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Note

1,5-Anhydro-D-fructose: regioselective acylation with fatty acids

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Abstract

Regioselective acylation of 1,5-anhydro-D-fructose was performed with dodecanoic acid to give 1,5-anhydro-6-*O*-dodecanoyl-D-fructose, chemically in 50% yield and enzymatically in quantitative yield. Quantitative conversions were also obtained using hexadecanoic and octadecanoic acids as acyl donors.

Keywords: 1,5-Anhydro-D-fructose; Antioxidant; Regioselective acylation; Lipases

1. Introduction

1,5-Anhydro-D-fructose (**1**) can tautomerise into an enediol (Fig. 1) and might therefore be a potential antioxidant (similar to L-ascorbic acid [1]). Antioxidants are important both in food and biological systems. In food, they are particularly advantageous to prevent oxidation of unsaturated fatty acids, while in biological systems they protect cell components, such as membranes, proteins and DNA, from oxidative damage. Since 1,5-anhydro-D-fructose is easily produced as a degradation product from α-(1→4)-glucans such as starch [2–4], it is an attractive alternative to already existing antioxidants.

Food systems typically consist of a heterogeneous suspension of water and oil. Due to its hydrophilicity, 1,5-anhydro-D-fructose (**1**) will partition into the aqueous phase, and thus does not have the potential to protect the lipid phase from oxidation. Since it is known that fatty acid esters of carbohydrates have amphiphilic properties [5] and tend to be concentrated on the surface between an aqueous and a lipid phase, these compounds might protect a lipid phase better than **1** against oxidation. This class of compounds, non-ionic surfactants based upon sugars, has widespread uses besides food applications, e.g., extraction of proteins from membranes and solubilisation of hydrophobic molecules. They have also shown anti-HIV and anti-*Aspergillus fumiga-*

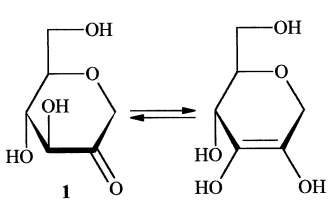

Fig. 1. Enolisation of 1,5-anhydro-D-fructose.

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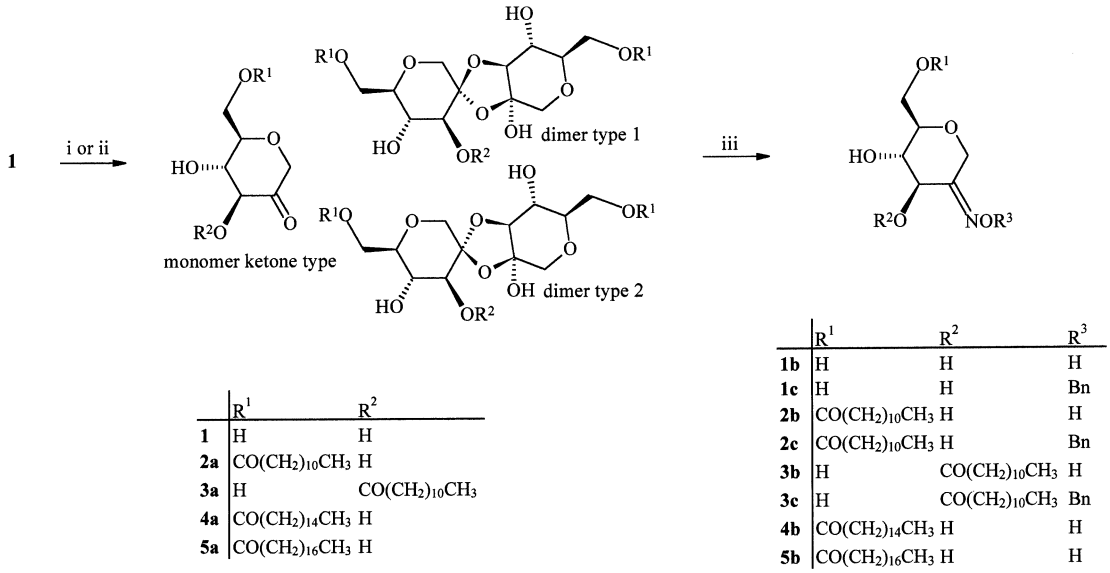

