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Synthesis of non-natural carbohydrates from glycerol and aldehydes in a one-pot four-enzyme cascade reaction

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A simple procedure has been developed for the synthesis of enantio- and diastereomerically pure carbohydrate analogues from glycerol and a variety of aldehydes in one pot using a four-enzyme cascade reaction. As a proof of concept of the usefulness of this enzymatic catalytic cascade the naturally occurring azasugar D-fagomine was synthesized. This work highlights the potential value of using enzymes in cascade reactions to selectively form complex products that by previous traditional organic chemistry could only be obtained *via* repeated isolation and purification of intermediates.

Introduction

In the search for efficient, sustainable synthetic strategies, chemists increasingly pursue approaches that are inspired by Nature.[1] The ultimate goal is to mimic biosynthetic pathways and construct complex products *via* ingenious sequences of multiple enzymatic conversions in one pot, preferably with both high chemo- and stereoselectivity.[2] This concept is demonstrated in the synthesis of complex carbohydrate building blocks,[3] which may serve amongst others as intermediates for iminosugars.[4] Besides impressive achievements in the chemical synthesis of carbohydrate building blocks using asymmetric catalysis[5] and organocatalysis,[6] use of aldolases could provide direct stereoselective access to carbohydrate structures without going through protecting group manipulations.[7] In order to explore the full potential of existing aldolases in C–C bond formation, it is crucial to have facile access to phosphorylated substrates.[8] Dihydroxyacetone phosphate (DHAP) is one of the key compounds in aldol condensation using DHAP dependant aldolases and the research groups of Whitesides, Wong and Fessner have used several enzymatic procedures to arrive at the formation of DHAP starting from glycerol,[9] glycerol phosphate[10] or dihydroxyacetone.[11,12] These methods have the disadvantage that

kinases are needed and that regeneration of ATP is required. The group of Sheldon was among the first to explore the formation of a heptose sugar using a multi-enzyme system in which a phosphorylated intermediate was continuously generated using pyrophosphate as a phosphate donor and in which the DHAP formed was coupled enzymatically to an aldehyde. However, a pH switch was needed to turn off the activity of one of the enzymes and also a very high concentration of glycerol (85%) had to be present.[13]

In our group, we recently reported the efficient phosphorylation of dihydroxyacetone by the acid phosphatase from *Shigella flexneri* (PhoN-Sf) using pyrophosphate (PP$_i$) as the phosphate source. In addition, it was shown that this phosphorylation process could be applied in one pot in combination with dihydroxyacetone phosphate (DHAP)-dependent rabbit muscle aldolase (RAMA) to give the desired aldol product.[14] Motivated by this outcome, we now report a one-pot synthesis of chiral carbohydrate fragment **1** by the simultaneous action of four different enzymes starting from inexpensive glycerol **2** and an appropriate aldehyde (Scheme 1).
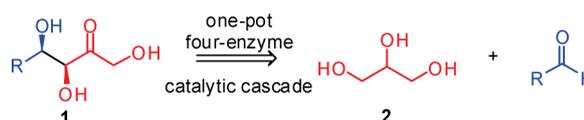
Scheme 1 One-pot retrosynthesis of carbohydrate fragments **1**.

The newly envisaged cascade was based on initial phosphorylation of glycerol into glycerol-3-phosphate,[9,13] followed by L-glycerol-3-phosphate oxidase (GPO)-mediated oxidation to DHAP. Subsequent RAMA-catalyzed C–C bond formation and dephosphorylation with PhoN-Sf should then give rise to target molecules **1**. Having PhoN-Sf available which operates at

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pH 6, we envisioned that we should be able to carry out the glycerol-DHAP cascade in a single pot with all enzymes acting simultaneously.[14,15] Furthermore, we show that it is possible to obtain all the stereocomplementary products by using all four DHAP-dependent aldolases present in nature.

