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Synthesis of 5,6-dimodified open-chain D-fructose derivatives and their properties as substrates of bacterial polyol dehydrogenase

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Abstract

5-Deoxy-5-fluoro-D-xylulose as well as a range of new 5,6-dimodified open-chain analogues of D-fructose, namely the 5,6-diazido-5,6-dideoxy, 6-azido-5,6-dideoxy, 6-azido-5,6-dideoxy-5-fluoro, 5,6-dideoxy-5-fluoro, 5,6-dideoxy-6-fluoro and 5,6-dideoxy-5,6-difluoro derivatives, were synthesised employing glucose isomerase catalysed isomerisation of the corresponding D-xylo- and D-glucofuranoses as a key step. New compounds as well as some previously reported analogues such as 5-azido-5,6-dideoxy-6-fluoro-D-fructose were shown to be excellent substrates of polyol dehydrogenase from *Burkholderia cepacia* DSM 50181 with K_m values two orders of magnitude smaller than the corresponding natural substrates.

1. Introduction

Polyol dehydrogenases are widespread in Nature and catalyse the reversible oxidation of a polyol into a ketose. Many polyol dehydrogenases utilise NAD(H) as the redox co-enzyme, and therefore the reaction of sorbitol dehydrogenase with D-sorbitol is:

D-sorbitol+$NAD^+ \rightleftharpoons$ D-fructose+NADH+H^+

NAD-dependent polyol dehydrogenases (PDHs) appear to cope with a number of different physiological functions. For example, they are involved in mainstream carbohydrate catabolism in which energy for growth and maintenance is generated,[1] as well as in detoxification metabolism.[2] Several important details of enzyme/substrate interactions of PDHs are not well understood, and structural information at high resolution is not available for this class of enzymes. We would like to have a better understanding of how binding energy is utilised by PDHs to bring about catalytic efficiency, and related this to the proposed roles of these enzymes in vivo. The use of substrate analogues in kinetic studies is instrumental

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to identify important enzyme/substrate interactions and to quantify the contributions of these interactions to transition state stabilisation. Here, we report experiments in which open-chain derivatives of D-fructose have been used to characterise the specificity of a bacterial PDH (from *B. cepacia* DSM 50181). Generally, microbial PDHs are considered interesting biocatalysts in the stereospecific reduction of carbonyl substrates.[3]

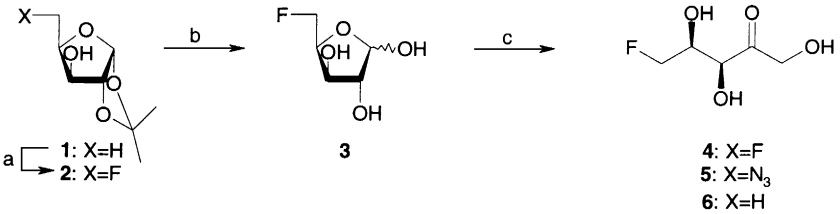
Very recently, we successfully employed 5,6-dimodified D-glucose derivatives, which are characterised by a significantly increased amount of acyclic aldehyde tautomer in aqueous solution, as valuable tools to probe the active sites as well as the substrate specificities of microbial aldose reductases.[4] A similar biochemical application was envisaged for 5,6-dimodified derivatives of D-fructose and D-xylulose as open-chain substrate analogues of microbial polyol dehydrogenases.

Available methodology for the preparation of the desired 5,6-dimodified ketoses has been fairly limited. An important approach is the aldolase catalysed addition of 2,3-dimodified glyceraldehyde derivatives to dihydroxyacetone phosphate.[5] Following pioneering work by Bock and co-workers,[6] a viable alternative to the aldolase based method was recently found in the glucose isomerase (EC 5.3.1.5)[7] mediated conversion of 5,6-dimodified aldofuranoses into open-chain ketoses[8] and other analogues of D-fructose, L-sorbose[9] as well as D-psicose and L-tagatose.[10]

2. Results and discussion

2.1. Syntheses

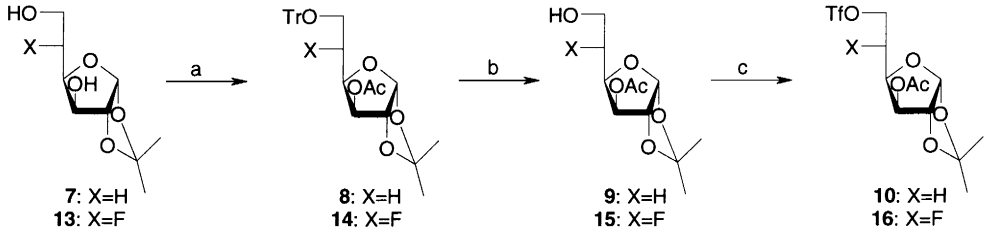
For the preparation of 5-modified D-xyluloses, 1,2-*O*-isopropylidene-D-xylofuranose **1**[11] served as the starting material (Scheme 1). Regioselective reaction with diethylaminosulfur trifluoride (DAST) gave the corresponding known[12] 5-deoxyfluoro derivative **2** which was deprotected to yield 5-deoxy-5-fluoro-D-xylose **3**. The latter was isomerised employing the standard procedure to give 5-deoxyfluoro-D-xylulose **4**, a pentulose which was previously synthesised with the aid of yeast transketolase and racemic 3-fluoro-2-hydroxypropanal.[13] 5-Azido-5-deoxy-D-xylulose **5**[9a] and 5-deoxy-D-xylulose **6**[14] were obtained according to published approaches.



Scheme 1. Reagents and conditions: (a) DAST, CH_2Cl_2, −40°C, inverse addition; (b) IR 120, H_2O/CH_3CN, 50°C; (c) EC 5.3.1.5, $MgSO_4$, H_2O, 60°C

Based on the previously established and proven route,[15] 1,2-*O*-isopropylidene-α-D-glucofuranurono-6,3-lactone[16] was employed as the starting material for syntheses of 5,6-dimodified D-glucofuranoses. From easily accessible 5-deoxy-1,2-*O*-isopropylidene-D-*xylo*-hexofuranurono-6,3-lactone,[17] 5-deoxy-1,2-*O*-isopropylidene-α-D-xylohexofuranose **7**[18] was prepared by sodium tetrahydridoborate reduction. 6-*O*-Tritylation and subsequent acetylation of O-3 were achieved in a simple one-pot procedure to give fully protected intermediate **8**. Chemoselective deprotection[19] of O-6 yielded compound **9** which was converted into the corresponding 6-triflate **10** (Scheme 2). For the preparation of the 5,6-dideoxy-6-fluoro derivative **11**, this was reacted with 'dry tetrabutylammonium fluoride'[20] in acetonitrile at ambient

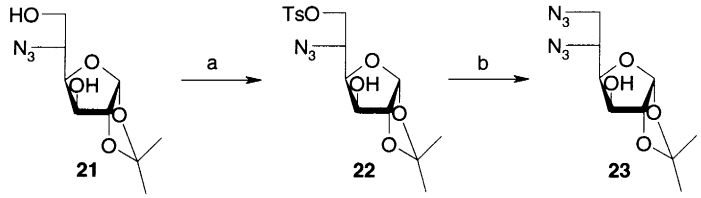
temperature. For the synthesis of the corresponding 6-azido-5,6-dideoxy compound **12**, triflate **10** was treated with sodium azide in acetone.



Scheme 2. Reagents and conditions: (a) (i) TrCl, Py, 45°C, (ii) Ac_2O; (b) BF_3Et_2O, MeOH, CH_2Cl_2; (c) Tf_2O, Py, CH_2Cl_2, −15°C

Likewise, 5-deoxy-5-fluoro-1,2-*O*-isopropylidene-α-D-glucofuranurono-6,3-lactone[21] was employed as an intermediate for compounds of the 5-deoxyfluoro series following essentially the same approach: conventional reduction of the lactone moiety led to 5-deoxy-5-fluoro-1,2-*O*-isopropylidene-α-D-glucofuranose **13**.[21] Compound **13** was regioselectively protected at O-6 with chlorotriphenylmethane in pyridine followed by acetylation of O-3 in a one-pot procedure. Deprotection of O-6 in **14** led to the fluoroalcohol **15** which was transformed into the corresponding triflate **16**. Its reaction with dry tetrabutylammonium fluoride[20] in acetonitrile at ambient temperature gave 5,6-dideoxy-5,6-difluoroglucose derivative **17**, whereas 6-azido-5,6-dideoxy-5-fluoro-1,2-*O*-isopropylidene-α-D-glucofuranose **18** was formed with sodium azide in acetone. Reaction of intermediate **16** with sodium iodide gave intermediate **19** which was further processed by catalytic hydrogenation to furnish 5,6-dideoxy-5-fluorosugar **20**.