	R^1	R^2
1	H	H
2a	$CO(CH_2)_{10}CH_3$	H
3a	H	$CO(CH_2)_{10}CH_3$
4a	$CO(CH_2)_{14}CH_3$	H
5a	$CO(CH_2)_{16}CH_3$	H

	R^1	R^2	R^3
1b	H	H	H
1c	H	H	Bn
2b	$CO(CH_2)_{10}CH_3$	H	H
2c	$CO(CH_2)_{10}CH_3$	H	Bn
3b	H	$CO(CH_2)_{10}CH_3$	H
3c	H	$CO(CH_2)_{10}CH_3$	Bn
4b	$CO(CH_2)_{14}CH_3$	H	H
5b	$CO(CH_2)_{16}CH_3$	H	H

i) $CH_3(CH_2)_{10}COCl$ / pyridine; ii) $CH_3(CH_2)_nCOOH$ / Novozym 435 / 3 Å m.s. / acetone; iii) NH_2OR^3·HCl, pyridine.

Scheme 1. Synthesis of 6-*O*-acyl-1,5-anhydro-D-fructose.

tus activity [6]. Furthermore, fatty acid esters of carbohydrates are biodegradable, harmless and inexpensively made from renewable sources [7].

Monoacylation of 1,5-anhydro-D-fructose (**1**) could in principle be accomplished at OH-3, OH-4 or OH-6, but to avoid tedious protection and deprotection of **1** as well as to preserve the antioxidative activity [8,9], it is desirable to selectively acylate the primary hydroxyl group. It was recently found that 1,5-anhydro-D-fructose exists as a hydrated monomer in aqueous solutions. In non-aqueous systems, **1** exists as two dimeric spiroketals in equilibrium with the monomeric ketoform [10,11]. An incomplete regioselective acylation of the primary hydroxyl group could therefore theoretically afford seven products. To make the monitoring of the reaction easier, the reaction mixture was derivatised with hydroxylamine, which quantitatively converted the products to the corresponding monomeric oximes.

At low temperature, 1,5-anhydro-D-fructose (**1**) was regioselectively acylated with dodecanoyl (lauroyl) chloride in pyridine. After derivatisation with hydroxylamine and chromatographic separation, 6-*O*-dodecanoyl-1,5-anhydro-D-fructose oxime (**2b**) (50%) and 3-*O*-dodecanoyl-1,5-anhydro-D-fructose oxime (**3b**) (11%) were isolated. Alternatively the carbonyl group was protected with *O*-benzylhydroxylamine to give the corresponding *O*-benzyloximes **2c** and **3c**. By the relative downfield shift of the proton on the acyloxylated carbon, the position of the acyl group was assigned by ^1H NMR spectroscopy. For comparison, methyl α-D-glucopyranoside can be regioselectively acylated at the primary hydroxyl group with dodecanoyl chloride in pyridine at low temperature in 27% yield [12]. The improved regioselectivity in the acylation of **1** was probably due to steric hindrance in the dimeric forms of 1,5-anhydro-D-fructose (**1**). Employing activated esters such as *N*-lauroylthiazolidine-2-thiones [13] or Mitsunobu conditions [14] did not improve the regioselectivity, but caused some degradation of 1,5-anhydro-D-fructose (**1**), probably caused by the addition of NaH, DMAP or PPh_3. The only product isolated from the regioselective acylation of **1** with *N*-lauroylthiazolidine-2-thiones followed by derivatisation was the 3-*O*-dodecanoyl-1,5-anhydro-D-fructose oxime (**3b**). 3-O-Acylation therefore stabilises **3a** against degradation, as known for similar derivatives of L-ascorbic acid [8,9].

An attractive alternative to the chemical procedures is the use of lipases and proteases for the regioselective acylation of the primary

Table 1
Enzymatic acylation of 1,5-anhydro-D-fructose (**1**) with dodecanoic acid [a]

Dodecanoic acid (mol/mol **1**)	Solvent	3 Å Molecular sieve (w/w **1**)	Temperature (°C)	Reaction time (h)	Conversion
1	*tert*-BuOH	–	40	24	21%
1	*tert*-BuOH	1 (powd.)	40	24	56%
1	*tert*-BuOH	1 (powd.)	40	72	62%
1	acetone	1 (powd.)	20	24	55%
1	*tert*-BuOH	5	45	24	56%
1	*tert*-BuOH	10	45	24	61%
1	*tert*-BuOH	20	45	24	66%
3	*tert*-BuOH	20	45	24	73%
3	*tert*-BuOH	20 (powd.)	45	24	78%
3	*tert*-BuOH	20 (powd.)	45	48	quantitative
3	acetone	20	20	72	quantitative

