Fluorogenic Stereochemical Probes for Transaldolases

Eva González-García,[a] Virgil Helaine,[b] Gérard Klein,[a] Melanie Schuermann,[c] Georg A. Sprenger,[c] Wolf-Dieter Fessner,*[b] and Jean-Louis Reymond*[a]

Abstract: Transaldolase catalyzes the transfer of dihydroxyacetone from, for example, fructose 6-phosphate to erythrose 4-phosphate. As a potential probe for assaying fluorescent transaldolase, 6-*O*-coumarinyl-fructose (**1**) was prepared in six steps from D-fructose. The corresponding 6-*O*-coumarinyl-5-deoxy derivative **2** was prepared stereoselectively from acrolein and *tert*-butyl acetate by a chemoenzymatic route involving *Amano PS* lipase for the kinetic resolution of *tert*-butyl 3-hydroxypent-4-enoate (**7**) and *E. coli* transketolase for assembly of the final product. The corresponding stereoisomer related to D-tagatose was obtained by a chemical synthesis starting from D-ribose. Indeed, transaldolases catalyze the retro-aldolization of substrate **1** to give dihydroxyacetone and 3-*O*-coumarinyl-glyceraldehyde. The latter primary product undergoes a β-elimination in the presence of bovine serum albumin (BSA) to give the strongly fluorescent product umbelliferone. A similar reaction is obtained with the 5-deoxy analogue **2**, but there is almost no reaction with its stereoisomer **3**. The stereoselectivity of transaldolases can be readily measured by the relative rates of fluorescence development in the presence of the latter pair of diastereomeric substrates.

Keywords: aldolases · enzyme catalysis · enzyme evolution · fluorogenic assays · high-throughput screening

Introduction

Enzyme engineering has made rapid progress in recent years, mainly due to the invention of genetic variation techniques such as error-prone PCR and gene shuffling.[1] Directed evolution has yielded libraries of modified biocatalysts possessing remarkably improved properties such as improved substrate specificity, enantioselectivity, or activity under various demanding reaction conditions (organic solvents, high temperature, pH, etc.).[2] At present, the bottleneck for enzyme engineering is often the lack of a simple assay for catalysis that is applicable to high-throughput mode. Such an assay should not only give a readily recordable signal, but combine simplicity of use with selectivity and specificity. One of the most practical solutions is to use fluorogenic enzyme substrates.[3]

Initially, the first choice for studies in directed evolution experiments had been hydrolytic enzymes, because of practical issues such as their synthetic utility and relative operational simplicity.[2] In comparison, the function of stereochemically and mechanistically more intricate catalysts such as those involved in carbon–carbon bond generation are more difficult to assess. Here we report on the stereoselective synthesis of fluorogenic substrates for transaldolases and their evaluation as probes for enzyme stereospecificity. Transaldolases belong to the class I aldolases, which operate by an enamine mechanism.[4] Aldolases offer complete catalyst control in the generation of two adjacent chiral centers, and are of general use in asymmetric synthesis.[4, 5] The new substrates represent the first stereospecific fluorogenic assay system for this class of enzymes, which should be suitable for screening of libraries of mutant transaldolases for altered stereoselectivity.

[a] Prof. Dr. J.-L. Reymond, Dr. E. González-García,[+] Dr. G. Klein[+]
Departement für Chemie und Biochemie, Universität Bern
Freiestrasse 3, 3012 Bern (Switzerland)
Fax: (+41) 31-631-80-57
E-mail: jean-louis.reymond@ioc.unibe.ch

[b] Prof. Dr. W.-D. Fessner, Dr. V. Helaine[+]
Institut für Organische Chemie und Biochemie
Technische Universität Darmstadt
Petersenstrasse 22, 64287 Darmstadt (Germany)
Fax: (+49) 6151-166636
E-mail: fessner@tu-darmstadt.de

[c] Dr. M. Schuermann, Prof. Dr. G. A. Sprenger
Institut für Biotechnologie 1, Forschungszentrum Jülich
52425 Jülich (Germany)
Fax: (+49) 2461-61-2710
E-mail: g.sprenger@fz-juelich.de

[+] These authors contributed equally to the work.

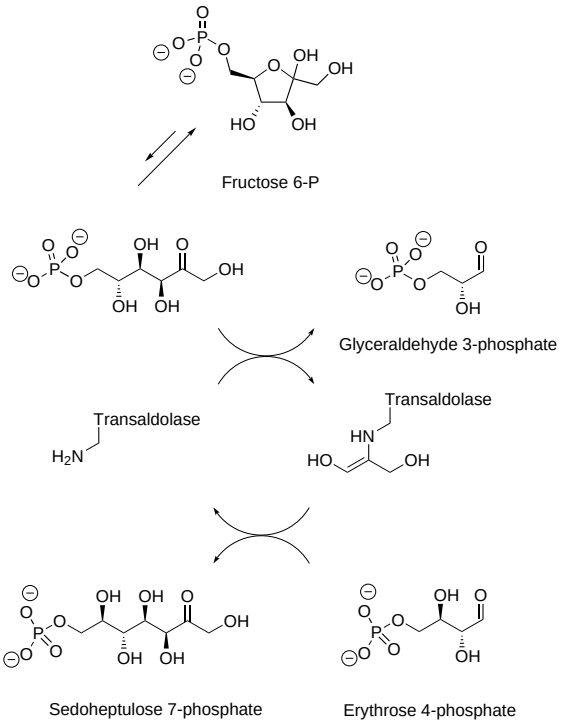
Supporting information for this article is available on the WWW under http://www.chemeurj.org/ or from the author.

Results and Discussion

Substrate design: Transaldolases (EC 2.2.1.2) catalyze the transfer of a dihydroxyacetone moiety between sugar phos-

  0947-6539/03/0904-0893 $ 20.00+.50/0

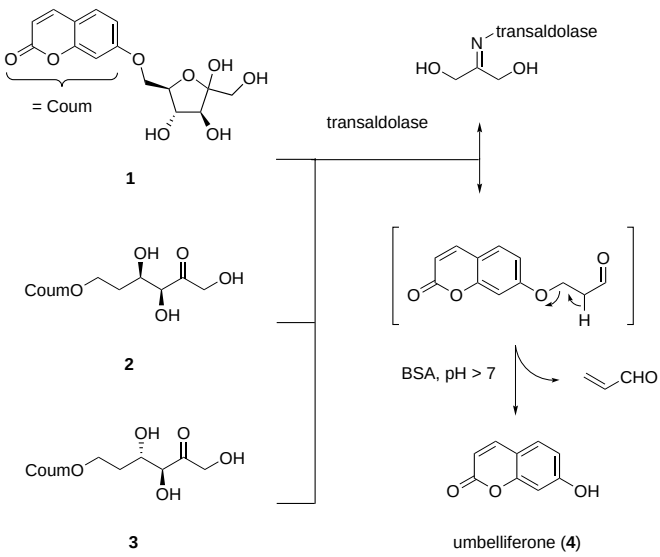
phates (e.g., from D-fructose 6-phosphate to D-erythrose 4-phosphate, yielding D-glyceraldehyde 3-phosphate and D-sedoheptulose 7-phosphate; Scheme 1) by an enamine mechanism typical for class I aldolases.[6] The reaction is completely stereospecific for the *threo* configuration at the diol being transformed by the aldol reaction. In direct relation with their function, transaldolases possess a strong specificity for dihydroxyacetone, but may accept aldehydes other than glyceraldehyde 3-phosphate or erythrose 4-phosphate. Although this class of enzymes is well studied structurally and mechanistically, preparative use of transaldolases is so far limited.[4]



Scheme 1. Natural reactions catalyzed by transaldolases.