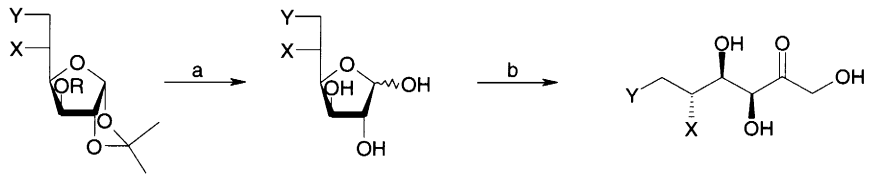
Conversely, the synthesis of 5,6-diazido-5,6-dideoxy-1,2-*O*-isopropylidene-α-D-glucofuranose **23** (Scheme 3) did not require the standard protection–deprotection sequence described but was readily achieved from 5-azido-5-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranose **21** which was synthesised from 5-azido-5-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranurono-6,3-lactone.[22] Regioselective tosylation of O-6 followed by treatment with sodium azide in *N*,*N*-dimethylformamide (DMF) gave access to the desired derivative **23**. All attempts to employ the same approach with compound **7** invariably led to the formation of the corresponding 3,6-anhydro derivatives.



Scheme 3. Reagents and conditions: (a) TsCl, Py; (b) NaN_3, DMF, 45°C

5,6-Dideoxy-1,2-*O*-isopropylidene-α-D-*xylo*-hex-5-eno-furanose **40**[9a] as well as the corresponding 5,6-dideoxy derivative **24** were synthesised from suitable 3-*O*-protected 1,2-*O*-isopropylidene-α-D-glucofuranose derivatives via Tipson–Cohen elimination of the corresponding 5,6-dimesylates.[23]

Acidic hydrolysis of the protected 5,6-dimodified aldofuranoses (Scheme 4) furnished the corresponding free sugars **25**–**31** in good yields. All modified aldose derivatives were accepted as substrates by immobilised glucose isomerase (Sweetzyme T) at 65°C and pH 7 and yielded the desired open-chain ketoses **32**–**39** in isolated yields ranging between 22 and 63%. 5-Azido-5,6-dideoxy-6-fluoro-D-fructose **39** was synthesised by a similar route.[24]



11: R=Ac, X=H, Y=F
12: R=Ac, X=H, Y=N_3
17: R=Ac, X=Y=F
18: R=Ac, X=F, Y=N_3
19: R=Ac, X=F, Y=I
20: R=H, X=F, Y=H
23: R=H, X=Y=N_3
24: R=H, X=Y=H

25: X=H, Y=F
26: X=H, Y=N_3
27: X=Y=F
28: X=F, Y=N_3
29: X=F, Y=H
30: X=Y=N_3
31: X=Y=H

32: X=H, Y=F
33: X=H, Y=N_3
34: X=Y=F
35: X=F, Y=N_3
36: X=F, Y=H
37: X=Y=N_3
38: X=Y=H
39: X=N_3, Y=F

Scheme 4. Reagents and conditions: (a) IR 120, H_2O/CH_3CN, 50°C; (b) EC 5.3.1.5, $MgSO_4$, H_2O, 60°C

2.2. Biochemical studies

Open-chain ketoses were employed as substrates with *B. cepacia*. Data obtained from these experiments are listed in Table 1.

Table 1
Kinetic parameters of polyol dehydrogenase from *Burkholderia cepacia* determined in 50 mM potassium phosphate buffer, pH 7.5 and 25°C. The concentration of NADH was 200 μM and constant at apparent saturation of the enzyme

Compound	V_{max} (U mg^{-1})[a]	K_m (mM)[a]	(V_{max}/K_m) x 100 (U mg^{-1} mM^{-1})	DDG[b] (kJ mol^{-1})
D-fructose	39 ± 2	183 ± 28	21	-
5,6-dideoxy-6-fluoro **32**			= 0.2[c]	= 12
5,6-dideoxy-5-eno **40**			= 0.2	= 12
5,6-dideoxy **38**			= 0.2	= 12
5,6-dideoxy-5-fluoro **36**	8.0 ± 0.5	6.0 ± 1.3	133	- 4.6
5,6-dideoxy-5,6-difluoro **34**			~ 300[d]	~ - 6.6
6-azido-5,6-dideoxy **33**			= 0.2	= 12
6-azido-5,6-dideoxy-5-fluoro **35**	20 ± 1	0.8 ± 0.3	2500	- 11.8
5,6-diazido-5,6-dideoxy **37**	16 ± 1	2.0 ± 0.6	800	- 9.0

[a] Calculated from non-linear fits of the experimental data to the eqn. $V = V_{max}[S]/(K_m+[S])$, where V is the initial velocity, $[S]$ is the substrate concentration, V_{max} is the initial velocity at saturation with substrate, and K_m is the Michaelis constant for S; [b] calculated according to the eqn. $DDG = -RT \ln [(V_{max}/K_m)_{derivative}/(V_{max}/K_m)_{fructose}]$ using 298.15 K for T and 8.3144 J mol^{-1} for R; [c] corresponding to the smallest catalytic efficiency that would have been detectable in our measurements; evidence for binding was obtained from inhibition of derivatives against D-fructose; [d] only V_{max}/K_m is given because of the strong substrate inhibition by this compound; the apparent substrate inhibition constant was estimated to be about 0.5 times the K_m; V_{max}/K_m was calculated from the part of the curve where V is linearly dependent on $[S]$.

Steady state kinetic parameters were determined for the NADH-dependent reduction of compounds **32**–**40** catalysed by PDH. They are summarised in Table 1 and compared with the corresponding kinetic parameters for the reduction of D-fructose. Preliminary results obtained reveal important interactions between the enzyme or, presumably, the binary complex between the enzyme and NADH, and the positions 5 and 6 of the substrates. They also indicate that the open-chain derivatives of D-fructose probed are valuable substrate analogues for the evaluation of mechanistic aspects in the NADH-dependent reduction of carbonyl groups catalysed by polyol dehydrogenases such as the PDH from *B. cepacia*. Polar functionalities, for example azido and fluoro substituents, at C-5 of the ketose (compounds **34**–**37**) result in clearly superior substrate properties as compared with D-fructose as well as various open-chain 5-deoxy derivatives thereof, such as compounds **32**, **33**, **38** and the 5,6-eno analogue **40**. A detailed interpretation of the results regarding substrate recognition and catalysis by PDH will be presented elsewhere.

3. Experimental

3.1. General methods

Melting points were recorded on a Tottoli apparatus and are uncorrected. Optical rotations were measured on a Perkin–Elmer 341 with a path length of 10 cm. NMR spectra were recorded at 200 as well as 300 MHz (^{1}H), and at 50.29 and 75.47 MHz (^{13}C). $CDCl_3$ was employed for protected compounds and D_2O or CD_3OD for free sugars. Chemical shifts are listed in δ employing residual, not deuterated, solvent as the internal standard. TLC was performed on precoated aluminium sheets (E. Merck 5554). TLC plates were stained with concd H_2SO_4 containing 5% vanillin. For column chromatography silica gel 60 (E. Merck) was used. Mixtures of ethyl acetate:cyclohexane (1:10 to 3:1) were used for TLC and column chromatography of protected compounds, ethyl acetate as well as ethyl acetate:MeOH mixtures (10:1 to 4:1) were employed for TLC and chromatography of unprotected sugars. Saturated aqueous bicarbonate for washing was freshly prepared.

3.2. PDH

The enzyme was isolated from crude cell extracts of *B. cepacia* DSM 50181, grown on medium containing D-sorbitol as the main carbon source. The full purification procedure will be published elsewhere. An enzyme preparation with a specific activity of approx. 40 units (μmol min^{-1}) per mg protein showed a single band in SDS–PAGE and in non-denaturing anionic PAGE. Staining for enzyme activity with D-sorbitol as the substrate in non-denaturing gels unequivocally identified the protein band as PDH. The preparation did not contain any detectable contamination by mannitol dehydrogenase. Standard assays for PDH activity and mannitol dehydrogenase activity were carried out in 50 mM tris buffer, pH 9.0, at 25°C using 200 mM D-sorbitol or D-mannitol as substrates, respectively, and 2 mM NAD as co-enzyme. One unit refers to 1 μmol NADH produced per minute under these conditions.

