as to the unusual enzymatic degradation of starch by α-(1→4)-glucan lyase.

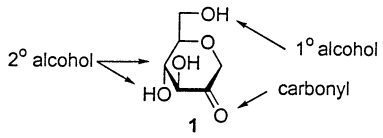


Fig. 1. 1,5-Anhydro-D-fructose.

2. Biochemistry

Occurrence of 1,5-anhydro-D-fructose in biological systems.—In 1986, 1,5AnFru was for the first time found to occur naturally in fungi (*Morchella* sp.).[4,5] At the same time it was observed that a subcellular membranous fraction from a soil bacterium (*Pseudomonas* sp.) catalyzed the oxidation of 1,5AnGlc-ol (**2**) to 1,5AnFru.[6,7] Since then, 1,5AnFru has been detected in a variety of organisms, comprising morels, in particular *Morchella* species,[5,8] red algae,[9,10] rat liver tissue,[11] cell lines of human erythroleukemia,[12] and *Escherichia coli*.[13] Typical concentration levels are listed in Table 1. In natural sources, 1,5AnFru has exclusively been detected as a monomeric compound and not as part of more complex polysaccharides.

Biosynthesis of 1,5-anhydro-D-fructose.—The biosynthesis of 1,5AnFru can occur either by an enzyme-catalyzed degradation of α-(1→4)-D-glucans or by an enzyme-catalyzed oxidation of 1,5AnGlc-ol.

Table 1
Occurrence of 1,5-anhydro-D-fructose (**1**) in selected organisms

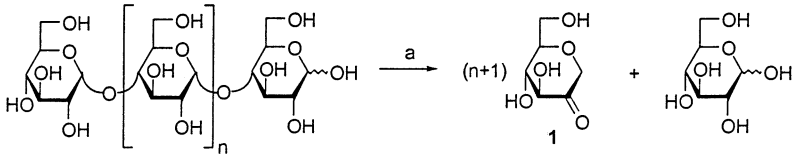
Isolated from	Concentration (μg/g fresh tissue)	Reference
E. coli	16.2 [a]	13
G. lemaneiformis	37–1900	10
Ceramium gobii	1.4	10
Ceramium rubrum	0.16	10
Furcellaria lubricalis	1.7	10
Cladophora rupestris	0.31	10
Ulva lactuca	0.062	10
Pilayella littoralis	0.10	10
Scytosiphon lomentaria	0.016	10
Rat liver	0.43	11

[a] μg/L culture medium.

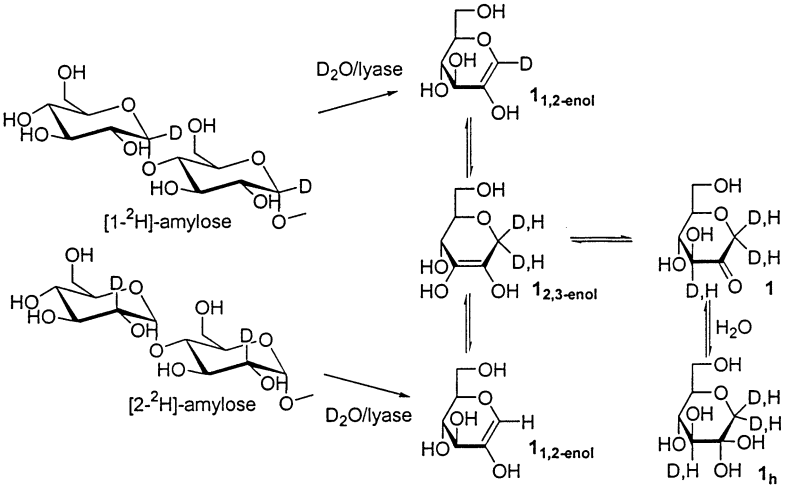
Enzyme-catalyzed degradation of α-(1→4)-D-glucans.—Maltosaccharides like starch and glycogen, which are substrates for the α-(1→4)-glucan lyase (EC 4.2.2.13), are partly degraded to 1,5AnFru (Scheme 1).[14]

When a linear α-(1→4)-glucan having $n+2$ glucose residues (e.g., maltose, maltosaccharides or amylose) was used as a substrate, $n+1$ mol of 1,5AnFru were formed together with 1 mol of glucose (Scheme 1). When an α-(1→4)-linked glucan with a branching point at a C-6 position (e.g., amylopectin or glycogen) was used as substrate, 1,5AnFru was produced together with a limit dextrin, as the degradation terminates at the branch point.[3,15] The mechanism of the enzyme-catalyzed cleavage has been studied by treating unlabeled, [1-^{2}H]- and [2-^{2}H]-labeled amylose, respectively, with the lyase in either water or D_2O.[15] The 1,5AnFru formed was analyzed by ^{1}H NMR spectroscopy to determine the amount and the position of deuterium labeling. It was found that by degradation of [1-^{2}H]- and [2-^{2}H]-labeled amylose in water and D_2O, ^{2}H was incorporated in the 1,5AnFru formed both at C-1 and C-3. A similar incorporation of ^{2}H at C-1 and C-3 was observed when unlabeled amylose was degraded in D_2O. Furthermore, a decreased reaction rate was observed in D_2O compared to water. Based on these observations the following mechanism for the exo-cleavage was proposed (Scheme 2).

The lyase cleaves the glycosidic bond simultaneously with abstraction of H-2, thereby forming a double bond between C-1 and C-2 to give the enol $\mathbf{1}_{1,2\text{-enol}}$. Incorporation of deuterium at C-1 and C-3 in the 1,5AnFru moiety was observed when unlabeled amylose was reacted with the lyase in D_2O. This indicates a tautomerization between the enol $\mathbf{1}_{1,2\text{-enol}}$ and the enediol $\mathbf{1}_{2,3\text{-enol}}$. The tautomerization between the two enols takes place in the active site of the lyase, since little or no exchange of H-1 or H-3 with deuterium took place when **1** was kept in D_2O, even in the presence of lyase. This model does not account for the incorporation of ^{2}H at C-3 into 1,5AnFru when [1-^{2}H]-amylose was reacted with



Scheme 1. (a) α-(1→4)-D-Glucan lyase (EC 4.2.2.13), H_2O.



Scheme 2. Degradation of [1-^{2}H]- and [2-^{2}H]-amylose by α-(1→4)-glucan lyase in D_2O.

the lyase in water. Neither does the model explain the results obtained when the degradation of [2-^{2}H]-amylose was carried out in water, nor the decreased reaction rate observed in D_2O. A more detailed catalytic mechanism of the α-(1→4)-glucan lyase has recently been proposed in analogy with α-glucosidases (Scheme 3).[16] Compared to other starch-degrading hydrolases, this enzyme is unique, as it yields an unsaturated carbohydrate.[9,15,17]

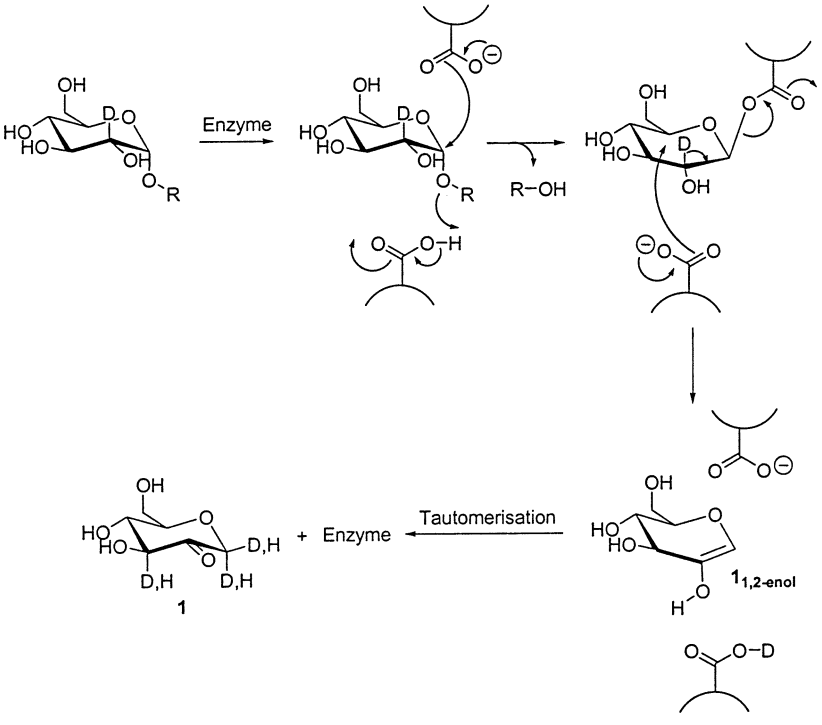
The glycosidic oxygen is initially protonated by the carboxylic acid residue in the active site of the enzyme. Due to the absence of water near the catalytic residues, 1,2-elimination occurs by aid of the second carboxylate group present in the active site resulting in formation of the enol $\mathbf{1}_{1,2\text{-enol}}$. In the active site the enol $\mathbf{1}_{1,2\text{-enol}}$ can tautomerize into the 2,3-enediol $\mathbf{1}_{2,3\text{-enol}}$ and into 1,5AnFru, as shown in Scheme 2. The tautomerization is catalyzed by the deuterated carboxylate acting in a proton/deuterium transfer relay (Scheme 3). This accounts for the incorporation of ^{2}H at C-1 and C-3 of **1** when [1-^{2}H]- or [2-^{2}H]-amylose was used as substrate and when unlabeled amylose was degraded in D_2O. A decreased reaction rate was observed in D_2O, as compared to the reaction rate in water. This observation would be in accordance with this mechanistic suggestion. In D_2O the carboxylic acid residues in the catalytic site of the lyase are probably deuterated. Since the dissociation energy for an O–D bond is higher than for an O–H bond, the rate of protonation of the glycosidic oxygen will be decreased, thus explaining the observed deuterium isotope effect.

