Fluorogenic Polypropionate Fragments for Detecting Stereoselective Aldolases

Raquel Pérez Carlón,[a, b] Nathalie Jourdain,[a, b] and Jean-Louis Reymond*[a]

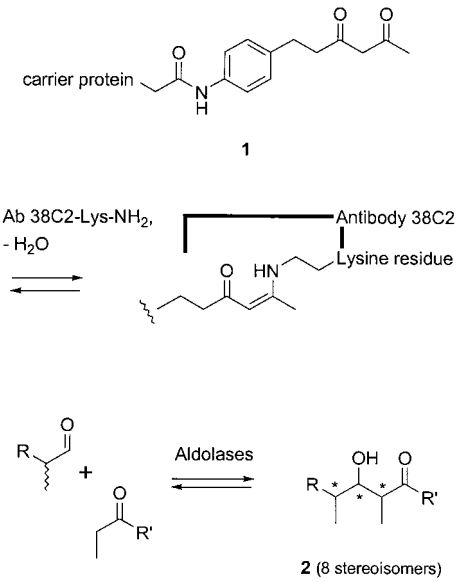
Abstract: A series of fluorogenic polypropionate fragments has been prepared. These undergo retroaldolization to an intermediate aldehyde that liberates the fluorescent product umbelliferone by a secondary β-elimination reaction, leading to a >20-fold increase in fluorescence ($\lambda_{em} = 460 \pm 20$ nm, $\lambda_{ex} = 360 \pm 20$ nm). By applying the principle of microscopic reversibility to the reversible aldol reaction, we can use these substrates to detect stereoselective aldolases. Test substrates are available to probe the classical cases of *syn*- and *anti*-selective aldolization (**11a–d**), Cram/anti-Cram-selective aldolization (**10a–d**), and double stereoselective aldolization (**3a–h**). The selectivity of aldolase antibody 38C2 for these substrates is demonstrated as an example. The assay is suitable for high-throughput screening for catalysis in microtiter plates, and therefore provides a convenient tool for the isolation of new stereoselective aldolases from catalyst libraries.

Keywords: aldolases · aldol reactions · enzyme catalysis · fluorescence · high-throughput screening · polypropionates

Introduction

The aldol reaction is one of the key carbon–carbon bond-forming processes. One of its most important applications is the preparation of polypropionate natural products.[1] Recently Barbas et al. have shown that it is possible to obtain very efficient aldolase catalytic antibodies that catalyze stereo- and enantioselective aldol reactions.[2] The Barbas approach involves immunization with 1,3-diketones such as **1** as reactive antigens. These diketones create a lysine residue in the active site of antibodies; that was the case for the commercially available aldolase 38C2 (Scheme 1).

Although efficient catalysis in terms of yields was achieved, this reactive inmunization approach for inducing catalysis does not permit direction of the stereoselectivities of the resulting antibodies owing to the configurational lability of the 1,3-diketone hapten **1**. For the most complex case of the double stereoselective aldolization with a substrate such as **2**, eight stereoisomers are possible, which would require eight corresponding stereoselective aldolases to catalyze the formation of each of them. Herein we report a series of fluorogenic polypropionate fragments that allow the assay of aldolase catalysts for such stereoselective aldolizations by simple fluorescence measurement.[3] This assay should be useful for screening large libraries of antibodies,[4] chemical catalysts,[5] or mutant enzymes[6] for stereoselective aldolization.



Scheme 1. Achiral hapten **1** induces highly active aldolase antibodies. Prototypical polypropionate fragment **2** would require eight selective, different aldolases.

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Supporting information for this article (^{1}H and ^{13}C NMR spectra for all compounds prepared) is available on the WWW under http://www.wiley-vch.de/home/chemistry or from the author.

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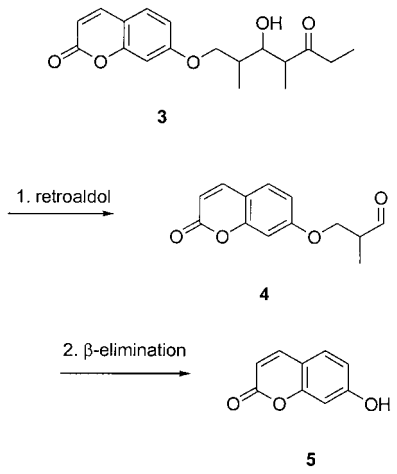
Results

Cross-aldolization of ketones with aldehydes under aqueous conditions is a reversible process.[7] By virtue of microscopic reversibility, the stereoselectivities observed for a catalyst in the aldol addition and in the retroaldolization are identical, and we have verified this experimentally with an aldolase catalytic antibody.[8] Therefore it is possible to assess the stereoselectivity of an aldolase catalyst by measuring its reactivity with all possible stereoisomeric aldol products in the retroaldol reaction.

Following a concept that we have developed to assay alcohol dehydrogenases and esterases,[9] we reasoned that aldols such as **3** would undergo a retroaldolization to liberate aldehyde **4** (Scheme 2). This product, like the aldol substrate,

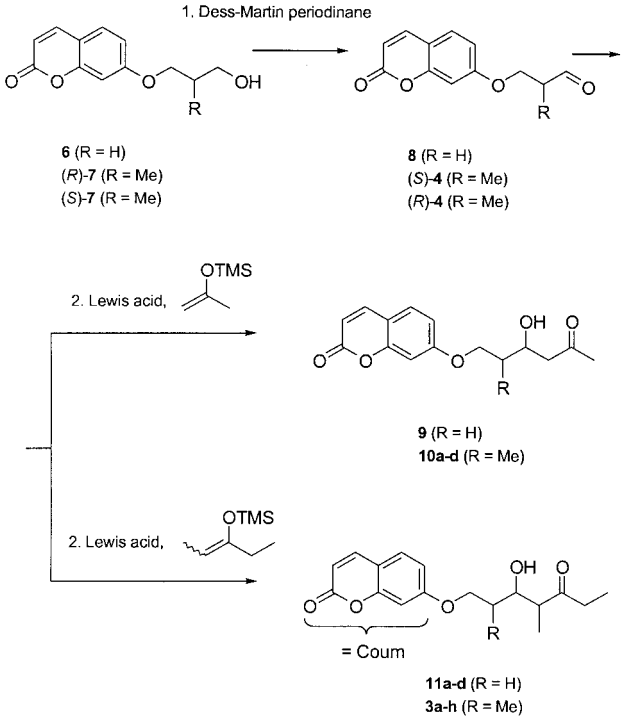


Scheme 2. Principle of fluorogenic aldolase assay.

is only weakly fluorescent, but would undergo a facile β-elimination to liberate the strongly fluorescent product umbelliferone (**5**). Measurements with isolated stereoisomers of **3** would provide a panel of retroaldolization rates from which the stereoselectivity of a potential catalyst could be directly measured for the case of the assembly of a simple polypropionate fragment.