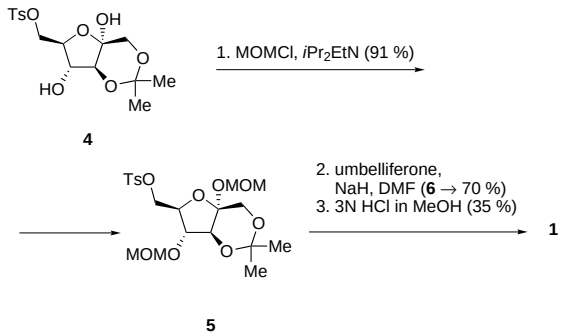
In connection with our interest in developing enzyme evolution experiments involving transaldolases, we set out to develop a simple stereospecific assay for these enzymes, based on Reymond's fluorogenic principle. In these substrates, fluorescence release is based on the secondary release of umbelliferone by β-elimination from a primary or secondary carbonyl reaction product.[7] This principle has been demonstrated for fluorogenic substrates of a variety of enzymes, including alcohol dehydrogenases,[8] lipases, esterases, amidases, phosphatases, and epoxide hydrolases,[9] as well as for fluorogenic polypropionate fragments that react stereospecifically with aldolase catalytic antibodies.[10]

Thus, an immediate target was 6-*O*-coumarinyl-D-fructose (**1**), a straightforward analogue of D-fructose 6-phosphate that should be accessible from the parent ketose by simple synthetic manipulation (Scheme 2). We also set out to prepare the corresponding 5-deoxy analogue **2**, which advantageously could exist in solution only in the reactive open-chain form and was therefore expected to display enhanced retroaldolization reactivity. Given the known tolerance of aldolases for aldehydic substrate components but their distinct bias for the C3/C4-aldol stereochemistry,[4, 5, 11] substrate **2** should function properly as a fluorogenic substrate. We also envisioned the preparation of the C4-stereoisomeric fluorogenic 5-deoxy-substrate **3**, corresponding to the D-tagatose configuration. With respect to the microscopic reversibility of aldolizations, the stereoisomeric fluorogenic pair **2**/**3** would provide a useful tool for rapid measurement of the *Re*/*Si* stereoselectivity of transaldolases for enamine addition to the accepting aldehyde carbonyl.



Scheme 2. Fluorogenic stereochemical probes for transaldolases.

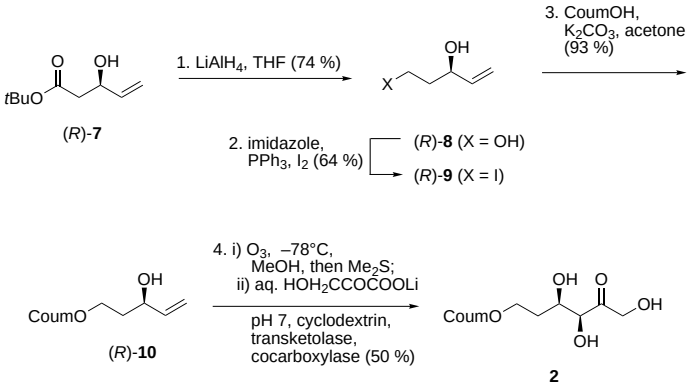
Synthesis: The preparation of substrate **1** required a protected derivative of D-fructose that would allow the selective substitution of the C6-OH group by a coumarinyl ether (Scheme 3). The known fructofuranose derivative **4**, accessible in two steps from D-fructose, was chosen as starting material. Protection of the free hydroxyl groups as methoxymethyl ethers gave derivative **5**. Substitution of the tosylate in **5** with the sodium salt of umbelliferone in DMF proceeded cleanly to give ether **6**. Finally, acidic deprotection and preparative reversed-phase HPLC purification gave substrate **1**.



Scheme 3. Synthesis of fluorogenic substrate **1** from protected D-fructofuranoside **4**.

0947-6539/03/0904-0894 $ 20.00+.50/0 *Chem. Eur. J.* **2003**, *9*, No. 4

Substrate **2** was prepared by a chemoenzymatic route that made use of the common constitutional and stereochemical relationship that substrates of transaldolase share with those of transketolase.[4] A plausible alternative route based on the synthetic capacity of dihydroxyacetone phosphate-dependent fructose 1,6-diphosphate aldolases[4, 5, 12] was abandoned, due to the anticipated lability of the required precursor aldehyde (identical to the transient assay intermediate; Scheme 2). The preparation started with racemic **7**, obtained by addition of the lithium enolate of *tert*-butyl acetate to acrolein (Scheme 4).[13] The optically pure β-hydroxy-ester (*R*)-**7** (*ee* 99%) was

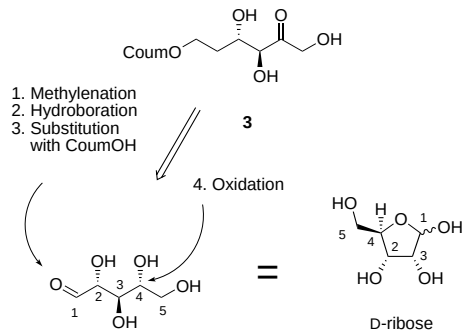


Scheme 4. Biocatalytic stereoselective synthesis of fluorogenic substrate **2**.

prepared by the procedure of Vrielynck and Vandewalle, by enantioselective esterification of its antipode with vinyl acetate in the presence of *Amano PS* lipase.[14] Hydride reduction gave diol **8**, which was selectively activated at the primary alcohol function to give iodide **9**, to be used directly for alkylation of umbelliferone to provide ether **10**. The hydroxyaldehyde generated by ozonolysis from (*R*)-**10** was immediately treated with lithium hydroxypyruvate with transketolase catalysis (in the presence of thiamin diphosphate in aqueous solution) to yield the desired aldol **2**.

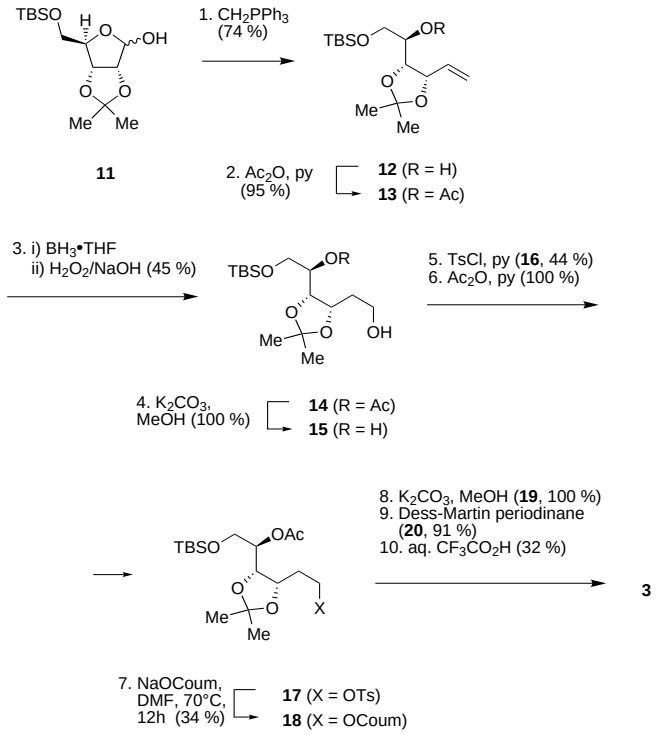
The addition of modified cyclodextrin was necessary to solubilize the hydroxyaldehyde substrate in the aqueous buffer and to facilitate the enzymatic chain-extension.[15] Initially, a fully racemic sequence to the (*RS*)-2-hydroxyaldehyde intermediate was followed (generated from (*R*,*S*)-**10**, no lipase resolution step). This, in the final enzymatic step, made use of the practically complete kinetic selectivity of transketolase for *R*-configured 2-hydroxyaldehydes[4, 16] to give enantio- and diastereomerically pure **2**. Because of the difficulties in solubilizing the hydroxyaldehyde, however, preparation of the enantiomerically enriched precursor proved more economical.

Substrate **3** was prepared from D-ribose by a synthetic sequence involving methylenation at C1, hydroboration, substitution of the newly formed primary alcohol with umbelliferone, and finally oxidation at the original C5. Thus, C1 of D-ribose became the C5-deoxy position in the target, while the chirality at C4 of D-ribose was lost in becoming the ketose carbon C2 in substrate **3**. The C2/C3 stereochemical elements of D-ribose were preserved during the synthesis, giving rise to the *erythro*-configured diol at the aldol linkage (Scheme 5).