The lyase has been isolated from several sources (Table 2). The major differences between algal and fungal lyases are the pH-optimum and substrate specificity. Algal lyases have a pH optimum around pH 4, while fungal lyases have pH optimum at pH 6.5. Furthermore, compared to algal lyases, the fungal lyases show much lower activity towards maltose than towards starch and glycogen.[3]

1,5-AnFru has been produced in 40–50% yield by lyasic degradation of starch,[2,22] and in 55–80% yield from soluble starch and amylopectin.[3] The process has attracted commercial interest and has been patented.[23–26] The enzyme has furthermore been cloned and expressed in *Aspergillus niger*,[27,28] which accomplishes a more rational, industrially compatible, production strategy.

*Enzyme-catalyzed oxidation of 1,5-anhydro-*D*-glucitol.*—The second route for biosynthesis of 1,5AnFru is the enzyme-catalyzed oxidation of 1,5AnGlc-ol by pyranose 2-oxidase (EC 1.1.3.10) in the presence of an electron acceptor, such as molecular oxygen (Scheme 4).[29]

The pyranose 2-oxidase from the fungus *Coriolus versicolor* KY 2912,[30] as well as from *Peniophora gigantea*,[31] has been immobilized for 1,5AnFru production from 1,5AnGlc-ol. The substrate is quantitatively converted into **1**. Compared with starch, 1,5AnGlc-ol is considered a rarer chemical.[32–34] However, the enzyme-catalyzed oxidation of 1,5AnGlc-ol to 1,5AnFru is of interest as an analytical tool, since **2** has been found in



Scheme 3. The α-(1→4)-glucan lyase enzymatic degradation of [2-^{2}H]-amylose in H_2O.

Table 2
Occurrence of lyase

Origin of lyase	pH optimum	Isolated as	Reference
G. lemaneiformis *Isozymes GLq1, GLq2, GLq3.*	3.8	purified form	3, 18
G. lemaneiformis *Isozymes GLs1, GLs2.*	4.1	purified form	3
Gracilaria verrucosa	not assayed	purified form	9
Gracilaria chorda	not assayed	purified form	19
Morchella costata	6.5	purified form	20
Morchella vulgaris	6.4	purified form	20
Peziza ostracoderma	not assayed	cell-free extract	20
Endophyte of *Gracilariopsis* sp.	not assayed	detected	21

human cerebrospinal fluid[35] and may be used as a clinical marker of diabetes.[36] Pyranose oxidase is a non-specific enzyme that accepts several hexoses as substrates.[31] Therefore, the presence of this enzyme in an organism does not always implement a biosynthetic production of 1,5AnFru.

Recently it has further been shown that also α-glucosidases are able to produce **1** by degradation of maltosaccharides.[37]

Metabolism of 1,5-anhydrofructose

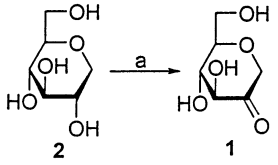
In mammals and humans. In mammals and humans, glycogen seems to be degraded by α-(1→4)-glucan lyase to 1,5AnFru,[11,12] which subsequently is reduced to 1,5AnGlc-ol by a NADPH-dependent 1,5AnFru specific reductase (Scheme 5).[38] This constitutes a third glycogenolytic pathway, in addition to the phosphorolytic and hydrolytic degradation sequences.[11]

The reverse reaction, oxidation of 1,5AnGlc-ol to 1,5AnFru, has neither been observed in human erythroleukemia cells nor in mammalian cells.[12] 1,5AnGlc-ol has been detected in the cerebrospinal fluid and plasma of humans,[35] and may be phosphorylated by an ATP-dependent hexokinase (Scheme 5) or directly secreted to urine.[39]

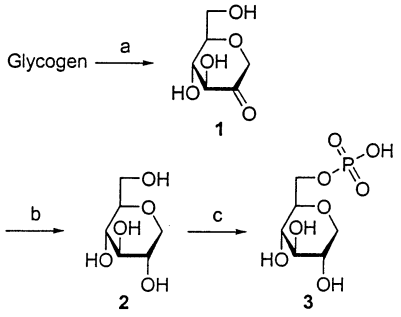
The physiological role of 1,5AnFru (**1**) and 1,5AnGlc-ol (**2**) in mammals is not well understood at present. It is known that **2** in the human body originates mainly from food. It has been shown that there is an equilibrium between oral intake, a small but steady de novo synthesis, and urine secretion.[39] Oral intake and de novo synthesis of 1,5AnGlc-ol contribute to increase the concentration of 1,5AnGlc-ol in the body, while secretion through urine decreases the amount of 1,5AnGlc-ol. Thereby the body maintains a constant level of 1,5AnGlc-ol.

Radioactive tracing experiments using [U-^{14}C]-1,5AnFru at a dosage of 100 mg/kg body weight, showed that most of **1** administered orally was absorbed in the digestive tract in male rats (S. Yu and J. Marcussen, unpublished data). Radioactive material was subsequently found secreted in urine (68.9%) and expired in air (CO_2, 11.7%), in cage wash (contributed from urine and faeces) (6.7%) and faeces (4.9%). The radioactivity remaining in the body 7 days after administration was 7.8%. The conversion of **1** to CO_2 in the expired air may indicate the participation of 1,5AnFru in the general carbon metabolism in rats. This may, however, also be contributed from the intestinal bacterial flora. Further studies to identify the nature of the radioactive material in urine and to study the contribution by intestinal bacteria in 1,5AnFru metabolism are needed.

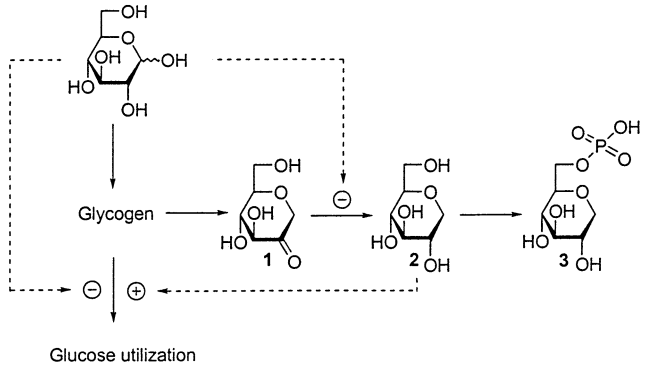
In bacteria. The same glycogenolytic pathway as found in mammals has been observed in *E. coli*, and the bacterium was used to provide further details of the regulation mechanism of glycogen metabolism (Scheme 6).[13]



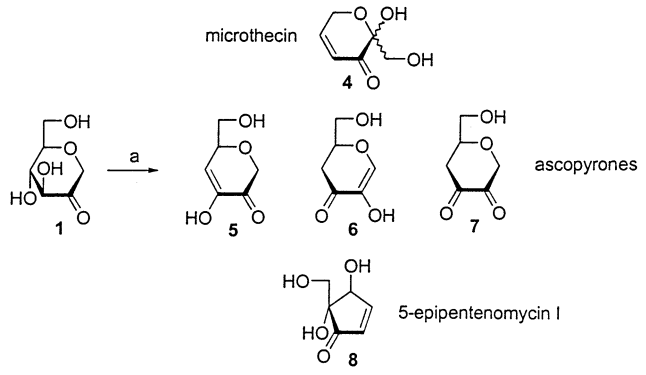
Scheme 4. (a) Pyranose 2-oxidase (EC 1.1.3.10), catalase (EC 1.11.1.6), O_2, H_2O, pH 7, 98%.



Scheme 5. The third glycogenolytic pathway. (a) α-(1→4)-Glucan lyase, H_2O; (b) 1,5-anhydro-D-fructose reductase (NADPH-dependent) (EC number not assigned yet); (c) hexose kinase (EC 2.7.1.1).



Scheme 6. Regulation of glycogen metabolism in *E. coli*. + and − indicate that the reaction rate is either stimulated or inhibited by the related metabolite.



Scheme 7. The metabolism of 1,5-anhydro-D-fructose in fungi and red algae. (a) 1,5-Anhydro-D-fructose dehydratase (a spectrum of secondary products are formed depending on the origin of the dehydratase).

Table 3
Compounds produced by metabolization of **1**

Organism	Precursor of	Reference
Morchella costata	microthecin	4, 40
Morchella vulgaris	microthecin	5, 40
G. lemaneiformis	microthecin	10
Plicaria leiocarpa	ascopyrone P	43
Terfezia sp.	ascopyrone T	43
Peziza echinospora	5-epipentenomycin I	42

1,5AnFru accumulates in the growth medium as glycogen accumulates in the cells of *E. coli*. When glucose is depleted in the medium, **1** is transported across the cell membrane into the cell and converted to 1,5AnGlc-ol, which serves as a messenger molecule that triggers the glycogen mobilization to supply the cells with glucose. 1,5AnGlc-ol is further converted to 1,5-anhydro-D-glucitol 6-phosphate (**3**) and secreted into the medium, no longer triggering the glycogen degradation. In the presence of glucose, the formation of 1,5AnGlc-ol from 1,5AnFru is inhibited and glycogen biosynthesis is favored.

