Enzymes in Organic Synthesis, 15[+]
Short Enzymatic Synthesis of L-Fucose Analogs

Wolf-Dieter Fessner,*[a,b] Claudius Goße,[b] Georg Jaeschke,[b] and Oliver Eyrisch[b]

Dedicated to Prof. George M. Whitesides on the occasion of his 60th birthday

Keywords: Aldol reactions / Carbohydrates / C–C coupling / Isomerases / Controlled ozonolysis

A short enzymatic route for the synthesis of L-fucose analogs modified at the nonpolar terminus is reported. In particular, fucose derivatives bearing extended linear (**1b**) and branched (**1e**) saturated, or various unsaturated (**1c**, **1d**) aliphatic chains have been prepared, in order to increase hydrophobic contacts. The rather general approach involves a sequential application of the recombinant enzymes L-fuculose 1-phosphate aldolase (FucA) and L-fucose ketol isomerase (FucI) from *E. coli*. Enantiomerically pure L-fucose analogs have been prepared in up to 30% overall yield starting from the appropriate hydroxyaldehyde precursors and dihydroxyacetone phosphate as readily available components. Unsaturated 2-hydroxyaldehydes have been efficiently prepared by alk(en/yn)yl Grignard addition to cinnamaldehyde followed by controlled ozonolysis of the styrene fragment.

Introduction

In nature, complex oligosaccharides serve to encode information for a broad spectrum of biological recognition processes.[1] Of the sugars occurring most frequently in mammalian oligosaccharides, L-fucose **1a** is the most hydrophobic residue. Fucosylated oligosaccharides are of importance as partial structures of bacterial polysaccharides[2] as well as of glycoproteins[3] and glycolipids[4] useful for cancer diagnostics[5] or immunotyping.[1b] The fucosylated blood group antigens Le^b and H-1 mediate adherence to the human gastric epithelium of *Helicobacter pylori*,[6] the causative agent of chronic gastritis and peptic ulceration[7] and associated with the development of gastric adenocarcinoma.[8] The sialyl Lewis X (sLe^x) determinant has been identified as a tetrasaccharide ligand (Figure 1) of the selectins,[9] a family of cell adhesion molecules involved in the early stages of the inflammation process.[10]

Studies using synthetic carbohydrates with specific structural modifications of the sLe^x epitope have shown that the specific hydroxylation pattern on the fucose residue is mandatory for ligand recognition by E- and L-selectins.[11] Elaborate NMR studies on sLe^{x}[12–14] have revealed that, in addition to interactions of the key polar groups, the fucose methyl group is involved in van der Waals contacts with the ring surface of the galactose moiety, which are likely to stabilize the active conformation (Figure 1).[15] In fact, it has been demonstrated that removal of this methyl group, i.e. replacement of L-fucose by D-arabinose, leads to a 5-fold decrease in binding potency.[16]

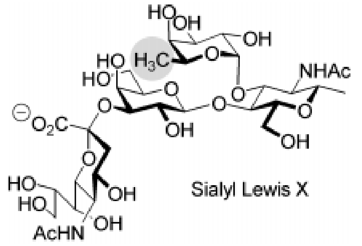


Figure 1. Major conformation of the sLe^x tetrasaccharide in solution and complexed with *E*-selectin, as determined by NMR studies

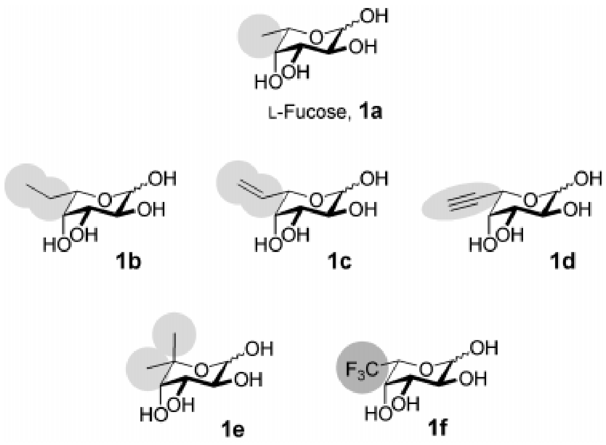
To facilitate investigations on the significance of hydrophobic contacts of fucosylated structures with regard to intramolecular stabilization of oligosaccharide conformations and intermolecular contacts during receptor binding, we now report the synthesis of a number of L-fucose analogs in which the methyl terminus of **1a** (Scheme 1) is augmented by hydrocarbon fragments (**1b**, **1e**), including some having conformationally less flexible unsaturated chains (**1c**, **1d**). In addition, a novel synthesis of the 6,6,6-trifluoro derivative **1f**[17] has been pursued. It should be pointed out that a rather broad substrate specificity has been documented for fucosyl transferases in relation to modifications of fucose at C-6,[18] which renders such derivatives particularly attractive for biological studies.

Our synthetic approach was based on a two-step enzymatic sequence (Scheme 2), consisting of a C−C bond forming reaction catalyzed by a dihydroxyacetone phosphate (DHAP, **3**) dependent aldolase to effect asymmetric chain extensions of appropriate aldehydes to ketoses (**5**), followed by a biocatalytic ketol isomerization to give the target aldose analogs (**1**). This strategy was originally developed by Wong et al. for the synthesis of certain D-glucose derivatives

---

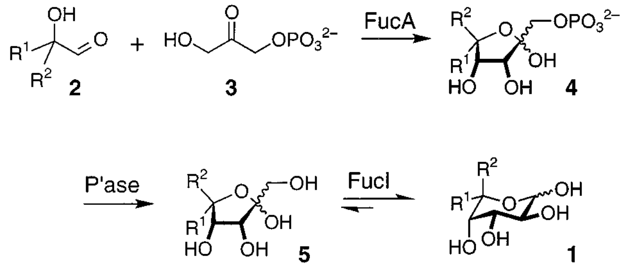
[+] Part 14: Ref.[22]

[a] Technische Universität Darmstadt, Institut für Organische Chemie,
Petersenstraße 22, D-64287 Darmstadt, Germany
Fax: (internat.) +49 (0)6151/166636
E-mail: fessner@tu-darmstadt.de
[b] RWTH Aachen, Institut für Organische Chemie,
Professor-Pirlet-Straße 1, D-52056 Aachen, Germany



Scheme 1. L-Fucose analogs modified by chain termini of increased hydrophobicity

using commercial fructose 1,6-bis(phosphate) aldolase (FruA; EC 4.1.2.13) in conjunction with glucose isomerase (GlcI; EC 5.3.1.5).[19] Recently, we have studied several other microbial DHAP aldolases and isomerases, which are readily available by recombinant expression,[20–22] and we have shown that these enzymes are highly stereoselective yet display sufficient tolerance towards substrate analogs to support a general strategy for the *de novo* synthesis of a range of unusual and nonnatural sugars.[21][23] In particular, the L-fuculose 1-phosphate aldolase (FucA; EC 4.1.2.17) and L-fucose isomerase (FucI; EC 5.3.1.3) from *E. coli* have proved to be of utility in the synthesis and conversion of L-fucose, as well as of its derivatives and analogs.[20,21,23–26] More recently, Wong's group has reported similar findings.[27]



	R^1	R^2
a	CH_3	H
b	$CH_2{-}CH_3$	H
c	$CH{=}CH_2$	H
d	$C{\equiv}CH$	H
e	CH_3	CH_3
f	CF_3	H