Scheme 5. Retrosynthetic analysis of fluorogenic substrate **3**.

The six-carbon chain was first established by methylation of protected D-ribose derivative **11** with methylene triphenyl phosphorane (Scheme 6). Subsequent acetylation to give **13** and subsequent hydroboration gave **14**, which was deacetylated to provide the diol **15**. The umbelliferyl group was



Scheme 6. Synthesis of fluorogenic substrate **3** from protected D-ribofuranose **11**.

introduced at this stage by selective tosylation of the primary alcohol to give **16**. As direct alkylation of umbelliferone with **16** under a variety of conditions gave no results, **16** was first converted into its acetate **17**, which reacted smoothly with the sodium salt of umbelliferone in DMF to yield the aryl ether **18**. Finally, acidic deprotection and purification by reversed-phase HPLC gave substrate **3**.

Enzyme assays: Substrates were diluted from 5 mM stock solution in water, under which conditions they were all perfectly soluble and stable in the frozen state for long periods

of time. Assays were conducted at neutral or basic pH with three different enzymes from various sources.

Assay conditions were first optimized with yeast transaldolase and substrate **1**, which is structurally closest to the natural compound fructose 6-phosphate. A time-dependent increase in fluorescence was observed at either neutral or basic pH in the presence of bovine serum albumin (BSA). There was no reaction in the absence of transaldolase even when BSA was present, showing that the fluorogenic substrate was highly specific for transaldolase catalytic activity and was not susceptible to chemical decomposition or non-specific degradation by BSA. This observation parallels our previous experiments with fluorogenic polypropionate fragments.[10] Remarkably, there was no fluorescence increase in the absence of BSA. BSA is unlikely to be essential for the activity of transaldolase, and is most probably essential to promote the secondary β-elimination of umbelliferone from the intermediate aldehyde.

Erythrose 4-phosphate, which is a natural acceptor for dihydroxyacetone for this enzyme, was investigated as a possible additive to shift the reaction equilibrium further towards retroaldolization of substrate **1**, and thus increase the steady rate of fluorescence increase, which appeared to level off after an initial burst of activity. Indeed, addition of erythrose 4-phosphate at an optimized concentration of 100 μM resulted in a sustained reaction, suggesting that the expected effect was operative. A decrease in reaction rate was observed at higher concentrations of erythrose 4-phosphate; this might be due to competition of this natural substrate with the fluorogenic substrate **1** for occupancy of the enzyme's catalytic site. Three different enzymes were tested with the fluorogenic substrates **1**–**3** under the optimized conditions in the presence of BSA and erythrose 4-phosphate (Figure 1, Table 1). This included transaldolases from yeast (commercial) and *E. coli* (recombinant *talB*),[11] as well as the fructose 6-phosphate aldolase from *E. coli* (recombinant *fsa*). The last of these is a novel enzyme that has recently been shown to cleave fructose 6-phosphate in a manner resembling that of transaldolase but without requiring an acceptor co-substrate.[17] All three enzymes tested positive with assay compounds **1** and **2**. The reaction with the 5-deoxy substrate **2** was generally either somewhat faster than with substrate **1** or very similar, indicating that deletion of the hydroxyl group at C5 of fructose was neither strongly favorable nor disfavorable for the overall fluorogenic process. While the 5-deoxy substitution should accelerate the reaction by supporting the reactive acyclic form, and also by destabilizing the aldehyde produced by the retroaldolization reaction towards β-elimination, it is probably also recognized less specifically and bound by transaldolase enzymes with lower affinity than the simple fructose analogue **1**, due to the lack of hydrogen bonding to a 5-hydroxyl group, and overall the two effects seem to compensate each other. Remarkably, the reaction rates of the *E. coli* transaldolase with both substrate analogues were more than one order of magnitude lower than for the corresponding yeast enzyme. This seems to indicate a relatively lower binding preference of the bacterial enzyme for the non-phosphorylated assay compounds **1** and **2**, which in fact would be in line with the very low affinity of the *E. coli*

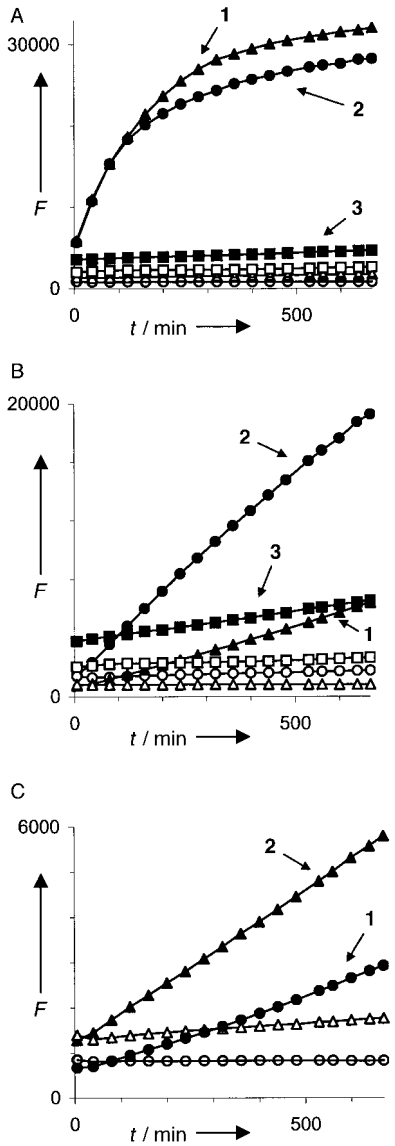


Figure 1. Time course of fluorescence increase in transaldolase assay with substrates **1**–**3**. The increase in fluorescence at $\lambda_{em} = 460$ nm ($\lambda_{ex} = 365$ nm) is shown for the reaction at 26 °C in aq. Tris buffer pH 8.0, 100 μM substrate, 0.1 mg mL^{-1} enzyme. A) Transaldolase from yeast, B) TalB transaldolase from *E. coli*, C) fructose 6-phosphate aldolase from *E. coli*. The data are shown for substrate **1** with (▲) and without (△) enzyme, substrate **2** with (●) and without (○) enzyme, substrate **3** with (■) and without (□) enzyme. Assays (0.1 mL) were followed at 26 °C in individual wells of round-bottomed polypropylene 96-well plates (Costar) by using a Cytofluor II Fluorescence Plate Reader (Perseptive Biosystems, filters $\lambda_{ex} = 360 \pm 20$, $\lambda_{em} = 460 \pm 20$ nm).

Table 1. Activity and stereoselectivity determinations with fluorogenic substrates **1**–**3**.[a]

Enzyme	conc. [U mL^{-1}]	V(**1**) [pM s^{-1}]	V(**2**) [pM s^{-1}]	V(**3**) [pM s^{-1}]	*de*[b] [%]
yeast transaldolase	0.23	600.0	615.4	10.7	97
E. coli transaldolase B	5.8	17.5	34.3	n.d.	n.d.
fructose 6-phosphate aldolase, *E. coli*	0.6	43.0	135.7	20.6	74

[a] Apparent rate of release of umbelliferone when using 100 μM substrate in Tris buffer (20 mM, pH 8.0) containing BSA (2.0 mg mL^{-1}) and 100 μM erythrose 4-phosphate. [b] Diastereoselectivity predicted for the aldol addition step from the apparent retroaldolization rates with **2** and **3**.

0947-6539/03/0904-0896 $ 20.00+.50/0 *Chem. Eur. J.* **2003**, *9*, No. 4

transaldolase for D-fructose ($K_m = 2$ M) as compared to that for fructose 6-phosphate ($K_m = 1.2$ mM.[11]).