Abstract in French: *Nous avons préparé une série de fragments fluorogéniques de type polypropionate. La rétro-aldolization de ces fragments est suivie d'une élimination-β de l'aldéhyde intermédiaire, qui libère l'umbelliférone, produisant ainsi une forte augmentation (>20×) de la fluorescence ($\lambda_{em}=460\pm20$ nm, $\lambda_{ex}=360\pm20$ nm). Selon le principe de réversibilité microscopique, on peut utiliser ces fragments fluorogéniques pour détecter des aldolases stéréosélectives. Des substrats tests sont disponibles pour les cas classiques d'aldolizations syn et anti (**11a–d**), Cram et anti-Cram (**10a–d**), et doublement stéréosélectives (**3a–h**). Comme exemple, nous avons déterminé la sélectivité de l'abzyme aldolase 38C2 pour ces substrats. Notre test est adapté au criblage à haut débit en microplaques, et peut être utilisé pour l'identification de nouvelles aldolases stéréosélectives à partir de bibliothèques de catalyseurs.*

The known alcohols **6**, (*R*)-**7**, and (*S*)-**7** were obtained by alkylation of umbelliferone (**5**) by 3-bromo-1-propanol, (*R*)- or (*S*)-3-bromo-2-methyl-1-propanol. Dess–Martin periodinane oxidation gave aldehydes **8**, (*R*)-**4**, and (*S*)-**4**. These aldehydes were only moderately stable but underwent Lewis-acid-promoted aldol reaction with the TMS enol ether of either acetone or 3-pentanone to produce stereoisomeric mixtures of aldols **9**, **10a–d**, **11a–d**, and **3a–h** (Scheme 3).



Scheme 3. Synthesis of the target aldols.

The yields of the reactions with the linear aldehyde **8** were moderate. By contrast, all reactions with the branched aldehydes (*R*)-**4** and (*S*)-**4** gave good yields, probably because of the fact that these aldehydes were much less sensitive than aldehyde **8** towards β-elimination. The aldolization leading to **3a–h** was also carried out using the tin enolate of 3-pentanone. Both procedures gave good yields of a mixture of four stereoisomers containing the *syn*, *syn* aldol **3a** or **3h** as major product.

The crude aldol products were purified by preparative reverse-phase HPLC (Table 1). Aldol **9** was obtained as a racemate that could not be resolved by any of our chiral columns. Aldols **11a–d** were obtained as a 4:1 mixture of *syn* and *anti* diastereoisomers. Individual aldol stereoisomers were separated either by semipreparative reverse-phase HPLC to obtain 5–20 mg amounts of racemic mixture of *syn* and *anti* aldols **11a**+**d** and **11b**+**c**, or by chiral-phase HPLC to obtain each individual stereoisomer (3–6 mg, Table 2). The diastereoisomeric mixtures of either **10a**+**b** or **10c**+**d**, obtained from aldolization with aldehyde (*R*)-**4** or (*S*)-**4**, respectively, were separated by semipreparative reverse-phase HPLC to give single stereoisomers (5–10 mg each). Preparative reverse-phase HPLC of the crude aldols **3a–d** and **3e–h** allowed separation of the main stereoisomer **3a** or

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Table 1. Analytical RP-HPLC conditions for aldehydes and aldols.[a]

Compound	A [%]	B [%]	t_R [min]
8	80	20	21.9
4	60	40	12.4
9	60	40	8.3
11a/d	50	50	13.5
11b/c	50	50	14.6
10a/d	60	40	16.3
10b/c	60	40	17.3
3a/h	55	45	30.2
3b/g	55	45	38.7
3c/f	55	45	41.6
3d/e	55	45	38.9

[a] Isocratic elution at 1.5 mL min^{-1}, detection by UV at 325 nm, A = 0.1 % TFA in H_2O, B = 50/50 CH_3CN/H_2O, analytical column: Vydac 218TP-54 (C_{18}, pore size 300 Å), 0.45 × 22 cm.

Table 2. Chiral-HPLC conditions for aldols 11 and 3.[a]

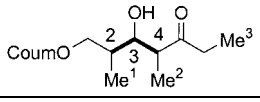
Compound	Daicel col.	hexane [%]	*i*PrOH [%]	t_R [min]
11a	AS	50	50	35.2
11b	AS	50	50	19.4
11c	AS	50	50	27.3
11d	AS	50	50	16.0
3b	OD-H	75	25	24
3c	OD-H	75	25	18
3d	OD-H	75	25	22
3e	OD-H	75	25	24
3f	OD-H	75	25	16
3g	OD-H	75	25	24

[a] Isocratic elution at 1.0 mL min^{-1}, detection by UV at 325 nm, Daicel analytical columns (0.45 × 22 cm).

3h (20–30 mg each). Further separation of diastereoisomers **3b–d** or **3e–g** was achieved on a small scale using a combination of chiral-phase HPLC and semipreparative RP-HPLC to yield 3–6 mg of single stereoisomers.

The relative configurations of aldols **10a–d**, **11a–d**, and **3a–h** were assigned on the basis of the ^{13}C NMR shifts of the methyl carbon atoms, a method that has been shown to be reliable for a variety of aldols (Table 3).[10] The ^{13}C-NMR-based assignment of *syn* stereochemistry for the major product of the $SnCl_4$-promoted aldolization was consistent with the known *syn* selectivity of this protocol. The stereochemistry assigned from the NMR data was confirmed by crystal structure analysis for compound **3b** (Figure 1).

Table 3. ^{13}C NMR chemical shifts δ for methyl groups in aldols **3**, **10**, and **11**.