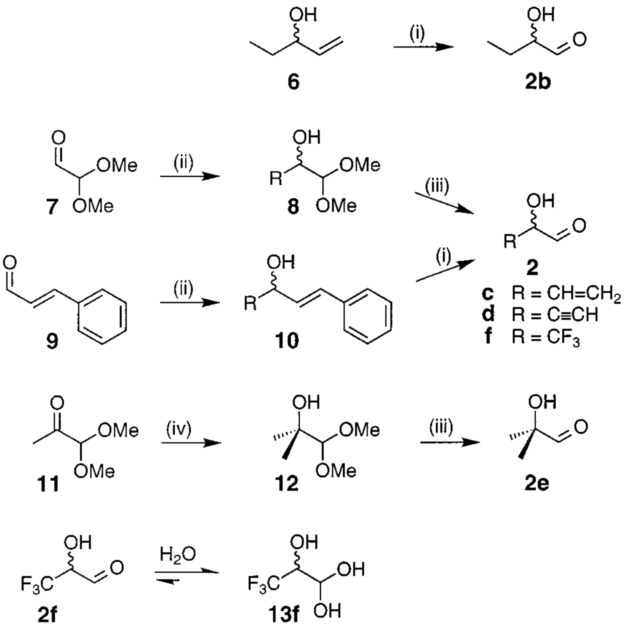
Scheme 2. Enzymatic synthesis of L-fucose analogs by aldolization−isomerization; FucA = L-fuculose 1-phosphate aldolase, P'ase = phosphatase, FucI = L-fucose isomerase

Results and Discussion

Our synthetic plan required α-hydroxylated aldehydes **2b−f** with various side chains as starting materials for the aldol reactions (Scheme 3). 2-Hydroxy-2-methylpropanal **2e** was synthesized by reaction of 1,1-dimethoxyacetone **11**[19] with methylmagnesium bromide and subsequent acid hydrolysis.[28] At first, dimethoxyethanal **7** was used as a building block to prepare the remaining components, which were obtained by addition of suitable alk(en/yn)yl Grignard reagents followed by cleavage of the dimethyl acetals **8**. This furnished the aldehydes **2** as aqueous solutions that could be used directly for the enzymatic steps. However, the yields from the Grignard additions were often less than satisfactory (ca. 50%) owing to the fact that commercial **7** is sold as a 45% aqueous solution, is difficult to dry rigorously, and shows a high propensity to undergo oligomerization. Moreover, the trifluoromethyl derivative **8f**, generated by treatment of **7** with $TMSCF_3$ in the presence of catalytic amounts of TBAF,[29] proved surprisingly resistant to acetal hydrolysis by strong mineral acids, even at elevated temperatures, as a result of the strong electron-withdrawing effect of the CF_3 group. In view of the applications envisaged for the fucose analogs, routes from **7** thus proved impractical.

More conveniently, 2-hydroxybutyraldehyde **2b** could be generated from commercial 3-hydroxypentene **6** by ozonolysis in methanol followed by reductive workup. Because the formaldehyde formed as a by-product is an excellent competing substrate in the aldolization,[20] this had to be removed by repeated concentration of the aqueous product solution. Since alkene ozonolysis at −78 °C has proved to be a most versatile and efficient method for the synthesis of aldehydes under mild conditions[30] and is compatible with a wide range of functional groups, we chose to assess the utility of cinnamaldehyde **9** as a common precursor for the remaining aldehydes **2c**,**d**,**f**. The regioselective ozonolysis of compounds with multiple sites of unsaturation seemed a feasible aim in the light of earlier kinetic studies, which had demonstrated a significant retardation in the rate of ozone capture by alkenes having reduced electron density and/or polarity.[31] Thus, we anticipated that the conjugated, internal styrene π-bond would be considerably more reactive than that of a terminal alkene, and particularly that of an alkyne. In practice, nucleophilic additions to **9** proved unremarkable. Controlled ozonolyses of **10c**,**d** were performed by restricting the amount of ozone to equal to or slightly less than one equivalent per mol of substrate. The kinetic selectivity seemed to be practically complete, as determined by high-field ^{1}H-NMR analysis of the crude material obtained from an incomplete conversion of **10c**, which showed no detectable trace of the styrene-containing hydroxy aldehyde (≤2%). Thus, the styrene π-system was evidently far more reactive than the vinyl fragments in both the starting diene and the initially formed ozonide. 3,3,3-Trifluoro-2-hydroxypropanal **2f** was also cleanly generated from the corresponding styrene precursor **10f** by ozonolysis.

After standard reductive workup with dimethyl sulfide, the resulting aqueous aldehyde solutions were extracted with diethyl ether to remove benzaldehyde. The hydroxyaldehyde solutions could then be used directly without any further purification for the enzymatic aldol addition since the by-product DMSO formed in the ozonide reduction is known to be generally well-tolerated by aldolases.[20][32]



Scheme 3. Synthesis of 2-hydroxy aldehydes **2**: (i) O_3 (1 equiv.), MeOH, −78°C; Me_2S, room temp., (ii) **c**,**d**: R^1MgX; NH_4Cl, **f**: $TMSCF_3$, cat. TBAF; HCl, (iii) H_3O^+, (iv) CH_3MgBr; NH_4Cl

The natural substrate of the FucA from *E. coli* is L-lactaldehyde **2a**. In previous studies,[20,24,26] we established that hydroxylated aldehydes are good substrates for the recombinant FucA from *E. coli*, resulting in complete stereocontrol at the newly created C−C bond with respect to both asymmetric centers. Moreover, we showed that the microbial aldolase displays an overwhelming kinetic preference for the L-configured antipodes of 2-hydroxyaldehydes[24][26] (in contrast, e.g., to the negligible preference shown by rabbit muscle aldolase[32]), which allows efficient and complete kinetic resolution of racemic precursors.

In practice, aldehydes **2b−d** were found to be accepted well with formation of the corresponding ketose 1-phosphates **4b−d** (Scheme 2). DHAP (**3**) was synthesized enzymatically from glycerol 3-phosphate by using the convenient procedure[33] based on glycerol phosphate oxidase (EC 1.1.3.21). With **2d**, it was found essential that the pH was kept precisely at 7.0 to prevent acid- or base-catalyzed rearrangement with formation of highly electrophilic enzyme deactivators. When *rac*-aldehydes were used in ⩾ 3-fold excess, single diastereomers were indeed obtained, having a relative and absolute configuration identical to that of authentic natural L-fuculose 1-phosphate (**4a**),[26] as shown by ^{1}H-NMR analysis of the crude sugar phosphates. Thus, the kinetic enantioselectivity was not impaired by the steric bulk of the hydrophobic side chain (*de* ≥95%), which allowed effective control over three adjacent stereogenic centers in a single step. These results further corroborate our mechanistic hypothesis for the FucA catalysis as derived from inhibition[34] and X-ray[35] investigations, which requires an intrinsic recognition of a 2-hydroxyl group of the aldehyde moiety in the transition state.[36]

The sterically more demanding branched dimethyl substrate **2e** was converted more slowly and, for preparative purposes, required larger quantities of the catalyst to achieve a sufficient reaction rate. The trifluoromethylated analog **2f**, however, did not serve as a substrate at all. Upon prolonged incubation in the presence of DHAP, a decrease in the pH was indicative of base-catalyzed HF elimination. No conversion of **2f** could be realized, neither by high catalyst loading nor by strict pH control. According to its ^{1}H-NMR spectrum, the aldehyde exists in water exclusively in its hydrated form **13f**, owing to the electron-withdrawing trifluoromethyl group, so that the free aldehyde is seemingly not available to act as a substrate for the aldolase.

The sugar phosphates were hydrolyzed enzymatically to furnish the free ketoses. Acid phosphatase (EC 3.1.3.2) at pH 6.0 was preferred initially because of the sensitive nature of the all-*cis* substitution pattern in the furanose ring isomers that predominate in solution for both **4** and **5**.[26] However, it was subsequently found that dephosphorylation could also be achieved without complications using a more economical alkaline phosphatase (EC 3.1.3.2) at pH 8.5 since all compounds proved sufficiently stable towards epimerization under these conditions. For analytical purposes, free ketoses **5** were purified by chromatography on silica gel. The unsatisfactory material balance recovered following purification (e.g. ca. 30% yield of **5b**) reflects the sensitivity of the ketoses, but this can be obviated by direct submission of the crude material to the ensuing step.