Results and discussion

Scheme 2 illustrates the sequence of events occurring in the cascade. The PhoN-Sf-mediated phosphorylation of glycerol **2** with the phosphate donor PP_i produces glycerol-3-phosphate **3** in a chemoselective manner, due to the known selectivity of PhoN-Sf for primary hydroxy functions (Scheme 2).[15] The L-enantiomer is then oxidized by GPO in the presence of oxygen to produce DHAP **4**.[10] The oxidation takes place with concomitant formation of hydrogen peroxide, which is converted into water and oxygen by a third enzyme, catalase. In the next step of the cascade, DHAP reacts with the aldehyde catalyzed by RAMA to provide the phosphorylated aldol product **5**. Under the reaction conditions, the aldol product is ultimately dephosphorylated by PhoN-Sf leading to enantio- and diasteromerically pure diol **1**. The resulting enzyme-bound phosphate may then be either transferred to water (hydrolysis) or to glycerol (transphosphorylation).[14] Hydrolysis is generally preferred over transphosphorylation so that P_i is released unless a suitable substrate is present. This essentially irreversible step fortuitously shifts the thermodynamic equilibrium of the cascade to aldol product **1** once PP_i becomes exhausted.
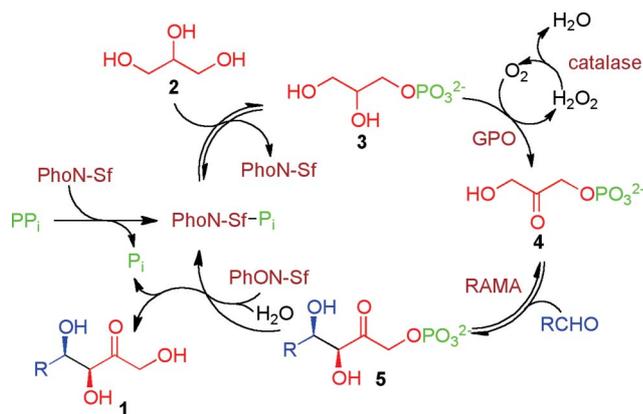
Scheme 2 Four-step one-pot catalytic cascade.

Supposedly, PhoN-Sf-induced phosphorylation of glycerol gives rise to a racemic mixture of D- and L-glycerol-3-phosphate. Since GPO would oxidize only the L-enantiomer[10] the D-isomer might accumulate. However, the acid phosphatase possesses strong transphosphorylation activity[14] and most likely PhoN-Sf will transfer the phosphate group from D-glycerol-3-phosphate to glycerol resulting in racemic glycerol-3-phosphate so that accumulation of the D-isomer will not occur.

The first cascade was conducted at 0.5 mL scale (Table 1, entry 1) with propanal as acceptor aldehyde.[14] A solution of 500 mM glycerol, 250 mM PP_i and 100 mM propanal at pH 6 was incubated with the four enzymes at 30 °C, resulting in 42% conversion after 24 h. By HPLC it was possible to monitor at selected time intervals the ratio of PP_i to P_i, the formation of

Table 1 Optimization of the four-enzyme cascade

Entry	R	Product	PhoN-Sf (U mL^{-1})	GPO (U mL^{-1})	Conv. (%)^a	Reaction time (h)
1	Et	**1a**	1.5	2	42	24
2	Et	**1a**	1.5	5	50	24
3	Et	**1a**	1.5	10	58	24
4	Et	**1a**	1.5	50	84	24
5	Et	**1a**	3.0	50	87	8
6	Et	**1a**	3.0	50	95^b	8
7	Et	**1a**	3.0	50	100^{b,c}	8
8	i-Pr	**1b**	3.0	50	48	8

^{a} Conversions were based on the aldehyde and determined using HPLC on aliquots of the reaction mixture. ^{b} Reaction was performed at 20 °C. ^{c} Reaction was performed in a closed vessel.

the phosporylated product, the dephosphorylated end product, and the amount of aldehyde converted. This allowed us to study in detail the effect of changing the amount of activity of GPO from 2 U mL^{-1} to 50 U mL^{-1} in the cascade. Optimal conversion was obtained with 50 U mL^{-1} GPO (entry 4).

Encouraged by these results the effect of changing the activity of the acid phosphatase was also investigated. When the activity of PhoN-Sf in the cascade was doubled, within 8 h a conversion of 87% was reached (entry 5). Changing the activity of GPO or PhoN-Sf had no effect on the diastereoselectivity, a d.r. of 14 : 1 ((3S,4R) $vs.$ (3S,4S)) was observed in all cases. Lowering the temperature to 20 °C resulted in 95% conversion and increased diastereoselectivity (entry 6). Performing the reaction in a closed vessel for 8 h without taking samples led to a conversion of 100% (entry 7). These effects are probably due to evaporation of the rather volatile propanal.

A branched aldehyde was also successfully used as a substrate, albeit that the yield was lower compared to propanal (Table 1, entry 8), which is in line with the aldehyde specificity of RAMA.[16] The cascade reaction using propanal was also performed on a 10 mL scale in the presence of 3 U mL^{-1} of PhoN-Sf and 50 U mL^{-1} of GPO resulting in 87% conversion and an isolated yield of 97 mg (65%).

Fig. 1 shows the time course of the reaction and a comparison between the currently described cascade and the previous cascade starting from DHA instead of glycerol.[14] It is obvious that the glycerol cascade is superior as compared to the DHA one. This may relate to the value of the K_m for glycerol which is 0.7 M (not shown), whereas the K_m for DHA is 3.6 M.[14]

This may represent an important factor for the cascade reaction resulting in higher conversions compared to the DHA route. Further glycerol stabilizes most enzymes. The glycerol cascade has additional advantage that since DHA is prone to oxidation and side reactions significantly fewer by-products are formed.