[a] A typical experiment: 1,5-anhydro-D-fructose (200 mg), dodecanoic acid, Novozym 435 (200 mg), 3 Å molecular sieves and solvent (10 mL) were stirred for the time and at the temperature indicated. The solids were filtered off and washed with pyridine. The filtrate was concentrated, pyridine (2 mL) and NH_2OH, HCl (200 mg) were added and stirred for 1 h, followed by ^{13}C NMR analysis.

hydroxyl group of unprotected sugars with fatty acids [15–17]. The enzymatic acylation of sugars with fatty acids can be accomplished in organic solvents [18], in a minimum of organic solvent (adjuvant technique) [19] or solvent-free [20,21]. Recently, supercritical CO_2 has also been used [22].

Initially, commercially available lipases from nine different organisms were screened for the regioselective acylation of 1,5-anhydro-D-fructose (**1**) using dodecanoyl *O*-acetoxime as the acylating agent. In pyridine lipases isolated from *Candida antarctica*, *Pseudomonas cepacia*, *Pseudomonas fluorescens* and hog pancreas were active. Since polar solvents such as pyridine can inhibit the lipase activity, the screening was also performed in a mixture of 2:1 *tert*-BuOH–pyridine . It was found that the same lipases were active together with the lipase from *Candida cylindracea*. Since the lipase from *C. antarctica* showed higher activity compared with the others in both screenings, this lipase was chosen for further studies.

The enzymatic (Novozym 435[1]) acylation of 1,5-anhydro-D-fructose (**1**) with dodecanoic acid to 1,5-anhydro-6-*O*-dodecanoyl-D-fructose (**2a**) was studied in different solvents by varying the sugar–fatty acid ratio, the amount of drying agents (molecular sieves), temperature and reaction time (Table 1). Simply stirring equimolar amounts of **1**, lauric acid and lipase in *tert*-BuOH at 40 °C for 24 h afforded 21% conversion. The reaction is reversible. Several factors can shift the equilibrium towards complete conversion: product crystallisation, removal of water and the sugar–fatty acid ratio. Since the acylation products from 1,5-anhydro-D-fructose (**1**) did not crystallise from the reaction mixture, the removal of water and the **1**–fatty acid ratio were investigated. Addition of excess drying agent (3 Å molecular sieves) increased the conversion to 66% after 24 h. It is noteworthy that a large excess of molecular sieves was necessary in order to optimise the enzymatic reaction, much more than the capacity (20%, w/w) of the molecular sieves. Increasing the amount of fatty acid to 3 molar equiv, together with an excess of molecular sieves, afforded 73% conversion. By using powdered molecular sieves, 78% conversion was obtained after 24 h. Extending the reaction time to 48 h afforded a quantitative conversion of **1** to the 6-*O*-dodecanoyl ester **2a**, as shown by ^{13}C NMR. When the enzymatic acylation was carried out in refluxing *tert*-BuOH or simply in melted fatty acid at 70 °C under low pressure in order to remove water [20,21], several by-products were formed, such as elimination and diacylation products.

[1] Novozym 435 is the trademark for an immobilised *C. antarctica* lipase from Novo Nordisk A/S.

To further simplify the reaction, acetone was used as solvent and the enzymatic acylation was performed at ambient temperature. Under these reaction conditions, **1** was converted in quantitative yields to the corresponding 6-*O*-dodecanoyl-, 6-*O*-hexadecanoyl and 6-*O*-octadecanoyl esters (**2a**, **4a**, and **5a**, respectively) in three to seven days.

Like anhydrous 1,5-anhydro-D-fructose (**1**), the 6-O-acylated products **2a**, **4a**, and **5a** form dimeric ketals in pyridine solution [10,11]. Thus the acylation products were present as mixtures of monomeric ketone, dimer type 1 and dimer type 2 as seen by ^{13}C NMR (Scheme 1).

In conclusion, a promising enzymatic synthesis of 6-O-acylated derivatives of 1,5-anhydro-D-fructose has been achieved. These types of biodegradable fatty acid esters of 1,5-anhydro-D-fructose (**1**) will be tested in food systems both as antioxidants and emulsifiers.