For isomerization of the ketoses, L-fucose isomerase (FucI) from *E. coli*[26] was employed. This enzyme, for which recent X-ray determinations suggest an enediol mechanism rather than a hydride shift,[37] catalyzes reversible ketol isomerization between sugars having absolute (2*S*,3*R*)-stereochemistry, but tolerates modifications at further centers permitting the use of epimeric, functionalized, or deoxy sugars.[21,25,38] The recombinant isomerase was produced and purified as described previously.[26] All the terminus-modified fuculose analogs **5b−e** indeed proved to be FucI substrates. Reactions could readily be monitored by TLC on silica plates by adding phenylboronic acid to the eluent to facilitate separation by progressive elution of *cis*-*vic* diol compounds. Isomerization rates decreased considerably with increasing steric bulk and rigidity of the chain terminus (**5b** < **5c** < **5d** < **5e**), which indicates reduced substrate recognition by the FucI. In fact, the *E. coli* enzyme has a rather weak affinity for even its natural substrate L-fucose (**1a**; K_m = 37 mM[38]) and an earlier detailed study using a variety of substrate analogs showed that K_m values increase rapidly upon even small structural modifications.[38] Thus, it proved to be advantageous to dissolve the keto sugars **5** in the enzyme preparation to achieve high substrate concentrations, to increase the catalyst quantity (up to 350 U mmol^{-1}), and to extend reaction times (up to several days). The aldose/ketose mixture proved to be readily separable by complexation chromatography on a Ca^{2+}-charged cation-exchange resin, providing the aldoses **1** as crystalline solids. Alternatively, aldose separation from the mixtures may be greatly facilitated, particularly on a larger scale, by a group-specific conversion of the ketose isomers to sugar phosphates by using recombinant rhamnulose kinase (RhuK; EC 2.7.1.5), which displays adequate substrate tolerance.[24][26]

All equilibrations were found to be strictly stereospecific with formation of only a single aldose diastereomer, as shown by high-field ^{1}H-NMR analysis, thus confirming the complete stereocontrol anticipated for enzymes of this type.[39] The equilibrium fucose/fuculose ratio of 89:11 in favor of the aldose[40] reflects the relative instability of the all-*cis* substitution pattern in the ketofuranose and the higher stability of the six-membered aldopyranose ring system. In the **1b**/**5b** couple, with an ethyl side chain in place of the methyl group of the natural substrate, a virtually identical ratio of ca. 90:10 was obtained and the crystalline *homo*-fucose **1b** was isolated in high yield (83%). Although similar ratios were also expected for the remaining analogs investigated, only a 75:25 ratio was seen with **1c**/**5c** and even smaller fractions were obtained with the corresponding **d**/**e** pairs after comparable reaction times. Presumably, the inferior substrate quality of the sterically more rigid derivatives precluded the attainment of overall equilibrium in the preparative reactions and thus limited the yields of the corresponding aldoses **1c**,**d**,**e** (58, 40, 34%). Similar observations have recently been reported for related compounds bearing other substituents in the 6-position.[27] However, the facile access to large quantities of FucI from recombinant clones[26] and its sufficiently high protein stability compensate for this disadvantage if a rapid synthesis of these or related L-fucose analogs is required.

Proton and carbon NMR spectra of the fucose analogs **1b**−**e** show gross overall similarity to those of the parent compound in terms of coupling constants and anomer ratios. This shows that the analogs adopt very similar structures in solution and hence can be expected to serve as suitable surrogates of L-fucose in a biological environment.

Conclusion

We have described herein the short and efficient enzymatic synthesis of four new L-fucose analogs modified at the hydrophobic tail. The route employs inexpensive (racemic) starting materials and readily available enzymes and is completely stereoselective at each step in controlling the configuration at all four consecutive centers of chirality. Whereas this strategy seems to be sufficiently flexible to be used in the synthesis of other L-fucose structural analogs,[25][38] the low substrate affinity shown by FucI upon structural modification of the substrate remains a shortcoming. However, this is not a general feature of the strategy, but of the *E. coli* enzyme in particular, and may thus be overcome by appropriate protein engineering on the basis of the known 3D structure[37] or by screening for more tolerant FucI enzymes if demand exists. The aldolization−isomerization route nicely complements other chemical or enzymatic methods for deoxy sugar synthesis.[41] The intrinsic reactivity of the unsaturated sugar derivatives **1c**−**d** is particularly attractive with regard to investigations into the metabolism of fucose-containing natural products and may be suitable for the covalent restriction of oligosaccharide conformation through intramolecular bridging reactions. Work in these areas is currently in progress.

Experimental Section

General Remarks: NMR spectra were recorded on Varian VXR 300 and Unity 500 or Bruker AC 250 and WM 400 spectrometers; chemical shifts are referenced to internal TMS ($CDCl_3$) or TSP (D_2O; $\delta = 0.00$). − Mass spectra were recorded on a Finnigan MAT 212 system and elemental analyses were performed on a Heraeus CHN-O-Rapid system. A Fischer 502 ozone generator was used for ozonolyses. A Schott TitroLine alpha autotitrator was employed for pH control. − Column chromatography was performed on Merck 60 silica gel (0.063−0.200 mesh); analytical thin-layer chromatography was performed on Merck silica gel plates 60 GF_{254} using anisaldehyde staining for detection. Analytical grade ion-exchange media (100−200 mesh) were purchased from Bio-Rad. Recombinant fuculose 1-phosphate aldolase and fucose isomerase from *E. coli* were prepared according to published procedures.[20][26] Acid phosphatase was purchased from Boehringer Mannheim; alkaline phosphatase from bovine intestinal mucosa (synthetic purity grade) was purchased from Fluka. Dihydroxyacetone phosphate was prepared from glycerol phosphate.[33]

(±)-2-Hydroxybutanal (2b): Ozone was passed through a solution of 3-hydroxypentene **6** (0.86 g, 10.0 mmol) in methanol (50 mL) at −78 °C until a blue color persisted. The excess ozone was then removed by nitrogen purging, Me_2S (5.0 mL, 68.6 mmol) was added, and the mixture was stirred at room temp. for 3 h. In order to remove the formaldehyde produced, the solution was repeatedly concentrated to a quarter of its original volume in vacuo and rediluted with methanol. Water (100 mL) was then added, and methanol was removed in vacuo. The resulting aqueous solution of **2b** was used directly for enzymatic reactions without further purification. − ^{1}H NMR (250 MHz, D_2O); carbonyl form (35%): δ = 9.66 (d, 1-H), 3.67 (q, 2-H), 1.47 (qdd, 3-H), 1.38 (qdd, 3-H′), 0.97 (t, 4-H); hydrate (65%): δ = 4.86 (d, 1-H), 3.43 (ddd, 2-H), 1.71 (qdd, 3-H), 1.65 (qdd, 3-H′), 0.97 (t, 4-H).

6,7-Dideoxy-L-*lyxo*-heptulose 1-Phosphate (4b): An aqueous solution of dihydroxyacetone phosphate (**3**) (30 mL, 1.0 mmol) was combined with a solution of **2b** (50 mL, 5.0 mmol) and the mixture was adjusted to pH 6.8. It was then incubated with FucA (200 U) for 2 d at room temp. The product was isolated by anion-exchange chromatography (Dowex AG1-X8, HCO_3^- form), eluting with 0.2 M triethylammonium bicarbonate buffer. Repeated concentration from water (3 × 10 mL), ion-exchange to give the free acid (Dowex AG50 W-X8, H^+ form), neutralization with cyclohexylamine, followed by crystallization from 90% aqueous ethanol provided the colorless bis(cyclohexylammonium) salt of **4b** (411 mg, 90%). − **α anomer:** ^{1}H NMR (400 MHz, D_2O): δ = 4.48 (d, 3-H), 4.26 (dd, 4-H), 3.92 (td, 5-H), 3.88 (m, 1-H), 1.70 (qd, 6-H), 1.69 (qd, 6-H′), 0.95 (t, 7-H); $J_{3,4}$ = 6.8 Hz, $J_{4,5}$ = 4.5 Hz, $J_{5,6}$ = 7.5 Hz, $J_{5,6'}$ = 7.5 Hz, $J_{6,7}$ = 7.5 Hz, $J_{6',7}$ = 7.5 Hz. − **β anomer:** ^{1}H NMR (400 MHz, D_2O): δ = 4.28 (d, 3-H), 4.23 (dd, 4-H), 4.10 (td, 5-H), 3.88 (m, 1-H), 1.65 (qd, 6-H), 1.69 (qd, 6-H′), 0.94 (t, 7-H); $J_{3,4}$ = 5.0 Hz, $J_{4,5}$ = 3.0 Hz, $J_{5,6'}$ = 7.5 Hz, $J_{6,7}$ = 7.5 Hz, $J_{6',7}$ = 7.5 Hz; anomer ratio α:β = 1:2. − ^{13}C NMR (100.6 MHz, D_2O): δ = 101.9

(C-2), 82.0 (C-5), 72.3 (C-4), 71.3 (C-3), 66.5 (C-1), 22.8 (C-6), 9.7 (C-7, $J_{C-2,P}$ = 7.7 Hz).