Compound	Stereochemistry	Me^1	Me^2	Me^3
11a/d	*syn*	–	10.2	7.5
11b/c	*anti*	–	14.4	7.7
10a/d	*syn*	10.9	–	–
10b/c	*anti*	13.7	–	–
3a/h	*syn*, *syn*	11.9 and 11.4		7.2
3b[a]**/g**	*syn*, *anti*	9.8[b]	14.0[b]	7.6
3c/f	*anti*, *anti*	15.2 and 15.1		7.4
3d/e	*anti*, *syn*	13.7[b]	9.0[b]	7.6

[a] The relative configuration of **3b** is confirmed by X-ray structure determination. [b] Signals were assigned on the basis of 2D NMR: H/H COSY was used to assign the ^{1}H NMR signal of Me^1 and Me^2, both doublets at $\delta \approx 1$. They were correlated with the adjacent hydrogen (signals at $\delta \approx 2$ (H-C(2)) and at $\delta \approx 2.8$ (H-C(4)). C/H COSY was used afterwards to identify the corresponding ^{13}C NMR signal.

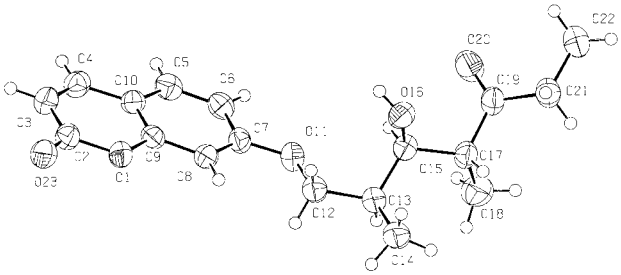
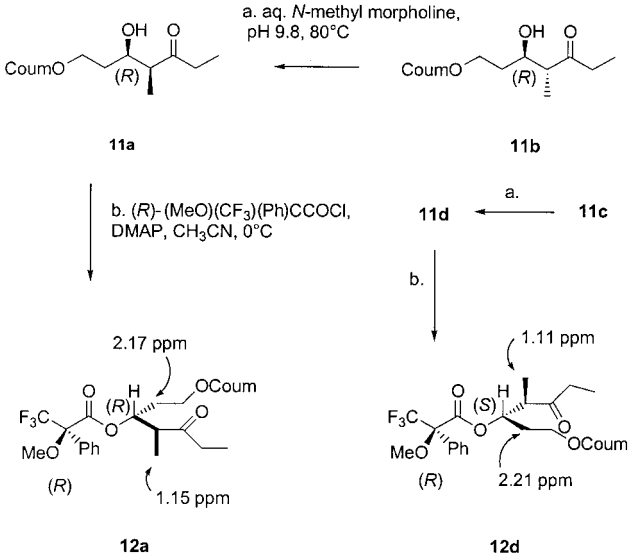


Figure 1. ORTEP drawing of compound **3b**.

The absolute configuration of the *syn* aldols **11a** and **11d** was attributed according to the ^{1}H NMR shift of the H_2-C(2) and CH_3-C(4) protons on the corresponding Mosher esters **12a** and **12d**.[11] Following Mosher's correlations, the ^{1}H NMR chemical shift of either the H_2-C(2) or CH_3-C(4) is shifted upfield when the atom is eclipsed by the phenyl group relative to the chemical shift when the atom is eclipsed by the methoxy group, in the conformations shown in Scheme 4 for **12a** and



Scheme 4. Assignment of absolute configuration for aldols **11a–d**.

12d. The assignment was confirmed by the optical rotation of the *syn* aldols **11a** and **11d**, which was that expected from literature data for similar aldols.[12] The minor *anti* aldols **11b** and **11c** were assigned by chemical correlation to the *syn* aldols (Scheme 4). Thus, treatment of the *anti* stereoisomer **11c** with base gave the *syn* stereoisomer **11d** stereoselectively by epimerization at the α-carbon. The same experiment starting with **11b** gave only **11a**. The absolute configuration of aldols **10a–d** and **3a–h** was known from the optically pure starting aldehydes (*R*)-**4** and (*S*)-**4**. The relative stereochemistry of the different aldols as determined by these experiments is shown in Scheme 5.

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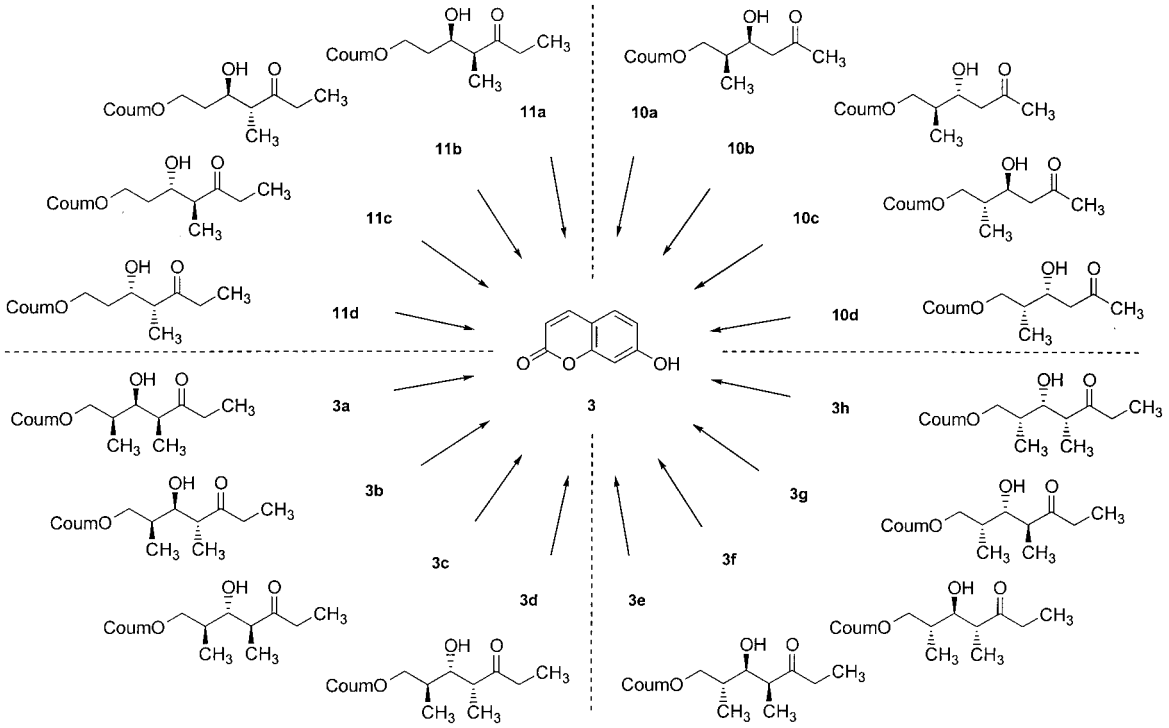


Scheme 5. Fluorogenic polypropionates all release umbelliferone upon retroaldolization.