2. Experimental

General methods.—1,5-Anhydro-D-fructose (**1**) was freeze-dried from an aqueous solution and residual water co-evaporated with toluene three times before use. Molecular sieves were activated with a heatgun in a flow of Ar. Pyridine and *tert*-BuOH were distilled from CaH_2 onto 3 Å molecular sieves, acetone was dried over $MgSO_4$ and dodecanoyl chloride was distilled prior to use. The enzymes used in the screenings were dried in vacuo over 'Blue Gel' for two days. The reactions were performed in an Ar atmosphere, unless otherwise stated. ^1H NMR and ^{13}C NMR spectra were recorded on Bruker instruments AC 250 (ambient temperature) or AM 500 (300 K). For ^1H and ^{13}C NMR spectra in pyridine the solvent peak was used as reference. For ^{13}C NMR in D_2O, acetone ($\delta = 30.89$ ppm) was used as internal reference. Reported melting points are uncorrected. Optical rotations were measured on a Perkin–Elmer 241 polarimeter. Microanalyses were carried out by the Microanalytical Department, University of Copenhagen and Institut für Physikalische Chemie, University of Vienna. HRMS was carried out by the Chemistry Department, University of Copenhagen. TLC was performed on precoated Kieselgel 60 F_{254} and spots were visualised by spraying with a mixture of 1.5% (w/w) $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, 1% (w/w) $Ce(SO_4)_2\cdot 4H_2O$ and 10% (v/v) H_2SO_4, followed by heating. Flash chromatography was performed on Silica Gel 60 (Grace AB Amicon, 35–70 μm). HPLC was performed on a Waters 8NVC186 reverse-phase column, with an evaporative light scattering detector (ELSD). Evaporations were performed in vacuo at temperatures below 45 °C. In the screenings the lipases from *Aspergillus niger*, *C. antarctica*, *C. cylindracea*, *Mucor miehei*, *P. cepacia*, *P. fluorescens*, *Rhizopus arrhizus*, *Rhizopus niveus* and hog pancreas were tested (Lipase Basic Kit from Fluka).

1,5-Anhydro-6-O-dodecanoyl-D-fructose oxime (**2b**) *and 1,5-anhydro-3-O-dodecanoyl-D-fructose oxime* (**3b**).—1,5-Anhydro-D-fructose (**1**, 1.0 g, 6.3 mmol) was dissolved in pyridine (30 mL) and the solution was cooled to 0 °C. Dodecanoyl chloride (1.50 mL, 6.3 mmol) was added dropwise and the reaction mixture was stirred for 1 h at 0 °C and 3 h at ambient temperature. Then MeOH (1 mL) was added and, after 1 h, $NH_2OH\cdot HCl$ (660 mg, 9.5 mmol) was added and the mixture was stirred for 24 h. The reaction mixture was concentrated and the products were separated by column chromatography (64 g silica, eluted with 3:1 to 1:2 hexane–EtOAc) to give 1,5-anhydro-6-*O*-dodecanoyl-D-fructose oxime (**2b**, 1.1 g, 50%), mp 89 – 96 °C, and 1,5-anhydro-3-*O*-dodecanoyl-D-fructose oxime as a syrup (**3b**, 250 mg, 11%). Analytical samples of **2b** and **3b** were prepared by further column chromatography.

Table 2
^{13}C NMR of isolated compounds in pyridine-d_5 [a]

Compound	C-1	C-2	C-3	C-4	C-5	C-6
1b	61.9	155.7	74.8	74.0	82.7	63.2
1c	62.2	157.8	74.4	73.5	82.8	63.1
2b	61.6	155.0	74.9	73.8	79.2	64.8
2c	61.9	157.1	74.4	73.4	79.2	64.6
3b	62.1	151.3	75.5	70.4	82.9	62.8
3c	62.1	152.5	75.0	70.1	82.8	62.4
4b	61.6	155.0	74.9	73.8	79.2	64.8
5b	61.6	155.0	74.9	73.8	79.2	64.8

[a] δ-Values (ppm) for **1b**, **1c**, **2b**, **2c**, **3b** (125 MHz), **3c**, **4b**, **5b** (62.9 MHz); signals were assigned by C–H correlated NMR; solvent peaks used as reference (δ 149.9, 135.5 and 123.5 ppm).