The effect of the glycerol concentration on the rate of formation was also investigated (Fig. 2). A concentration of 0.5 M glycerol was optimal. At 1 M glycerol both the rate

Table 2 Four-enzyme cascade with four different aldolases and 3 different aldehydes

Aldehyde	Conv. (%)[a]	Aldolase[17]	d.r.	Configuration[17]
1a propanal	87	RAMA	14 : 1	$(3S,4R) : (3S,4S)$
	72	RhuA	2 : 1	$(3R,4S) : (3R,4R)$
	15	TagA	8 : 1	$(3S,4S) : (3S,4R)$
	77	FucA	2 : 1	$(3R,4R) : (3R,4S)$
1b isobutanal	61	RAMA	>20 : 1	$(3S,4R) : (3S,4S)$
	25	RhuA	13 : 1	$(3R,4S) : (3R,4R)$
	—	TagA	—	
	16	FucA	>20 : 1	$(3R,4R) : (3R,4S)$
1c Alloc-amino-propanal	61/87[b]	RAMA	11 : 1	$(3S,4R) : (3S,4S)$
	44	RhuA	2 : 1	$(3R,4S) : (3R,4R)$
	8	TagA	2 : 1	$(3S,4S) : (3S,4R)$

[a] Conversions were based on the aldehyde and determined with HPLC on aliquots of the reaction mixture. The diastereoisomeric ratio was determined based upon the peak areas in the HPLC profile and the assignments of the stereochemistry on the reported specificity of these aldolases in literature.[17] [b] It was possible to increase the yield by addition of an extra portion of PP_i.
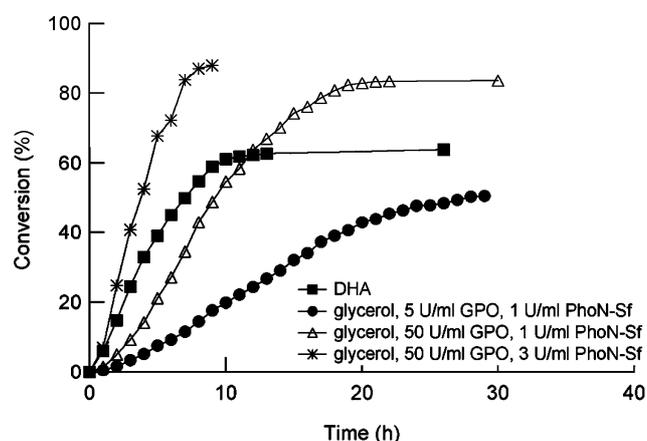

Fig. 1 Time course of the cascade reaction starting from DHA or glycerol. The reactions were carried out with 1.5 U mL^{-1} PhoN-Sf, 6 U mL^{-1} RAMA, 250 mM PP$_i$, 100 mM propanal and 0.5 M glycerol or 0.5 M DHA.

Fig. 2 Effect of glycerol concentration on the yield. 250 mM PPi, 20 mM acetate pH 6, 100 mM propanal, 6 U ml^{-1} RAMA, 50 U ml^{-1} GPO, 10 U ml^{-1} catalase, 1 or 3 U ml^{-1} of PhoN-Sf.

and the yields were lower. The lower activity at low glycerol concentrations probably relates to the K_m value of PhoN-Sf for glycerol of about 0.7 M.

To probe the broader applicability of the glycerol cascade, the reaction was also carried out with three other aldolases in combination with propanal (Table 2). Rhamnulose-1-phosphate aldolase (RhuA, entry 2) and fuculose-1-phosphate aldolase (FucA, entry 4) gave similar conversions as compared to RAMA (entry 1), although the d.r. in both cases was significantly lower. In all cases the enantioselectivity with respect to the C3 center was complete, while C4 gave a 2 : 1 mixture in favor of the anticipated diastereoisomer.[17]

Tagatose-1,6-diphosphate aldolase (TagA) showed higher diastereoselectivity (entry 3), but in this case the conversion was decreased, probably due to lower activity of the aldolase (entry 3).