6,7-Dideoxy-L-*lyxo*-heptulose (5b): An aqueous solution of **4b** (370 mg, 0.81 mmol) was incubated at room temp. with acid phosphatase (30 U) at pH 6.0. After 3 d, the product was purified by chromatography on silica (methanol/chloroform, 5:1) to give **5b** as a colorless, glassy solid (41 mg, 29%). − **α anomer:** ^{1}H NMR (400 MHz, D_2O): δ = 4.23 (d, 3-H), 4.21 (dd, 4-H), 3.88 (td, 5-H), 3.60 (d, 1-H), 3.53 (d, 1-H′), 1.70 (qd, 6-H), 1.69 (qd, 6-H′), 0.95 (t, 7-H); $J_{1,1'}$ = 11.7 Hz, $J_{3,4}$ = 4.8 Hz, $J_{4,5}$ = 2.8 Hz, $J_{5,6}$ = 7.2 Hz, $J_{5,6'}$ = 7.2 Hz, $J_{6,7}$ = 7.5 Hz, $J_{6',7}$ = 7.5 Hz. − **β anomer:** ^{1}H NMR (400 MHz, D_2O): δ = 4.32 (d, 3-H), 4.20 (dd, 4-H), 4.10 (td, 5-H), 3.67 (d, 1-H), 3.56 (d, 1-H′), 1.65 (qd, 6-H), 1.69 (qd, 6-H′), 0.94 (t, 7-H); anomer ratio α:β = 1:2.

6,7-Dideoxy-L-*galacto*-heptose (1b): An aqueous solution (0.5 mL) of **5b** (24 mg, 0.13 mmol) was incubated with FucI (20 U) for 3 d at room temp. Protein was removed by filtration through charcoal and the sugars were separated by chromatography on cation-exchange resin (Dowex AG50 W-X8, Ca^{2+} form) eluting with water. Fractions containing the aldose product **1b** were eluted first, which was crystallized from ethanol/ethyl acetate (20 mg, 83%). − **α anomer:** ^{1}H NMR (400 MHz, D_2O): δ = 5.27 (d, 1-H), 3.84 (dd, 4-H), 3.63 (dd, 3-H), 3.53 (td, 5-H), 3.47 (dd, 2-H), 1.51 (qd, 6-H), 0.95 (t, 7-H); $J_{1,2}$ = 7.8 Hz, $J_{2,3}$ = 9.8 Hz, $J_{3,4}$ = 3.5 Hz, $J_{4,5}$ = 0.3 Hz, $J_{5,6}$ = 7.2 Hz, $J_{6,7}$ = 7.5 Hz. − ^{13}C NMR (100.6 MHz, D_2O): δ = 96.8 (C-1), 76.8 (C-4), 73.6 (C-3), 72.4 (C-2), 70.1 (C-5), 23.4 (C-6), 9.7 (C-7). − **β anomer:** ^{1}H NMR (400 MHz, D_2O): δ = 4.55 (d, 1-H), 3.92 (dd, 4-H), 3.91 (td, 5-H), 3.85 (dd, 3-H), 3.78 (dd, 2-H), 1.52 (qd, 6-H), 0.94 (t, 7-H); $J_{1,2}$ = 3.7 Hz, $J_{2,3}$ = 10.2 Hz, $J_{3,4}$ = 3.0 Hz, $J_{4,5}$ = 0.2 Hz, $J_{5,6}$ = 7.2 Hz, $J_{6,7}$ = 7.5 Hz; anomer ratio α:β = 31:69. − ^{13}C NMR (100.6 MHz, D_2O): δ = 92.6 (C-1), 72.2 (C-4), 70.6 (C-3), 70.0 (C-2), 68.8 (C-5), 23.4 (C-6), 9.7 (C-7). − $C_7H_{14}O_5$ (178.186): calcd. C 47.19, H 7.92; found C 47.12, H 8.00.

(±)-1,1-Dimethoxybut-3-en-2-ol (8c): To a stirred solution of dimethoxyethanal **7** (18.2 g, 175 mmol) in dry diethyl ether (200 mL) at 0 °C was added dropwise a solution of 1.0 M vinylmagnesium bromide in THF (140 mL). Stirring was continued at room temp. for 2 h, and then the reaction was quenched by the addition of satd. aq. NH_4Cl solution (5.0 mL). The organic layer was decanted off, and the remaining precipitate was dissolved in aq. NH_4Cl solution and extracted twice with diethyl ether. The combined organic layers were dried ($MgSO_4$) and concentrated. Distillation yielded the alcohol **8c** as a colorless liquid (9.03 g, 49%); b.p. 58−60 °C/15 Torr. − ^{1}H NMR (250 MHz, $CDCl_3$): δ = 5.93 (ddd, 3-H), 5.42 (ddd, 4-H_Z), 5.17 (ddd, 4-H_E), 4.18 (d, 1-H), 4.14 (ddddd, 2-H), 3.47, 3.44 (2 s, 2 CH_3), 2.35 (d, OH); $J_{1,2}$ = 2.7 Hz, $J_{2,3}$ = 5.0 Hz, $J_{2,4E}$ = 1.5 Hz, $J_{2,4Z}$ = 1.5 Hz, $J_{2,OH}$ = 1.7 Hz, $J_{3,4E}$ = 10.5 Hz, $J_{3,4Z}$ = 17.3 Hz, $J_{4E,4Z}$ = 1.5 Hz.

(±)-2-Hydroxy-3-butenal (2c): (a) By Acetal Hydrolysis: A solution of **8c** (1.20 g, 9.1 mmol) in 50% aq. acetone (80 mL) was stirred with ion-exchange resin (Dowex AG50 W-X8, H^+ form) for 2 d at room temp. After removal of the resin by filtration, water (40 mL) was added and the acetone was removed by vacuum distillation. The volume was adjusted to 90 mL and a faint cloudiness was separated by centrifugation. The resulting clear, colorless solution of **2c** was used directly for the enzymatic step without further purification.

(b) By Controlled Ozonolysis: A solution of **10c**[42] (11.0 g, 68.6 mmol) in 90% aq. methanol (200 mL) was cooled to −78 °C, whereupon a stream of ozone was passed through it at a rate of ca. 30 mmol/h until ca. 90% conversion (62 mmol, previously calibrated by ozonolysis of a reference compound). Me_2S (25 mL, 345 mmol) was then added and stirring was continued for 3 h at room temp., after which a peroxide test proved negative. The solution was then diluted with water (80 mL) and the volatile organic components were removed in vacuo. The remaining aqueous solution containing **2c** was successively extracted with diethyl ether and cyclohexane prior to submission to the enzymatic reaction. − ^{1}H NMR (250 MHz, D_2O; hydrate): δ = 5.94 (ddd, 3-H), 5.39 (ddd, 4-H_Z), 5.33 (ddd, 4-H_E), 4.91 (d, 1-H), 4.02 (dddd, 2-H); $J_{1,2}$ = 5.3 Hz, $J_{2,3}$ = 6.6 Hz, $J_{2,4E}$ = 1.4 Hz, $J_{2,4Z}$ = 1.4 Hz, $J_{3,4E}$ = 10.5 Hz, $J_{3,4Z}$ = 17.3 Hz, $J_{4E,4Z}$ = 1.5 Hz.