3.3. Initial velocity studies

Measurements of the initial rate of carbonyl reduction were performed with a Beckman DU-650 spectrophotometer at 25°C by using NADH as the co-enzyme. The oxidation of co-enzyme upon carbonyl reduction was monitored at 340 nm (1 to 5 min, rate of 0.05 to 0.1 DA·min^{-1}). All rates were corrected

for the appropriate blank readings, lacking either the substrate or the enzyme. The standard reaction mixture had a total volume of 0.3 ml. It contained approx. 10 μg mL^{-1} PDH and a constant concentration of 200 μ*M* NADH, dissolved in 50 mM potassium phosphate, pH 7.5. The substrate was varied, typically in 6 to 7 different concentration points, over a concentration range covering approximately 0.5 to 5 times the K_m.

3.4. Chemical procedures

3.4.1. General procedure for the introduction of heterosubstituents

To a solution of the OH-6 unprotected alcohol in dichloromethane containing 1.5 equivalents pyridine, a 10% solution of 1.1 equivalents trifluoromethanesulfonic anhydride in CH_2Cl_2 was added dropwise at −15°C. When TLC indicated the quantitative formation of a faster moving product, the solution was diluted with CH_2Cl_2 and washed consecutively with 5% aq. HCl and satd aq. $NaHCO_3$ and dried over Na_2SO_4. After filtration and removal of the solvent under reduced pressure the residue was dissolved in the appropriate solvent and the respective nucleophile (3 to 5 equivalents) was added (fluorosugars were prepared with dried tetrabutylammonium fluoride in acetonitrile, azidosugars were synthesised with sodium azide in acetone and for the introduction of iodine, sodium iodide in *N*,*N*-dimethylformamide was employed). The reaction mixture was stirred at ambient temperature until TLC indicated quantitative conversion of the triflate, after which it was diluted with dichloromethane and washed with water. After drying (Na_2SO_4), filtration and evaporation of the solvents the residue was purified by chromatography.

3.4.2. General procedure for the one-pot protection of diols with chlorotriphenylmethane and acetic anhydride

To a solution (20%) of the respective diol in pyridine, 1.5 equivalents chlorotriphenylmethane were added and the solution was heated to 45°C until TLC indicated the quantitative conversion of the starting material. The reaction mixture was allowed to reach ambient temperature when acetic anhydride was added. Upon completion (TLC), the mixture was concentrated under reduced pressure. The residue was partitioned between dichloromethane and 5% aq. HCl; the organic layer was washed with satd aq. $NaHCO_3$ and dried over Na_2SO_4. Filtration and evaporation of the solvent under reduced pressure gave the crude product which was purified by chromatography.

3.4.3. General procedure for the cleavage of trityl ethers[19]

To a solution of the fully protected furanose in dichloromethane containing 5% methanol, boron trifluouride etherate (1.1 equivalents) was added and the mixture was kept at room temperature until TLC indicated the complete consumption of the starting material. The reaction mixture was diluted with CH_2Cl_2 and washed with satd aq. $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by chromatography.

3.4.4. General procedure for the removal of acetate and isopropylidene protecting groups

An aqueous acetonitrile solution (CH_3CN:H_2O 1:1 v/v) containing the respective 3-*O*-acetyl-1,2-*O*-isopropylidene derivative was stirred with Amberlite IR-120 [H^+] ion-exchange resin, which had been washed with distilled water before use, at 50°C before use. When TLC indicated the completion of the reaction, the resin was removed by filtration and the solution was concentrated under reduced pressure. The free aldofuranose was purified by chromatography.

3.4.5. General procedure for isomerisation reactions with immobilised glucose isomerase

To a 5% solution of the respective free sugar in distilled water, a few drops of a 1% aqueous solution of $MgSO_4$ and 4 equiv. (w/w) of immobilised glucose isomerase (Sweetzyme T) were added and the mixture was spun on a rotary evaporator or shaken at 60°C for 3 to 8 h. After the appropriate reaction time, the solids were filtered off, the solution was concentrated under reduced pressure and the residue was chromatographed on silica gel. Alternatively, in case of similar polarities of product and starting material, the aqueous solution was treated with bromine in the presence of barium carbonate to oxidise the remaining aldose to the corresponding lactone to allow easier separation. Nitrogen was bubbled through the brown reaction mixture, the solids were removed by filtration, the solution was concentrated under reduced pressure and the residue subjected to chromatography. The aldonolactone can be recycled by conventional reduction to the free aldose.[25]

*3.5. 5-Deoxy-5-fluoro-1,2-*O*-isopropylidene-α-*D*-xylofuranose **2***

Diethylaminosulfur trifluoride (DAST, 10.2 g, 63.1 mmol, 4 equiv.) was dissolved in 100 mL CH_2Cl_2, which had been rinsed over basic aluminium oxide (Aldrich, 150 mesh),[26] and cooled to −40°C. Then, 1,2-*O*-isopropylidene-α-D-xylofuranose (**1**, 3.0 g)[11] dissolved in 20 mL dichloromethane was added dropwise and the reaction mixture was left overnight at 4°C. After cooling to −50°C the reaction was quenched by adding 4 mL methanol and stirring for 20 min. The mixture was diluted with satd aq. $NaHCO_3$ and extracted with dichloromethane. The organic layer was dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified by chromatography to yield 1.02 g (34%) of compound **2**. Mp: 82–84°C [lit.[12] mp: 86°C]; $[\alpha]_D^{20}$ −11.4 (*c* 2.25, CH_2Cl_2) [lit.[12] $[\alpha]_D^{20}$ −20.6 (*c* 1, $CHCl_3$)]; ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.98 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 4.75 (ddd, 1H, $J_{5,F}$ 46.8 Hz, $J_{5,5'}$ 10.0 Hz, H-5), 4.60 (ddd, 1H, H-5′), 4.53 (dd, 1H, $J_{2,F}$ 1.5 Hz, H-2),[27] 4.41 (dddd, 1H, $J_{4,5}$ 5.2 Hz, $J_{4,5'}$ 5.1 Hz, $J_{4,F}$ 16.6 Hz, H-4), 4.33 (m, 1H, $J_{3,4}$ 2.8 Hz, H-3); ^{13}C NMR: δ 112.1 (4°C, Isp), 105.0 (C-1), 85.4 (C-2), 81.3 ($J_{5,F}$ 166 Hz, C-5), 78.4 ($J_{4,F}$ 22.0 Hz, C-4), 75.2 ($J_{3,F}$ 4.7 Hz, C-3), 26.8, 26.2 (2×CH_3, Isp).

*3.6. 5-Deoxy-5-fluoro-*D*-xylofuranose **3***

Compound **2** (980 mg, 5.10 mmol) was hydrolysed according to the respective general procedure and gave xylofuranose **3** (668 mg, 86%). $[\alpha]_D^{20}$ +36.4 (*c* 1.55, MeOH) [lit.[12] $[\alpha]^{20}_D$ +52.7 (*c* 1.7, H_2O)]; ^{1}H NMR (300 MHz, D_2O): δ 5.61 (s, 1H, H-1α), 5.42 (s, 1H, H-1β), α:β-ratio: 1.2:1; ^{13}C NMR: δ 102.5, 96.5 (C-1α/C-1β), 84.5, 83.8 ($J_{5,F}$ 163.6 Hz, $J_{5,F}$ 163.6 Hz, C-5α/C-5β), 81.2 (C-2α/C-2β), 80.5 ($J_{4,F}$ 19.5 Hz, C-4α/C-4β), 77.6 ($J_{4,F}$ 17.6 Hz, C-4α/C-4β), 76.8 (C-2α/C-2β), 75.7 ($J_{3,F}$ 6.3 Hz, C-3α/C-3β), 75.4 ($J_{3,F}$ 4.5 Hz, C-3α/C-3β).

*3.7. 5-Deoxy-5-fluoro-*D*-threo-pentulose **4** (5-deoxy-5-fluoro-*D*-xylulose)*

The general procedure for the isomerisation was applied. The ketose **4** is less polar than the starting material and the aldose **3** could be recycled without derivatisation by column chromatography. Deoxyfluoroaldose **3** (543 mg, 3.57 mmol) yielded 261 mg (48%) of desired ketose **4**. $[\alpha]_D^{20}$ −36.7 (c 2.25, MeOH); [lit.[13] $[\alpha]_D^{20}$ −2.4 (*c* 0.3, D_2O)]; ^{1}H NMR spectra data were identical with values given in Ref. 13; ^{13}C NMR: δ 213.2 (C-2), 84.7 ($J_{5,F}$ 165.9 Hz, C-5), 75.8 ($J_{3,F}$ 5.1 Hz, C-3), 71.0 ($J_{4,F}$ 20.6 Hz, C-4), 67.0 (C-1).