Substrate **3**, with the non-natural L-*erythro* configuration (instead of D-*threo*) at C3/C4, gave a weak but measurable fluorescence signal upon incubation with yeast transaldolase. Comparison of the reaction rates of substrate **2** and of its stereoisomer **3**, with correction for any detectable background reaction in the latter case, allows the diastereoselectivity for this pair of substrates to be determined (Table 1). Interestingly, for the Fsa enzyme this correlation of kinetic rates with probes **2** and **3** amounts to a stereoselectivity of only 74% *de*. Although this analysis points out a potential weakness of the enzyme for synthetic applications, such deductions still require further experimental verification. The observation of a non-absolute stereospecificity for this class of enzymes is consistent with known limitations for certain other aldolases in handling distinct non-natural substrate analogues, in particular for the related *fructo*/*tagato*-stereoselective pair of dihydroxyacetone phosphate-dependent aldolases (FruA, TagA).[18]

Conclusion

Three stereochemical fluorogenic probes for transaldolases and related enzymes have been prepared by stereoselective synthesis. 6-*O*-Coumarinyl-D-fructose (**1**) is the most straightforward probe to synthesize, and provides a viable fluorogenic assay for transaldolases in the presence of BSA and erythrose 4-phosphate. A similar assay is available with the structurally and stereochemically simpler, yet synthetically more demanding, 6-*O*-coumarinyl-5-deoxy derivative **2**. Together with its stereoisomer **3**, this opens the door to effective screening of directed evolution experiments aimed at inverting the stereoselectivity of transaldolases. Given the strong specificity of these enzymes for dihydroxyacetone, switching of the stereochemistry at C4, which corresponds to the aldehyde carbonyl, is probably the most reasonable stereochemical manipulation to attempt with transaldolases.[19] This experiment would involve mutations that would enable reorientation of the aldehyde carbonyl to expose its *Si* face instead of its *Re* face towards an attack by the enamine at the enzyme's active site. Experiments towards this goal, using fluorogenic and chromogenic probes **2** and **3** as reporters for catalytic activity, are in progress.

Experimental Section

General: Transaldolase (D-sedoheptulose-7-phosphate: D-glyceraldehyde-3-phosphate dihydroxyacetone transferase, EC 2.2.1.2) from baker's yeast was purchased from Sigma (T6008); the transketolase, transaldolase B, and the fructose 6-phosphate aldolase, the latter three all from *E. coli*, were produced from recombinant clones and purified according to published protocols.[11, 17] Reagents were purchased in the highest quality available from Fluka, Sigma, or Aldrich. All solvents used in reactions were bought in p.a. quality or distilled and dried prior to use. Solvents for extractions were distilled from technical quality. Sensitive reactions were carried out under nitrogen or argon, the glassware being heated under HV. Chromatographic purifications (flash) were performed with silica gel 60 from Merck or Fluka (0.04–0.063 nm; 230–400 mesh ASTM). Preparative HPLC was performed with a Waters Delta Prep 4000 system with a Waters Prepak Cartridge (500 g) as column and Waters 486 Tunable Absorbance Detector. Analytical normal-phase HPLC was performed on Waters 6000 systems with Vydac C18 columns (218-TP-54) as stationary phase (column dimensions 0.5×22 cm, pore size of stationary phase 300 Å, flow rate 1.5 mL min^{-1}, UV detection with Waters 996 photodiode array detector). TLC monitoring was performed with Alugram SIL G/UV$_{254}$ silica gel sheets (Macherey–Nagel), followed by coloration with cerium solution (10.5 g Ce^{IV} sulfate, 21 g phosphomolybdic acid, 60 mL conc. H_2SO_4 in 900 mL water) or anisaldehyde stain and heating. Optical rotations were determined in a polarimeter (Perkin Elmer 241) in a 10 cm cell. Infrared spectra were recorded in a Perkin Elmer 1600 series FTIR. MS and HRMS analyses were provided by the mass spectrometry service of the Department of Chemistry and Biochemistry, University of Bern. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AC 300 (300 MHz) and DRX 500 or Avance 500 (500 MHz) instruments. Chemical shifts δ are given in ppm, coupling constants (J) in Hertz (Hz).

6-*O*-(2-Oxo-2*H*-chromen-7-yl)-D-fructose (1): Intermediate **6** was heated for 5 h at 60 °C in 6N HCl. The crude product was purified by preparative reversed-phase HPLC (RP C18, gradient 0–10% acetonitrile in H_2O) to give **1** (10 mg, 35%) as a colorless oil. ^{1}H NMR (300 MHz, CD_3OD): $\delta =$ 7.88 (d, $J = 9.6$ Hz, 1H), 7.36 (d, $J = 8.5$ Hz, 1H), 6.84 (dd, $J = 8.5$, 2.4 Hz, 1H), 6.81 (d, $J = 2.4$ Hz, 1H), 6.25 (d, $J = 9.6$ Hz, 1H), 4.25 (m, 3H), 4.13 (s, 1H), 4.09 (m, 2H), 3.51 (s, 2H) ppm; ^{13}C NMR (75 MHz, CD_3OD): $\delta =$ 163.8, 163.7, 157.1, 145.8, 130.5, 114.3, 114.2, 113.5, 103.6, 102.5, 80.6, 77.4, 77.1, 71.7, 64.4 ppm; IR (KBr): $\tilde{\nu} = 3392$, 1702, 1618, 1131, 1048, 837 cm^{-1}; HRMS: calcd for $C_{15}H_{16}O_8$: 325.0918; found: 325.0925 $[M+H]^+$

5-Deoxy-6-*O*-(2-oxo-2*H*-chromen-7-yl)-D-*threo*-hexulose (2): Compound **10** (2.16 g, 8.8 mmol) and hydroxypropyl-β-cyclodextrin (25.7 g, 2 equiv, 18.6 mmol) were dissolved in methanol (150 mL) and the resulting solution was treated with ozone at -78 °C until no more starting material was detectable by TLC (CH_2Cl_2/MeOH 95:5). Dimethylsulfide (6 mL) was then added dropwise and the solution was allowed to stir at -78 °C for 1 h. The mixture was allowed to warm to room temperature and stirred for 4 h. Water (90 mL) was added, and methanol was removed by rotary evaporation. The pH was carefully adjusted to 7.5 by addition of diluted aq. NaOH solution, and then lithium hydroxypyruvate (1.0 g, 9.1 mmol, 1 equiv), *E. coli* transketolase (50 U), thiamin diphosphate (80 mg, 2 mM), and $MgCl_2 \cdot 6H_2O$ (55 mg, 3 mM) were added. The slightly yellow solution was shaken for 72 h, with identical quantities of lithium hydroxypyruvate and transketolase being added after 24 h. After evaporation to dryness, the crude solid was purified by chromatography on silica gel (CH_2Cl_2/MeOH 95:5) to give **2** (1.36 g, 50%). $[\alpha]_D^{20} = +14.8$ (CH_3OH, $c = 0.48$); ^{1}H NMR (CD_3OD, 500 MHz): $\delta = 7.89$ (d, $J = 11.9$ Hz, 1H; H_{arom}), 7.54 (d, $J =$ 10.6. Hz, 1H; H_{arom}), 6.97–6.94 (m, 2H; H_{arom}), 6.24 (d, $J = 11.9$ Hz, 1H; H_{arom}), 4.54 ($2 \times$ d, $J = 23.8$ Hz, 2H; H_6), 4.31 (s, 1H; H_1), 4.24–4.19 (m, 2H; H_3 and H_4), 1.99–1.95 (m, 2H; H_5) ppm; ^{13}C NMR (CD_3OD, 125 MHz): $\delta = 213.5$, 163.4, 157.2, 145.8, 130.4, 114.2, 113.3, 102.3, 79.6, 70.1, 67.9, 66.5, 33.9 ppm; HRMS: calcd for $C_{15}H_{16}O_7$: 307.0817; found: 307.0830 $[M-H]^-$.