6,7-Dideoxy-L-*lyxo*-hept-6-enulose 1-Phosphate (4c): An aqueous solution (60 mL) of **3** (1.1 mmol) and aldehyde **2c** (6.0 mmol) at pH 6.8 was incubated with FucA (150 U) for 2 d at room temp. The product was then isolated by anion-exchange techniques as described above for **4b** and was crystallized as the colorless bis(-cyclohexylammonium) salt **4c** (646 mg, 84%). − **α anomer:** ^{1}H NMR (400 MHz, D_2O): δ = 5.99 (ddd, 6-H), 5.44 (ddd, 7-H_Z), 5.38 (ddd, 7-H_E), 4.53 (dddd, 5-H), 4.32 (d, 3-H), 4.28 (dd, 4-H), 3.75−3.85 (m, 1-H); $J_{3,4}$ = 4.6 Hz, $J_{4,5}$ = 4.3 Hz, $J_{5,6}$ = 7.0 Hz, $J_{5,7E}$ = 1.0 Hz, $J_{5,7Z}$ = 1.0 Hz, $J_{6,7E}$ = 17.3 Hz, $J_{6,7Z}$ = 10.5 Hz, $J_{7E,7Z}$ = 1.0 Hz Hz. − **β anomer:** ^{1}H NMR (400 MHz, D_2O): δ = 5.96 (ddd, 6-H), 5.41 (ddd, 7-H_Z), 5.35 (ddd, 7-H_E), 4.70 (dddd, 5-H), 4.48 (dd, 4-H), 4.21 (d, 3-H), 4.06 (ddd, 1-H), 3.75−3.85 (m, 1-H′); $J_{1,P}$ = 7.8 Hz, $J_{3,4}$ = 5.0 Hz, $J_{4,5}$ = 5.4 Hz, $J_{5,6}$ = 7.8 Hz, $J_{5,7E}$ = 1.0 Hz, $J_{5,7Z}$ = 1.0 Hz, $J_{6,7E}$ = 17.4 Hz, $J_{6,7Z}$ = 10.5 Hz, $J_{7E,7Z}$ = 1.0 Hz ; anomer ratio α:β = 32:68.

6,7-Dideoxy-L-*lyxo*-hept-6-enulose (5c): An aqueous solution (200 mL) of **3** (6.5 mmol) and aldehyde **2c** (20.0 mmol) at pH 6.8 was treated with FucA (1000 U). The mixture was incubated for 2 d at room temp., after which TLC indicated complete conversion. After removal of the aldolase by filtration through a pad of charcoal, the pH was adjusted to 8.5, alkaline phosphatase (500 U) was added, and the resulting mixture was incubated for a further 2 d at room temp. After protein removal, the solution was desalted by passing it successively through anion- and cation-exchange resins. Concentration of the resulting eluate provided **5c** as a colorless syrup (580 mg, 51%). − **α anomer:** ^{1}H NMR (D_2O, 300 MHz): δ = 5.93 (ddd, 6-H), 5.40 (dd, 7-H_Z), 5.32 (dd, 7-H_E), 4.70 (dd, 5-H), 4.46 (dd, 4-H), 4.28 (d, 3-H), 3.62 and 3.55 (AB, 1-H); $J_{1,1'}$ = 10.5 Hz, $J_{3,4}$ = 4.5 Hz, $J_{4,5}$ = 5.4 Hz, $J_{5,6}$ = 7.5 Hz, $J_{6,7Z}$ = 10.0 Hz, $J_{6,7E}$ = 17.2 Hz, $J_{7E,7Z}$ = 1.0 Hz. − ^{13}C NMR (D_2O, 75 MHz): δ = 135.6 (C-6), 122.5 (C-7), 107.7 (C-2), 83.4, 80.4, 75.7 (C-3, C-4, C-5), 65.3 (C-1) − **β anomer:** ^{1}H NMR (D_2O, 300 MHz): δ = 5.99 (ddd, 6-H), 5.43 (dd, 7-H_Z), 5.38 (dd, 7-H_E), 4.50 (dd, 5-H), 4.32 (d, 3-H), 4.27 (dd, 4-H), 3.59 and 3.58 (AB, 1-H); $J_{1,1'}$ = 10.0 Hz, $J_{3,4}$ = 4.5 Hz, $J_{4,5}$ = 4.3 Hz, $J_{5,6}$ = 7.0 Hz, $J_{6,7Z}$ = 10.0 Hz, $J_{6,7E}$ = 17.2 Hz, $J_{7E,7Z}$ = 1.0 Hz; anomer ratio α:β = 32:68. − ^{13}C NMR (D_2O, 75 MHz): δ = 136.1 (C-6), 122.2 (C-7), 105.4 (C-2), 84.3, 75.4, 73.7 (C-3, C-4, C-5), 65.5 (C-1). − FAB-MS (SIMS, DTE/DTT); *m/z* (%): 183.1 [M + Li]$^+$ (100.0).

6,7-Dideoxy-L-*galacto*-hept-6-enose (1c): A sample of ketose **5c** (130 mg, 0.47 mmol) was dissolved in a FucI stock solution (1.5 mL, 105 U) and the mixture was incubated for 4 d at room temp. Protein was then removed by charcoal filtration and the remaining solution was applied to a column of Dowex AG50 W-X8 (Ca^{2+} form). Elution with water furnished **1c** in a first fraction, which was obtained as a colorless syrup that slowly crystallized on standing (76 mg, 58%). − **α anomer:** ^{1}H NMR (D_2O, 300 MHz): δ = 5.94 (ddd, 6-H), 5.41 (dd, 7-H_Z), 5.34 (dd, 7-H_E), 5.38 (d, 1-H), 4.58 (dd, 5-H), 3.97 (dd, 4-H), 3.93 (dd, 3-H), 3.82 (dd, 2-H); $J_{1,2}$ = 3.7, $J_{2,3}$ = 10.2, $J_{3,4}$ = 3.1, $J_{4,5}$ = 1.2, $J_{5,6}$ = 5.7, $J_{6,7Z}$ =

10.5, $J_{6,7E}$ = 17.2, $J_{7E,7Z}$ = 1.5 Hz. − ^{13}C NMR (D_2O, 75 MHz): δ = 136.6 (C-6), 120.7 (C-7), 95.1 (C-1), 74.2, 73.9, 71.8, 70.8 (C-2, C-3, C-4, C-5). − **β anomer:** ^{1}H NMR (D_2O, 300 MHz): δ = 5.94 (ddd, 6-H), 5.41 (dd, 7-H_Z), 5.35 (dd, 7-H_E), 4.63 (d, 1-H), 4.23 (dd, 5-H), 3.91 (dd, 4-H), 3.69 (dd, 3-H), 3.50 (dd, 2-H); $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 9.8 Hz, $J_{3,4}$ = 3.6 Hz, $J_{4,5}$ = 1.5 Hz, $J_{5,6}$ = 5.7 Hz, $J_{6,7Z}$ = 10.5 Hz, $J_{6,7E}$ = 17.2 Hz, $J_{7E,7Z}$ = 1.5 Hz; anomer ratio α:β = 33:67. − ^{13}C NMR (D_2O, 75 MHz): δ = 136.1 (C-6), 120.7 (C-7), 99.1 (C-1), 78.3, 75.5, 74.3, 73.7 (C-2, C-3, C-4, C-5). − FAB-MS (SIMS, DTE/DTT); *m/z* (%): 183.1 [M + Li]$^+$ (100.0). − $C_7H_{12}O_5$ (176.170): calcd. C 47.73, H 6.87; found C 47.60, H 6.99.

(±)-2-Hydroxy-3-butynal (2d): Ozone was passed through a solution of **10d**[42][43] (0.82 g, 9.8 mmol) in methanol (40 mL) at −78 °C at a rate of ca. 30 mmol/h until one equivalent had been absorbed (10 mmol, previously calibrated by ozonolysis of a reference compound). Me_2S (5.0 mL, 69 mmol) was then added and the resulting mixture was stirred at room temp. for 3 h. After dilution with water (100 mL), the volatile organic components were removed in vacuo and the remaining aqueous solution of **2d** was extracted twice with diethyl ether. − ^{1}H NMR (300 MHz, D_2O; hydrate): δ = 5.00 (d, 1-H), 4.29 (dd, 2-H), 2.87 (d, 4-H); $J_{1,2}$ = 5.0 Hz, $J_{2,3}$ = 2.1 Hz.