In algae and fungi. In red algae and fungi, 1,5AnFru is predominantly formed by degradation of α-glucans, such as glycogen and starch, catalyzed by the enzyme α-(1→4)-glucan lyase.[2,8,9] In some algae and fungi, **1** is further metabolized to biologically active compounds. The pyrone, microthecin (**4**), is formed in several fungi[5,40] and in the red algae, *Gracilariopsis lemaneiformis*,[41] while 5-epipentenomycin I (**8**) is found in some Discomycetes,[42] and ascopyrones (**5**, **6**, and **7**) are detected in many Ascomycetes[43] (Scheme 7, Table 3).

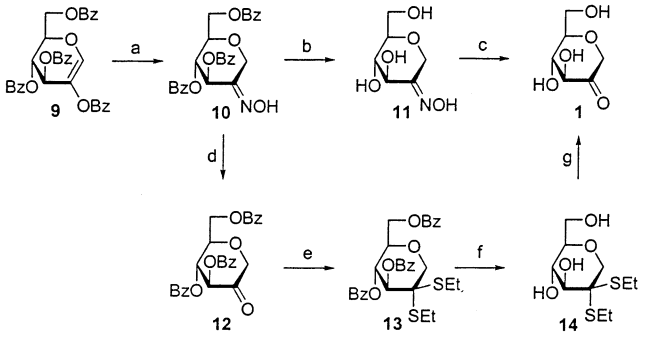
The conversion of 1,5AnFru to the previously mentioned biologically active compounds is catalyzed by species-specific 1,5-anhydrofructose dehydratases.[42] The formation of these secondary products from glycogen and starch via **1**, the so-called *Anhydrofructose Pathway*,[15] is induced under abiotic and biotic stress conditions, such as freeze-thaw, and exposure to organic solvents.[2,5,42] The depletion of nutrients and accumulation of metabolites in the fungal growth medium may also induce the formation of these secondary products (S. Yu, unpublished data). This is further supported by the fact that there are two regulatory sites in the promoter regions of the fungal lyase genes, the AREA and CREA sites. This may indicate that in the presence of glutamine, ammonium salts or glucose, it is unlikely that the lyase gene is expressed. This may reflect an interaction of glutamine or ammonium salts with AREA protein and that glucose interacts with the CREA protein inside the cell by modifying their conformations. The conformation modified AREA and CREA proteins may act negatively towards the expression of the lyase gene.[20]

Toxicology of 1,5-anhydro-D-fructose.—1,5-AnFru does not exhibit mutagenic activity, as shown by the Ames test[44,45] with four strains of *Salmonella*, which were sensitive to mutagens causing base-pair substitution and frameshift mutations (S. Yu and J. Marcussen, unpublished data). Neither does **1** seem to be toxic for filamentous fungi nor towards yeast. *A. niger* was able to grow in a minimal medium complemented with **1** at concentrations of 0.2, 0.5, 0.8, and 1.0% as the sole carbon source (S. Yu, unpublished data). The growth on a 1,5AnFru-supplemented medium was not as strong as the growth on a glucose-supplemented medium. At higher concentrations of **1** (1.0%), the growth was partially retarded. In contrast to *A. niger*, Baker's yeast was unable to assimilate **1**, as it could not grow on a medium containing **1** as the sole carbon source. 1,5-Anhydro-D-fructose (**1**) is apparently non-toxic to baker's yeast, as it could still grow after 12 days exposure to 1,5AnFru in concentrations ranging from 0.2 to 1.0%. Additional experiments indicate that the yeast could still grow on glucose, even in the presence of **1** at concentrations up to 0.47 M (S. Yu, unpublished data).

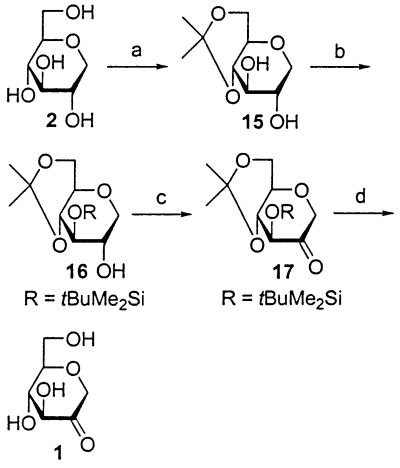
The acute toxicity of 1,5AnFru after oral administering was investigated in a group of five male and five female rats at a single dosage of 5 g/kg body weight (S. Yu and J. Marcussen, unpublished data). No toxic symptoms and sign of treatment were observed over a 14-day period, and the rats achieved normal body weight gains. Further experiments showed that orally administered **1** stimulates glucose utilization in mice by stimulating the hormone secretion of insulin and glucagon-like peptide 1 (GLP 1).[46]

3. Chemistry

*Synthesis of 1,5-anhydro-*D*-fructose.*—1,5-AnFru was synthesized in 1980 by Lichtenthaler et al.[1] via two different routes from 1,5-anhydro-2,3,4,6-tetra-*O*-benzoyl-D-*arabino*-hex-1-enitol (**9**) (Scheme 8). The benzoylated hydroxyglucal **9** is available from D-glucose in three steps.[47]



Scheme 8. (a) $NH_2OH{\cdot}HCl$, pyridine, 93%; (b) MeOH–MeONa, 65%; (c) CH_3CHO, HCl (aq), 60%; (d) CH_3CHO, HCl (aq), 88%; (e) EtSH, $BF_3{\cdot}Et_2O$, 85%; (f) MeOH–MeONa, 91%; (g) $HgCl_2$, $CdCO_3$, 73%.



Scheme 9. (a) $Me_2C(OMe)_2$, toluene-4-sulfonic acid, 92%; (b) *t*-$BuMe_2SiCl$, imidazole, 43%; (c) PDC, 86%; (d) 70% AcOH (aq), 69%.

Attempts to synthesize 1,5AnFru directly by base-catalyzed debenzoylation of **9** have been unsuccessful. Probably liberation of the carbonyl group and a subsequent β elimination of benzoic acid prior to deacylation at C-3 and C-4 accounts for the results obtained.[48] However, a successful conversion of **9** into the oxime, 1,5-anhydro-3,4,6-tri-*O*-benzoyl-D-fructose oxime (**10**), proceeded in an excellent yield without any β elimination.[49] The oxime **10** was debenzoylated using NaOMe–MeOH, followed by transoximination with acetaldehyde, giving 1,5AnFru in a low yield. It is noteworthy that no 3,4-elimination took place from the base-catalyzed deacylation of the perbenzoylated oxime **10**.

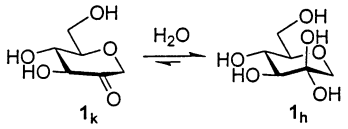
The second approach, which was more tedious[1] (Scheme 8), involved liberation of the keto function of **10** by transoximation with acetaldehyde. This afforded 1,5-anhydro-3,4,6-tri-*O*-benzoyl-D-fructose (**12**) in an excellent yield. Like the attempts to deacylate the protected 2-hydroxyglucal **9** under alkaline conditions, base-catalyzed deacylation of the perbenzoylated 1,5-anhydro-D-fructose **12** gave a complex mixture. This was probably again due to a 3,4-elimination, followed by an aldol condensation and/or benzilic acid rearrangements of the 2,3-diuloses formed. Thus, the carbonyl group was protected as the thioketal **13**, which could be debenzoylated to afford 1,5-anhydro-D-fructose diethyl dithioketal (**14**) in a good yield. Finally, demercaptalization of **14** gave 1,5AnFru.

The exact structure of anhydrous 1,5AnFru has been a topic of ongoing discussions. Lichtenthaler et al. showed, however, that the compound they had synthesized contained a carbonyl group, since reduction gave both the D-*gluco*- as well as the D-*manno*-1,5-anhydrohexitol.[1] Similarly, the corresponding 6-deoxy derivative, 1,5-anhydro-3,4-di-*O*-benzoyl-6-deoxy-D-fructose, was synthesized starting from 1,5-anhydro-2,3,4-tri-*O*-benzoyl-6-deoxy-D-*arabino*-hex-1-enitol.[1] Later, in 1986, another synthetic route to 1,5AnFru was published.[50] The method was based on oxidation at C-2 of 1,5AnGlc-ol, inspired by the observation that **1** was an oxidation product of the diabetes related-alditol, 1,5AnGlc-ol.[6]

The preparative four-step synthesis of 1,5AnFru from 1,5AnGlc-ol is shown in Scheme 9.

Acid-catalyzed 4,6-*O*-isopropylidene protection of 1,5AnGlc-ol, followed by monosilylation, gave **16** in a moderate isolated yield, probably due to formation of two monosilylated products. Oxidation of the 1,5-anhydro-alditol **16** using pyridinium dichromate in dichloromethane[51] gave the protected 1,5-anhydro-D-fructose derivative **17** (86%), followed by deprotection with aqueous acetic acid, to afford 1,5AnFru (69%).

Besides these three chemical procedures developed for the synthesis of 1,5AnFru, two enzymatic routes have also been developed. One is based on an enzyme-



Scheme 10. Structure of 1,5-anhydro-D-fructose in aqueous solution.

catalyzed oxidation at C-2 of 1,5AnGlc-ol, and the other one is based on an enzyme-catalyzed degradation of α-(1→4)-glucans.

Nippon Kayaku Co. Ltd. (Japan) developed a quantitative assay for 1,5AnGlc-ol on an analytical scale.[6,7] By treating 1,5AnGlc-ol with a subcellular membranous fraction isolated from *Pseudomonas* NK-85001 and other microorganisms, **2** was oxidized to 1,5AnFru. Quite recently the enzyme-catalyzed oxidation has been repeated on a preparative scale using a pyranose 2-oxidase from the basidiomycete fungus *P. gigantea* (EC 1.1.3.10) to give **1** in 98% yield[31] (Scheme 4).