5-Deoxy-6-*O*-(2-oxo-2*H*-chromen-7-yl)-L-*erythro*-hexulose (3): Compound **20** (14 mg, 0.03 mmol) was dissolved in water (0.2 mL) and trifluoroacetic acid (0.1 mL). After 1 h at 25 °C, the solvents were evaporated in vacuo to give 11 mg of crude product. Preparative reversed-phase HPLC gave **3** (3 mg, 32%) as a colorless oil: $[\alpha]_D^{20} = -9.4$ (CH_3OH, $c = 0.48$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.92$ (d, $J = 9.2$ Hz, 1H), 7.53 (d, $J = 9.2$ Hz, 1H), 6.99–6.92 (m, 2H), 6.28 (d, $J = 9.5$ Hz, 1H), 4.56 (d, $J = 7.0$ Hz, 2H), 4.38 (d, $J = 5.1$ Hz, 1H), 4.23 (t, $J = 5.9$ Hz, 2H), 4.17–4.14 (m, 1H), 3.29 (br s, 3H), 2.00–1.98 (m, 2H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): 213.3, 164.2, 163.7, 157.4, 146.0, 130.7, 114.5, 114.3, 113.6, 102.6, 79.9, 70.9, 68.4, 66.6, 33.3 ppm; IR (film): $\tilde{\nu} = 3393$ (br m), 2927 (w), 1706 (s), 1615 (s), 1132 (s), 837.9 (w) cm^{-1}; HRMS: calcd for $C_{15}H_{16}O_7$: 307.0817; found: 307.0817 $[M-H]^-$.

1,3-*O*-Isopropylidene-2,4-di-*O*-(methoxymethyl)-6-*O*-(*p*-tosyl)-D-fructofuranose (5): A solution of tosylate **4** (80 mg, 0.26 mmol) in anhydrous dichloromethane (1 mL) was treated at 0 °C with *N*,*N*-diisopropylethylamine (0.45 mL, 2.6 mmol) and chloromethyl methyl ether (0.165 mL, 2.2 mmol). After 24 hours at 25 °C, the solution was diluted with CH_2Cl_2 and washed with aq. HCl (1N). Evaporation of the residue and flash chromatography (EtOAc/hexane 3:2, $R_f = 0.55$) gave **5** (19.5 mg, 30%).

^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.82$ (d, $J = 8.3$, 2H), 7.34 (d, $J = 8.3$, 2H), 4.97 (m, 2H), 4.65 (m, 2H), 4.18 (s, 4H), 3.87 (m, 3H), 3.38 (s, 3H), 3.35 (s, 3H), 2.45 (s, 3H), 1.27 (s, 3H), 1.43 (s, 3H) ppm; ^{13}C NMR (75 MHz, CD_3OD): $\delta = 144.8$, 132.7, 129.8, 128.1, 101.9, 98.6, 96.4, 89.9, 83.2, 81.5, 79.1, 69.3, 63.8, 55.9, 55.6, 27.7, 21.6, 19.7 ppm; IR ($CHCl_3$): $\tilde{\nu} = 2952$, 2253, 1373, 1170, 990, 915, 746 cm^{-1}; HRMS: calcd for $C_{20}H_{30}O_{10}S$: 463.1629; found: 463.1623 $[M+H]^+$.

1,3-*O*-Isopropylidene-2,4-di-*O*-(methoxymethyl)-6-*O*-(2-oxo-2*H*-chromen-7-yl)-D-fructofuranose (6): Tosylate **5** (45 mg, 0.098 mmol) was added to a solution of the sodium salt of umbelliferone, prepared from umbelliferone (24 mg, 0.15 mmol) and sodium hydride (6.5 mg, 0.16 mmol) in DMF (1.5 mL). After 24 h at 80 °C, the reaction mixture was diluted with ethyl acetate and washed with aq. NaOH (1N). Evaporation of the organic phase and flash chromatography (AcOEt/hexane 1:1, $R_f = 0.35$) gave **6** (36 mg, 70 %) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.64$ (d, $J =$ 9.6 Hz, 1H), 7.38 (d, $J = 8.5$ Hz, 1H), 6.91 (dd, $J = 8.5$, 2.4 Hz, 1H), 6.86 (d, $J = 2.4$ Hz, 1H), 6.26 (d, $J = 9.6$ Hz, 1H), 4.97 (m, 2H), 4.65 (m, 2H), 4.18 (s, 4H), 3.87 (m, 3H), 3.38 (s, 3H), 3.35 (s, 3H), 2.45 (s, 3H), 1.49 (s, 3H), 1.38 (s, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 161.8$, 163.7, 155.8, 143.3, 129.7, 113.4, 112.9, 113.5, 102.4, 101.9, 98.6, 89.9, 80.6, 77.4, 77.1, 73.0, 68.8, 63.9, 55.9, 55.6, 27.7, 19.8 ppm.

Enzymatic resolution of *tert*-butyl 3-hydroxypent-4-enoate (7): Compound **7** (22 g, 0.128 mol) and vinyl acetate (23.6 mL, 0.256 mol, 2 equiv) were dissolved in pentane (800 mL). *Amano PS* lipase (10 g) was added, and the suspension was heated at reflux for 45 h. The mixture was filtered and evaporated, and the acetylated *S* isomer was removed by silica gel chromatography (cyclohexane/ethyl acetate 8:2) to give (*R*)-**7** (9.53 g, 43 %) with *ee* > 95 % (measured by ^{1}H NMR in the presence of Eu(hfc)$_3$).

(*R*)-Pent-4-ene-1,3-diol (8): Lithium aluminium hydride (2.31 g, 61 mmol, 1.1 equiv) was suspended in dry THF (300 mL) at 0 °C. A solution of (*R*)-**7** (9.53 g, 55 mmol) in dry THF (20 mL) was then added dropwise at 0 °C. After 20 min at room temperature, the reaction mixture was quenched with water until evolution of hydrogen ceased, followed by few drops of conc. aq. HCl. The solution was dried ($MgSO_4$), filtered, and concentrated. Chromatography on silica gel (cyclohexane/ethyl acetate 3:7) afforded **8** (4.2 g, 74 %) as a colorless liquid. ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.8$ (m, 2H; C2H$_2$), 2.5 (br s, 2H; 2 × OH), 3.85 (m, 2H; C1H$_2$), 4.4 (m, 1H; CH), 5.1 (d, $J = 10.4$ Hz, 1H; CH *trans*), 5.3 (d, $J = 17.1$ Hz, 1H; CH *cis*), 5.9 (ddd, $J = 5.7$, 10.4, 17.1 Hz, 1H; CH) ppm; ^{13}C NMR (300 MHz, $CDCl_3$): $\delta = 38.1$ (*C*2), 61.1 (*C*1), 72.8 (*C*3), 114.7 (*C*5), 140.5 (C4) ppm.

(3*R*)-1-Iodopent-4-en-3-ol (9): Compound **7** (4.8 g, 47 mmol), imidazole (4.8 g, 70.6 mmol, 1.5 equiv), and triphenylphosphine (18.5 g, 70.6 mmol, 1.5 equiv) were dissolved in dry CH_2Cl_2 (200 mL). The solution was cooled to 0 °C, and iodine (23.9 g, 94.1 mmol, 2 equiv) was added in small portions, with the temperature maintained below 0 °C. After 30 minutes the mixture was allowed to warm to room temperature, the Ph_3PO precipitate was filtered off, and the filtrate was evaporated. The residue was purified by double flash chromatography on silica gel (CH_2Cl_2/MeOH 99:1, then cyclohexane/EtOAc 8:2) to give **9** (6.4 g, 64 %) as a slightly yellow liquid. ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.7$ (br s, 1H; OH), 2.0 (m, 2H; CH_2), 3.2 (m, 2H; CH_2), 4.2 (m, 1H; CH), 5.1 (d, $J = 10.4$ Hz, 1H; CH *trans*), 5.25 (d, $J = 17.2$ Hz, 1H; CH *cis*), 5.8 (ddd, $J = 6.2$, 10.4, 17.2 Hz, 1H; CH) ppm; ^{13}C NMR (300 MHz, $CDCl_3$): $\delta = 2.2$ (C1), 40.2 (C2), 73.0 (C3), 115.8 (C5), 139.9 (C4) ppm.