6,7-Dideoxy-L-*lyxo*-hept-6-ynulose (5d): A solution of freshly prepared aldehyde **2d** (60 mL, 6 mmol) was treated with **3** (1.0 mmol) and FucA (500 U) while the pH was kept constant at 7.0 by means of an autotitrator. The solution was kept at room temp. until TLC indicated complete conversion (2 d). The dark solution was then filtered through charcoal and the filtrate was treated with alkaline phosphatase (100 U) for a further 2 d. Workup as described above provided **5d** as a slightly yellow syrup (100 mg, 57%). − **α anomer:** ^{1}H NMR (D_2O, 300 MHz): δ = 4.99 (dd, 5-H), 4.48 (dd, 4-H), 4.21 (d, 3-H), 3.58 and 3.54 (AB, 1-H), 3.56 (d, 7-H); $J_{1,1'}$ = 12.0 Hz, $J_{3,4}$ = 5.0 Hz, $J_{4,5}$ = 5.0 Hz, $J_{5,7}$ = 2.7 Hz. − ^{13}C NMR (D_2O, 75 MHz): δ = 108.0 (C-2), 80.9 (C-6), 81.3, 77.5, 77.4, 74.5 (C-3, C-4, C-5, C-7), 64.1 (C-1). − **β anomer:** ^{1}H NMR (D_2O, 300 MHz): δ = 4.64 (dd, 5-H), 4.33 (dd, 4-H), 4.23 (d, 3-H), 3.75 and 3.66 (AB, 1-H), 3.56 (d, 7-H); $J_{1,1'}$ = 11.8 Hz, $J_{3,4}$ = 4.7 Hz, $J_{4,5}$ = 5.0 Hz, $J_{5,7}$ = 2.7 Hz; anomer ratio α:β = 35:65. − ^{13}C NMR (D_2O, 75 MHz): δ = 105.9 (C-2), 80.6 (C-6), 78.9, 74.5, 74.3, 72.8 (C-3, C-4, C-5, C-7), 65.2 (C-1). − FAB-MS (SIMS, DTE/DTT); *m/z* (%): 175 [M + 1]$^+$ (100.0).

6,7-Dideoxy-L-*galacto*-hept-6-ynose (1d): Ketose **5d** (30 mg, 0.17 mmol) was incubated in a stock solution containing FucI (1.0 mL, 60 U) for 2 days at room temp. After concentration, the residue was subjected to chromatography on a silica gel column, eluting with chloroform/methanol (5:1), which gave colorless, syrupy **1d** (12 mg, 40%). − **α anomer:** ^{1}H NMR (D_2O, 300 MHz): δ = 5.27 (d, 1-H), 4.89 (d, 5-H), 3.98 (dd, 4-H), 3.88 (dd, 3-H), 3.80 (dd, 2-H), 3.58 (s, 7-H); $J_{1,2}$ = 3.7 Hz, $J_{2,3}$ = 9.7 Hz, $J_{3,4}$ = 3.3 Hz, $J_{4,5}$ = 1.2 Hz. − ^{13}C NMR (D_2O, 75 MHz): δ = 95.3 (C-1), 74.9, 74.0, 73.6, 71.2, 70.5, 65.1 (C-2, C-3, C-4, C-5, C-6, C-7). − **β anomer:** ^{1}H NMR (D_2O, 300 MHz): δ = 4.59 (d, 1-H), 4.53 (d, 5-H), 4.05 (dd, 4-H), 3.67 (dd, 3-H), 3.59 (s, 7-H), 3.51 (dd, 2-H); $J_{1,2}$ = 7.4 Hz, $J_{2,3}$ = 9.7 Hz, $J_{3,4}$ = 3.4 Hz, $J_{4,5}$ = 1.5 Hz; anomer ratio α:β = 40:60. − ^{13}C NMR (D_2O, 75 MHz): δ = 99.2 (C-1), 74.9, 74.0, 73.9, 73.6, 69.2, 65.1 (C-2, C-3, C-4, C-5, C-6, C-7). − $C_7H_{10}O_5$ (174.154): calcd. C 48.28, H 5.79; found C 48.11, H 6.08.

2-Hydroxy-2-methylpropanal (2e): To a stirred solution of 1,1-dimethoxypropanone **11**[19] (10.0 g, 84.6 mmol) in dry diethyl ether (60 mL) was slowly added a 3.0 M solution of methylmagnesium bromide (33.9 mL, 101.7 mmol). After refluxing for 2 h, the cooled reaction mixture was hydrolyzed by the addition of satd. aq. NH_4Cl solution. The aqueous phase was extracted with diethyl ether, and the combined organic layers were successively washed with satd. aq. $NaHSO_3$ solution, satd. aq. $NaHCO_3$ solution, and brine. After drying with $MgSO_4$ and concentration in vacuo, 1,1-dimethoxy-2-methylpropan-2-ol **12** was obtained as a colorless liquid (3.0 g, 81%). − ^{1}H NMR (250 MHz, $CDCl_3$): δ = 4.0 (s, 1-H), 3.38 (s, OCH_3), 3.20 (s, O*H*), 1.10 (s, CH_3). This compound was hydrolyzed by stirring an aqueous solution (200 mL) with ion-exchange resin (Dowex AG50 W-X8, H$^+$ form; ca. 2 g) for 3 h at room temp. After filtration, the solution containing **2e** was concentrated in vacuo to remove most of the methanol and then was adjusted to 100 mM prior to the enzymatic reaction. − ^{1}H NMR (250 MHz, $CDCl_3$): δ = 9.49 (s, 1-H), 3.20 (s, O*H*), 1.24 (s, CH_3).

6-Deoxy-5−*C*-methyl-L-*lyxo*-hexulose (5e): Aqueous solutions of **2e** (10.0 mmol) and **3** (5.7 mmol) were combined and the mixture was adjusted to pH 6.8 and a volume of 100 mL. FucA (800 U) was then added and the mixture was allowed to stand at room temp. for 2 d. After dephosphorylation with alkaline phosphatase (500 U) for 2 d, the sugar was isolated in the standard manner to furnish **5e** as a colorless syrup (344 mg, 34%). − **α anomer:** ^{1}H NMR (D_2O, 300 MHz): δ = 4.29 (d, 3-H), 4.14 (d, 4-H), 3.68 and 3.58 (AB, 1-H), 1.37, 1.24 (2 s, 2 CH_3); $J_{1,1'}$ = 11.8 Hz, $J_{3,4}$ = 5.0 Hz. − ^{13}C NMR (D_2O, 75 MHz): δ = 107.3 (C-1), 76.7, 75.9, 67.7, 65.6 (C-2, C-3, C-4, C-5), 27.2, 25.8 (2 CH_3). − **β anomer:** ^{1}H NMR (D_2O, 300 MHz): δ = 4.34 (d, 3-H), 3.94 (d, 4-H), 3.54 and 3.46 (AB, 1-H), 1.34, 1.23 (2 s, 2 CH_3); $J_{1,1'}$ = 11.8 Hz, $J_{3,4}$ = 5.5 Hz; anomer ratio α:β = 28:72. − ^{13}C NMR (D_2O, 75 MHz): δ = 105.5 (C-1), 86.2, 79.2, 73.1, 65.5 (C-2, C-3, C-4, C-5), 29.7, 25.9 (2 CH_3). − FAB-MS (SIMS, DTE/DTT); *m/z* (%): 201.0 [M − 1 + LiOH]$^+$ (100.0)