Kinetic measurements were carried out to demonstrate the utility of these aldol substrates as stereochemical fluorogenic probes of aldolase activity. The commercially available aldolase antibody 38C2 was tested for its catalytic activity with stereochemically pure substrates **10a–d**, **11a–d**, and **3a–h**. For all aldols, the rate of umbelliferone release was very small in the absence of catalytic antibody or in the presence of bovine serum albumin (BSA) only. In these assays BSA is used to accelerate the secondary β-elimination of umbelliferone (**5**) from the intermediate aldehydes **4** or **8** ($t_{1/2} < 1$ min for both **4** and **8** with 2 mg mL^{-1} BSA), so that the retroaldolization step is rate-limiting. HPLC analysis of the unreacted samples of the aldols confirmed that these substrates did not undergo nonspecific degradation in aqueous buffer.

Among all fluorogenic aldol substrates, the reaction in the presence of aldolase antibody 38C2 was fastest with the (*S*)-*anti* aldol **11c** (Figure 2, Table 4). When compared with the reaction rates within the parent stereoisomeric series **11a–d**, antibody 38C2 displayed an *S* enantioselectivity for chirality of the secondary alcohol (87.5% *ee*), in agreement with

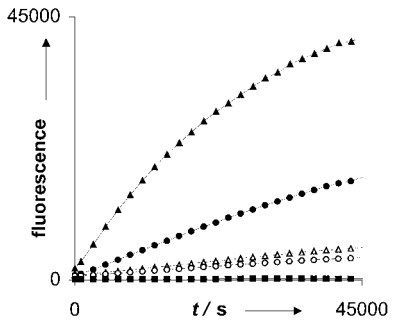
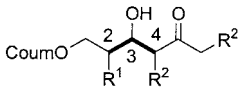


Figure 2. Fluorescence signal for antibody 38C2-catalyzed retroaldolization for aldols (▲) **11c**, (●) **11d**, (△) **11b**, (○) **11a**, (■) control **11c** without antibody. Conditions: 100 μM substrate **11**, 20 mM aq. borate pH 8.8, 2 mg mL^{-1} BSA, 1 mg mL^{-1} Ab 38C2. 200 mL assays were run in 96-well round-bottomed polypropylene plates with a Cytofluor II plate reader (Perseptive Biosystems), with $\lambda_{em} = 460 \pm 20$ nm, $\lambda_{ex} = 360 \pm 20$ nm.

Table 4. Rate of retroaldolization of fluorogenic substrates **3**, **10**, and **11** with aldolase antibody 38C2. Substrates sorted according to reaction rate in descending order.[a]



(R^{1,2} = Me or H)

Compound	V_{app} [10^{-6} s^{-1}]	C(2)[b]	C(3)[c]	C(4)[b]
11c	17	–	down	up
3h	6.8	down	down	down
10d	6	down	down	
3a	5.6	up	up	up
3d[d]	5.5	up	down	down
10c	5.5	down	up	–
3c	5.1	up	down	up
3e	4.7	down	up	up
11d	4.1	–	down	down
10b	3.6	up	down	–
3g	1.9	down	down	up
3b[e]	1.7	up	up	down
10a	1.2	up	up	–
11b	0.83	–	up	down
11a	0.6	–	up	up
3f	0.15	down	up	down

[a] Apparent first-order rate constant for the release of umbelliferone from the corresponding aldol. Measured at 26 °C in 20 mM aq. borate pH 8.8, 1 mg mL^{-1} Ab 38C2, 2 mg mL^{-1} BSA. Fluorescence readings at $\lambda_{em} = 460 \pm 20$ nm ($\lambda_{ex} = 360 \pm 20$) were converted to product concentration according to a calibration curve with pure umbelliferone in the same buffer. [b] At C(2) and C(4), "down" is always *R*. [c] At C(3), "up" is *R* for **3a–h** and **11a–d**, and "up" is *S* for **10a–d**. [d] Sample of **3d** is 81% pure, contains 19% of a mixture of **3b** + **3c**. [e] Sample of 3b is 86% pure, contains 14% of a mixture of **3c** + **3d**.

previous observations for this catalytic antibody. The overall *anti* diastereoselectivity (59.1% *de*) within this series is noteworthy since *anti* aldols are usually difficult to obtain.

Catalytic antibody 38C2 showed no significant stereoselectivity for aldols **10a–d**. For the series of aldols **3a–h**, all stereoisomeric aldol substrates displayed very similar reactivities, with the exception of the *anti*, *anti* aldol **3f**, which was not accepted as substrate. Overall, the observed rates of retroaldolization with aldolase 38C2 correlate only partially with the stereochemistry (Table 4). The retroaldolization rates are larger when the C(3)-hydroxyl group is "down" in each of the three series **3**, **11**, and **10** (*S* for **3** and **11**, and *R* for **10**), with the stereochemistry being conversely "up" at C(3)-OH for the four least reactive stereoisomers **10a**, **11a**, **11b**, and **3f**. Also, the nonreactive *anti* stereoisomers **11b** and **3f** possess the same configuration at carbons C(3) and C(4). However, the enantiomeric aldols **3b** and **3g** display similar reactivities. These observations support the general *S* enantioselectivity of antibody 38C2, which has been previously observed,[2] but also clearly show that a general rule of stereoselectivity cannot be established for this catalyst and this class of substrates.