The efficiency of the newly developed four-enzyme cascade was evaluated in a two-step synthesis of the naturally occurring
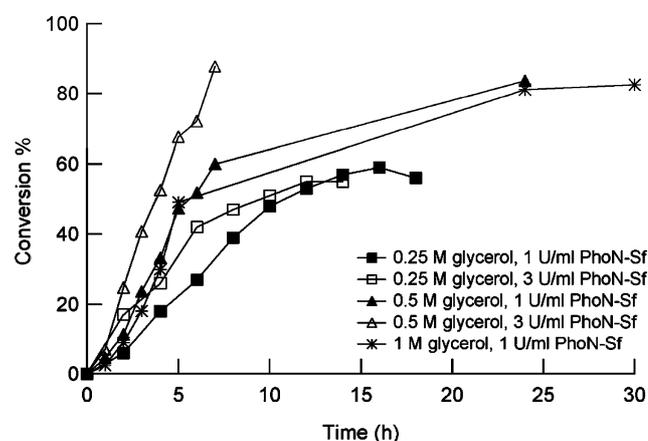
iminosugar D-fagomine (**8**, Scheme 3). D-Fagomine was isolated from buckwheat seeds (*Fagopyrum esculentum* Moench), characterized for the first time in 1974, and shown to possess relevant biological activity.[18] It is a potent inhibitor of isomaltase and specific α- and β-galactosidases. Furthermore, D-fagomine has a potent antihyperglycemic effect in streptozocin-induced diabetic mice. Over the years, several pathways have been developed
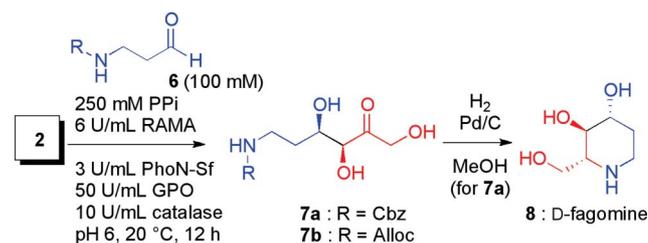
Scheme 3 Two-step synthesis of D-fagomine (**8**).

for the synthesis of D-fagomine,[19] including chemoenzymatic approaches involving aldolases.[20]

We envisioned that the cascade with glycerol **2**, RAMA and aldehyde **6** under the optimized reaction conditions would result in the formation of the intermediate product **7** (Scheme 3), which may then be converted into D-fagomine **8**. The first attempt using Cbz-protected aldehyde **6** resulted in 21% isolated yield of aldol product **7a**. Due to the low solubility of the aldehyde, the experiment was repeated in the presence of 10% DMSO, which resulted in a significant increase in yield to 49%. Besides, an improvement of the d.r. was observed from 6 : 1 to 10 : 1. This could be due to a solvent effect on the rate of formation of one of the diastereoisomers caused by active site perturbation of the aldolase. With the more soluble Alloc-protected aldehyde **6** under the optimal conditions (without cosolvent) a conversion of 75% into **7b** was observed and a d.r. of 5 : 1. Since it was noted that the temperature of incubation had an effect on the diastereoselectivity (*cf.* Table 1, entry 6), we also investigated the effect of temperature on the conversion and the diastereoselectivity of the resulting Alloc-modified carbohydrate **7b**. There was a marked increase in the isomeric ratio from 5 : 1 to 30 : 1 when the temperature was lowered from 30 to 10 °C though the conversion dropped to approximately 50%. Optimal results in terms of isolated yield were obtained at 20 °C giving product **7b** in in 69% yield and a d.r. of 11 : 1. When instead of RAMA the RhuA aldolase derived from the thermophilic organism *Thermatoga maritima* was used and the temperature lowered to 10 °C the conversion dropped considerably (not shown), but unfortunately the isomeric ratio remained 2 : 1.

Subsequent Pd-mediated Alloc-deprotection of **7b** (Pd on C, Et$_3$SiH), immediately followed by intramolecular reductive amination led to the diastereoselective formation of D-fagomine **8** in quantitative yield, thereby realizing a two-step synthesis of the natural product in 69% overall yield.[21] These yields are in the same range as previously reported by the Clapés group for a two-step approach using DHA and D-fructose-6-phosphate aldolase (FSA),[22] but the four-enzyme cascade has the advantage that it can also be applied to the stereocomplementary DHAP-dependent aldolases.

Conclusions

A convenient procedure was developed for the production of carbohydrates in a highly enantio- and diastereoselective manner using an efficient one-pot four-enzyme catalytic cascade. This cascade starts from inexpensive reagents without need for protection-deprotection steps. The primary step is chemoenzymatic phosphorylation of glycerol and subsequent oxidation to the energy rich phosphate ester DHAP at the expense of pyrophosphate. In the same pot, the formed DHAP is coupled to a variety of aldehydes by DHAP-dependent aldolases allowing the synthesis of all four possible stereoisomers. Dephosphorylation to the final aldol product occurs once pyrophosphate is fully consumed. The value of the catalytic cascade was demonstrated in an efficient two-step synthesis of the naturally occurring azasugar D-fagomine. Thus, this approach may lead to the simple and inexpensive synthesis of a variety of non-natural heterocycles that presently can only be produced at high costs.