Using a fungal enzyme the degradation of α-(1→4)-glucans to 1,5AnFru was reported by Baute et al. in 1988 (Scheme 1).[2] By this procedure 1,5AnFru could be prepared from starch in 40–50% yield. This process has attracted commercial interest, as previously mentioned.[23–26]

Structure of 1,5-anhydro-D-fructose.—Concerning the structure of 1,5AnFru: when referring to 1,5AnFru, 1,5-anhydro-D-fructose or **1**, we refer to the monomeric as well as the dimeric forms of 1,5AnFru, unless otherwise stated.

In aqueous solution. The structure of 1,5AnFru in aqueous solution has been elucidated by Göckel in 1984 by NMR spectroscopic studies[52] and by Okuda and co-workers in 1993 by NMR spectroscopy and FABMS studies.[30] Both investigations concluded that **1** is completely hydrated to 1,5-anhydro-D-fructose hydrate ($\mathbf{1}_h$), which adopts a $^4C_1$ chair conformation (Scheme 10). Other conceivable structures, such as a cyclic hemiketal,[5] were rejected.

Our investigations of an aqueous solution of 1,5AnFru by ^{13}C NMR spectroscopy showed that the hydration of **1** proceeded slowly.[53] From the ^{13}C NMR spectra it was obvious that besides **1** and $\mathbf{1}_h$ several other compounds were present. We later identified the additional compounds as dimeric forms of 1,5AnFru.[54] After dissolving anhydrous **1** in D_2O (100 mg in 1 mL), the complete hydration took approximately 5 h (Fig. 2). The signal at 93.5 ppm corresponds to C-2 in the hydrated form $\mathbf{1}_h$, while no signal was present around 200 ppm, corresponding to the absence of the carbonyl carbon of **1**.

Anhydrous 1,5-anhydro-D-fructose. Recently, the structure of anhydrous 1,5AnFru has been elucidated, both by NMR spectroscopic studies[55] and by X-ray crystallography.[54] A ^{13}C NMR spectrum of freeze-dried 1,5AnFru (**1**) in pyridine showed a complex mixture of compounds (Fig. 3).

In order to derivatize **1**, Lundt and co-workers acetylated the compound under acidic conditions and obtained, besides the peracetylated **1**, two crystalline compounds. X-ray analysis revealed that these compounds were both acetylated dimeric forms of 1,5AnFru (Fig. 4).[54] The dimeric forms are two C-2 epimeric spiroketals formed between C-2 and C-2′/C-3′, shown as dimer $\mathbf{1}_{d1}$ and dimer $\mathbf{1}_{d2}$ (Figs. 4 and 5).

Similarly, Köpper and Freimund have isolated and assigned the structures of the two acetylated dimeric forms of 1,5-anhydro-D-fructose (**1**) by NOESY NMR

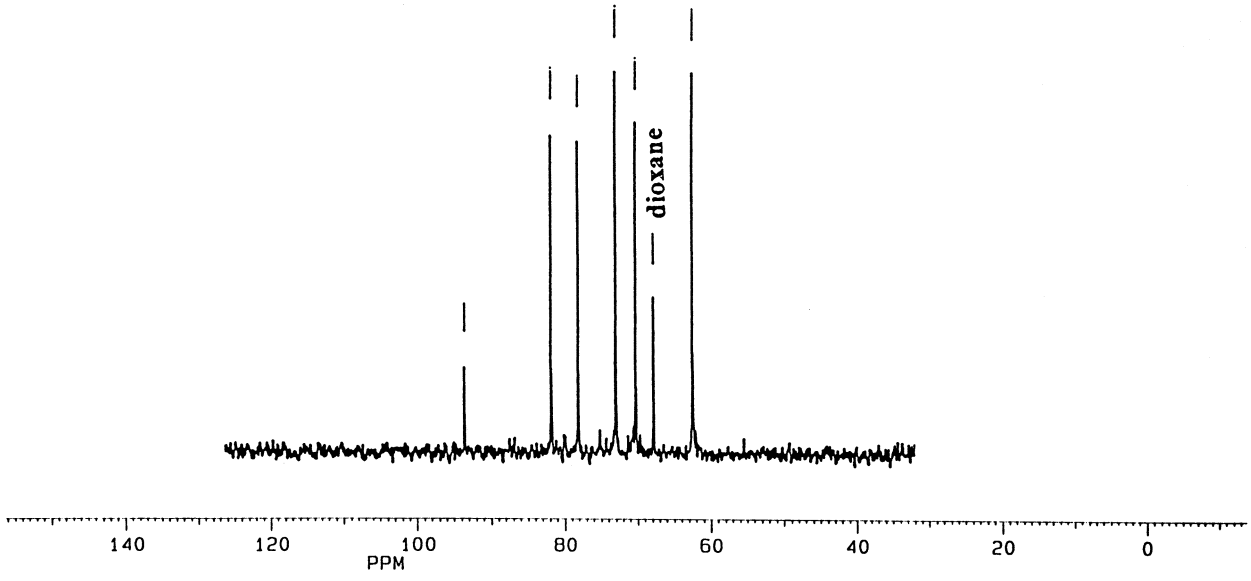


Fig. 2. A ^{13}C NMR spectrum of an aqueous solution of 1,5-anhydro-D-fructose.

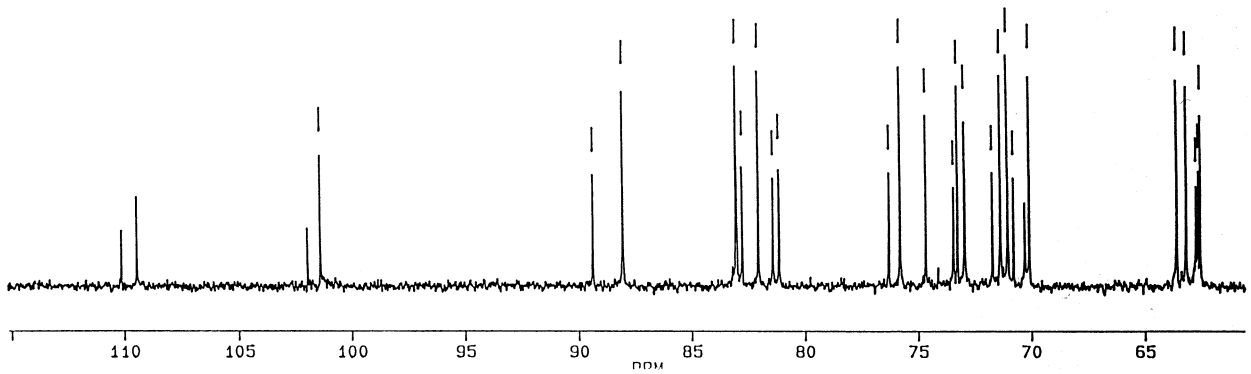


Fig. 3. A ^{13}C NMR spectrum of freeze-dried 1,5-anhydro-D-fructose (**1**) in pyridine-d_5 clearly demonstrates the complex nature of the anhydrous compound.

experiments.[55] Thus anhydrous 1,5AnFru consists of a monomeric ketone $\mathbf{1}_k$ and two isomeric dimeric forms, $\mathbf{1}_{d1}$ and $\mathbf{1}_{d2}$.

By analyzing the ^{13}C NMR spectra of anhydrous **1**, Lundt and co-workers have also shown that the composition of freeze-dried 1,5AnFru immediately after dissolving in pyridine-d_5, was 35% monomeric ketone $\mathbf{1}_k$, 43% dimer $\mathbf{1}_{d1}$ and 22% dimer $\mathbf{1}_{d2}$. After 1 week in pyridine the composition of anhydrous **1** was shifted to 28% of the monomeric ketone $\mathbf{1}_k$, 3% of dimer $\mathbf{1}_{d1}$ and 69% of dimer $\mathbf{1}_{d2}$. Therefore, dimer $\mathbf{1}_{d1}$ is the kinetically favored dimer, which is formed predominantly during the isolation of 1,5AnFru by freeze-drying, and dimer $\mathbf{1}_{d2}$ is the thermodynamically more stable dimer, which is formed slowly during equilibration of 1,5AnFru in pyridine.[54]

Köpper et al. found that solutions of **1** in Me_2SO-d_6 contained 76% monomeric ketone $\mathbf{1}_k$, 15% of dimer $\mathbf{1}_{d1}$ and 9% of dimer $\mathbf{1}_{d2}$ and that solutions of **1** in pyridine-d_5 contained 40% monomeric ketone $\mathbf{1}_k$, 50% of dimer $\mathbf{1}_{d1}$ and 10% of dimer $\mathbf{1}_{d2}$.[55]

The variations of the composition of **1** in pyridine is probably due to small differences in the isolation and workup procedures for 1,5AnFru.

Physicochemical properties.—Anhydrous 1,5AnFru is a hygroscopic amorphous solid that consists of monomeric ketone $\mathbf{1}_k$, dimer $\mathbf{1}_{d1}$ and dimer $\mathbf{1}_{d2}$. The optical rotation of anhydrous **1** is obviously dependent on the ratio between $\mathbf{1}_k$:$\mathbf{1}_{d1}$:$\mathbf{1}_{d2}$, and therefore on the workup procedure, and may thus vary. Upon measurement of the optical rotation of an aqueous solution of **1**, mutarotation is observed as **1** is slowly hydrated. Baute et al.[5] have reported $[\alpha]_D$ −40° (*c* 0.5, water) whereas Lichtenthaler et al.[1] have reported $[\alpha]_D$ −13° (*c* 0.5, water). These reported specific optical rotations vary due to differences in the composition of **1**, but also due to different degrees of hydration. Göckel[52] has reported the variation in optical rotation for anhydrous **1** when dissolved in water: $[\alpha]_D$ −18.9° (4 min), $[\alpha]_D$ −15.1° (20 min), $[\alpha]_D$ −10.5° (30 min) and $[\alpha]_D$ −9.3° (50 min, end-value, *c* 0.5, water). The final value corresponds to the optical rotation for 1,5-anhydro-D-fructose hydrate: $[\alpha]_D$ −9.3° (*c* 0.5, water).