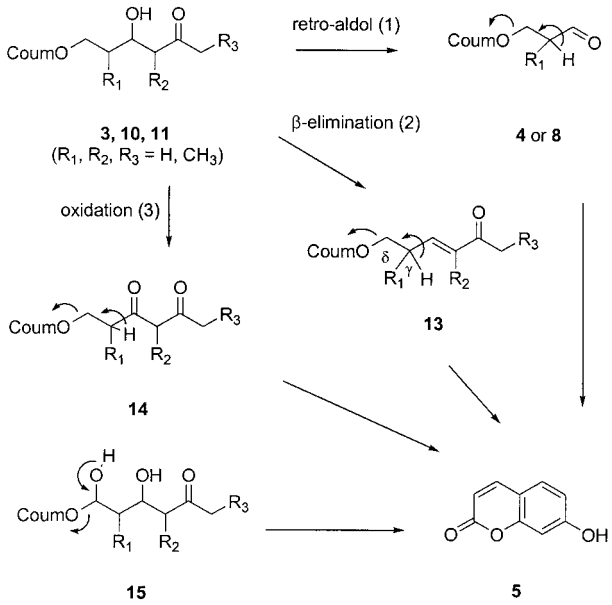
Discussion

The above experiment demonstrates that fluorogenic aldol stereoisomers such as **11a–d**, **10a–d**, and **3a–h** can serve as stereochemical probes for assaying aldolases by fluorescence. Their synthesis by a nonstereoselective route combined with HPLC separation gave rapid access to all possible stereoisomers in stereochemically pure form. Even though this synthesis produces only a few milligrams of each isomer, such amounts are sufficient to carry out many assays due to the small concentration (10^{-5} M) and small volume required. The solubility of these umbelliferone derivatives in aqueous buffer is sufficient at the concentration needed for the assay, so that substrate precipitation does not interfere in the measurement.

Aldolization selectivities: As for the *E* value determining enantioselectivity, stereoselectivity of a catalyst is given microscopically by the ratio of specificity constants k_{cat}/K_M of the individual stereoisomers, and not by the ratio of catalytic constants k_{cat}.[13] The ratio of specificity constants k_{cat}/K_M can be approximated from the ratio of apparent reaction rates at low substrate concentration if all these concentrations are below the respective K_M. In this assay we used substrate concentrations in the range 10–100 μM, which should indeed be below K_M for most catalysts since for many enzymes K_M is in the millimolar range. A similar analytical assay based on secondary release of umbelliferone was found to predict the enantioselectivity of lipases on preparative scale with good accuracy.[9b] In the present aldolization study, we cannot carry out the aldolization in the forward direction due to the β-elimination reactivity of aldehydes **4** and **8**. However, as pointed out above, we have previously demonstrated experimentally that stereoselectivities measured by retroaldolization are identical to those in the aldol addition by the example of an aldolization catalyzed by aldolase antibody 72D4 and producing four different stereoisomeric aldol products.[8]

BSA and β-elimination: Bovine serum albumin plays as central role in the assay by catalyzing the β-elimination of umbelliferone (**5**) from the aldehydes produced by retroaldolization. The catalytic activity of BSA for related deprotonation processes has been described.[14] Although the β-elimination described here is also catalyzed by a number of weak bases and buffers,[15] BSA turns out to be more active and convenient to use. BSA also induces a very small rate of release of umbelliferone (**5**) from the aldol substrates themselves. This interference is probably due to the action of some of its 30 surface lysine residues, presumably by an enamine mechanism promoting retroaldolization, since general base catalysis of this process does not occur in aqueous media.[8]

Alternative pathways that could give a fluorescence signal: Release of umbelliferone from the aldol substrate could in principle occur by three different pathways (Scheme 6). The



Scheme 6. Possible decomposition pathways for aldols, including enone **13**, diketone **14**, and hemiacetal **15**.

first pathway is the expected process of retroaldolization liberating the parent aldehyde, which subsequently undergoes β-elimination of umbelliferone. In the second pathway elimination of water from the aldols could lead to the corresponding enone **13**, and a subsequent γ,δ-elimination could then release umbelliferone. The initial β-elimination in this sequence would however require deprotonation at the α-carbon of the aldol. We have shown that epimerization at the α-carbon is observable for cyclic aldols under β-elimination conditions.[16] HPLC analysis of the assays with aldols **11a–d** and **10a–d** after reaction shows only stereochemically pure aldols without any epimerization at C(3), suggesting that this second pathway does not occur. For the case of antibody 38C2, elimination products have never yet been reported in

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any of the retroaldolizations induced by this catalyst,[2] suggesting that our assay reflects the actual aldolization reactivity of the catalyst. Nevertheless we cannot exclude the possibility that a catalyst promoting an aldol–elimination pathway might be found using our aldol substrates.

A third pathway could involve oxidative reaction either at the secondary alcohol to yield diketone **14** or at the aryl ether carbon to give hemiacetal **15**, which would also release umbelliferone by subsequent cleavage. The related alcohols **6** and **7** can be used as control substrates to check for this oxidative degradation pathway, which could be promoted by oxidative enzymes present in a crude medium. For example these alcohols are found to be completely stable in the hybridoma cell culture supernatants we are using for screening antibodies.

Substrate selectivity: One limitation of the catalysis assays that use chromogenic or fluorogenic substrates is that the activity pattern identified with these substrates for a given catalyst might not extend to other substrates. This is an inherent limitation for any catalysis assay that uses single substrates to report catalytic activity, and is the case for almost all existing catalysis assays[17]. This problem also applies, of course, to the pattern of stereoselectivity whenever a family of stereoisomeric substrates is used, as is the case in the aldol assay described here. Extending the set of fluorogenic aldol stereoisomers reported herein to a broader library of structurally diverse, yet stereochemically defined and addressable aldols would be extremely challenging. The fluorogenic aldol substrates discussed here result from the aldolization of aliphatic aldehydes wherein a fluorogenic aromatic nucleus is placed in a position remote from their reactive center. This situation, which is made possible by releasing the fluorophore through a secondary β-elimination, does not lead to any obvious bias either in reactivity or in stereoselectivity, as is often the case for aromatic aldehydes. We therefore believe that our fluorogenic aldols represent a fair selection for testing for stereoselective aldolases.

High-throughput screening: Any fluorescence-based assay is optimally suited for high-throughput screening owing to the ease with which fluorescence can be recorded, even in miniaturized reaction vessels. If the appropriate robotic system is available, the need to split each sample in up to sixteen different compartments for our aldolase assay does not represent an insurmountable obstacle, even for handling thousands of samples. Alternatively, one could choose to screen for retroaldolization catalysis with one particular aldol stereoisomer of interest, for example one of the *anti*, *anti* aldols **3c** or **3f**, which are difficult to make by standard methods. Any hit identified could then be retested for stereoselectivity in a second round, where catalyst purification could also be addressed.