3.8. 3-O-Acetyl-5-deoxy-1,2-O-isopropylidene-6-O-triphenylmethyl-α-D-xylo-hexofuranose **8**

Compound **7** (3.57 g, 17.5 mmol) was processed according to the general procedure for the one-pot protection sequence and led to compound **8** (8.25 g, 99%) as a slightly yellow foam. $[\alpha]_D^{20}$ −17.1 (*c* 4.6, CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.47–7.19 (m, 15H, Ar), 5.89 (d, 1H, $J_{1,2}$ 3.9 Hz, H-1), 5.08 (d, 1H, $J_{3,4}$ 2.7 Hz, H-3), 4.58 (m, 1H, $J_{4,5}$ 6.2 Hz, H-4), 4.51 (d, 1H, H-2), 3.21 (m, 2H, $J_{6,6'}$ 15.9 Hz, H-6, H-6′), 2.06 (s, 3H, CH_3, Ac), 1.91 (m, 2H, H-5, H-5′), 1.53 (s, 3H, CH_3, Isp), 1.32 (s, 3H, CH_3, Isp); ^{13}C NMR: δ 169.9 (CO, Ac), 144.2, 128.7, 127.8, 127.0 (Ar), 111.8 (4°C, Isp), 104.4 (C-1), 86.6 (4°C, Tr), 83.7, 77.4, 76.3 (C-2/C-3/C-4), 60.4 (C-6), 28.7 (C-5), 26.6, 26.3 (2×CH_3, Isp), 20.8 (CH_3, Ac).

3.9. 3-O-Acetyl-5-deoxy-1,2-O-isopropylidene-α-D-xylo-hexofuranose **9**

The general procedure for the removal of the trityl ether group was applied. Compound **8** (2.15 g, 4.40 mmol) was deprotected and afforded **9** (876 mg, 81%) as a colourless oil. [α] +4.3 (*c* 0.85, CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.89 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1), 5.16 (d, 1H, $J_{3,4}$ 2.8 Hz, H-3), 4.50 (d, 1H, H-2), 4.42 (ddd, 1H, $J_{4,5}$ 4.8 Hz, $J_{4,5'}$ 8.2 Hz, H-4), 3.76 (m, 2H, H-6, H-6′), 2.25 (s, 1H, OH), 2.09 (s, 3H, CH_3, Ac), 1.84 (m, 2H, H-5, H-5′), 1.51 (s, 3H, CH_3, Isp), 1.30 (s, 3H, CH_3, Isp); ^{13}C NMR: δ 170.0 (CO, Ac), 112.0 (4°C, Isp), 104.5 (C-1), 83.5, 77.6, 77.4 (C-2/C-3/C-4), 60.2 (C-6), 30.7 (C-5), 26.6, 26.2 (2×CH_3, Isp), 20.8 (CH_3, Ac).

3.10. 3-O-Acetyl-5,6-dideoxy-6-fluoro-1,2-O-isopropylidene-α-D-xylo-hexofuranose **11**

Compound **9** (876 mg, 3.56 mmol) was triflated to furnish intermediate **10**. The subsequent displacement of the leaving group with dry TBAF according to the respective general procedure afforded 627 mg (71%) **23** as a colourless oil. $[\alpha]_D^{20}$ −1.6 (*c* 0.7, CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.89 (dd, 1H, $J_{1,2}$ 3.8 Hz, H-1), 5.16 (d, 1H, $J_{3,4}$ 2.8 Hz, H-3), 4.7–4.4 (m, 3H, $J_{6,F}$ 46.7 Hz, H-4, H-6, H-6′), 4.52 (d, 1H, H-2), 4.48 (ddd, 1H, $J_{6',F}$ 47.4 Hz, H-6′), 2.09 (s, 3H, CH_3, Ac), 2.05–1.88 (m, 2H, H-5, H-5′), 1.51 (s, 3H, CH_3, Isp), 1.31 (s, 3H, CH_3, Isp); ^{13}C NMR: δ 169.9 (CO, Ac), 112.1 (4°C, Isp), 104.6 (C-1), 83.8 (C-2), 81.0 ($J_{6,F}$ 165.5 Hz, C-6), 77.2 (C-3), 75.6 ($J_{4,F}$ 4.5 Hz, C-4), 29.5 ($J_{5,F}$ 20.8 Hz, C-5), 26.7, 26.3 (2×CH_3, Isp), 20.8 (CH_3, Ac).

3.11. 6-Azido-3-O-acetyl-5,6-dideoxy-1,2-O-isopropylidene-α-D-xylo-hexofuranose **12**

Intermediate **10** was synthesised from **9** (1.15 g, 4.67 mmol) according to the respective general procedure. Nucleophilic substitution with sodium azide led to the desired compound **12** in 79% yield (998 mg). Compound **12** was a colourless oil. $[\alpha]_D^{20}$ −3.7 (*c* 1.25, CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.89 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1), 5.16 (d, 1H, $J_{3,4}$ 2.9 Hz, H-3), 4.52 (d, 1H, H-2), 4.34 (ddd, 1H, $J_{4,5}$ 5.1 Hz, $J_{4,5'}$ 8.2 Hz, H-4), 3.44 (m, 2H, $J_{6,6'}$ 9.5 Hz, H-6, H-6′), 2.11 (s, 3H, CH_3, Ac), 1.84 (m, 2H, H-5, H-5′), 1.51 (s, 3H, CH_3, Isp), 1.30 (s, 3H, CH_3, Isp); ^{13}C NMR: δ 169.9 (CO, Ac), 112.1 (4°C, Isp), 104.4 (C-1), 83.6, 77.0, 76.4 (C-2/C-3/C-4), 48.5 (C-6), 27.9 (C-5), 26.6, 26.2 (2×CH_3, Isp), 20.8 (CH_3, Ac).

3.12. 3-O-*Acetyl-5-deoxy-5-fluoro-1,2*-O-*isopropylidene-6*-O-*triphenylmethyl*-α-D-*glucofuranose* ***14***

5-Deoxy-5-fluoro-1,2-*O*-isopropylidene-α-D-glucofuranose **8**[21] **(**1.85 g, 8.32 mmol) was reacted according to the general one-pot protection sequence to yield 3.39 g (80.0%) **14** as a faintly yellow foam. $[\alpha]_D^{20}$ −16.7 (*c* 2.2, CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.52–7.24 (15H, Ar), 5.86 (dd, 1H, $J_{1,2}$ 3.6 Hz, $J_{1,F}$ 1.7 Hz, H-1), 5.36 (d, 1H, $J_{3,4}$ 2.8 Hz, H-3), 4.78 (dddd, 1H, $J_{5,6}$ 2.8 Hz, $J_{5,6'}$ 4.5 Hz, $J_{5,F}$ 46.4 Hz, H-5), 4.70–4.54 (m, 1H, $J_{4,5}$ 8.6 Hz, H-4), 4.53 (d, 1H, H-2), 3.53 (ddd, 1H, $J_{6,6'}$ 12.7 Hz, $J_{6,F}$ 29.2 Hz, H-6), 3.39 (ddd, 1H, H-6′), 1.47 (s, 3H, CH_3, Isp), 1.30 (s, 3H, CH_3, Isp), 2.09 (s, 3H, CH_3, Ac), 1.52 (s, 3H, CH_3, Isp), 1.50 (s, 3H, CH_3, Isp); ^{13}C NMR: δ 169.6 (CO, Ac), 143.8, 128.8, 128.2, 127.9, 127.3, 127.1 (Ar), 112.5 (4°C, Isp), 105.1 (C-1), 88.8 ($J_{5,F}$ 172.7 Hz, C-5), 86.7 (4°C, Tr), 83.2 (C-2), 76.0 ($J_{4,F}$ 30.7 Hz, C-4), 75.8 (C-3), 63.7 ($J_{6,F}$ 18.8 Hz, C-6), 26.9, 26.4 (2×CH_3, Isp), 26.7, 26.3 (2×CH_3, Isp), 20.8 (CH_3, Ac).