6-Deoxy-5−*C*-methyl-L-*galacto*-hexose (1e): A sample of the ketose **5e** (73 mg, 0.41 mmol) was treated with FucI (2.0 mL, 140 U) for 5 d at room temp. After concentration of the mixture in vacuo, the residue was purified by chromatography on silica gel (chloroform/methanol, 5:1) to provide pure aldose **1e** (17 mg, 23%). − **α anomer:** ^{1}H NMR (D_2O, 300 MHz): δ = 5.21 (d, 1-H), 4.33 (d, 4-H), 3.99 (dd, 3-H), 3.95 (dd, 2-H), 1.32, 1.24 (2 s, 2 CH_3); $J_{1,2}$ = 3.3 Hz, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 4.7 Hz. − ^{13}C NMR (D_2O, 75 MHz): δ = 95.4 (C-1), 79.0, 76.8, 75.0, 73.1 (C-2, C-3, C-4, C-5), 28.2, 26.6 (2 CH_3). − **β anomer:** ^{1}H NMR (D_2O, 300 MHz): δ = 5.26 (d, 1-H), 4.08 (dd, 2-H), 3.87 (d, 4-H), 3.65 (dd, 3-H), 1.28, 1.27 (2 s, 2 CH_3); $J_{1,2}$ = 4.7 Hz, $J_{2,3}$ = 7.0 Hz, $J_{3,4}$ = 6.3 Hz; anomer ratio α:β = 58:42. − ^{13}C NMR (D_2O, 75 MHz): δ = 97.2 (C-1), 79.7, 76.6, 73.9, 73.8 (C-2, C-3, C-4, C-5), 28.0, 26.5 (2 CH_3). − FAB-MS (SIMS, DTE/DTT); *m/z* (%): 200.9 [M − 1 + LiOH]$^+$ (100.0). − $C_7H_{14}O_5$ (178.186): calcd. C 47.19, H 7.92; found C 47.03, H 8.05.

1,1,1-Trifluoro-3,3-dimethoxypropan-2-ol (8f): A mixture of a commercial 45% aqueous solution of dimethoxyethanal (20 mL) and dichloromethane (100 mL) was refluxed in a Soxhlet apparatus charged with $CaCl_2$ (30 g) until a single organic phase persisted (6 h). Refluxing was continued for a further 2 h, then the solvent was distilled off under reduced pressure and the residue was fractionated in vacuo using a Vigreux column to give pure dimethoxyethanal (8.6 g, 92.0%); b.p. 65−68 °C/160 Torr. − ^{1}H NMR (250 MHz, $CDCl_3$): δ = 3.46 (s, CH_3), 4.50 (d, 2-H), 9.48 (d, 1-H, $J_{1,2}$ = 1.5 Hz). A solution of the freshly distilled aldehyde (1.13 g, 10.7 mmol) and $TMSCF_3$ (3.0 mL, 20.0 mmol) in dry THF (10 mL) was cooled to −20 °C under nitrogen. After the addition of 1.0 M TBAF in THF (200 µL, 0.2 mmol), the mixture was allowed to slowly warm to 0 °C, whereupon an exothermic reaction commenced. The solution was kept at 0 °C for 30 min, then allowed to warm to room temp. and stirring was continued for a further 5 h.

The yellow solution thus obtained was quenched by the addition of 1.0 M HCl (3 mL) under vigorous stirring. After 3 h, the layers were separated and the aqueous phase was extracted with diethyl ether (5 × 10 mL). The combined organic phases were dried over $MgSO_4$ and carefully concentrated. Distillation of the residue provided the colorless product (712 mg, 38%); b.p. 65−69°C/10 Torr. − ^1H NMR (250 MHz, $CDCl_3$): δ = 4.47 (d, 1-H), 3.93 (dq, 2-H), 3.47, 3.41 (2 s, OCH_3), 3.23 (br, OH); $J_{1,2}$ = 4.4 Hz, $J_{2,F}$ = 7.4 Hz. − ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 124.0 (q, C-3), 101.0 (C-1), 70.7 (q, C-2), 55.5, 54.9 (2 OCH_3); $J_{C\text{-}2,F}$ = 29.9 Hz, $J_{C\text{-}3,F}$ = 282 Hz.

(±)-*E*-1,1,1-Trifluoro-4-phenylbut-3-en-2-ol (10f): A solution of cinnamaldehyde (13.2 g, 100 mmol) and $TMSCF_3$ (15.0 mL, 100 mmol) in dry THF was treated at −20°C under nitrogen with 1.0 M TBAF in THF (500 µL, 0.5 mmol). Upon slow warming to −5°C, an exothermic reaction was noticed. Stirring was continued at 0°C for 0.5 h and then at 20°C for a further 2 h, after which the yellow mixture was quenched by the addition of 1.0 M HCl (30 mL). Workup as described above yielded **10f** as a colorless oil (19.5 g, 97%), b.p. 85−88°C/20 Torr, which solidified below 0°C. − ^1H NMR (250 MHz, $CDCl_3$): δ = 7.45−7.39 (m, H_{ar}), 6.84 (d, 4-H), 6.19 (dd, 3-H), 4.60 (q, 2-H), 2.64 (s, OH); $J_{2,F}$ = 7.5 Hz, $J_{2,3}$ = 6.3 Hz, $J_{3,4}$ = 15.8 Hz. − ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 135.5 (C-4), 136.3, 128.8, 128.7, 126.9 (C_{ar}), 124.4 (q, C-1), 120.9 (C-3), 71.6 (q, C-2); $J_{C\text{-}1,F}$ = 282 Hz, $J_{C\text{-}2,F}$ = 32 Hz.

(±)-3,3,3-Trifluoro-2-hydroxypropanal (2f): A solution of **10f** (10.0 g, 49.5 mmol) in methanol (350 mL) was treated with ozone until a blue color persisted (ca. 2.5 h). Excess ozone was then removed by purging with nitrogen. After the addition of Me_2S (25 mL, 350 mmol) at −78°C, the solution was allowed to warm to room temp. over a period of 2 h. Water (100 mL) was then added and the mixture was concentrated to half of its original volume. After extraction with diethyl ether, the clear aldehyde solution was adjusted to 200 mM with water and used directly for the subsequent enzymatic reactions. − ^1H NMR (400 MHz, D_2O): δ = 5.16 (d, 1-H), 4.00 (dq, 2-H, $J_{1,2}$ = 4.9 Hz, $J_{2,F}$ = 7.3 Hz). − ^{13}C NMR (100.6 MHz, D_2O): δ = 124.8 (q, C-3), 88.2 (C-1), 72.4 (q, C-2); $J_{C\text{-}2,F}$ = 28.6 Hz, $J_{C\text{-}3,F}$ = 282 Hz.

Acknowledgments

This research was supported by the Deutsche Forschungsgemeinschaft (grant Fe 244/6−1) and by the Fonds der Chemischen Industrie. We are grateful to Dr. A. Peyman of Hoechst AG for a gift of dimethoxyethanal and to Dr. W. Tischer of Boehringer Mannheim GmbH for a generous supply of enzymes.

---

[1] [1a] A. Varki, *Glycobiology* **1993**, *3*, 97−130. − [1b] M. Fukuda in *Molecular Glycobiology* (Eds.: M. Fukuda, O. Hindsgaul), Oxford University Press, Oxford, **1994**, p. 1−52. − [1c] R. A. Dwek, *Biochem. Soc. Trans.* **1995**, *23*, 1−25.

[2] K. Jann, B. Jann in *Surface Carbohydrates of the Procaryotic Cell* (Ed.: I. W. Sutherland), Academic Press, New York, **1977**, p. 247−287.

[3] R. Kornfeld, S. Kornfeld, *Ann. Rev. Biochem.* **1976**, *45*, 217−237.

[4] S. Hakomori, *Prog. Biochem. Pharmacol.* **1975**, *10*, 167−196.

[5] S. Hakomori, *Ann. Rev. Immunol.* **1984**, *2*, 103−126.

[6] [6a] T. Boren, P. Falk, K. A. Roth, G. Larson, S. Normark, *Science* **1993**, *262*, 1892−1895. − [6b] D. Ilver, A. Arnqvist, J. Ogren, I. M. Frick, D. Kersulyte, E. T. Incecik, D. E. Berg, A. Covacci, L. Engstrand, T. Boren, *Science* **1998**, *279*, 373−377.