In any event, one of the limiting factors will be the reaction time allowed before product formation is recorded. This assay time depends on the efficiency requirements for the aldolase to be identified, combined with the concentration of aldolase present in the samples assayed. For aldolase 38C2 and its best substrate in our set, **11c**, a clear signal is recorded visually after only 30 minutes incubation with 1 mg mL^{-1} antibody 38C2, but this catalyst might not be particularly efficient. One might expect that the newer aldolases described by Barbas et al. might yield a signal within minutes at lower concentrations.[2g] Eventually, the present assay should help identify very efficient aldolases.

Conclusion

Sixteen fluorogenic polypropionate fragments have been prepared as single stereoisomers from the parent aldehydes by nonstereoselective aldolization followed by separation by means of HPLC. These aldols release the fluorescent product umbelliferone upon retroaldolization, and can be used to probe the catalytic activity and stereoselectivity of aldolase catalysts for the classical cases of *syn*- and *anti*-selective aldolization (**11a–d**), Cram/anti-Cram-selective aldolization (**10a–d**), and double stereoselective aldolization (**3a–h**). The commercially available aldolase catalytic antibody 38C2 was found to display an interesting *anti* selectivity for aldol **11c**, as well as a selective lack of reactivity with aldol **3f**.

From the various methods known for screening catalysis,[18] fluorogenic substrates are the most attractive in terms of simplicity of use and sensitivity, and their use effectively allows the isolation of catalytic antibodies.[19] The fluorogenic polypropionate fragments reported here offer a convenient tool for the isolation of new stereoselective aldolases by screening of catalyst libraries.

Experimental Section

All reagents and enzymes were purchased from Aldrich or Fluka. Chromatography (flash) was performed with Merck silica gel 60 (0.040–0.063 mm). CH_2Cl_2 was distilled from P_2O_5 prior to use. Pentanone silyl enol ether was prepared following a literature procedure.[20] Preparative HPLC was carried out with HPLC-grade acetonitrile and MilliQ deionized water using a Waters prepak cartridge 500 g (RP-C18 20 μm, 300 Å pore size) installed on a Waters Prep LC 4000 system from Millipore, flow rate 100 mL min^{-1}, gradient +0.5 % min^{-1} CH_3CN, detection by UV at 325 nm. Semipreparative RP-HPLC was performed on a Vydac Protein & Peptide C18 column (1.0 × 22 cm, 5 μm, 300 Å pore size), 5 mL min^{-1}, isocratic elution (analytical conditions as given in Table 1). TLC was performed with fluorescent F254 glass plates. MS and HRMS (high-resolution mass spectrometry) spectra were provided by Dr. Thomas Schneeberger (University of Bern). Fluorescence measurements were carried out with a Cytofluor II Plate-Reader from Perseptive Biosystems. The X-ray crystal structure analysis of compound **3b** water solvate was carried out by the BENEFRI Small Molecule Crystallography Service, Institute of Chemistry, University of Neuchâtel, Switzerland (http://www.unine.ch/chim/chp2/smcs/SMCS.HTML).

7-(3-Oxoprop-1-yloxy)-2*H*-benzopyran-2-one (8): A solution of alcohol **6** (840 mg, 3.81 mmol) in 8 mL dichloromethane was added to a suspension of Dess–Martin periodinane (1.7 g, 4.2 mmol) in CH_2Cl_2 (8 mL). After 10 min the reaction mixture was poured into cold water and extracted with CH_2Cl_2. After evaporation to dryness, the residue was redissolved in CH_2Cl_2 and filtered through cotton to give aldehyde **8** (560 mg, 60 %) as a white solid. TLC (hexane/ethyl acetate 2:1): $R_f = 0.77$ (UV: 365 nm, dinitrophenyl hydrazine yellow); IR ($CDCl_3$): $\tilde{\nu} = 2254$, 1727, 1614, 1508, 1404, 1351, 1281, 1230, 1159, 1125 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta =$ 9.90 (s, 1H), 7.60 (d, $J = 9.6$ Hz, 1H), 7.34 (d, $J = 8.5$ Hz, 1H), 6.79 (d, $J =$ 8.5 Hz, 2H), 6.21 (d, $J = 9.6$ Hz, 1H), 4.35 (t, $J = 5.9$ Hz, 2H), 2.96 (t, $J =$

5.9 Hz, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 199.2$, 143.3, 128.9, 113.4, 112.8, 101.5, 62.0, 42.9.

(*R*)- and (*S*)-7-(2-Methyl-4-oxoprop-1-yloxy)-2*H*-benzopyran-2-ones (4): A solution of alcohol (*R*)-**7** (226 mg, 0.96 mmol) in CH_2Cl_2 (4 mL) was added dropwise to a stirred suspension of Dess–Martin periodinane (450 mg, 1.06 mmol) in CH_2Cl_2 (4 mL). After 10 min at 20 °C, the suspension was hydrolyzed with water (20 mL) and the dichloromethane evaporated under vacuum. The residue was taken up in 50% aqueous acetonitrile and purified by preparative RP-HPLC. The pure RP-HPLC fractions were extracted with ethyl acetate (4 × 200 mL). The organic phase was then washed once, fast, with 1N NaOH to remove traces of carboxylic acid formed by overoxidation, washed once more with water, dried (Na_2SO_4), and concentrated to yield aldehyde (*S*)-**4** (156 mg, 0.67 mmol, 70%) as a colorless oil. The same procedure was applied for its enantiomer. These aldehydes may be stored in acetonitrile/water solution at −80 °C for months, but they decompose upon concentration within some hours. IR (neat): $\tilde{\nu} = 1731$, 1614, 1123 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): $\delta = 9.76$ (s, 1H), 7.66 (d, $J = 9.5$ Hz, 1H), 7.40 (d, $J = 9.2$ Hz, 1H), 6.87 (m, 2H), 6.29 (d, $J = 9.5$ Hz, 1H), 4.25 (m, 2H), 2.92 (m, 1H), 1.32 (d, $J = 7.3$ Hz, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): $\delta = 202.1$, 161.5, 161.0, 155.5, 143.3, 128.7, 113.1, 112.7, 112.6, 101.3, 67.9, 45.8, 10.6; EIMS: 232 $[M]^+$ (40%), 162 (80%), 134 (100%).