(3′*R*)-7-(3′-Hydroxypent-4′-enoxy)-chromen-2-one (10): A solution of **9** (2 g, 9.4 mmol) and umbelliferone (1.53 g, 9.4 mmol) in acetone (100 mL) was treated with K_2CO_3 (1.43 g, 10.4 mmol), and the mixture was heated at reflux overnight. After cooling to room temperature, the solution was filtered and concentrated. The crude yellow product was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH 99:1) to give **10** (2.16 g, 93 %) as a colorless liquid. ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 2.05$ (m, 2H; C2′H$_2$), 2.1 (br s, 1H; OH), 4.2 (m, 2H; C1′H$_2$), 4.45 (m, 1H; CH), 5.2 (d, $J =$ 10.5 Hz, 1H; CH *trans*), 5.3 (d, $J = 17.2$ Hz, 1H; CH *cis*), 5.95 (ddd, $J = 6.1$, 10.4, 17.2 Hz, 1H; CH), 6.2 (d, $J = 9.5$ Hz, 1H; C3H), 6.85 (m, 2H; C6H + C8H), 7.35 (d, $J = 8.5$ Hz, 1H; C5H), 7.65 (d, $J = 9.5$ Hz, 1H; C4H) ppm; ^{13}C NMR (300 MHz, $CDCl_3$): $\delta = 36.0$ (C2′), 65.5 (C1′), 70.1 (C3′), 101.6 (C6), 112.7 (C10), 113.0 (C8), 113.1 (C3), 115.3 (C5′), 140.5 (C4′), 144.0 (C4), 155.9 (C7), 161.5 (C9), 162.2 (C2) ppm.

6-*O*-*tert*-Butyldimethylsilyl-1,2-dideoxy-3,4-*O*-isopropylidene-D-*ribo*-hex-1-enitol (12): In a modification of an established method,[20a] a yellow mixture of methyltriphenylphosphonium bromide sodium amide ("instant ylide", 3.7 g, 8.85 mmol) in THF (100 mL) was stirred for 30 minutes under N_2 at RT. A solution of **11** (1.8 g, 5.91 mmol) in THF (3 mL) was added, and the mixture became white and was stirred for a further 6 h. The reaction mixture was quenched with water and extracted with EtOAc (3 × 100 mL), and the combined organic extracts were washed with brine (3 × 50 mL), dried (Na_2SO_4), and evaporated under reduced pressure. The crude product was purified by flash chromatography (hexane/ether/CH_2Cl_2 7:2:1) yielding compound **12** as a colorless oil (1.3 g, 74 %). Spectral data were in agreement with the published data: ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 6.09 - 5.98$ (m, 1H), 5.39 (dt, $J = 15.8$, 1.7 Hz, 1H), 5.26 (dt, $J = 11.8$, 1.7 Hz, 1H), 4.69 (t, $J = 6.6$ Hz, 1H), 4.08 – 4.03 (m, 1H), 3.83 – 3.80 (m, 1H), 3.70 – 3.65 (m, 2H), 1.47 (s, 3H), 1.36 (s, 3H), 0.91 (s, 9H), 0.09 (s, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 134.8$, 118.2, 109.4, 79.5, 78.0, 70.2, 64.9, 28.5, 26.5, 26.1, 18.9, −4.68, −4.77 ppm.

5-*O*-Acetyl-6-*O*-*tert*-butyldimethylsilyl-1,2-dideoxy-3,4-*O*-isopropylidene-D-*ribo*-hex-1-enitol (13): Acetic anhydride (15 mL) was slowly added at 0 °C to a solution of **12** (1.3 g, 4.30 mmol) in dry pyridine (15 mL). The mixture was stirred for 1 h at 0 °C and at 25 °C overnight, then quenched with water and extracted with EtOAc (3 × 50 mL). The combined organic extracts were dried (Na_2SO_4) and evaporated under reduced pressure to give **13** as a colorless oil (1.4 g, 95 %). $[\alpha]_D^{20} = -3.3$ (CH_3OH, $c = 0.24$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 5.81 - 5.75$ (m, 1H), 5.33 (dt, $J = 16.9$, 1.09 Hz, 1H), 5.22 (dt, $J = 11.7$, 1.09 Hz, 1H), 4.84 – 4.80 (m, 1H), 4.65 – 4.61 (m, 1H), 4.43 – 4.38 (m, 1H), 3.89 – 3.82 (m, 2H), 2.01 (s, 3H), 1.48 (s, 3H), 1.38 (s, 3H), 0.89 (s, 9H), 0.05 (s, 3H), 0.04 (s, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 169.7$, 133.3, 118.1, 108.8, 79.5, 78.7, 75.0, 72.2, 62.0, 27. 25.8, 25.2, 21.1, 18.3, −5.48 ppm; IR (film): $\tilde{\nu} = 2933$ (s), 1747 (s), 1372 (m), 1234 (br s), 1123 (m), 1058 (s), 837 (s) cm^{-1}; HRMS: calcd for $C_{17}H_{32}O_5Si$: 345.2097; found: 345.2108 $[M+H]^+$.

5-*O*-Acetyl-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-3,4-*O*-isopropylidene-D-*ribo*-hexitol (14): Compound **13** (790 mg, 2.3 mmol) was dissolved in THF (40 mL), and $BH_3 \cdot$THF (≈1M solution in THF, 23 mL) was added at 0 °C. The mixture was kept at 0 °C for 1 h and was allowed to warm to 25 °C and stirred for a further 12 h. NaOH (5N, 4.1 mL), H_2O_2 (30 %, 2.6 mL), and ethanol (13 mL) were then added, and the mixture was stirred at 55 °C for 2 h. Aqueous workup (water/EtOAc) and FC (hexane/EtOAc 8:2 to 1:1) gave **14** (376 mg, 45 %) as a colorless oil. $[\alpha]_D^{20} = -20.2$ ($CHCl_3$, $c = 0.37$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 4.92 - 4.87$ (m, 1H), 4.40 – 4.29 (m, 2H), 3.92 – 3.77 (m, 4H), 2.28 (br s, 1H), 2.07 (s, 3H), 1.85 – 1.60 (m, 2H), 1.45 (s, 3H), 1.35 (s, 3H), 0.89 (s, 9H), 0.05 (s, 3H), 0.04 (s, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 169.9$, 108.4, 75.7, 74.6, 72.1, 62.2, 60.5, 31.7, 27.9, 25.7, 25.5, 21.1, 18.5, −5.54 ppm; IR (film): $\tilde{\nu} = 3447$ (br m), 2933 (s), 1740 (s), 1473 (m), 1370 (s), 1252 (s), 1055 (s), 835(s) cm^{-1}; HRMS: calcd for $C_{17}H_{34}O_6Si$: 363.2203; found: 363.2193 $[M+H]^+$.

6-*O*-*tert*-Butyldimethylsilyl-2-deoxy-3,4-*O*-isopropylidene-D-*ribo*-hexitol (15): Compound **14** (37 mg, 0.10 mmol) and K_2CO_3 (28 mg, 0.20 mmol) were dissolved in aq. MeOH (50 %, 1.2 mL) and stirred overnight at 25 °C. Aqueous workup (water/AcOEt) and evaporation of the residue quantitatively gave **15** as a colorless oil. $[\alpha]_D^{20} = -17.2$ (CH_3OH, $c = 0.27$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 4.41 - 4.37$ (m, 1H), 4.00 – 3.95 (m, 1H), 3.78 – 3.17 (m, 3H), 3.69 – 3.62 (m, 2H), 2.67 (br s, 1H), 2.11 – 2.02 (m, 1H), 1.94 – 1.89 (m, 1H), 1.42 (s, 3H), 1.33 (s, 3H), 0.91 (s, 9H), 0.09 (s, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 108.3$, 77.5, 77.3, 69.3, 64.8, 61.3, 31.9, 28.4, 26.0, 25.8, 18.9, −5.20 ppm; IR (film): $\tilde{\nu} = 3403$ (br s), 2933 (s), 1472 (m), 1381 (m), 1255 (s), 1058 (s), 837(s) cm^{-1}; HRMS: calcd for $C_{15}H_{32}O_5Si$: 321.2097; found: 321.2087 $[M+H]^+$.