Molecular dynamics simulations of 1,5AnFru in aqueous solution have shown that the water structure generated around the molecule was significantly different from the water structures generated around either α-D-glucose or α-D-mannose. This can be ascribed to a

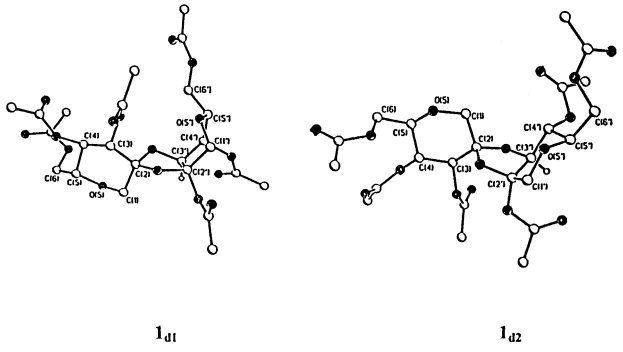


Fig. 4. X-ray structures of acetylated dimeric forms of 1,5-anhydro-D-fructose. Black atoms correspond to oxygens. The only illustrated hydrogen: H-3′.

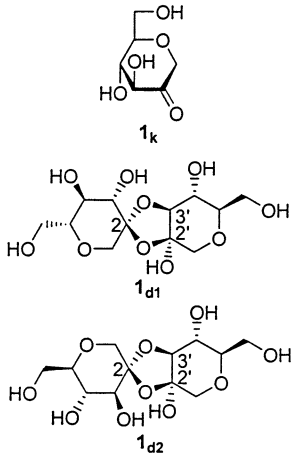
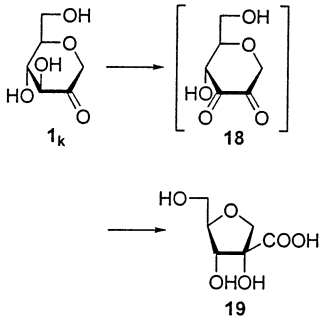
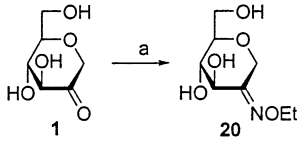


Fig. 5. Composition of anhydrous 1,5-anhydro-D-fructose.



Scheme 11. 3,5-Dinitrosalicylic acid assay of 1,5-anhydro-D-fructose. See text.



Scheme 12. (a) $EtONH_2 \cdot HCl$, quant.

more hydrophilic ring oxygen and a clearly hydrophobic ring methylene group in 1,5AnFru compared to the reference sugar molecules.[56]

It is important to obtain knowledge about hydration of carbohydrates in biological media since the conformation of the hydrated molecules can be altered due to hydrogen-bond formation. The carbohydrate–water structures thus play a definite role in enzymatic recognition processes.

Analytical assays.—Several assays for the detection and quantification of 1,5AnFru have been reported: TLC,[5] colorimetric methods,[57] ^{1}H NMR spectroscopy,[57] HPLC,[57] and GC–MS.[10,58]

On silica gel plates using 7:3 $CHCl_3$–MeOH as an eluent, 1,5AnFru has a an R_f value of 0.5. Deffieux et al.[5] found anisaldehyde to be the best reagent for detection, showing **1** as blue spots.

A colorimetric assay, in which 1,5AnFru was treated with 3,5-dinitrosalicylic acid (DNS) at pH 13–14 and at room temperature for 10 min, has been reported. Yu et al.[57] showed that the absorbance of the reaction mixture at 550 nm was proportional to the concentration of **1** in the range of 0.5–16 μmol/mL (80 μg–2.6 mg/mL). Ahmad has followed this reaction by ^{1}H and ^{13}C NMR spectroscopy and has shown that **1** was oxidized to 1′,4-anhydro-2-*C*-(hydroxymethyl)-D-arabonic acid (**19**) in 95% yield[59] (Scheme 11). In this reaction DNS is reduced to 3-amino-5-nitrosalicylic acid.[60] Ahmad[59] also showed that the carboxylic acid **19** could not be obtained using **6** or **7** as starting material (Scheme 7); therefore, **1** is probably oxidized by DNS to the hexo-2,3-diulose **18** initially, followed by a benzilic acid rearrangement to **19**.

Other colorimetric assays were tested by Yu et al.,[57] such as the alkaline ferricyanide,[61] the Somogyi-Nelson assay,[62] the BCA,[63] and the anthrone–tryptophan–sulfuric acid method;[64] however, the DNS assay was found to be superior since it was specific, due to the fact that reducing sugars such as glucose, maltose, maltosaccharides, starch and glycogen do not interfere.

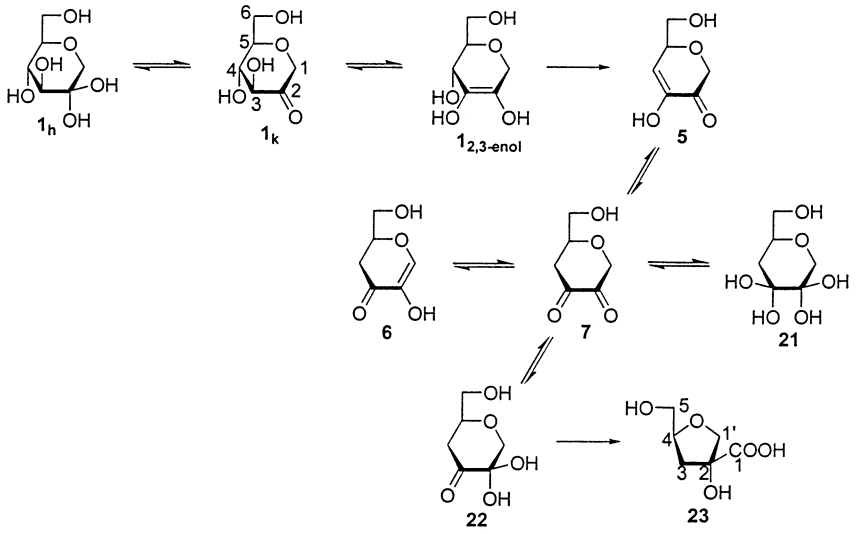
^{1}H NMR spectroscopy has been used for quantification of 1,5AnFru in an aqueous solution with *N*,*N*-dimethylformamide as the internal standard.[57] In water, both the monomeric and dimeric forms of 1,5AnFru are completely hydrated to $\mathbf{1}_h$, thereby making the quantification possible. For this method to be valid, it is important to leave the aqueous 1,5AnFru solution for several hours to assure complete conversion of the dimeric forms of **1** into the hydrate ($\mathbf{1}_h$).[53]

1,5AnFru has been quantified by HPLC with a carbohydrate Ca^{2+}-column and a differential refractometer, using water as eluent. The response was linear in a range of 1–384 μmol **1**/mL (0.16–62.26 mg **1**/mL) with an injection volume of 50 μL.[57] In this assay both monomeric and dimeric forms of 1,5AnFru were hydrated to 1,5-anhydro-D-fructose hydrate ($\mathbf{1}_h$), which was quantified. A short time allowed for formation of the hydrate might cause deviations, since the dimeric forms of 1,5-anhydro-D-fructose, $\mathbf{1}_{d1}$ and $\mathbf{1}_{d2}$, have a retention time different from that of 1,5-anhydro-D-fructose hydrate ($\mathbf{1}_h$). By Dionex anion-exchange chromatography with a pulsed amperometric detection unit, using 0.2 M NaOH as eluent, the linear range for 1,5AnFru was 12.5–250 nmol/mL (2.0–40.5 μg/mL) with an injection volume of 20 μL.[57] Due to the alkaline conditions applied in this assay, 1,5AnFru was probably transformed into the isosaccharinic acid **23** (Scheme 13), which then was quantified.

Silylated 1,5AnFru (pyridine–hexamethyldisilazane–chlorotrimethylsilane) has been detected by GC–MS in selected ion-monitoring (SIM) mode.[10] By monitoring the ions of m/z 273, 288 and 306, 70 pg of 1,5AnFru (as Me_3Si derivative) has been detected with an injection volume of 1 μL. This makes it possible to detect 14 ng of **1**/g fresh wt. algae if a 5-μL silylated sample is injected. The silylation conditions obviously result in formation of a substantial amount of silylated $\mathbf{1}_{d1}$ and $\mathbf{1}_{d2}$, which is not quantified. Since the workup conditions of 1,5AnFru have an impact on the ratio of $\mathbf{1}_k$:$\mathbf{1}_{d1}$:$\mathbf{1}_{d2}$, it is important that the sample is handled in exactly the same way each time. The authors also observed a deviation of 20–30% when they altered the workup conditions.

A complementary GC–MS method for quantification of 1,5AnFru[58] is based on the hydroxylaminolysis of **1** with *O*-ethylhydroxylamine to afford 1,5-anhydro-D-fructose *O*-ethyloxime (**20**) (Scheme 12).

Both monomeric as well as dimeric forms are converted into the oxime (see below), and thus the reaction is quantitative. If samples contain other ketones than **1**, the mixture of oximes may subsequently be separated



Scheme 13. Reaction of 1,5-anhydro-D-fructose (**1**) in strong aqueous base (pH 12–14).

by HPLC, followed by silylation and quantification by GC–MS in SIM mode. Using this methodology, 15 ng of **1**/g rat tissue has been detected.[11] In contrast to the above-mentioned GC–MS method,[10] both monomeric 1,5AnFru, $\mathbf{1}_k$ and dimeric 1,5AnFru, $\mathbf{1}_{d1}$ and $\mathbf{1}_{d2}$, are detected. Thus this method is probably the most sensitive one; however, it is also the most laborious.