7-(3-Hydroxy-2,4-dimethyl-5-oxohept-1-yloxy)-2*H*-benzopyran-2-ones (3a–h)

A. Synthesis with silyl enol ethers: Aldehyde (*S*)-**4** (75 mg, 0.32 mmol) was dissolved in dry CH_2Cl_2 (7 mL) and cooled to −78 °C. Boron trifluoride etherate (47 µL, 0.38 mmol) was added, followed after 5 min by 3-trimethylsilyloxy-2-pentene (0.42 mmol, 81 µL). After 1 h at −78 °C, the reaction was quenched at that temperature with water (0.1 mL) and warmed very slowly to room temperature (1 h). Water (20 mL) was added and the mixture extracted with ethyl acetate. Organic layers were washed once with water, filtered and concentrated. Separation of the crude product by preparative RP-HPLC gave the (2′*S*,3′*R*,4′*S*)-aldol **3a** (42 mg, 41%) and an equal mixture of aldols **3b**+**c**+**d** (26 mg, 26%). The same procedure was applied for the enantiomeric aldehyde.

B. Synthesis with tin enol ether: Triethylamine (47 µL, 0.33 mmol) was added to a suspension of $Sn(OSO_2CF_3)_2$ (113 mg, 0.27 mmol) in dry CH_2Cl_2 (2 mL) The mixture was cooled to −78 °C and 3-pentanone (25 µL, 0.21 mmol) was added. Enolization was allowed at that temperature for 2 h, after which a solution of aldehyde (*S*)-**4** (44 mg, 0.19 mmol in 1 mL CH_2Cl_2 + 1 mL washings) was added. After 1 h, the reaction was warmed to room temperature. Aqueous phosphate buffer (pH 7, 20 mL) was added and the mixture extracted with CH_2Cl_2 (4 × 25 mL). The combined organic layers were washed once with buffer and once with water, dried over Na_2SO_4, filtered and concentrated to yield a mixture of aldols (52 mg). Preparative RP-HPLC gave the (2*S*,3*R*,4*S*) aldol **3a** (21 mg, 35%), and an equal mixture of aldols **3b**+**c**+**d** (22 mg, 36%).

Separation of anti *isomers* **3b–d**: Small amounts (0.1 mg/injection) of the mixture of **3b**+**c**+**d** were separated using chiral-phase HPLC (Table 2), yielding pure **3c** and **3b**+**d**. Finally **3b** and **3d** were separated using semipreparative RP-HPLC (Table 1). The same procedure as above was used for the preparation of aldols **3e–h**. Aldol **3h** was separated from **3e**+**f**+**g** by preparative RP-HPLC. Aldol **3f** was then separated from **3e**+**3g** using chiral columns (Table 2). Finally **3e** and **3g** were separated by semipreparative RP-HPLC. All compounds were obtained in >3 mg amounts, with >98% purity (HPLC) except for **3b** (86%, 14% mixture of **3c**+**d**), and **3d** (81%, 19% mixture **3b**+**c**). **3a**+**b**+**c**+**d:** HRMS: calcd for $C_{18}H_{22}O_5$ $[M]^+$, 318.146850; found, 318.146724; IR (neat): $\tilde{\nu} = 3500$, 1729, 1707, 1124 cm^{-1}.

(2*S*,3*R*,4*S*)-7-(3-Hydroxy-2,4-dimethyl-5-oxohept-1-yloxy)-2*H*-benzopyran-2-one (3a): $[\alpha]_D^{25} = 0.0 \pm 1.0$ ($CDCl_3$, $c = 0.19$); ^{1}H NMR ($CDCl_3$, 200 MHz): $\delta = 7.64$ (d, $J = 9.5$ Hz, 1H), 7.38 (d, $J = 9.2$ Hz, 1H), 6.82 (m, 2H), 6.26 (d, $J = 9.5$ Hz, 1H), 3.97 (m, 3H, H_2-C(1) + H-C(3)), 2.80 (dq, $J = 5.1$, 7.0 Hz, 1H, H-C(4)), 2.53 (m, 2H, H_2-C(6)), 2.05 (m, 1H, H-C(2)), 1.23 (d, $J = 7.2$ Hz, 3H, CH_3), 1.13 (d, $J = 6.9$ Hz, 3H, CH_3), 1.06 (t, $J = 7.3$ Hz, 3H, H_3-C(7)); ^{13}C NMR ($CDCl_3$, 50 MHz): $\delta = 215.2$, 161.6, 160.7, 152.5, 142.9, 128.4, 112.9, 112.4, 112.3, 101.1, 72.1, 71.0, 48.0, 35.4, 34.6, 11.9, 11.4, 7.24.

Aldol 3h: $[\alpha]_D^{25} = 0.0 \pm 1.0$ ($CDCl_3$, $c = 0.21$). NMR data were measured and found identical to those for the enantiomer **3a**.

(2*S*,3*R*,4*R*)-7-(3-Hydroxy-2,4-dimethyl-5-oxohept-1-yloxy)-2*H*-benzopyran-2-one (3b): ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 7.64$ (d, $J = 9.6$ Hz, 1H), 7.38 (d, $J = 8.1$ Hz, 1H), 6.84 (m, 2H), 6.26 (d, 9.6 Hz, 1H), 4.04 (dd, $J = 9.0$, 5.3 Hz, 1H, H-C(1)), 3.95 (dd, $J = 8.5$, 3.0 Hz, 1H, H-C(3)), 3.94 (dd, $J = 9.0$, 5.3 Hz, 1H, H-C(1)), 2.80(dq, $J = 8.4$, 7.3 Hz, 1H, H-C(4)), 2.57 (m, 2H, H_2-C(6)), 2.18 (m, 1H, H-C(2)), 1.13 (d, $J = 7.4$ Hz, 3H, CH_3), 1.08 (t, $J = 7.1$ Hz, 3H, H_3-C(7)), 1.04 (d, $J = 7.0$ Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): $\delta = 216.2$, 162.1, 161.1, 155.9, 128.7, 113.2, 112.8, 112.6, 101.6, 72.9, 71.4, 43.7, 35.7, 34.9, 14.0, 9.75, 7.57.