[7] A. Dubois, *Emerg. Infect. Dis.* **1995**, *1*, 79−85.

[8] M. J. Blaser, G. I. Perez-Perez, H. Kleanthous, T. L. Cover, R. M. Peek, P. H. Chyou, G. N. Stemmermann, A. Nomura, *Cancer Res.* **1995**, *55*, 2111−2115.

[9] A. Varki, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 7390−7397.

[10] [10a] C. Foxall, S. R. Watson, D. Dowbenko, C. Fennie, L. A. Lasky, M. Kiso, A. Hasegawa, D. Asa, B. K. Brandley, *J. Cell. Biol.* **1992**, *117*, 895−902. − [10b] L. A. Lasky, *Science* **1992**, *258*, 964−969.

[11] B. K. Brandley, M. Kiso, S. Abbas, P. Nikrad, O. Srivasatava, C. Foxall, Y. Oda, A. Hasegawa, *Glycobiology* **1993**, *3*, 633−641.

[12] B. J. Graves, R. L. Crowther, C. Chandran, J. M. Rumberger, S. Li, K. S. Huang, D. H. Presky, P. C. Familletti, B. A. Wolitzky, D. K. Burns, *Nature* **1994**, *367*, 532−538.

[13] [13a] G. E. Ball, R. A. O'Neill, J. E. Schultz, J. B. Lowe, B. W. Weston, J. O. Nagy, E. G. Brown, C. J. Hobbs, M. D. Bednarski, *J. Am. Chem. Soc.* **1992**, *114*, 5449−5451. − [13b] Y. Ichikawa, Y. C. Lin, D. P. Dumas, G. J. Shen, E. Garcia-Junceda, M. A. Williams, R. Bayer, C. Ketcham, L. E. Walker, J. C. Paulson, C.-H. Wong, *J. Am. Chem. Soc.* **1992**, *114*, 9283−9298. − [13c] T. J. Rutherford, D. G. Spackman, P. J. Simpson, S. W. Homans, *Glycobiology* **1994**, *4*, 59−68.

[14] [14a] R. M. Cooke, R. S. Hale, S. G. Lister, G. Shah, M. P. Weir, *Biochemistry* **1994**, *33*, 10591−10596. − [14b] K. Scheffler, B. Ernst, A. Katopodis, J. L. Magnani, W. T. Wang, R. Weisemann, T. Peters, *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1841−1844. L. Poppe, G. S. Brown, J. S. Philo, P. V. Nikrad, B. H. Shah, *J. Am. Chem. Soc.* **1997**, *119*, 1727−1736.

[15] H. C. Kolb, B. Ernst, *Chem. Eur. J.* **1997**, *3*, 1571−1578.

[16] J. Y. Ramphal, Z. L. Zheng, C. Perez, L. E. Walker, S. A. Defrees, F. C. A. Gaeta, *J. Med. Chem.* **1994**, *37*, 3459−3463.

[17] R. C. Bansal, B. Dean, S. Hakomori, T. Toyokuni, *J. Chem. Soc., Chem. Commun.* **1991**, 796−798.

[18] [18a] G. Srivastava, K. J. Kaur, O. Hindsgaul, M. M. Palcic, *J. Biol. Chem* **1992**, *267*, 22356−22361. − [18b] G. Baisch, R. Öhrlein, A. Katopodis, B. Ernst, *Bioorg. Med. Chem. Lett.* **1996**, *6*, 759−762. − [18c] G. Baisch, R. Öhrlein, A. Katopodis, M. Streiff, F. Kolbinger, *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2447−2450. − [18d] C. Vogel, C. Bergemann, A.-J. Ott, T. K. Lindhorst, J. Thiem, W. V. Dahlhoff, C. Hällgren, M. M. Palcic, O. Hindsgaul, *Liebigs Ann.* **1997**, 601−612. − [18e] G. Baisch, R. Öhrlein, M. Streiff, *Bioorg. Med. Chem. Lett.* **1998**, *8*, 161−164.

[19] J. R. Durrwachter, D. G. Drueckhammer, K. Nozaki, H. M. Sweers, C.-H. Wong, *J. Am. Chem. Soc.* **1986**, *108*, 7812−7818.

[20] W.-D. Fessner, G. Sinerius, A. Schneider, M. Dreyer, G. E. Schulz, J. Badia, J. Aguilar, *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 555−558.

[21] W.-D. Fessner, in *Microbial Reagents in Organic Synthesis* (Ed.: S. Servi), Kluwer Academic Press, Dordrecht, **1992**, p. 43−55.

[22] M. T. Zannetti, C. Walter, M. Knorst, W.-D. Fessner, *Chem. Eur. J.* **1999**, *5*, 1882−1890.

[23] W.-D. Fessner, C. Walter, *Top. Curr. Chem.* **1997**, *184*, 97−194.

[24] W.-D. Fessner, J. Badia, O. Eyrisch, A. Schneider, G. Sinerius, *Tetrahedron Lett.* **1992**, *33*, 5231−5234.

[25] W.-D. Fessner, *GIT Fachz. Lab.* **1993**, *37*, 951−956.

[26] W.-D. Fessner, A. Schneider, O. Eyrisch, G. Sinerius, J. Badia, *Tetrahedron: Asymmetry* **1993**, *4*, 1183−1192.

[27] [27a] R. Alajarin, E. Garcia-Junceda, C.-H. Wong, *J. Org. Chem.* **1995**, *60*, 4294−4295. − [27b] C.-H. Wong, R. Alajarin, F. Moris-Varas, O. Blanco, E. Garcia-Junceda, *J. Org. Chem.* **1995**, *60*, 7360−7363. These authors presented their work as an original development but − despite their better knowledge − failed to adequately quote our earlier investigations in this area.

[28] [28a] W.-D. Fessner, C. Walter, *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 614−616. − [28b] A. Franke, *Monatsh. Chem.* **1900**, *21*, 210−215. − [28c] R. Dworzak, J. Pierri, *Monatsh. Chem.* **1929**, *52*, 141−150.

[29] G. K. S. Prakash, R. Krishnamurti, G. A. Olah, *J. Am. Chem. Soc.* **1989**, *111*, 393−395.

[30] M. Petersen, M. T. Zannetti, W.-D. Fessner, *Top. Curr. Chem.* **1997**, *186*, 87−117.

[31] W. A. Pryor, D. Giamalva, D. F. Church, *J. Am. Chem. Soc.* **1983**, *105*, 6858−6861.

[32] M. D. Bednarski, E. S. Simon, N. Bischofberger, W.-D. Fessner, M.-J. Kim, W. Lees, T. Saito, H. Waldmann, G. M. Whitesides, *J. Am. Chem. Soc.* **1989**, *111*, 627−635.

[33] W.-D. Fessner, G. Sinerius, *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 209−212.

[34] W.-D. Fessner, A. Schneider, H. Held, G. Sinerius, C. Walter, M. Hixon, J. V. Schloss, *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2219−2221.

[35] M. K. Dreyer, G. E. Schulz, *J. Mol. Biol.* **1996**, *259*, 458−466.
[36] A. Jörger, C. Goße, W.-D. Fessner, G. E. Schulz, submitted for publication.
[37] J. E. Seemann, G. E. Schulz, *J. Mol. Biol.* **1997**, *273*, 256−268.
[38] O. Eyrisch, *Dissertation*, University of Freiburg, **1994**.
[39] P. A. Bash, M. J. Field, R. C. Davenport, G. A. Petsko, D. Ringe, M. Karplus, *Biochemistry* **1991**, *30*, 5826−5832.
[40] M. Green, S. S. Cohen, *J. Biol. Chem.* **1956**, *219*, 557−568.
[41] A. Kirschning, A. F.-W. Bechthold, J. Rohr, *Top. Curr. Chem.* **1997**, *188*, 1−84.
[42] K. Burgess, L. D. Jennings, *J. Am. Chem. Soc.* **1991**, *113*, 6129−6139.
[43] L. Skattebøl, E. R. H. Jones, M. Whiting, *Org. Synth. Coll. Vol. IV*, **1963**, 792−795.

Received March 5, 1999
[O99130]