Experimental

Chemicals and enzymes

All chemicals were purchased from commercial suppliers and used without purification. Recombinant *Shigella flexneri* acid phosphatase (PhoN-Sf) was expressed in *E.coli* and purified as previously described.[23] Catalase from bovine liver and L-glycerol-3-phosphate oxidase were purchased from Sigma-Aldrich and Toyobo, respectively.

Recombinant rhamnulose-1-phosphate aldolase from the thermophilic organisms *T. maritima* (pBAD/gIII plasmid provided by DSM) was expressed in *E. coli* strain TOP10 by inducing with 0.02% L-arabinose overnight at 28 °C. The resulting culture broth was centrifuged at 6000 rpm for 15 min at 4 °C and the cells were resuspended in 100 mM Bis-Tris pH 6.5, 0.1 mM ZnCl$_2$. Cell disruption was performed by sonication followed by centrifugation at 14 000 rpm for 30 min at 4 °C. The thermophilic enzyme RhuA was isolated from the cell free extract by heat-shock in a 70 °C water bath for 20 min, followed by centrifugation at 14 000 rpm for 30 min.

Tagatose-1,6-diphosphate aldolase from *E. coli* (pBAD plasmid supplied by DSM) was expressed in *E. coli* TOP10 cells and induced by 0.02% L-arabinose in the presence of 0.3 mM ZnCl$_2$. Cells were harvested by centrifugation at 6000 rpm for 15 min and resuspended in 20 mM Tris/HCl pH 7.5. Cell disruption was performed by sonication. From the cell-free extract, TagA was recovered by 40% ammonium-sulfate precipitation and centrifugation at 13 000 rpm for 30 min. The supernatant containing TagA was dialyzed overnight against 20 mM Tris/HCl pH 7.5 and loaded onto HiTrap DEAE FF 1 mL FPLC column for further purification. TagA was eluted at 200 mM NaCl and dialyzed against 20 mM Tris/acetate pH 7.5, 0.1 mM ZnCl$_2$.

Recombinant fuculose-1-phosphate (FucA) aldolase from the thermophilic bacterium *T. ethanolicus* (pBAD/gIII plasmid provided by DSM) was expressed in *E. coli* strain TOP10 by inducing with 0.02% L-arabinose overnight at 37 °C. The resulting culture was centrifuged at 7000 rpm for 15 min at 4 °C and the cells were resuspended in 100 mM Tris-HCl (pH 7.5), 150 mM NaCl and 0.1 mM ZnCl$_2$. Cell disruption was performed by French Press followed by centrifugation at 17 000 rpm for 30 min at 4 °C. The thermophilic enzyme was isolated from the cell free extract by heat-shock in a 70 °C water bath for 20 min, followed by centrifugation at 17 000 rpm for 30 min at 4 °C. The supernatant containing FucA was loaded onto a Q-Sepharose FF FPLC column (40 mL) and eluted with a linear gradient of NaCl (0–1 M NaCl in 20 column volumes). The purified fractions were collected and desalted using 50 mM Tris-HCl pH 7.5 and 150 mM NaCl buffer.

Enzyme activity assays

Aldolase activity was assayed by measuring the decrease of DHAP concentration in a reaction in which the aldolase performs the aldol coupling in the presence of DHAP (50 mM), *rac*-glyceraldehyde (100 mM), 0.1 mM ZnCl$_2$, 50 mM acetate pH 6, 30 °C. The decrease in DHAP concentration was assayed every 5 min with a coupled-enzyme system in presence of NADH-consuming glycerol-3-phosphate dehydrogenase by

measuring NADH concentration by UV spectroscopy. Samples of the aldolase reaction mixture were diluted and incubated in 100 mM Tris/acetate pH 7.5, 0.16 mM NADH, and 1 U mL^{-1} glycerophosphate dehydrogenase. The absorption was monitored at 340 nm at 20 °C (molar absorption coefficient 6.22 mM^{-1} cm^{-1}).

The K_m value of PhoN-Sf for glycerol was determined with a coupled enzyme system as described by Bergmeyer.[24] L-Glycerol-3-phosphate resulting from the phosphatase reaction was assayed by coupling with glycerol-3-phosphate dehydrogenase in the presence of hydrazine and NAD$^+$ at pH 9.5 and 25 °C. The reaction was spectrophotometrically monitored at 340 nm.