Table 3
^{1}H NMR of isolated compounds in pyridine-d_5 [a]

Compound	H-1a	H-1b	H-3	H-4	H-5	H-6a	H-6b
1b	4.27 (d) $J_{1a,1b}$ 14.5	5.68 (d)	4.89 (d) $J_{3,4}$ 7.5	4.38 (broad t) $J_{4,5}$ 8.5	3.99 (ddd) $J_{5,6a}$ 6.0 $J_{5,6b}$ 2.5	4.31 (dd) $J_{6a,6b}$ 11.5	4.49 (dd)
1c	4.21 (d) $J_{1a,1b}$ 15.0	5.42 (d)	4.83 (d) $J_{3,4}$ 7.5	4.39 (dd) $J_{4,5}$ 8.5	3.95 (ddd) $J_{5,6a}$ 5.5 $J_{5,6b}$ 2.5	4.29 (dd) $J_{6a,6b}$ 12.0	4.45 (dd)
2b	4.24 (d) $J_{1a,1b}$ 14.5	5.68 (d)	4.87 (d) $J_{3,4}$ 8.0	4.22 (broad t) $J_{4,5}$ 8.5	4.06 (ddd) $J_{5,6a}$ 6.5 $J_{5,6b}$ 2.0	4.77 (dd) $J_{6a,6b}$ 12.0	4.97 (dd)
2c	4.17 (d) $J_{1a,1b}$ 14.5	5.40 (d)	4.80 (d) $J_{3,4}$ 7.5	4.21 (broad t) $J_{4,5}$ 8.5	4.02 (ddd) $J_{5,6a}$ 6.5 $J_{5,6b}$ 2.5	4.75 (dd) $J_{6a,6b}$ 11.5	4.91 (dd)
3b	4.24 (d) $J_{1a,1b}$ 14.5	5.63 (d)	6.16 (d) $J_{3,4}$ 7.5	4.53 (broad t) $J_{4,5}$ 8.0	3.95 (ddd) $J_{5,6a}$ 5.5 $J_{5,6b}$ 2.5	4.29 (dd) $J_{6a,6b}$ 12.0	4.41 (dd)
3c	4.08 (d) $J_{1a,1b}$ 15.0	5.38 (d)	6.10 (d) $J_{3,4}$ 7.0	4.52 (broad t) $J_{4,5}$ 8.5	3.92 (ddd) $J_{5,6a}$ 5.0 $J_{5,6b}$ 2.5	4.28 (dd) $J_{6a,6b}$ 12.0	4.39 (dd)
4b	4.23 (d) $J_{1a,1b}$ 14.5	5.70 (d)	4.87 (d) $J_{3,4}$ 8.0	4.23 (broad t) $J_{4,5}$ 8.0	4.07 (ddd) $J_{5,6a}$ 6.5 $J_{5,6b}$ 2.0	4.77 (dd) $J_{6a,6b}$ 12.0	4.98 (dd)
5b	4.21 (d) $J_{1a,1b}$ 14.5	5.68 (d)	4.86 (d) $J_{3,4}$ 8.0	4.21 (broad t) $J_{4,5}$ 8.5	4.05 (ddd) $J_{5,6a}$ 6.5 $J_{5,6b}$ 2.0	4.76 (dd) $J_{6a,6b}$ 12.0	4.97 (dd)

[a] δ-Values (ppm) and coupling constants (J, Hz) for **1b**, **1c**, **2b**, **2c**, **3b** (500 MHz), **3c**, **4b**, **5b** (250 MHz); signals were assigned by 2D-COSY NMR; solvent peaks were used as reference (δ 8.71, 7.55 and 7.19 ppm).

1,5-Anhydro-6-*O*-dodecanoyl-D-fructose oxime (**2b**): mp 90 – 101 °C; $[\alpha]_D$ −13.5° (*c* 1.1, MeOH); ^{13}C and ^{1}H NMR: Tables 2 and 3. Anal. Calcd for $C_{18}H_{33}NO_6$: C, 60.14; H, 9.25; N, 3.90. Found: C, 60.35; H, 9.18; N, 4.10.

1,5-Anhydro-3-*O*-dodecanoyl-D-fructose oxime (**3b**): $[\alpha]_D$ −45.7° (*c* 1.1, MeOH); ^{13}C and ^{1}H NMR: Tables 2 and 3. HRMS Anal. Calcd for $C_{18}H_{33}NO_6$: 359.2309 [M]. Found: 359.2297.