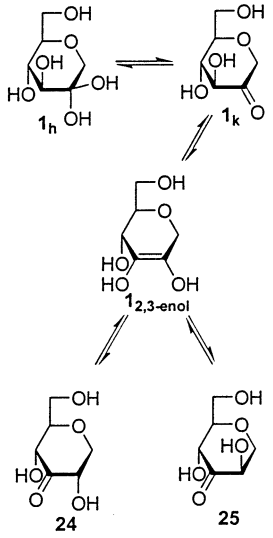
*Behavior of 1,5-anhydro-*D*-fructose in base.*—The reaction between reducing sugars and base, which has been a topic of intense studies,[65] has revealed that enolization, β elimination, benzilic acid rearrangement and retro-aldol reactions are the typical degradation pathways under basic conditions.

Ahmad has studied the behavior of 1,5AnFru in aqueous alkaline solution.[59] The reaction between 1,5AnFru and aqueous base was studied by monitoring samples of **1**, and of ^{13}C-labeled **1**, in water or D_2O at pH 12–14 by ^{1}H and ^{13}C NMR spectroscopy. Several compounds were formed, and some of the products were isolated by HPLC and identified as compounds **5**–**7**, **21** and **23** (Scheme 13). In an experiment performed in D_2O at pH 12–14, it was found that H-3 of 1,5AnFru was replaced by deuterium, indicating equilibrium between **1** and the enediol $\mathbf{1}_{2,3\text{-enol}}$ in aqueous NaOH. Based on these results, the mechanism shown in Scheme 13, was proposed: elimination of water from $\mathbf{1}_{2,3\text{-enol}}$ gave the enolone **5**, which was shown to be in equilibrium with the hexo-2,3-diulose **7**. A subsequent benzilic acid rearrangement gave the branched tetrahydrofuran carboxylic acid **23**, 1′,4-anhydro-3-deoxy-2-*C*-(hydroxymethyl)-D-*threo*-pentonic acid, as the main product.

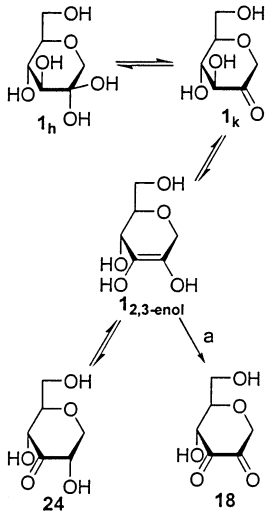
To further prove the pathway, Ahmad repeated the experiments using [1-^{13}C]- and [2-^{13}C]- substituted analogues of **1**. The results showed that C-1′ of the isosaccharinic acid **23** originated from C-1 of 1,5AnFru and C-1 of **23** originated from C-2 of **1**, thus supporting the proposed pathway. The isosaccharinic acid **23** has also been reported as an alkaline degradation product from cellulose.[66]

By means of ^{13}C NMR spectroscopy, our group has studied the behavior of 1,5AnFru in a weakly alkaline aqueous solution at pH 8.5 with the exclusion of oxygen (Scheme 14).[53] After 24 h the ^{13}C NMR spectra showed the presence of $\mathbf{1}_h$ and an isomer of **1** in the ratio 3:1. The isomer was assigned as a hex-3-ulose based on the spectra, and the ribo configuration on the analogy with the isomerization of 2-oxo-glucopyranosides in a pyridine solution to similar types of compounds.[67]

Analogous experiments performed in D_2O showed that H-3 of 1,5AnFru was completely replaced by



Scheme 14. Isomerization of 1,5-anhydro-D-fructose in weakly alkaline aqueous solution (pH 8.5).



Scheme 15. (a) O_2, $NaHCO_3$.

deuterium after 24 h and that deuterium had been incorporated at C-2 in the isomeric product. These findings suggested a 2,3-enediol as an intermediate in the formation of the thermodynamically more stable hex-3-ulose, 1,5-anhydro-D-*ribo*-hex-3-ulose (**24**). No β elimination, retro-aldol or benzilic acid rearrangement reactions were observed under these reaction conditions.[53] In a non-aqueous base, such as pyridine, a slow equilibration between the monomeric and dimeric forms of 1,5AnFru takes place as discussed above.[54]

*Behavior of 1,5-anhydro-*D*-fructose in acid.*—In contrast to base, we have observed that 1,5AnFru is quite stable toward aqueous acid. Dissolving **1** in aqueous 1 M HCl resulted in an immediate formation of the hydrate $\mathbf{1}_h$. In a non-aqueous acid, such as acetic acid, 1,5AnFru was stable at room temperature, but after refluxing for 24 h, a substantial decomposition occurred to give elimination products, as observed by ^{13}C NMR spectroscopy.[53]

*Oxidation of 1,5-anhydro-*D*-fructose.*—Under neutral conditions we have observed that 1,5AnFru, in contrast to L-ascorbic acid,[68] is not oxidized by molecular oxygen. In the presence of $NaHCO_3$, however, **1** was smoothly oxidized to 1,5-anhydro-D-*erythro*-hexo-2,3-diulose (**18**) within a week (75% conversion as shown by ^{13}C NMR) (Scheme 15). Minor amounts of the isomerization product **24** were present during the oxidation of **1**.[53] 1,5-Anhydro-D-*erythro*-hexo-2,3-diulose (**18**) has also been reported to be formed from 2- or 3-oxo-glucopyranosides under alkaline conditions.[67,69]

*1,5-Anhydro-*D*-fructose as a chiral building block in organic synthesis*

*Using unprotected 1,5-anhydro-*D*-fructose*. The use of 1,5AnFru as a building block for organic synthesis is complicated by existence of the dimeric forms $\mathbf{1}_{d1}$ and $\mathbf{1}_{d2}$,[54,55] which has different reactivity compared to the monomeric ketone $\mathbf{1}_k$. Ketone $\mathbf{1}_k$ has a carbonyl functionality, acidic protons α to the carbonyl (H-1a, H-1b, H-3), two secondary hydroxyl groups and one primary hydroxyl group. In the dimers $\mathbf{1}_{d1}$ and $\mathbf{1}_{d2}$, the carbonyl function is masked as a ketal and a hemiketal, and the dimers have three secondary hydroxyl groups and two primary hydroxyl groups, which all are sterically hindered, compared to similar hydroxy groups in the monomeric ketone $\mathbf{1}_k$. Due to these structural differences many reactions would afford mixtures of monomeric and dimeric forms, if applied to anhydrous 1,5AnFru.

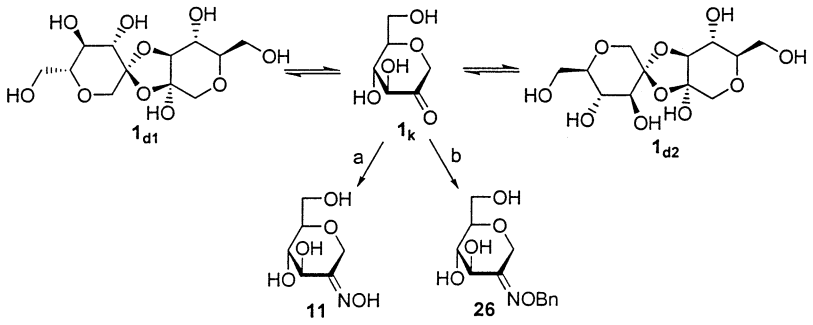
In order to simplify the mixture of mono- and dimeric forms of **1** it was shown that hydroxylaminolysis of **1** gave good to excellent yields of the corresponding oxime.[70] When treated with either hydroxylamine or *O*-benzylhydroxylamine, 1,5AnFru was quantitatively transformed into the corresponding monomeric oximes since hydroxylaminolysis only affected the carbonyl group, thereby shifting the equilibrium towards the monomeric ketone $\mathbf{1}_k$[70] (Scheme 16).

1,5AnFru oxime (**11**) was crystallized in a good yield and 1,5-anhydro-D-fructose *O*-benzyloxime (**26**) in an excellent yield, directly from the reaction mixture.

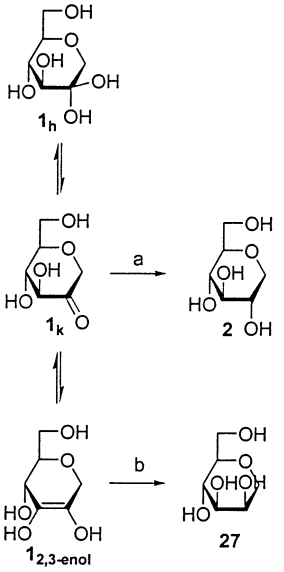
Hydroxylaminolysis of 1,5AnFru is an attractive and efficient derivatization procedure[71] which, as mentioned above, has been used for quantification of 1,5AnFru by a GC–MS method.[58]

Reductions:

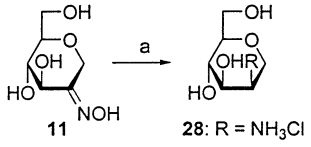
Synthesis of 1,5-anhydro-hexitols: Despite of the existence of isomeric forms, we have been able to reduce



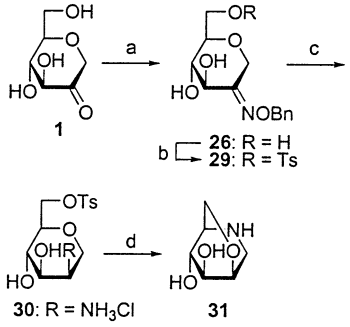
Scheme 16. (a) NH_2OH, EtOH, 66%; (b) NH_2OBn, EtOH, 91%.