6-*O*-*tert*-Butyldimethylsilyl-2-deoxy-3,4-*O*-isopropylidene-1-*O*-(*p*-tosyl)-D-*ribo*-hexitol (16): A solution of compound **15** (460 mg, 1.44 mmol) and *p*-toluenesulfonyl chloride (410 mg, 190.6 mmol) in dry pyridine (8 mL) was stirred at 0 °C for 12 h. Aqueous workup (water/AcOEt) and FC (hexane/EtOAc 8:2 to 1:1) gave compound **16** (300 mg, 44 %) as a yellowish oil. $[\alpha]_D^{20} = -4.4$ (CH_3OH, $c = 0.27$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.80$ (d, $J = 8.1$ Hz, 2H), 7.34 (d, $J = 8.1$ Hz, 2H), 4.26 – 4.19 (m, 3H), 3.92 – 3.87 (m, 1H), 3.80 (dd, $J = 9.9$, 3.3 Hz, 1H), 3.66 – 3.55 (m, 2H), 2.52 (br s, 1H), 2.45 (s, 3H), 2.26 – 2.15 (m, 1H), 1.93 – 1.81 (m, 1H), 1.33 (s, 3H), 1.26 (s, 3H), 0.90 (s, 9H), 0.08 (s, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 145.3$, 133.9, 130.5, 128.7, 109.1, 77.3, 74.4, 69.8, 68.9, 65.1, 30.1, 28.9, 26.6, 26.2, 22.2, 18.9, −4.65 ppm; IR (film): $\tilde{\nu} = 3549$ (w), 2932 (s), 1364 (s), 1178 (s), 837 (s) cm^{-1}; HRMS: calcd for $C_{22}H_{38}O_7SiS$: 475.2185; found: 475.2204 $[M+H]^+$.

0947-6539/03/0904-0898 $ 20.00+.50/0 *Chem. Eur. J.* **2003**, *9*, No. 4

5-*O*-Acetyl-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-3,4-*O*-isopropylidene-1-*O*-(*p*-tosyl)-D-*ribo*-hexitol (17): Compound **16** (133 mg, 0.28 mmol) was stirred overnight in pyridine/acetic anhydride (1:1, 8 mL). Aqueous workup (water/AcOEt) and evaporation of the residue gave the product **17** in a quantitative yield as a pale yellow oil. $[\alpha]_D^{20} = -27.9$ (CH_3OH, $c = 0.26$); ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.79$ (d, $J = 8.3$ Hz, 2H), 7.35 (d, $J = 8.3$ Hz, 2H), 4.83–4.78 (m, 1H), 4.29–4.15 (m, 4H), 3.87 (dd, $J = 11.4$, 2.6 Hz, 1H), 3.76 (dd, $J = 11.4$, 4.4 Hz, 1H), 2.46 (s, 3H), 2.06 (s, 3H), 1.90–1.65 (m, 2H), 1.34 (s, 3H), 1.27 (s, 3H), 0.88 (s, 9H); 0.03 (s, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 169.9$, 144.7, 133.0, 129.8, 127.9, 108.5, 74.2, 72.9, 71.9, 67.7, 62.2, 39.2, 27.9, 25.8, 25.5, 21.6, 21.1, 18.2, −5.50 ppm; IR (film): $\tilde{\nu} = 2933$ (s), 1743 (s), 1370 (s), 1222 (s), 1178 (s), 837 (s) cm^{-1}; HRMS: calcd for $C_{24}H_{40}O_8SiS$: 517.2291; found: 517.2309 $[M+H]^+$.

5-*O*-Acetyl-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-3,4-*O*-isopropylidene-1-*O*-(2-oxo-2*H*-chromen-7-yl)-D-*ribo*-hexitol (18): 7-Hydroxycoumarine (47 mg, 0.29 mmol) was added to a suspension of NaH (60%, 23 mg, 0.58 mmol) in DMF (1 mL) and the mixture was stirred for 1 h. A solution of **17** (150 mg, 0.29 mmol) in DMF (3 mL) was added, and the mixture was stirred at 70 °C overnight. Aqueous workup (water/AcOEt) and FC (hexane/EtOAc 7:3) gave **18** (50 mg, 34%) as a yellow oil. $[\alpha]_D^{20} = -14.2$ (CH_3OH, $c = 0.28$); ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.64$ (d, $J = 9.2$ Hz, 1H), 7.36 (d, $J = 9.2$ Hz, 1H), 6.86–6.83 (m, 2H), 6.25 (d, $J = 9.5$ Hz, 1H), 4.97–4.92 (m, 1H), 4.44–4.34 (m, 2H), 4.17 (t, $J = 5.5$ Hz, 2H), 3.95–3.90 (m, 1H), 3.82 (dd, $J = 11.4$, 4.4 Hz, 1H), 2.08 (s, 3H), 1.96–1.90 (m, 2H), 1.45 (s, 3H), 1.35 (s, 3H), 0.90 (s, 9H), 0.06 (s, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 170.0$, 162.8, 161.8, 156.6, 144.0, 129.4, 113.8, 113.5, 113.2, 109.2, 102.3, 75.2, 74.3, 72.9, 66.2, 62.9, 30.0, 28.7, 26.5, 26.3, 21.8, 18.9, −4.79 ppm; IR (film): $\tilde{\nu} = 2931$ (m), 1738 (s), 1614 (s), 1232 (m), 1124 (m), 836 (m) cm^{-1}; HRMS: calcd for $C_{26}H_{38}O_8Si$: 507.2414; found: 507.2426 $[M+H]^+$.

6-*O*-*tert*-Butyldimethylsilyl-2-deoxy-3,4-*O*-isopropylidene-1-*O*-(2-oxo-2*H*-chromen-7-yl)-D-*ribo*-hexitol (19): A solution of compound **18** (50 mg, 0.099 mmol) and K_2CO_3 (41 mg, 0.3 mmol) in MeOH (1 mL) and water (1 mL) was stirred at 25 °C overnight. Aqueous workup (water/AcOEt) and evaporation of the organic phase gave **19** quantitatively as a pale yellow oil: $[\alpha]_D^{20} = -23.7$ (CH_3OH, $c = 0.20$); ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.63$ (d, $J = 9.5$ Hz, 1H), 7.35 (d, $J = 9.2$ Hz, 1H), 6.87–6.84 (m, 2H), 6.24 (d, $J = 9.6$ Hz, 1H), 4.48–4.41 (m, 1H), 4.24–4.19 (m, 2H), 4.05–4.00 (m, 1H), 3.87–3.82 (m, 1H), 3.73–3.67 (m, 2H), 2.63 (brs, 1H), 2.45–2.29 (m, 1H), 2.10–1.95 (m, 1H), 1.44 (s, 3H), 1.34 (s, 3H), 0.92 (s, 9H), 0.11 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 163.3$, 162.2, 156.9, 144.3, 129.6, 113.9, 113.8, 113.4, 109.4, 102.5, 78.4, 75.3, 70.2, 66.8, 65.4, 30.5, 29.3, 26.8, 26.6, 19.2, −4.40, −4.49 ppm; IR (film): $\tilde{\nu} = 3426$ (s), 2932 (m), 1731 (s), 1614 (s), 1125 (m), 836 (m) cm^{-1}; HRMS: calcd for $C_{24}H_{36}O_7Si$: 465.230857; found: 465.230590 $[M+H]^+$.