*1,5-Anhydro-6-*O*-dodecanoyl-*D*-fructose* O-*benzyloxime* (**2c**) *and 1,5-anhydro-3-*O*-dodecanoyl-*D*-fructose* O-*benzyloxime* (**3c**).—Using the same procedure as above, 1,5-anhydro-D-fructose (**1**, 227 mg, 1.4 mmol) was acylated with lauroyl chloride (0.332 mL, 1.4 mmol) in pyridine (10 mL) followed by addition of $BnONH_2$·HCl (236 mg, 1.5 mmol). After stirring for 24 h, the mixture was concentrated. The products were separated by column chromatography (20 g silica, eluted with 3:1 to 1:1 hexane–EtOAc) to give 1,5-anhydro-6-*O*-dodecanoyl-D-fructose *O*-benzyloxime (**2c**, 287 mg, 46%) and 1,5-anhydro-3-*O*-dodecanoyl-D-fructose *O*-benzyloxime (**3c**, 43 mg, 7%). Analytical samples of **2c** and **3c** were prepared by further column chromatography.

1,5-Anhydro-6-*O*-dodecanoyl-D-fructose *O*-benzyloxime (**2c**): mp 45 – 50 °C; $[\alpha]_D$ −9.6° (*c* 1.0, MeOH); ^{13}C and ^{1}H NMR: Tables 2 and 3. HRMS Anal. Calcd for $C_{25}H_{39}NO_6$: 450.2856 [M + H$^+$]. Found: 450.2854.

1,5-Anhydro-3-*O*-dodecanoyl-D-fructose *O*-benzyloxime (**3c**): mp 48 – 51 °C; $[\alpha]_D$ −51.7° (*c* 1.3, MeOH); ^{13}C and ^{1}H NMR: Tables 2 and 3. HRMS Anal. Calcd for $C_{25}H_{39}NO_6$: 450.2856 [M + H$^+$]. Found: 450.2845.

*Screening of lipases for the regioselective esterification of 1,5-anhydro-*D*-fructose* (**1**).—Screening 1: A solution of 1,5-anhydro-D-fructose (**1**, 308 mg, 1.9 mmol), dodecanoyl *O*-acetoxime (480 mg, 1.9 mmol) and pyridine (13 mL) was prepared. A volume of 1 mL of this solution was added to approximately 10 mg of the corresponding lipase in an Eppendorf tube, and the mixture was shaken (1000

rpm) at 30 °C for four days and centrifuged. A sample (0.5 mL) of the supernatant was added to 0.5 M NH_2OH in pyridine (0.5 mL) and left for 24 h. The solution was analysed by HPLC. The lipases isolated from *C. antarctica*, *P. cepacia*, *P. fluorescens* and hog pancreas were active. Screening 2: The enzymatic reactions were performed in 2:1 *tert*-BuOH–pyridine using the same procedure as screening 1. The four lipases above and the lipase from *C. cylindracea* were active.

*Enzymatic regioselective acylation of 1,5-anhydro-*D*-fructose* (**1**). —*1,5-Anhydro-6-*O*-dodecanoyl-*D*-fructose* (**2a**). 1,5-Anhydro-D-fructose (**1**, 1.1 g, 6.7 mmol), dodecanoic acid (4.0 g, 20.2 mmol), Novozym 435 (1.1 g) and 3 Å molecular sieves (21.8 g) were suspended in acetone (95 mL) and stirred for 72 h. Pyridine (30 mL) was added to the reaction mixture and the solids were filtered off and washed with pyridine (2 × 30 mL). The filtrate was concentrated in vacuo to a syrup (6.3 g). To a sample (377 mg) was added pyridine (1.5 mL) and NH_2OH·HCl (116 mg). The solution was stirred for 1 h and was shown by ^{13}C NMR to consist of only 1,5-anhydro-6-*O*-dodecanoyl-D-fructose oxime (**2b**). The remaining syrup (5.9 g) was purified by column chromatography (100 g silica, eluted with 4:1:0 then 0:4:1 hexane–EtOAc–EtOH) to afford 1,5-anhydro-6-*O*-dodecanoyl-D-fructose (**2a**) as an amorphous solid (1.5 g, 67%). This preparation was shown by ^{13}C NMR (relative signal intensities) [11] to consist of monomeric ketone (δ 82.6, C-5) (20%), dimer type 1 (δ 88.3, C-3′) (60%) and dimer type 2 (δ 89.1, C-3′) (20%). To a sample of this mixture (72 mg) was added NH_2OH·HCl (75 mg) and pyridine (1 mL). The solution was stirred for 1 h and was shown by ^{13}C NMR to consist of only 1,5-anhydro-6-*O*-dodecanoyl-D-fructose oxime (**2b**), identical with the product described above.