Analytical methods and product characterization

The time-course of the cascade reaction and the conversions were determined by HPLC using an Alltech OA 1000 organic acid column (0.65 × 30 cm). 20 μl of the reaction mixture was diluted 10-fold before injection on the HPLC. Isocratic runs were performed using 4.5 mM H$_2$SO$_4$, with a flow rate of 0.4 ml min^{-1}. The effluent was monitored at 210, 215, 254, and 275 nm and by refractive index detector.

^{1}H NMR and ^{13}C NMR spectra were recorded at 300 (75) or 400 (100) MHz. Optical rotations were determined with a Perkin Elmer 241 polarimeter. IR spectra were recorded on a Thermo Mattson IR300 equipped with a Harrick split pea ATR unit. Fast Atom Bombardment (FAB) mass spectrometry was carried out using a JEOL JMS SX/SX 102A four-sector mass spectrometer coupled to a JEOL MS-MP9021D/UPD system program.

General procedure for the preparative scale one-pot four-enzyme cascade

To a solution of 500 mM glycerol, 250 mM PP$_i$ (3 : 2 Na$_2$PP$_i$/Na$_4$PP$_i$),[25] 20 mM sodium acetate (pH 6), and 100 mM aldehyde in water 10 U mL^{-1} catalase from bovine liver, 6 U mL^{-1} RAMA, and 50 U mL^{-1} GPO were added. The reactions were started by adding 3 U mL^{-1} PhoN-Sf and incubated at 20 °C under mild shaking until completion. Dephosphorylation to the end product was completed after 24 h and 4 g of silica gel was added. The reaction slurry was concentrated under reduced pressure, and the silica gel was poured on top of a silica gel column and eluted with EtOAc/MeOH (19 : 1). The pure fractions were collected and concentrated under reduced pressure to give the product as a light-yellow oil.

Two step synthesis of D-fagomine (8)

3-Alloc-aminopropanal (157 mg, 1.0 mmol) was dissolved in H$_2$O (5.47 mL). Subsequently, glycerol (2.00 mL, 2.5 M 5.0 mmol) and PP$_i$ (591 mg, 2.5 mmol) were added, followed by the addition of 1.00 mL GPO (50 U mL^{-1}), 20 μL (10 U mL^{-1}) catalase from bovine liver and 161 μL (6 U mL^{-1}) RAMA. The reactions were initiated by adding 600 μL (3U mL^{-1}) PhoN-Sf and incubated until completion at 20 °C under mild shaking. Silica gel (2 g) was added to the reaction and the resulting mixture was concentrated under reduced pressure. The product was purified by flash chromatography (EtOAc/MeOH/19 : 1), giving product **7b** in 69% yield.

(3*S*,4*R*) - 6 - [(Allyloxycarbonyl)amino] - 5,6 - dideoxy -2- hexulose (**7b**, 20 mg, 0.081 mmol) was dissolved in EtOH (10 mL) and trietylsilane (0.16 mL, 0.97 mmol) was added, followed by the addition of Pd on C (10 mg, 0.094 mmol). The reaction mixture was stirred overnight at room temperature and filtered over Celite. The filtrate was concentrated *in vacuo* and purified with solid phase extraction, giving rise to D-fagomine (**8**, 12 mg, 0.082 mmol, 100%. ^{1}H NMR (CD$_3$OD, 400 MHz) δ 3.87 (dd, *J* = 10.9, 3.1 Hz, 1H), 3.63–3.55 (m, 1H), 3.42–3.38 (m, 1H), 3.11 (t, *J* = 9.0 Hz, 1H), 3.02 (ddd, *J* = 12.7, 4.6, 2.4 Hz, 1H), 2.66–2.58 (m, 2H), 2.48–2.41 (m, 1H), 1.97–1.90 (m, 1H), 1.54–1.43 (m, 1H); ^{13}C NMR (CD$_3$OD, 75 MHz) δ 73.3, 73.1, 61.4, 61.2, 42.6, 32.8; $[\alpha]_D^{20}$ = +6.6 (*c* = 0.60, MeOH); LRMS 148.10 [M+H]$^+$. These data are in agreement with literature.[19]

Spectroscopic data

5,6-Dideoxy-D-*threo*-2-hexulose (1a). ^{1}H NMR (CD$_3$OD, 300 MHz) δ 4.53 (d, 1H, *J* = 19.2 Hz), 4.42 (d, 1H, *J* = 19.2 Hz), 4.14 (d, 1H, *J* = 2.4 Hz), 3.78 (dt, 1H, *J* = 2.4, 6.0 Hz), 1.65–1.50 (m, 2H), 0.96 (t, 3H, *J* = 7.4 Hz); ^{13}C NMR (CD$_3$OD, 75 MHz) δ 212.0, 77.1, 73.2, 66.0, 25.3, 8.7; $[\alpha]_D^{20}$ −17.7 (*c* 0.22, MeOH); IR (neat) 1723, 3397 cm^{-1}; LRMS 149.08 [M+H]$^+$, 171.09 [M+Na]$^+$. These data are in agreement with literature.[10]