1-*O*-*tert*-Butyldimethylsilyl-5-deoxy-3,4-*O*-isopropylidene-6-*O*-(2-oxo-2*H*-chromen-7-yl)-L-*erythro*-hexulose (20): A solution of **19** (22 mg, 0.05 mmol) in dry CH_2Cl_2 (1 mL) was added to a suspension of Dess–Martin periodinane (30 mg, 0.071 mmol) in dry CH_2Cl_2 (2 mL) and the reaction mixture was stirred for 4 h at 25 °C. The slurry was filtered through Celite to afford **20** (21 mg, 91%) as a pale yellow oil. $[\alpha]_D^{20} = -28.0$ (CH_3OH, $c = 0.35$); ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.64$ (d, $J = 9.6$ Hz, 1H), 7.36 (d, $J = 9.2$ Hz, 1H), 6.85–6.82 (m, 2H), 6.25 (d, $J = 9.6$ Hz, 1H), 4.85 (d, $J = 7.7$ Hz, 1H), 4.74–4.69 (m, 1H), 4.58–4.33 (m, 2H), 4.19–4.14 (m, 1H), 2.11–2.01 (m, 1H), 1.79–1.68 (m, 1H), 1.59 (s, 3H), 1.39 (s, 3H), 0.95 (s, 9H), 0.13 (s, 3H), 0.12 (s, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 207.8$, 162.7, 161.8, 156.5, 143.9, 130.3, 129.4, 113.8, 113.4, 110.6, 102.3, 81.8, 74.3, 69.4, 65.7, 31.3, 30.3, 26.5, 25.6, 19.1, −4.77, −4.82 ppm; IR (film): $\tilde{\nu} = 2931$ (m), 1738 (s), 1615 (s), 1123 (s), 839 (s) cm^{-1}; HRMS: calcd for $C_{24}H_{34}O_7Si$: 463.215207; found: 463.21555 $[M+H]^+$.

Enzyme measurements: Substrates **1**, **2**, and **3** were used as 5 mM stock solutions in 50% aqueous acetonitrile. The reactions were initiated by combining substrate (2 μL, final concentration 100 μM), erythrose 4-phosphate (0.4 mM stock solution in water, 20 μL, final concentration 100 μM), bovine serum albumin (40 mg mL^{-1} stock solution in phosphate-buffered saline (PBS, aq. 160 mM NaCl, 10 mM phosphate pH 7.4), final concentration 2 mg mL^{-1}), the purified transaldolase (stock solution 1 mg mL^{-1} protein in PBS, final concentration 100 μg mL^{-1} or 10 μg mL^{-1}), and 20 mM Tris buffer pH 8.0 up to a total volume of 100 μL. Assays (0.1 mL) were followed at 26 °C in individual wells of round-bottomed polypropylene 96-well plates (Costar) with a Cytofluor II Fluorescence Plate Reader (Perseptive Biosystems, filters $\lambda_{ex} = 360 \pm 20$, $\lambda_{em} = 460 \pm 20$ nm). Fluorescence data were converted to umbelliferone concentration by means of a calibration curve.

Acknowledgement

This work was supported by the University of Bern, the Swiss National Science Foundation, the European COST program D12, the Deutsche Forschungsgemeinschaft through SFB380 grants (B21 and B25), the Fonds der Chemischen Industrie, and the Swiss Office Fédéral de l'Education et de la Science. We would like to thank Prof. N. A. Dencher, University of Darmstadt, for use of his fluorescence spectrophotometer, as well as Prof. G. Schneider, Karolinska Institute, Sweden, and Dr. A. Berry, University of Leeds (UK), for helpful discussions.

[1] a) W. P. C. Stemmer, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 10747–10751; b) W. P. C. Stemmer, *Nature* **1994**, *370*, 389–391; c) L. You, F. H. Arnold, *Protein Eng.* **1996**, *9*, 77–83; d) M. T. Reetz, K.-E. Jaeger, *Chem. Eur. J.* **2000**, *6*, 407–412.

[2] a) M. T. Reetz, K.-E. Jaeger, *Top. Curr. Chem.* **1999**, *200*, 31–57; b) K. A. Powell, S. W. Ramer, S. B. del Cardayré, W. P. C. Stemmer, M. B. Tobin, P. F. Longchamp, G. W. Huisman, *Angew. Chem.* **2002**, *114*, 394; *Angew. Chem. Int. Ed.* **2002**, *41*, 382; c) H. Zhao, K. Chockalingam, Z. Chen, *Curr. Opin. Biotechnol.* **2002**, *13*, 104–110.

[3] a) M. T. Reetz, *Angew. Chem.* **2001**, *113*, 292–320; *Angew. Chem. Int. Ed.* **2001**, *40*, 284–310; b) D. Wahler, J.-L. Reymond, *Curr. Opin. Chem. Biol.* **2001**, *5*, 152–158; c) F. Beisson, A. Tiss, C. Rivière, R. Verger, *Eur. J. Lipid Sci. Technol.* **2000**, 133–153.

[4] W.-D. Fessner, C. Walter, *Top. Curr. Chem.* **1996**, *184*, 97–194.

[5] a) T. D. Machajewski, C.-H. Wong, *Angew. Chem.* **2000**, *112*, 1406–1430; *Angew. Chem. Int. Ed.* **2000**, *39*, 1352–1375; b) W.-D. Fessner, in *Stereoselective Biocatalysis* (Ed.: R. N. Patel), Marcel Dekker, New York **2000**, pp. 239–265; c) W.-D. Fessner, V. Helaine, *Curr. Opin. Biotechnol.* **2001**, *12*, 574–586.

[6] a) R. Venkataraman, E. Racker, *J. Biol. Chem.* **1961**, *236*, 1883–1886; b) J. Jia, U. Schörken, Y. Lindqvist, G. A. Sprenger, G. Schneider, *Protein Sci.* **1997**, *6*, 119–124.

[7] J.-L. Reymond, *Chimia* **2001**, *55*, 330–334.

[8] a) G. Klein, J.-L. Reymond, *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1113–1116; b) G. Klein, J.-L. Reymond, *Helv. Chim. Acta* **1999**, *82*, 400–407.

[9] a) F. Badalassi, D. Wahler, G. Klein, P. Crotti, J.-L. Reymond, *Angew. Chem.* **2000**, *112*, 4233–4236; *Angew. Chem. Int. Ed.* **2000**, *39*, 4067–4070; b) D. Wahler, F. Badalassi, P. Crotti, J.-L. Reymond, *Angew. Chem.* **2000**, *112*, 4589–4592; *Angew. Chem. Int. Ed.* **2001**, *40*, 4457–4460; c) D. Wahler, F. Badalassi, P. Crotti, J.-L. Reymond, *Chem. Eur. J.* **2002**, *8*, 3211–3228.

[10] a) N. Jourdain, R. Perez-Carlon, J.-L. Reymond, *Tetrahedron Lett.* **1998**, *39*, 9415–9418; b) R. Pérez Carlòn, N. Jourdain, J.-L. Reymond, *Chem. Eur. J.* **2000**, *6*, 4154–4162.

[11] G. A. Sprenger, U. Schörken, G. Sprenger, H. Sahm, *J. Bacteriol.* **1995**, *177*, 5930–5936.

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

[13] R. Zibuck, J. M. Streiber, *J. Org. Chem.* **1989**, *54*, 4717–4719.

[14] S. Vrielynck, M. Vandewalle, *Tetrahedron Lett.* **1995**, *36*, 9023–9026.

[15] V. Helaine, W.-D. Fessner, unpublished results.

[16] G. A. Sprenger, M. Pohl, *J. Mol. Catal. B: Enzymol.* **1999**, *6*, 145–159.

[17] M. Schürmann, G. A. Sprenger, *J. Biol. Chem.* **2001**, *276*, 11055–11061.

[18] W.-D. Fessner, O. Eyrisch, *Angew. Chem.* **1992**, *104*, 76–78; *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 56–58.

[19] Altered kinetic selectivity of an aldolase for aldehyde antipodes by protein sequence modification has recently been reported by Wong et al. for a mechanistically related class I aldolase: S. Fong, T. D. Machajewski, C. C. Mak, C.-H. Wong, *Chem. Biol.* **2000**, *7*, 873–883.

[20] a) B. Kaskar, G. Heise, R. Michalak, B. Vishuvajjala, *Synthesis* **1990**, 1031; b) F. Fillmore, D. Robarge, *Carbohydr. Res.* **1986**, *154*, 270–274; c) T. RajanBabu, W. Nugent, D. F. Taber, P. J. Fagan, *J. Am. Chem. Soc.* **1988**, *110*, 7128–7135.

Received: September 6, 2002 [F4400]

  0947-6539/03/0904-0899 $ 20.00+.50/0