3.13. 3-O-*Acetyl-5-deoxy-5-fluoro-1,2*-O-*isopropylidene*-α-D-*glucofuranose* ***15***

Compound **14** (1.90 g, 3.75 mmol) was deprotected according to the respective general procedure and furnished **15** as a white solid (855 mg, 86%). Mp: 78–79°C; $[\alpha]_D^{20}$ −30.1 (*c* 0.4, CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.91 (dd, 1H, $J_{1,2}$ 3.6 Hz, $J_{1,F}$ 1.8 Hz, H-1), 5.35 (d, 1H, $J_{3,4}$ 3.0 Hz, H-3), 4.70 (dddd, 1H, $J_{5,6}$ 2.6 Hz, $J_{5,6'}$ 4.9 Hz, $J_{5,F}$ 46.9 Hz, H-5), 4.54 (d, 1H, H-2), 4.46 (ddd, 1H, $J_{4,5}$ 8.7 Hz, $J_{4,F}$ 5.8 Hz, H-4), 4.01 (ddd, 1H, $J_{6,6'}$ 13.0 Hz, $J_{6,F}$ 25.1 Hz, H-6), 3.88 (ddd, 1H, $J_{6',F}$ 27.8 Hz, H-6′), 2.15 (s, 3H, CH_3, Ac), 1.53 (s, 3H, CH_3, Isp), 1.36 (s, 3H, CH_3, Isp); ^{13}C NMR: δ 169.6 (CO, Ac), 112.8 (4°C, Isp), 105.2 (C-1), 89.7 ($J_{5,F}$ 170.0 Hz, C-5), 83.4 (C-2), 76.4 ($J_{4,F}$ 31.2 Hz, C-4), 75.9 (C-3), 63.0 ($J_{6,F}$ 20.5 Hz, C-6), 26.9, 26.4 (2×CH_3, Isp), 20.9 (CH_3, Ac).

3.14. 3-O-*Acetyl-5,6-dideoxy-5,6-difluoro-1,2*-O-*isopropylidene*-α-D-*glucofuranose* ***17***

The general procedure for the introduction of a fluorine atom was applied. Compound **15** (1.02 g, 3.86 mmol) led to 793 mg (77%) of the dideoxydifluoro derivative **17**. $[\alpha]_D^{20}$ −25.3 (*c* 2.05, CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.89 (dd, 1H, $J_{1,2}$ 3.6 Hz, $J_{1,F}$ 1.8 Hz, H-1), 5.34 (d, 1H, $J_{3,4}$ 2.8 Hz, H-3), 5.05–4.56 (m, 3H, H-5, H-6, H-6′), 4.54 (d, 1H, H-2), 4.44 (m, 1H, H-4), 2.11 (s, 3H, CH_3, Ac), 1.51 (s, 3H, CH_3, Isp), 1.31 (s, 3H, CH_3, Isp); ^{13}C NMR: δ 169.3 (CO, Ac), 112.8 (4°C, Isp), 105.2 (C-1), 88.0 ($J_{5,F\text{-}5}$ 173.9 Hz, $J_{5,F\text{-}6}$ 18.8 Hz, C-5), 83.2 (C-2), 82.6 ($J_{6,F\text{-}6}$ 174.3 Hz, $J_{6,F\text{-}5}$ 19.5 Hz, C-6), 75.4 (C-3), 75.0 ($J_{4,F\text{-}5}$ 30.7 Hz, $J_{4,F\text{-}6}$ 7.7 Hz, C-4), 26.8, 26.2 (2×CH_3, Isp), 20.8 (CH_3, Ac).

3.15. 3-O-*Acetyl-6-azido-5,6-dideoxy-5-fluoro-1,2*-O-*isopropylidene*-α-D-*glucofuranose* ***18***

The respective general procedure applied to **15** (774 mg, 2.93 mmol) led to intermediate **16** which was immediately used for the displacement reaction with NaN_3 in acetone. 772 mg (91%) of compound **18** was obtained as colourless oil. $[\alpha]_D^{20}$ −37.8 (*c* 2.2, CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.87 (dd, 1H, $J_{1,2}$ 3.6 Hz, $J_{1,F}$ 1.8 Hz, H-1), 5.33 (d, 1H, $J_{3,4}$ 3.0 Hz, H-3), 4.76 (dddd, 1H, $J_{5,6}$ 2.9 Hz, $J_{5,6'}$ 5.6 Hz, $J_{5,F}$ 46.7 Hz, H-5), 4.53 (d, 1H, H-2), 4.39 (ddd, 1H, $J_{4,5}$ 8.5 Hz, $J_{4,F}$ 4.9 Hz, H-4), 3.68 (ddd, 1H, $J_{6,F}$ 27.0 Hz, $J_{6,6'}$ 12.6 Hz, H-6), 3.55 (ddd, 1H, H-6′), 2.11 (s, 3H, CH_3, Ac), 1.52 (s, 3H, CH_3, Isp), 1.30 (s, 3H, CH_3, Isp); ^{13}C NMR: δ 169.3 (CO, Ac), 112.8 (4°C, Isp), 105.1 (C-1), 88.3 ($J_{5,F}$ 173.6 Hz, C-5), 83.3 (C-2), 76.6 ($J_{4,F}$ 30.3 Hz, C-4), 75.5 (C-3), 52.4 ($J_{6,F}$ 19.0 Hz, C-6), 26.8, 26.2 (2×CH_3, Isp), 20.7 (CH_3, Ac).

3.16. 3-O-*Acetyl-5,6-dideoxy-5-fluoro-6-iodo-1,2*-O-*isopropylidene-α*-D-*glucofuranose* ***19***

The same procedure as for compound **18** applied to **15** (940 mg, 3.56 mmol) and subsequent treatment with sodium iodide in DMF led to **19** (1.16 g, 87%) as a white solid, mp: 62–63°C; $[\alpha]_D^{20}$ −24.7 (*c* 1.45, CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.91 (bs, 1H, H-1), 5.31 (d, 1H, $J_{3,4}$ 2.5 Hz, H-3), 4.64 (m, 1H, H-4), 4.52 (d, 1H, $J_{1,2}$ 3.5 Hz, H-2), 4.48 (dddd, 1H, $J_{5,6}$ 2.7 Hz, $J_{5,6'}$ 5.3 Hz, $J_{5,F}$ 47 Hz, H-5), 3.59 (ddd, 1H, $J_{6,6'}$ 11.6 Hz, $J_{6,F}$ 26.0 Hz, H-6), 3.39 (ddd, 1H, $J_{6',F}$ 24.5 Hz, H-6′), 2.15 (s, 3H, CH_3, Ac), 1.53 (s, 3H, CH_3, Isp), 1.36 (s, 3H, CH_3, Isp), 2.10 (s, 3H, CH_3, Ac), 1.51 (s, 3H, CH_3, Isp), 1.30 (s, 3H, CH_3, Isp); ^{13}C NMR: δ 169.3 (CO, Ac), 112.9 (4°C, Isp), 105.0 (C-1), 87.4 ($J_{5,F}$ 174.8 Hz, C-5), 83.3 (C-2), 79.2 ($J_{4,F}$ 30.7 Hz, C-4), 75.4 (C-3), 26.9, 26.4 (2×CH_3, Isp), 20.8 (CH_3, Ac), 5.1 ($J_{6,F}$ 21.0 Hz, C-6).

3.17. 3-O-*Acetyl-5,6-dideoxy-5-fluoro-1,2*-O-*isopropylidene-α*-D-*glucofuranose* ***20***

50 mL methanol containing **19** (1.03 g, 2.76 mmol), triethylamine (0.33 g, 3.31 mmol, 1.2 equiv.) and Raney-Ni was stirred under an atmosphere of hydrogen. When TLC indicated quantitative reaction the catalyst was removed by filtration and the solution concentrated under reduced pressure. The residue was dissolved in dichloromethane and consecutively washed with 5% aq. HCl and satd aq. $NaHCO_3$. The organic layer was dried (Na_2SO_4), filtered and evaporated and finally purified by column chromatography. Compound **20** was a white solid and was obtained in 87% (594 mg) yield. Mp 64–66°C; $[\alpha]_D^{20}$ −16.4 (*c* 1.55, CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.88 (dd, 1H, $J_{1,2}$ 3.8 Hz, $J_{1,F}$ 1.8 Hz, H-1), 5.31 (d, 1H, $J_{3,4}$ 3.0 Hz, H-3), 4.76 (ddq, 1H, $J_{5,6}$ 6.3 Hz, $J_{5,F}$ 47.0 Hz, H-5), 4.50 (d, 1H, H-2), 4.15 (ddd, 1H, $J_{4,5}$ 8.1 Hz, $J_{4,F}$ 6.2 Hz, H-4), 2.09 (s, 3H, CH_3, Ac), 1.46 (dd, 3H, $J_{6,F}$ 25.4 Hz, H-6), 1.51 (s, 3H, CH_3, Isp), 1.29 (s, 3H, CH_3, Isp); ^{13}C NMR: δ 169.5 (CO, Ac), 112.4 (4°C, Isp), 104.9 (C-1), 86.3 ($J_{5,F}$ 165.3 Hz, C-5), 83.5 (C-2), 80.6 ($J_{4,F}$ 30.5 Hz, C-4), 75.4 (C-3), 26.7, 26.2 (2×CH_3, Isp), 20.8 (CH_3, Ac), 18.7 ($J_{6,F}$ 21.2 Hz, C-6).