5,6,7-Trideoxy-D-*threo*-2-heptulose (1b). ^{1}H-NMR (CD$_3$OD, 300 MHz) δ 4.47 (q, 2H, *J* = 19.2 Hz), 4.11 (d, 1H, *J* = 2.3 Hz), 3.89 (dt, 1H, *J* = 2.4, 6.7 Hz), 3.30 (td, 1H, *J* = 1.6, 3.1 Hz), 1.56–1.40 (m, 4H), 0.95 (t, 3H, *J* = 7.2 Hz); ^{13}C-NMR (CD$_3$OD, 75 MHz) δ 211.9, 77.5, 71.4, 66.0, 34.5, 18.2, 12.4; $[\alpha]_D^{20}$ −14.1 (*c* 0.29, MeOH)); IR (neat) 1722, 3367 cm^{-1}; LRMS 163.10 [M+H]$^+$, 185.12 [M+Na]$^+$.

5,6-Dideoxy-5-methyl-D-*threo*-2-hexulose (1c). ^{1}H NMR (CD$_3$OD, 300 MHz) δ 4.53 (d, 1H, *J* = 19.2 Hz), 4.44 (d, 1H, *J* = 19.2 Hz), 4.31 (d, 1H, *J* = 1.8 Hz), 3.45 (dd, 1H, *J* = 2.0, 9.1 Hz), 1.90 (ddd, 1H, *J* = 2.3, 6.7, 13.4 Hz), 1.01 (d, 3H, *J* = 6.7 Hz), 0.93 (d, 3H, *J* = 6.7 Hz); ^{13}C NMR (CD$_3$OD, 75 MHz) δ 212.6, 77.4, 75.8, 65.9, 29.8, 17.8, 17.6; $[\alpha]_D^{20}$ −12.2 (*c* 0.16, MeOH); IR (neat) 1723, 3396 cm^{-1}; LRMS 163.10 [M+H]$^+$, 185.12 [M+Na]$^+$.

(3*S*,4*R*)-6-[(Benzyloxycarbonyl)amino]-5,6-dideoxy-2-hexulose (7a). ^{1}H-NMR (CD$_3$OD, 400 MHz) δ 7.48–7.21 (m, 5H), 5.19–5.02 (2H, m), 4.48 (q, 2H, *J* = 16.4 Hz), 4.19–4.09 (m, 1H), 3.97 (t, 1H, *J* = 7.2 Hz), 3.29–3.19 (m, 2H), 1.76 (q, 2H, *J* = 8.8 Hz); ^{13}C (CD$_3$OD, 75 MHz) δ 213.5, 159.2, 138.6, 129.6, 129.1, 129.0, 79.6, 71.3, 68.0, 67.6, 38.9, 34.6. These data are in agreement with literature.[19]

(3*S*,4*R*)-6-[(Allyloxycarbonyl)amino]-5,6-dideoxy-2-hexulose (7b). ^{1}H-NMR (CD$_3$OD, 400 MHz) δ 7.13–6.60 (1H, m), 6.12–5.67 (1H, m), 5.25 (d, 1H, *J* = 18 Hz), 5.17 (d, 1H, *J* = 9.0 Hz), 4.65–4.30 (m, 4H), 4.10 (d, 1H, *J* = 3.0 Hz), 3.96 (dt, 1H, *J* = 7.5, 3.0 Hz), 3.55 (s, 2H), 3.20 (s, 1H), 1.74 (q, 2H, *J* = 6 Hz); ^{13}C (CD$_3$OD, 75 MHz) δ 213.7, 134.8, 117.7, 79.7, 71.4, 68., 66.6, 38.9, 34.7; IR (neat) 1649, 1689, 3338 cm^{-1}; LRMS 248.10 [M+H]$^+$, 270.3 [M+Na]$^+$.

Acknowledgements

Notes and references

1 (*a*) A. Bruggink, R. Schoevaart and T. Kieboom, *Org. Process Res. Dev.*, 2003, **7**, 622–640; (*b*) R. A Sheldon, in *Multi-step enzyme catalysis: Biotransformations and chemoenzymatic synthesis*, ed. E. García-Junceda, Wiley-VCH, Weinheim, 2008, Ch. 6, pp. 109–136.