*1,5-Anhydro-6-*O*-hexadecanoyl-D-fructose* (**4a**). Using the same procedure as for **2a**, a mixture of 1,5-anhydro-D-fructose (**1**, 1.0 g, 6.3 mmol), hexadecanoic (palmitic) acid (4.9 g, 19.0 mmol), Novozym 435 (1.0 g), acetone (95 mL) and 3 Å molecular sieves (20.6 g) was stirred for 72 h, followed by workup, to give a crude residue (6.7 g). To a sample (340 mg) was added pyridine (1.5 mL) and NH_2OH·HCl (116 mg). The solution was stirred for 1 h and was shown by ^{13}C NMR to consist of only 1,5-anhydro-6-*O*-hexadecanoyl-D-fructose oxime (**4b**). The remaining syrup (6.4 g) was purified by column chromatography (100 g silica, eluted with 4:1:0 then 0:4:1 hexane–EtOAc–EtOH) to afford 1,5-anhydro-6-*O*-hexadecanoyl-D-fructose (**4a**) as an amorphous solid (1.6 g, 68%). This preparation was shown by ^{13}C NMR (relative signal intensities) [11] to consist of monomeric ketone (δ 82.6, C-5) (20%), dimer type 1 (δ 88.3, C-3′) (20%) and dimer type 2 (δ 89.1, C-3′) (60%). To a sample (58 mg) was added pyridine (1 mL) and NH_2OH·HCl (57 mg). The solution was stirred for 1 h and was shown by ^{13}C NMR to consist of only 1,5-anhydro-6-*O*-hexadecanoyl-D-fructose oxime (**4b**). An analytical sample of **4b** was prepared by column chromatography (eluted with 2:1 to 1:1 hexane–EtOAc): mp 97 – 104 °C; $[\alpha]_D$ – 13.4° (*c* 1.1, MeOH); ^{13}C and ^{1}H NMR: Tables 2 and 3. Anal. Calcd for $C_{22}H_{41}NO_6$: C, 63.59; H, 9.94; N, 3.37. Found: C, 63.80; H, 9.69; N, 3.65.

*1,5-Anhydro-6-*O*-octadecanoyl-D-fructose* (**5a**). By the same procedure as used for the preparation of **2a**, a mixture of 1,5-anhydro-D-fructose (**1**, 1.1 g, 6.6 mmol), octadecanoic (stearic) acid (5.7 g, 20.0 mmol), Novozym 435 (1.1 g), acetone (95 mL) and 3 Å molecular sieves (21.7 g) was stirred for 168 h. Workup gave a crude residue (7.5 g). To a sample (230 mg) was added pyridine (1.0 mL) and NH_2OH·HCl (110 mg). The solution was stirred for 1 h and was shown by ^{13}C NMR to consist of only 1,5-anhydro-6-*O*-octadecanoyl-D-fructose oxime (**5b**). The remaining residue (7.3 g) was purified by column chromatography (100 g silica, eluted with 8:1:0 then 0:4:1 hexane–EtOAc–EtOH) to afford 1,5-anhydro-6-*O*-octadecanoyl-D-fructose (**5a**) as an amorphous solid (1.8 g, 65%). This preparation was shown by ^{13}C NMR (relative signal intensities) [11] to consist of monomeric ketone (δ 82.6, C-5) (25%), dimer type 1 (δ 88.3, C-3′) (50%) and dimer type 2 (δ 89.1, C-3′) (25%). To a sample (69 mg) was added pyridine (0.7 mL) and NH_2OH·HCl (73 mg). The solution was stirred for 1 h and was shown by ^{13}C NMR to consist of only 1,5-anhydro-6-*O*-octadecanoyl-D-fructose oxime (**5b**). An analytical sample of **5b** was prepared

by column chromatography (eluted with 2:1 to 1:1 hexane–EtOAc): mp 99 – 105 °C; $[\alpha]_D$ – 13.0° (*c* 1.1, MeOH); ^{13}C and ^{1}H NMR: Tables 2 and 3. Anal. Calcd for $C_{24}H_{45}NO_6$: C, 64.98; H, 10.22; N, 3.16. Found: C, 64.70; H, 9.82; N, 3.15.

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