3.18. 5-Azido-5-deoxy-1,2-O-*isopropylidene-6*-O-*tosyl-α*-D-*glucofuranose* ***22***

5-Azido-5-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranose (**21**,[22] 1.12 g, 4.57 mmol) dissolved in 20 mL pyridine was treated with 1.31 g (6.85 mmol, 1.5 equiv.) *p*-toluenesulfonyl chloride at 0°C. Stirring was continued at room temperature until TLC indicated quantitative consumption of the starting material. Methanol (5 mL) was added and the mixture was concentrated under reduced pressure. The residue was partitioned between dichloromethane and 5% aq. HCl; the organic layer was washed with satd aq. $NaHCO_3$ and dried over Na_2SO_4. Filtration and evaporation of the solvent gave the crude product **22** which was purified by chromatography to yield 1.66 g (91%). Compound **22** was a colourless oil. $[\alpha]_D^{20}$ −24.8 (*c* 1.8, CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.87 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 4.48 (d, 1H, H-2), 4.41 (m, 1H, H-4), 4.29 (bs, 1H, H-3), 4.13–3.88 (m, 3H, H-5, H-6, H-6′) 2.45 (s, 3H, CH_3, Ts), 2.25 (d, 1H, $J_{3,OH\text{-}3}$ 4.9 Hz, OH), 1.45 (s, 3H, CH_3, Isp), 1.29 (s, 3H, CH_3, Isp); ^{13}C NMR: δ 145.2, 132.5, 130.0, 128.1 (Ar, Ts), 112.3 (4°C, Isp), 105.1 (C-1), 85.0, 78.5, 74.6 (C-2/C-3/C-4), 70.2 (C-6), 58.4 (C-5), 26.8, 26.2 (2×CH_3, Isp), 21.7 (CH_3, Ts).

3.19. 5,6-Diazido-5,6-dideoxy-1,2-O-*isopropylidene-α*-D-*glucofuranose* ***23***

A suspension of **22** (445 mg, 1.11 mmol) and sodium azide (361 mg, 5.55 mmol, 5 equiv.) in 20 mL DMF was stirred at 45°C. After complete reaction (judged by TLC) most of the solvent was evaporated

under reduced pressure and the residue was partitioned between CH_2Cl_2 and water. The organic layer was dried (Na_2SO_4), filtered and evaporated. Chromatography of the crude product afforded 266 mg (88%) of compound **23** as a colourless syrup. $[\alpha]_D^{20}$ −64.2 (*c* 4.0, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.90 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 4.50 (d, 1H, H-2), 4.32 (d, 1H, $J_{3,4}$ 2.7 Hz, H-3), 4.04 (dd, 1H, $J_{4,5}$ 9.2 Hz, H-4), 3.89 (ddd, 1H, $J_{5,6}$ 2.9 Hz, $J_{5,6'}$ 7.1 Hz, H-5), 3.67 (dd, 1H, $J_{6,6'}$ 12.8 Hz, H-6), 3.47 (dd, 1H, H-6′), 2.61 (s, 1H, OH), 1.47 (s, 3H, C*H*$_3$, Isp), 1.30 (s, 3H, C*H*$_3$, Isp); ^{13}C NMR: δ 112.3 (4°C, Isp), 105.0 (C-1), 85.2, 79.2, 74.5 (C-2/C-3/C-4), 59.5 (C-5), 52.8 (C-6), 26.8, 26.2 (2×CH_3, Isp).

3.20. 5,6-Dideoxy-6-fluoro-D-xylo-*hexofuranose* ***25***

Hydrolysis of **11** (510 mg, 2.05 mmol) according to the general procedure afforded 280 mg (82%) of compound **25** as a colourless oil. $[\alpha]_D^{20}$ +21.8 (*c* 2.35, MeOH); ^{1}H NMR (200 MHz, D_2O): δ 5.39 (d, 1H, $J_{1,2}$ 4.2 Hz, H-1α), 5.13 (s, 1H, H-1β), α:β-ratio: 1:1; ^{13}C NMR: δ 102.8, 96.7 (C-1α/C-1β), 83.5, 83.3 ($J_{6,F}$ 159.0 Hz, $J_{6,F}$ 158.5 Hz, C-6α/C-6β), 81.7 (C-2α/C-2β), 79.6 ($J_{4,F}$ 4.3 Hz, C-4α/C-4β), 77.1 (2 signals, C-2α/C-2β/C-3α/C-3β), 76.9 ($J_{4,F}$ 4.3 Hz, C-4α/C-4β), 76.4 (C-3α/C-3β), 31.1, 30.5 ($J_{5,F}$ 19.5 Hz, C-5α/C-5β).

3.21. 6-Azido-5,6-dideoxy-D-xylo-*hexofuranose* ***26***

Deprotection of **12** (820 mg, 3.02 mmol) led to desired aldose **26** (499 mg, 87%). $[\alpha]_D^{20}$ +14.4 (*c* 4.25, MeOH); ^{1}H NMR (200 MHz, CD_3OD): δ 5.34 (d, 1H, $J_{1,2}$ 4.0 Hz, H-1α), 5.05 (s, 1H, H-1β), α:β-ratio: 1:1; ^{13}C NMR: δ 104.1, 97.5 (C-1α/C-1β), 82.7, 80.3, 78.5, 78.0, 77.8, 77.7 (C-2α/C-2β/C-3α/C-3β/C-4α/C-4β), 49.7, 49.5 (C-6α/C-6β), 30.7, 29.9 (C-5α/C-5β).

3.22. 5,6-Dideoxy-5,6-difluoro-D-*glucofuranose* ***27***

Removal of the protecting groups in **17** (785 mg, 2.95 mmol) yielded compound **27** (451 mg, 83%) as a white solid. $[\alpha]_D^{20}$ −5.3 (*c* 1.4, MeOH); ^{1}H NMR (200 MHz, D_2O): δ 5.46 (d, 1H, $J_{1,2}$ 3.9 Hz, H-1α), 5.42 (d, 1H, $J_{1,2}$ 2.7 Hz, H-1β), α:β-ratio: 1:1; ^{13}C NMR: δ 102.4 (C-1β), 96.8 (C-1α), 89.5 ($J_{5\text{-}\alpha,F\text{-}5}$=$J_{5\text{-}\beta,F\text{-}5}$170.0 Hz, $J_{5\alpha,F\text{-}6}$=$J_{5\beta,F\text{-}6}$ 18.0 Hz, C-5α/C-5β), 83.4, 83.1 ($J_{6\alpha,F\text{-}6}$=$J_{6\beta,F\text{-}6}$ 167.6 Hz, $J_{6F\text{-}5}$ 19.3/19.2 Hz, C-6α/C-6β), 80.1, 75.6 (C-2α/C-2β), 77.9, 75.1 ($J_{4,F\text{-}5}$ 29.5/28.6 Hz, $J_{4,F\text{-}6}$ 7.2/6.9 Hz, C-4α/C-4β), 74.8 ($J_{3,F\text{-}5}$ 1.6 Hz, C-3α/C-3β), 74.4 (C-3α/C-3β).

3.23. 6-Azido-5,6-dideoxy-5-fluoro-D-*glucofuranose* ***28***

Acidic hydrolysis of the protecting groups in **18** (687 mg, 2.37 mmol) according to the respective general procedure led to **28** (451 mg, 92%) as a colourless oil. $[\alpha]_D^{20}$ +6.4 (*c* 2.0, MeOH); ^{1}H NMR (200 MHz, D_2O): δ 5.44 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1α), 5.22 (d, 1H, $J_{1,2}$ 2.7 Hz, H-1β), α:β-ratio: 1:1; ^{13}C NMR: δ 102.3, 96.8 (C-1α/C-1β), 90.0, 88.8 ($J_{5,F}$ 170.2 Hz, C-5α+C-5β), 80.3 (C-2α/C-2β), 79.7 ($J_{4,F}$ 28.8 Hz, C-4α/C-4β), 76.3 ($J_{4,F}$ 28.0 Hz, C-4α/C-4β), 75.8 (C-2α/C-2β), 74.8 ($J_{3,F}$ 2.0 Hz, C-3α/C-3β), 74.5 (C-3α/C-3β), 52.1 ($J_{6,F}$ 15.5 Hz, C-6α/C-6β), 51.7 ($J_{6,F}$ 15.9 Hz, C-6α/C-6β).