2 R. A. Sheldon, *Chem. Commun.*, 2008, 3352–3365.

3 S. M. Dean, W. A. Greenberg and C.-H. Wong, *Adv. Synth. Catal.*, 2007, **349**, 1308–1320.

4 (*a*) Y. Ichikawa, Y. Igarashi, M. Ichikawa and Y. Suhara, *J. Am. Chem. Soc.*, 1998, **120**, 3007–3018; (*b*) *Iminosugars: from synthesis to therapeutic applications*, ed. P. Compain and O. R. Martin, Wiley-VCH, Weinheim, 2007; (*c*) N. Asano, *Cell. Mol. Life Sci.*, 2009, **66**, 1479–1492.

5 (*a*) M. Movassaghi and E. N. Jacobsen, *Science*, 2002, **298**, 1904–1905; (*b*) S. Y. Ko, A. W. M. Lee, S. Masamune, L. A. Reed, K. B. Sharpless and F. J. Walker, *Science*, 1983, **220**, 949–951.

6 A. B. Northrup and D. W. C. MacMillan, *Science*, 2004, **305**, 1752–1755.

7 (*a*) H. J. M. Gijsen and C.-H. Wong, *J. Am. Chem. Soc.*, 1995, **117**, 7585–7591; (*b*) K. M. Koeller and C.-H. Wong, *Chem. Rev.*, 2000, **100**, 4465–4494; (*c*) P. Clapés, W.-D. Fessner, G. A. Sprenger and A. K. Samland, *Curr. Opin. Chem. Biol.*, 2010, **14**, 154–167.

8 (*a*) A. Bolt, A. Berry and A. Nelson, *Arch. Biochem. Biophys.*, 2008, **474**, 318–330; (*b*) M. G. Silvestri, G. Desantis, M. Mitchell and C.-H. Wong, *Top. Stereochem.*, 2003, **23**, 267–342; (*c*) M. Schumperli, R. Pellaux and S. Panke, *Appl. Microbiol. Biotechnol.*, 2007, **75**, 33–45.

9 D. C. Crans and G. M. Whitesides, *J. Am. Chem. Soc.*, 1985, **107**, 7019–7026.

10 W.-D. Fessner and G. Sinerius, *Angew. Chem., Int. Ed. Engl.*, 1994, **33**, 209–212.

11 O. Eyrisch, G. Sinerius and W.-D Fessner, *Carbohydr. Res.*, 1993, **238**, 287–306.

12 C. H. Wong and G. M Whitesides, *J. Org. Chem.*, 1983, **48**, 3199–3205.

13 R. Schoevaart, F. van Rantwijk and R. A. Sheldon, *J. Org. Chem.*, 2000, **65**, 6940–6943.

14 T. van Herk, A. F. Hartog, H. E. Schoemaker and R. Wever, *J. Org. Chem.*, 2006, **71**, 6244–6247.

15 T. van Herk, A. F. Hartog, A. M. van der Burg and R. Wever, *Adv. Synth. Catal.*, 2005, **347**, 1155–1162.

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

17 K. Faber, *Biotransformations in Organic Chemistry*, 5th ed., Springer-Verlag, Berlin, 2004, pp. 277–284.

18 A. Kato, N. Asano, H. Kizu and K. Matsui, *J. Nat. Prod.*, 1997, **60**, 312–314.

19 (*a*) N. Kumari, B. G. Reddy and Y. D. Vankar, *Eur. J. Org. Chem.*, 2009, 160–169; (*b*) L. Bartali, D. Scarpi, A. Guarna, C. Prandi and E. G. Occhiato, *Synlett*, 2009, 913–916.

20 M. Sugiyama, Z. Hong, P.-H. Liang, S. M. Dean, L. J. Whalen, W. A. Greenberg and C.-H. Wong, *J. Am. Chem. Soc.*, 2007, **129**, 14811–14817, and references cited therein.

21 P. K. Mandl and J. S. McMurray, *J. Org. Chem.*, 2007, **72**, 6599–6601.

22 J. A. Castillo, J. Calveras, J. Casas, M. Mitjans, M. Pilar Vinardell, T. Parella, T. Inoue, G. A. Sprenger, J. Joglar and P. Clapés, *Org. Lett.*, 2006, **8**, 6067–6070.

23 N. Tanaka, Z. Hasan, A. F. Hartog, T. van Herk and R. Wever, *Org. Biomol. Chem.*, 2003, **1**, 2833–2839.

24 H. U. Bergmeyer, K. Gawehn, M. Grassl, *Glucose-6-phosphate dehydrogenase from yeast, in Methods of Enzymatic Analysis*, ed. H. U. Bergmeijer, Verlag Chemie, vol. 1, 1986, pp. 458–459.

25 PP$_i$ was added as a mixture of 3.5 equiv. (350 mg) Na$_2$PP$_i$ and 2 equiv. (241 mg) Na$_4$PP$_i$ resulting in a buffered solution of pH 6.