Scheme 17. (a) $NaBH_4$, H_2O, 60%; (b) H_2–Pd/C, H_2O, 58%.



Scheme 18. (a) H_2–Pd/C, HCl, 72%.



Scheme 19. (a) NH_2OBn, 91%; (b) TsCl, pyridine, 68%; (c) H_2–Pd/C, HCl (MeOH), 82%; (d) Et_3N, quant.

the carbonyl function in 1,5AnFru stereoselectively, in good yields.[70] Dissolving **1** in water results in a slow hydration to 1,5AnFru hydrate ($\mathbf{1}_h$), in a nearly quantitative yield. However, since hydrate $\mathbf{1}_h$ is in equilibrium with the monomeric ketone $\mathbf{1}_k$ and the enediol $\mathbf{1}_{2,3\text{-enol}}$, it was possible to reduce an aqueous solution of **1** by catalytic hydrogenation and with $NaBH_4$[70] (Scheme 17).

Hydrogenation, using 5% Pd/C as a catalyst, gave a mixture of 1,5-anhydro-D-mannitol (**27**) and 1,5-anhydro-D-glucitol (**2**) in a 4:1 ratio. 1,5-Anhydro-D-mannitol (**27**) could be crystallized directly from this mixture in 58% yield upon addition of ethanol. The enediol $\mathbf{1}_{2,3\text{-enol}}$ is probably the reactive intermediate, since the hydrate $\mathbf{1}_h$ and the monomeric ketone $\mathbf{1}_k$ are relatively stable towards catalytic hydrogenation. Formation of the anhydromannitol as the main product indicated a cis addition to the double bond of $\mathbf{1}_{2,3\text{-enol}}$ from the least hindered site of the molecule, thus explaining the high stereoselectivity.[72] Reduction of 1,5AnFru in water using $NaBH_4$ gave predominantly the C-2 epimer, 1,5AnGlc-ol (**2**), which upon addition of ethanol crystallized in a 60% yield. In this case the monomeric ketone $\mathbf{1}_k$ is probably the reactive intermediate, since $NaBH_4$ reduces neither the hydrate $\mathbf{1}_h$ nor the enediol $\mathbf{1}_{2,3\text{-enol}}$. As reported by others in a similar reduction of cyclohexanones with $LiAlH_4$[73] an axial attack of the hydride was favored by torsional effects, thus explaining the stereoselectivity.

Both 1,5-anhydro-D-mannitol (**27**) (styracitol) and 1,5AnGlc-ol (polygalitol) belong to a rare class of naturally occurring sugars, many of which have shown biological activity.[74,75] Compared with previously reported procedures for the synthesis of 1,5-anhydro-D-mannitol (**27**) and 1,5-anhydro-D-glucitol (**2**),[32–34,76] the one-step procedure from 1,5AnFru seems to be very efficient.

Reductions: Synthesis of amino/hydroxy 1,5-anhydro-hexitols: Catalytic reduction of 1,5AnFru oxime (**11**) gave stereoselectively 2-amino-1,5-anhydro-2-deoxy-D-mannitol hydrochloride (**28**), which could be crystallized directly in a good yield (Scheme 18).[70] The formation of the manno-isomer, only, is similar to the result obtained by catalytic reduction of 1,5AnFru as discussed above (Scheme 17).

2-Aminodeoxy-1,5-anhydro-hexitols have been used for the synthesis of nucleoside analogues[77] and are biologically active compounds that have been shown to cause feeding suppression in rats.[78,79]

Synthesis of 2,6-anhydro-1-deoxymannojirimycin. Lundt et al. have synthesized the 2,6-anhydro analogue of the powerful mannosidase inhibitor 1,5-dideoxy-1,5-imino-D-mannitol (1-deoxymannojirimycin[80]) in four simple steps from 1,5AnFru using 1,5-anhydro-D-fructose *O*-benzyloxime (**26**) as a chiral building block (Scheme 19).[53]

Regioselective tosylation of **26** by standard conditions,[81] afforded the 6-*O*-tosylated oxime **29** in good yield, after purification by column chromatography. Hydrogenation of **29**, in the presence of a catalytic amount of 5% Pd/C, gave stereoselectively the manno configured amine **30**, which crystallized in excellent yield. To prevent deactivation of the catalyst by the amine formed,[82] the reaction was carried out in the presence of hydrochloric acid. 2-Amino-1,5-anhydro-2-deoxy-6-*O*-tosyl-D-mannitol hydrochloride (**30**) was finally treated with triethylamine, and intramolecular nucleophilic displacement of the tosyloxy group by the amine afforded the target compound, 2,6-anhydro-1,5-dideoxy-1,5-imino-D-mannitol (**31**) in a quantitative yield. Thus 2,6-anhydro-1-deoxymannojirimycin (**31**)

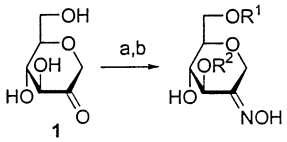
was prepared in four steps and 51% overall yield from 1,5AnFru.

Recently, the synthesis of **31** from 1,5AnGlc-ol **2** has been described in nine steps and in an overall yield of 11%.[83,84] Tanaka et al. showed that 2,6-anhydro-1-deoxymannojirimycin hydrochloride (**31**, HCl) was inactive ($K_i > 1$ mM) against α- and β-D-glucosidases and α-D-mannosidase.[83] We have shown that **31** was a weak inhibitor of α-L-fucosidase from bovine kidney, while other tested glycosidases were not inhibited to any significant extent.[53]

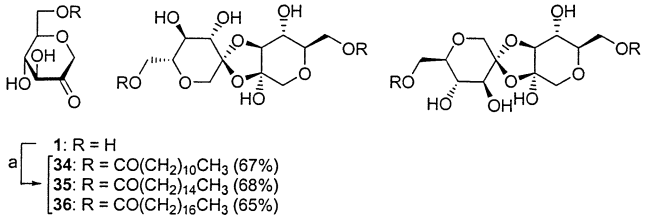
Since 2,6-anhydro-1-deoxymannojirimycin (**31**) is a conformationally restricted azasugar, it is forced to adopt a boat-like conformation ($^{2,5}B$). This conformation is different from the conformation of the natural substrates and thus also from their transition states in the active site of the glycosidases.[85]

*Synthesis of 6-*O*-acyl-1,5-anhydro-*D*-fructose*. In the discussion above on the structure and chemical reactions of 1,5AnFru, the $\mathbf{1}_{2,3\text{-enol}}$-form has been shown to be present in the equilibrium mixture. This 2,3-enediol structure is analogous to that in ascorbic acid, probably being responsible for the antioxidative properties of that compound. Thus a potential antioxidative property of 1,5AnFru was suggested.[22] Since 1,5AnFru is a degradation product from α-(1→4)-glucans, we investigated fatty acid esters of **1** as inexpensive antioxidants/surfactants with hitherto unknown properties.[86] Lundt and co-workers undertook the synthesis of fatty acid monoesters of **1**, both by a chemical procedure as well as by enzymatically catalyzed reactions.[71]

The synthesis was complicated by the dimeric nature of 1,5AnFru as discussed above.[54,55] Since both monomeric and dimeric forms of **1** can be acylated, a partial acylation of **1** could afford seven products, all of them containing at least one acylated primary hydroxyl group. But, since both monomeric $\mathbf{1}_k$ as well as $\mathbf{1}_{d1}$ and $\mathbf{1}_{d2}$ reacts with hydroxylamine or *O*-benzylhydroxylamine to yield the corresponding monomeric oximes, the acetylated mixture could be converted quantitatively into the corresponding oximes of selectively acylated monomers, as monitored by ^{13}C NMR spectroscopy.[71]



Scheme 20. (a) $CH_3(CH_2)_{10}COCl$ (1 equiv), pyridine; (b) NH_2OH, pyridine.



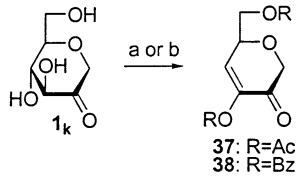
Scheme 21. (a) Fatty acid (3 equiv), Novozym 435, acetone, 3 Å molecular sieves.

Treatment of 1,5AnFru with one equivalent of lauroyl chloride in pyridine and subsequent treatment with hydroxylamine, afforded 1,5-anhydro-6-*O*-lauroyl-D-fructose oxime (**32**) (50%) and 1,5-anhydro-3-*O*-lauroyl-D-fructose oxime (**33**) (11%), after column chromatography (Scheme 20). For comparison, methyl α-D-glucopyranoside has been regioselectively acylated at the primary hydroxyl group in 27% yield.[87] The improved regioselectivity in the acylation of **1**, compared to methyl α-D-glucopyranoside, was probably due to steric hindrance of the secondary alcohol groups in the dimeric forms of **1**.