3.24. 5,6-Dideoxy-5-fluoro-D-*glucofuranose* ***29***

The general deprotection procedure was applied to compound **20** (550 mg, 2.21 mmol) and **29** was obtained in 86% yield. $[\alpha]_D^{20}$ −6.7 (*c* 3.25, MeOH); ^{1}H NMR (200 MHz, D_2O): δ 5.44 (d, 1H, $J_{1,2}$

4.0 Hz, H-1α), 5.20 (bs, 1H, H-1β), α:β-ratio: 1.2:1; ^{13}C NMR: δ 102.0, 96.2 (C-1α/C-1β), 89.0, 88.8 ($J_{5\alpha,F}$=$J_{5\beta,F}$ 162.0 Hz, C-5α/C-5β), 82.9 ($J_{4,F}$ 28.1 Hz, C-4α/C-4β), 80.3 (C-2α/C-2β), 79.7 ($J_{4,F}$ 26.4 Hz, C-4α/C-4β), 75.8 (C-2α/C-2β), 74.8 ($J_{3,F}$ 3.3 Hz, C-3α/C-3β), 74.4 ($J_{3,F}$ 2.1 Hz, C-3α/C-3β), 17.5 ($J_{6,F}$ 21.1 Hz, C-6α/C-6β), 16.8 ($J_{6,F}$ 21.5 Hz, C-6α/C-6β).

*3.25. 5,6-Diazido-5,6-dideoxy-*D*-glucofuranose **30***

Compound **23** (262 mg, 0.969 mmol) was deprotected according to the respective general procedure and furnished **30** (217 mg, 97%) as a colourless oil. $[\alpha]^{20}_D$ −80.3 (*c* 2.5, MeOH); ^{1}H NMR (200 MHz, CD_3OD_3): δ 5.41 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1α), 5.10 (s, 1H, H-1β), α:β-ratio: 1:1.2; ^{13}C NMR: δ 104.7, 98.8 (C-1α/C-1β), 82.2, 82.1 (C-2α/C-2β), 79.2, 78.0, 77.1, 76.6 (C-3α/C-3β/C-4α/C-4β), 62.5, 61.9 (C-5α/C-5β), 54.5, 54.3 (C-6α/C-6β).

*3.26. 5,6-Dideoxy-*D-xylo-*hexofuranose **31***

Hydrolysis of the isopropylidene group in **24**[23] (192 mg, 1.02 mmol) according to the general procedure gave 126 mg (83%) of compound **31** as a colourless oil. $[\alpha]_D^{20}$ −0.5 (*c* 2.65, MeOH); [lit.[28] $[\alpha]_D^{20}$ +2 (*c* 0.9, EtOH)]; ^{1}H NMR (300 MHz, CD_3OD): δ 5.37 (d, 1H, $J_{1,2}$ 4.1 Hz, H-1α), 5.05 (s, 1H, H-1β), α:β-ratio: ≈1:1; ^{13}C NMR: δ 104.0, 97.3 (C-1α/C-1β), 84.9, 82.6, 81.8, 78.4, 77.6, 76.9 (C-2α/C-2β/C-3α/C-3β/C-4α/C-4β), 23.8, 23.0 (C-5α/C-5β), 10.8, 10.6 (C-6α/C-6β).

*3.27. 5,6-Dideoxy-6-fluoro-*D-threo-*hex-2-ulose **32***

Following the respective general procedure **25** (212 mg, 1.28 mmol) was isomerised yielding **32** (82 mg, 39%) as a colourless syrup. $[\alpha]_D^{20}$ −11.0 (*c* 2.9, MeOH); ^{1}H NMR (200 MHz, D_2O): δ 4.73–4.43 (m, 2H, $J_{6',F}$ 46.9 Hz, H-6, H-6′), 4.59 (d, 1H, $J_{1,1'}$ 19.5 Hz, H-1), 4.42 (d, 1H, H-1′), 4.30 (d, 1H, $J_{3,4}$ 2.2 Hz, H-3) 4.17 (ddd, 1H, $J_{4,5}$ 5.0 Hz, $J_{4,5'}$ 7.1 Hz, H-4), 2.02–1.8 (m, 2H, $J_{5,F}$ 28.4, $J_{5',F}$ 28.2 Hz, H-5, H-5′); ^{13}C NMR: δ 212.8 (C-2), 81.8 ($J_{6,F}$ 158.9 Hz, C-6), 77.7 (C-3), 68.3 ($J_{4,F}$ 4.0 Hz, C-4), 66.1 (C-1), 33.2 ($J_{5,F}$ 19.2 Hz, C-5). Anal. calcd for $C_6H_{11}O_4F$: C, 43.37; H, 6.67; found: C, 43.15; H, 6.90.

*3.28. 6-Azido-5,6-dideoxy-*D-threo-*hex-2-ulose **33***

Enzymatic isomerisation according to the respective general procedure of **26** (183 mg, 0.967 mmol) gave 66 mg (36%) of the open-chain ketose **33** as a colourless syrup. $[\alpha]_D^{20}$ +19.3 (*c* 1.25, MeOH); ^{1}H NMR (200 MHz, CD_3OD): δ 4.56 (d, 1H, $J_{1,1'}$ 19.2 Hz, H-1), 4.43 (d, 1H, H-1′), 4.14 (d, 1H, $J_{3,4}$ 2.4 Hz, H-3) 4.05 (ddd, 1H, $J_{4,5}$ 5.1 Hz, $J_{4,5'}$ 8.0 Hz, H-4), 3.45 (m, 2H, H-6, H-6′), 1.83 (m, 2H, H-5, H-5′); ^{13}C NMR: δ 213.4 (C-2), 79.5, 70.6 (C-3/C-4), 67.9 (C-1), 49.3 (C-6), 33.5 (C-5). Anal. calcd for $C_6H_{11}O_4N_3$: C, 38.10; H, 5.86; found: C, 38.01; H, 5.97.

*3.29. 5,6-Dideoxy-5,6-difluor-*D-*fructose **34***

The general procedure for the enzymatic isomerisation was applied. Compound **27** (340 mg, 1.85 mmol) afforded 173 mg (51%) of syrupy compound **34**. $[\alpha]_D^{20}$ −28.0 (*c* 3.1, MeOH); ^{1}H NMR (200 MHz, D_2O): δ 5.0–4.5 (m, 4H, H-3, H-5, H-6, H-6′), 4.65 (d, 1H, $J_{1,1'}$ 19.8 Hz, H-1), 4.52 (d, 1H, H-1′), 4.29 (m, 1H, H-4); ^{13}C NMR: δ 212.9 (C-2), 89.9 ($J_{5,F\text{-}5}$ 174.8 Hz, $J_{5,F\text{-}6}$ 17.8 Hz, C-5), 82.3 ($J_{6,F\text{-}6}$ 168.3

Hz, $J_{6,F\text{-}5}$ 19.5 Hz, C-6), 74.6 (C-3), 67.9 ($J_{4,F\text{-}5}$ 26.6 Hz, $J_{4,F\text{-}6}$ 7.1 Hz, C-4), 66.2 (C-1). Anal. calcd for $C_6H_{10}O_4F_2$: C, 39.14; H, 5.47; found: C, 39.00; H, 5.61.

3.30. 6-Azido-5,6-didesoxy-5-fluor-D-fructose **35**

Application of the respective general procedure to starting material **28** (346 mg, 1.67 mmol) afforded 186 mg (54%) of desired fructose derivative **35**. $[\alpha]_D^{20}$ +9.1 (*c* 4.4, MeOH); ^{1}H NMR (200 MHz, D_2O): δ 4.76 (m, 1H, $J_{5,F}$ 46.3 Hz, $J_{5,6}$ 2.0 Hz, $J_{5,6'}$ 5.6 Hz, H-5), 4.56 (d, 1H, $J_{3,4}$ 1.5 Hz, H-3) 4.64 (d, 1H, $J_{1,1'}$ 19.5 Hz, H-1), 4.50 (d, 1H, H-1′), 4.22 (ddd, 1H, $J_{4,5}$ 4.7 Hz, $J_{4,F}$ 8.5 Hz, H-4), 3.82 (ddd, 1H, $J_{6,F}$ 25.5 Hz, $J_{6,6'}$ 14.1 Hz, H-6), 3.61 (ddd, 1H, $J_{6',F}$ 29.4 Hz, H-6′); the ^{13}C NMR data are essentially the same as reported in Ref. 29; ^{13}C NMR: δ 212.9 (C-2), 90.3 ($J_{5,F}$ 174.9 Hz, C-5), 74.6 ($J_{3,F}$ 2.0 Hz, C-3), 69.4 ($J_{4,F}$ 25.9 Hz, C-4) 66.2 (C-1), 51.2 ($J_{6,F}$ 19.0 Hz, C-6). Anal. calcd for $C_6H_{10}O_4FN_3$: C, 34.79; H, 4.87; found: C, 34.59; H, 4.98.

3.31. 5,6-Dideoxy-5-fluor-D-fructose **36**

Compound **29** (200 mg, 0.97 mmol) were reacted according to the general procedure to give desired fructose derivative **36** (126 mg, 63%). $[\alpha]_D^{20}$ −43.0 (*c* 2.65 MeOH); ^{1}H NMR (200 MHz, D_2O): δ 4.90–4.55 (m, 2H, $J_{5,6}$ 6.4 Hz, $J_{5,F}$ 44.0 Hz, H-5, H-3), 4.63 (d, 1H, $J_{1,1'}$ 19.5 Hz, H-1), 4.49 (d, 1H, H-1′), 3.95 (ddd, 1H, $J_{4,F}$ 7.5 Hz, $J_{4,5}$ 7.5 Hz, $J_{3,4}$ 1.6 Hz, H-4), 1.40 (dd, 3H, $J_{6,F}$ 25.9 Hz, H-6); ^{13}C NMR: δ 213.2 (C-2), 89.4 ($J_{5,F}$ 166.1 Hz, C-5), 74.7 ($J_{3,F}$ 2.9 Hz, C-3), 73.5 ($J_{4,F}$ 25.7 Hz, C-4), 66.1 (C-1), 16.8 ($J_{6,F}$ 21.5 Hz, C-6). Anal. calcd for $C_6H_{11}O_4F$: C, 43.37; H, 6.67; found: C, 43.35; H, 6.78.

3.32. 5,6-Diazido-5,6-dideoxy-D-fructose **37**

Starting from **30** (173 mg, 0.752 mmol) and applying the respective general procedure, 38 mg (22%) of compound **37** were obtained. Fructose derivative **37** was a faintly yellow, unstable oil which decomposed rapidly in the refrigerator. $[\alpha]_D^{20}$ −122.5 (*c* 0.65, $CHCl_3$); ^{1}H NMR (200 MHz, CD_3OD_3): δ 4.56 (d, 1H, $J_{1,1'}$ 19.2 Hz, H-1), 4.44 (d, 1H, H-1′), 4.44 (d, 1H, $J_{3,4}$ 1.5 Hz, H-3), 3.86 (dd, 1H, $J_{4,5}$ 9.2 Hz, H-4), 3.80–3.67 (m, 2H, $J_{6,6'}$ 13.0 Hz, $J_{5,6}$ 2.4 Hz, $J_{5,6'}$ 7.0 Hz, H-5, H-6), 3.51 (dd, 1H, H-6′); ^{13}C NMR: δ 213.6 (C-2), 77.0, 72.5 (C-3/C-4), 68.0 (C-1), 63.6 (C-5), 53.6 (C-6). Anal. calcd for $C_6H_{10}O_4N_6$: C, 31.31; H, 4.38; found: C, 31.22; H, 4.50.

3.33. 5,6-Dideoxy-D-threo-hex-2-ulose **38**

The general isomerisation procedure was applied. Compound **31** (110 mg, 0.742 mmol) led to 60 mg (55%) of compound **38**. $[\alpha]_D^{20}$ −16.8 (*c* 1.8, MeOH) [lit.[30] $[\alpha]_D^{20}$ −14.6 (*c* 1, MeOH)]. The NMR data are identical with previously published values.[30]

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References

1. Slatner, M.; Nidetzky, B.; Kulbe, K. D. *Biochemistry* **1999**, *38*, 10489–10498.
2. Jeffery, J.; Jörnvall, H. *Adv. Enzymol*. **1988**, *61*, 47–106.
3. (a) Devaux-Basseguy, R.; Bergel, A.; Comtat, M. *Enzyme Microb. Technol*. **1997**, *20*, 248–258; (b) Hummel, W.; Kula, M.-R. *Eur. J. Biochem*. **1989**, *184*, 1–13.
4. Nidetzky, B.; Mayr, P.; Hadwiger, P.; Stütz, A. E. *Biochem. J*. **1999**, in press.
5. Gijsen, H. J. M.; Qiao, L.; Fitz, W.; Wong, C.-H. *Chem. Rev*. **1996**, *96*, 443–473.
6. Bock, K.; Meldal, M.; Meyer, B.; Wiebe, L. *Acta Chim. Scand*. **1983**, *B37*, 101–108.
7. For a review, see: de Raadt, A.; Ekhart, C. W.; Stütz, A. E. *Adv. Detailed React. Mechan*. **1995**, *5*, 175–211.
8. Berger, A.; de Raadt, A.; Gradnig, G.; Grasser, M.; Löw, H.; Stütz, A. E. *Tetrahedron Lett*. **1992**, *33*, 7125–7128.
9. For reviews, see: (a) de Raadt, A.; Ebner, M.; Ekhart, C. W.; Fechter, M.; Lechner, A.; Strobl, M.; Stütz, A. E. *Catal. Today* **1994**, *22*, 549–561; (b) Fechter, M. H.; Stütz, A. E.; Tauss, A. *Curr. Org. Chem*. **1999**, *3*, 269–285.
10. Fechter, M. H.; Stütz, A. E. *Carbohydr. Res*. **1999**, *319*, 55–62.
11. Moravcova, J.; Capkova, J.; Stanek, J. *Carbohydr. Res*. **1994**, *263*, 7–16.
12. Kent, P. W.; Young, R. C. *Tetrahedron* **1971**, *27*, 4057–4064.
13. Effenberger, F.; Null, V.; Ziegler, T. *Tetrahedron Lett*. **1992**, *33*, 5157–5160.
14. Hadwiger, P.; Stütz, A. E. *J. Carbohydr. Chem*. **1998**, *17*, 1259–1267.
15. Hadwiger, P.; Mayr, P.; Tauss, A.; Stütz, A. E.; Nidetzky, B. *Bioorg. Med. Chem. Lett*. **1999**, *9*, 1683–1686.
16. Albert, R.; Dax, K.; Pleschko, R.; Stütz, A. E. *Carbohydr. Res*. **1985**, *137*, 282–290.
17. Dax, K.; Rauter, A. P.; Stütz, A. E.; Weidmann, H. *Liebigs Ann. Chem*. **1981**, 1768–1773.
18. Paulsen, H.; Stoye, D. *Chem. Ber*. **1966**, *99*, 908–919.
19. Dax, K.; Wolflehner, W.; Weidmann, H. *Carbohydr. Res*. **1978**, *65*, 132–138.
20. Cox, D. P.; Terpinski, J.; Lawrynowicz, W. *J. Org. Chem*. **1984**, *49*, 3216–3219.
21. Albert, R.; Dax, K.; Seidl, S.; Sterk, H.; Stütz, A. E. *J. Carbohydr. Chem*. **1985**, *4*, 513–520.
22. Dax, K.; Gaigg, B.; Grassberger, V.; Kölbinger, B.; Stütz, A. E. *J. Carbohydr. Chem*. **1990**, *9*, 479–499.
23. Hall, L. D.; Hough, L.; Pritchard, R. A. *J. Chem. Soc*. **1961**, 1537–1545.
24. Andersen, S. M.; Ebner, M.; Ekhart, C. W.; Gradnig, G.; Legler, G.; Lundt, I.; Stütz, A. E.; Withers, S. G.; Wrodnigg, T. *Carbohydr. Res*. **1997**, *301*, 155–166.
25. Bock, K.; Lundt, I.; Pedersen, C. *Carbohydr. Res*. **1981**, *90*, 7–16.
26. Hudlicky, M. *Org. React*. **1988**, *35*, 513–637.
27. Csuk, R.; Glänzer, B. I. *Adv. Carbohydr. Chem. Biochem*. **1988**, *46*, 73–177.
28. Gorin, P. A. J.; Hough, L.; Jones, J. K. N. *J. Chem. Soc*. **1955**, 2699–2705.
29. Kajimoto, T.; Liu, K. K.-C.; Pederson, R. L.; Zhong, Z.; Ichikawa, Y.; Porco Jr., J. A.; Wong, C.-H. *J. Am. Chem. Soc*. **1991**, *113*, 6187–6196.
30. Bednarski, M. D.; Simon, E. S.; Bischofsberger, N.; Fessner, W.-D.; Kim, M.-J.; Lee, W.; Saito, T.; Waldmann, H.; Whitesides, G. M. *J. Am. Chem. Soc*. **1989**, *111*, 627–635.