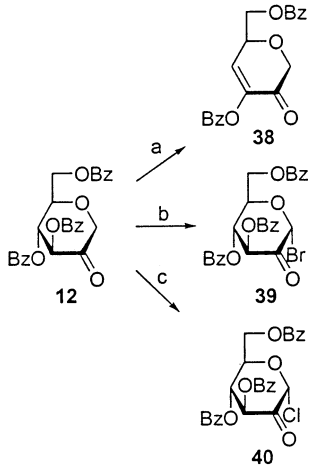
In an attempt to improve the regioselectivity in the acylation of 1,5AnFru, an activated ester of lauric acid, *N*-lauroylthiazolidine-2-thione, was used as acyl donor. Despite promising results from the regioselective acylation of methyl D-glycopyranosides under such conditions,[88,89] the acylation of **1** with the activated ester in pyridine, using a catalytic amount of 4-dimethylaminopyridine or NaH, resulted in degradation under the basic conditions. After derivatisation with hydroxylamine a small amount of the 3-*O*-acylated 1,5AnFru oxime **33** could be isolated. Preparation of 6-*O*-acylated derivatives from **1** with lauric acid under Mitsunobu conditions[90] was unsuccessful, probably due to degradation of **1** under the basic conditions.

Our interest was then turned to the lipase-catalyzed regioselective acylation[91–95] of 1,5AnFru. Among the enzymes studied, Novozym 435 was found to be superior, also on a preparative scale. The conversion of **1** was monitored by ^{13}C NMR spectroscopy after treatment of the reaction mixture with hydroxylamine. In this way the reaction has been optimized to give a quantitative conversion of **1** to the 6-*O*-acylated product with lauric, palmitic or stearic acid, respectively, as acyl donors.[71] The enzyme-catalyzed acylations were performed on a gram scale, and the products were further purified by column chromatography and isolated in good yields: 1,5-anhydro-6-*O*-lauroyl-D-fructose (**34**) (67%), 1,5-anhydro-6-*O*-palmitoyl-D-fructose (**35**) (68%), and 1,5-anhydro-6-*O*-steroyl-D-fructose (**36**) (65%). Obviously, the 6-*O*-acyl-1,5-anhydro-D-fructose derivatives **34**–**36** consist both of monomeric and dimeric forms (Scheme 21).

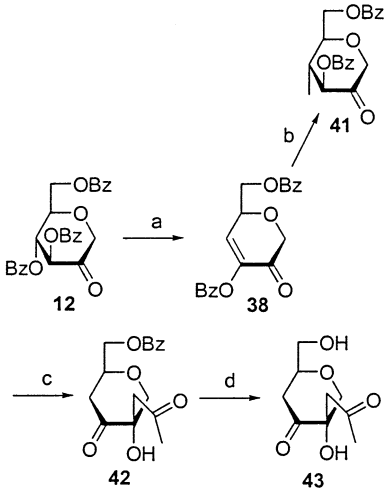
We have synthesized 1,5-anhydro-6-*O*-palmitoyl-D-fructose (**36**) on a 10-g scale, and the compound is currently being tested as an antioxidant/emulsifier in selected food products.



Scheme 22. (a) Ac_2O, pyridine, 55%; (b) BzCl, pyridine, 56%.



Scheme 23. (a) NaOAc, 92%; (b) $Br_2/h\nu$, 78%; (c) $SOCl_2$, AIBN, 87%.



Scheme 24. (a) $NaHCO_3$, $>84\%$; (b) $Li_2Cu_3Me_5$, 40%; (c) (i) $MeC(OLi)CH_2$; (ii) H_2O, 60%; (d) NaOMe–MeOH.

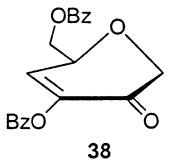


Fig. 6. The dihydropyranone **38** in the ^0E conformation.

*Using protected 1,5-anhydro-*D*-fructose*

Synthesis of new chiral building blocks. In connection with the structure elucidation of 1,5AnFru,[54] the compound was acetylated under acidic conditions (Ac_2O, $HClO_4$), giving low to moderate yields of the acetylated dimeric forms (Fig. 4). When **1** was acetylated under alkaline conditions, the elimination product, 3,6-di-*O*-acetyl-1,5-anhydro-4-deoxy-D-*glycero*-hex-3-en-2-ulose (**37**), was formed in a moderate yield, together with peracetylated dimeric forms of **1** as byproducts. By a similar methodology, base-catalyzed benzoylation of 1,5AnFru gave a moderate yield of the crystalline 1,5-anhydro-3,6-di-*O*-benzoyl-4-deoxy-D-*glycero*-hex-3-en-2-ulose (**38**) (Scheme 22). The pyranoid enolone ester **38**, and other unsaturated carbohydrate derivatives, have been thoroughly investigated as versatile chiral building blocks.[96]

Besides the routes leading to 1,5AnFru (Schemes 8 and 9), only a few preparative procedures using protected 1,5AnFru derivatives as building blocks have been described. Thus, perbenzoylated 1,5AnFru **12** (Scheme 8) has been treated with base to afford the enolone **38** in an excellent yield.[97] Photobromination of **12** gave 3,4,6-tri-*O*-benzoyl-α-D-*arabino*-hexopyranosyl-2-ulose bromide (**39**)[98,99] while treatment with thionyl chloride and AIBN as radical initiator gave the corresponding chloride **40**,[98,99] both in good yields (Scheme 23). In connection with the exploration of the reaction potential of **39**, this compound has been treated with Bu_3SnH and $h\nu$/AIBN to afford perbenzoylated 1,5AnFru **12**.[100]

Synthesis of bissetone and palythazin. Bissetone (**43**), which has shown a weak inhibitory activity towards the Gram-negative bacteria, *Pseudomonas aeruginosa* and *Xanthomonas campestris*, has been isolated from the encrusting gorgonian *Briareum polyanthes*. The relative stereochemistry was assigned by X-ray diffraction studies.[101] Lichtenthaler and co-workers synthesized (−)-bissetone (**43**) from 1,5-anhydro-3,4,6-tri-*O*-benzoyl-D-fructose (**12**) in three simple steps as shown in Scheme 24, thereby proving the absolute configuration.[102]

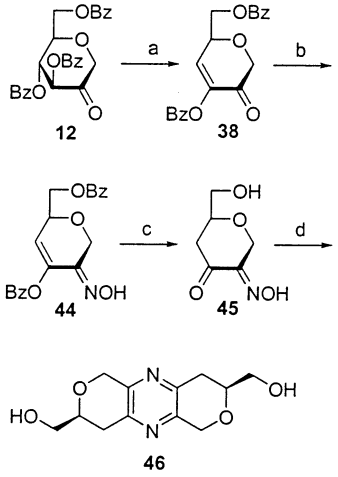
Perbenzoylated 1,5-anhydro-D-fructose (**12**) was prepared according to Scheme 8 and subsequently treated with base to afford the conjugated ketone **38** in an excellent yield, as discussed above (Scheme 23). 1,2-Addition of the lithium enolate of acetone, followed by a partial debenzoylation, gave stereoselectively the partially protected bissetone derivative **42**. The stereoselectivity was probably due to complexation between lithium and the ring and carbonyl oxygens of **38** in the ^0E conformation (Fig. 6).[102]

Debenzoylation of **42** with NaOMe–MeOH gave (−)-bissetone (**43**) in excellent yield. The ^1H and ^{13}C NMR spectra and the specific optical rotation were identical with the corresponding data of bissetone iso-

lated from *B. polyanthes*. Since the synthesized (−)-bissetone (**43**) originated from D-glucose, the absolute stereochemistry of bissetone isolated from *B. polyanthes* could thus be assigned as (2S,5S).

Nucleophilic addition of an organocuprate to **38** showed that in this case a 1,4-addition takes place. In this way 1,5-anhydro-3,6-di-*O*-benzoyl-4-deoxy-4-*C*-methyl-D-fructose (**41**) was synthesized in a moderate yield.[102]

Another natural product, (S,S)-palythazin (**46**), has been synthesized from perbenzoylated 1,5AnFru **12** (Scheme 25).[97] Palythazin was originally isolated from *Palythoa tuberculosa*,[103] and only the relative stereochemistry had been established. Synthesis of **46** from the 1,5AnFru derivative **12** established the absolute configuration of the palythazin. Thus, hydroxylaminolysis of **38** and subsequent debenzoylation gave the monooxime **45** in excellent yield. The coupling between two molecules of **45** was accomplished in three steps: reduction, condensation and finally oxidation, giving a moderate overall yield of (*S*,*S*)-palythazin (**46**), identical with the natural compound.



Scheme 25. (a) NaOAc, 92%; (b) NH_2OH, 96%; (c) NaOMe–MeOH, 89%; (d) (i) H_2–Pd/C, HCl; (ii) neutralization; (iii) O_2, 57%.

4. Conclusion and perspectives

The identification and isolation of the starch-degrading enzyme α-(1→4)-glucan lyase has facilitated the access to 1,5-anhydro-D-fructose. The lyases degrade starch and other α-(1→4)-glucans from the nonreducing end to give monomeric 1,5AnFru. The process can be further optimized to provide 1,5AnFru even in bulk quantities. 1,5AnFru is considered a valuable chiral building block, and the potential has already been shown by the use of protected derivatives for the synthesis of natural compounds. Recent investigations have led to a detailed knowledge of the structure of 1,5AnFru and its stereoselective conversions into amino/hydroxy 1,5-anhydro hexitols, and further to the synthesis of a modified noijirimycin derivative, a weak inhibitor of α-L-fucosidase. The synthesis of 6-*O*-acylated derivatives of 1,5AnFru using the commercially available Novozym 435, gave that compounds act as emulsifiers and are of potential interests as antioxidants.

The type of compounds that might be prepared from 1,5AnFru range from biologically active fine chemicals to bulk chemicals with potential applications in the food and pharmaceutical industry.

The possibilities for the enzyme-catalyzed conversion of starch to 1,5AnFru and further into a diversity of valuable compounds seem attractive. It is a great challenge to explore the possibilities of this now easily accessible chiral building block. The present work thus represents only a small step in the efficient utilization of the most abundant natural resources available to man: the carbohydrates.

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