(2*S*,3*S*,4*S*)-7-(3-Hydroxy-2,4-dimethyl-5-oxohept-1-yloxy)-2*H*-benzopyran-2-one (3c): ^{1}H NMR ($CDCl_3$, 200 MHz): $\delta = 7.64$ (d, $J = 9.5$ Hz, 1H), 7.38 (d, $J = 9.3$ Hz, 1H), 6.84 (m, 2H), 6.26 (d, $J = 9.5$ Hz, 1H), 4.22 (dd, $J = 9.1$, 4.8 Hz, 1H, H-C(1)), 4.00 (dd, $J = 9.1$, 6.2 Hz, 1H, H-C(1)), 3.63 (br t, $J = 5.6$ Hz, 1H, H-C(3)), 3.20 (br s, 1H, OH), 2.95 (dq, $J = 5.5$, 7.3 Hz, 1H, H-C(4)), 2.57 (m, 2H, H_2-C(6)), 2.14 (m, 1H, H-C(2)), 1.28 (d, $J = 7.3$ Hz, 3H, CH_3), 1.12 (d, $J = 6.8$ Hz, 3H, CH_3), 1.07 (t, $J = 7.0$ Hz, 3H, H_3-C(7)); ^{13}C NMR ($CDCl_3$, 50 MHz): $\delta = 217.3$, 162.2, 161.1, 155.9, 143.3, 128.7, 113.1, 112.6, 101.7, 76.1, 70.1, 47.3, 36.7, 36.0, 15.2, 15.1, 7.43.

(2*S*,3*S*,4*R*)-7-(3-Hydroxy-2,4-dimethyl-5-oxohept-1-yloxy)-2*H*-benzopyran-2-one (3d): ^{1}H NMR ($CDCl_3$, 200 MHz): $\delta = 7.63$ (d, $J = 9.4$ Hz, 1H), 7.37 (d, $J = 9.4$ Hz, 1H), 6.87 (m, 2H), 6.25 (d, 9.4 Hz, 1H), 4.21 (dd, $J = 8.8$, 5.1 Hz, 1H, H-C(1)), 4.08 (dd, $J = 8.8$, 6.2 Hz, 1H, H-C(1)), 3.93 (dd, $J = 9.4$, 2.2 Hz, 1H, H-C(3)), 3.20 (br s, 1H, OH), 2.78 (dq, $J = 2.24$, 7.4 Hz, 1H, H-C(4)), 2.59 (m, 2H, H_2-C(6)), 2.07 (m, 1H, H-C(2)), 1.18 (d, $J = 7.3$ Hz, 3H, CH_3), 1.09 (t, $J = 7.3$ Hz, 3H, $H_3C(7)$), 1.04 (d, $J = 7.0$ Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): $\delta = 216.7$, 162.3, 161.1, 155.8, 143.3, 128.6, 113.0, 112.6, 101.7, 71.5, 70.7, 46.4, 35.7, 34.8, 13.7, 8.99, 7.61.

7-(3-Hydroxy-5-oxohex-1-yloxy)-2*H*-benzopyran-2-one (9): $BF_3 \cdot OEt_2$ (85 mg, 0.6 mmol) was added to a solution of the aldehyde **8** (110 mg, 0.5 mmol) in CH_2Cl_2 (1 mL) at −78 °C under N_2. After 5 min the silyl enol ether of acetone (0.65 mmol) was added. After 1 h at −78 °C, water was added slowly and the mixture allowed to warm up to room temperature. The mixture was extracted with ether and the organic phases washed with aq. sat. $NaHCO_3$. Evaporation of the solvent and flash chromatography (hexane/ethyl acetate) gave aldol **9** (57 mg, 41%). IR ($CHCl_3$): $\tilde{\nu} = 3523$, 3019, 2956, 1727, 1711, 1614, 1557, 1469, 1428, 1392, 1293, 1159 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.63$ (d, $J = 9.5$ Hz, 1H); 7.36 (dd, $J = 9.2$, 2.2 Hz, 1H); 6.84 (m, 2H); 6.24 (d, $J = 9.5$ Hz); 4.15 (m, 3H, H_2-C(1) + H-C(3)); 3.20 (m, 1H, OH); 2.67 (m, 2H, H_2-C(4)); 2.22 (s, 3H, H_3-C(6)); 1.95 (q, $J = 6.2$ Hz, 2H, H_2-C(2)); ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 162.7$, 156.5, 144.1, 129.4, 113.8, 113.4, 113.3, 102.3, 65.8, 65.1, 50.5, 36.0, 31.4; HRMS: calcd. for $C_{15}H_{16}O_5$ $[M]^+$: 276.0998, found: 276.0995.

7-(3-Hydroxy-2-methyl-5-oxohex-1-yloxy)-2*H*-benzopyran-2-ones (10a–d): Aldehyde (*R*)-**4** (65 mg, 0.28 mmol) was dissolved in dry CH_2Cl_2 (6 mL) and cooled to −78 °C. $BF_3 \cdot OEt_2$ (42 µL, 0.34 mmol) was added, followed after 5 min by 2-trimethylsilyloxypropene (60 µL, 0.36 mmol). After 1 h at −78 °C, the reaction was quenched at that temperature with water (0.1 mL) and the solution was warmed very slowly (1 h) to room temperature. Water (25 mL) was added, and the mixture was extracted with AcOEt (2 × 25 mL). The combined organic layers were washed once, filtered, and concentrated. The crude mixture was chromatographed by preparative RP-HPLC to afford the pure aldol as a mixture of **10c** and **10d** (35 mg, 40%). A similar procedure starting with (*S*)-**4** gave **10a** and **10b**. The stereoisomers were separated by semipreparative RP-HPLC (see Table 1). **10a + 10b (1.5/1)**: $[\alpha]_D^{25} = -6.40$ (50% aq. acetonitrile, $c = 0.32$); IR (neat): 1727, 1707, 1612 cm^{-1}; HRMS: calcd. for $C_{16}H_{18}O_5$ $[M]^+$: 290.1154, found: 290.1152. **10c + 10d (1/1.5)**: $[\alpha]_D^{25} = +6.40$ (50% aq. acetonitrile, $c = 0.32$).

(3*S*,4*S*)-7-(3-Hydroxy-2-methyl-5-oxohex-1-yloxy)-2*H*-benzopyran-2-ones (10a): ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 7.63$ (d, $J = 9.6$ Hz, 1H), 7.37 (d, $J = 8.4$ Hz, 1H), 6.85 (m, 2H), 6.26 (d, 9.6 Hz, 1H), 4.32 (dt, $J = 9.5$, 3.0 Hz, 1H, H-C(3)), 4.10 (dd, $J = 9.2$, 7.0 Hz, 1H, H-C(1)), 3.94 (dd, $J = 9.2$, 5.5 Hz, 1H, H-C(1)), 2.75 (m, 2H, H_2-C(4)), 2.22 (s, 3H, H_3-C(6)), 2.04 (m, 1H, H-C(2)), 1.06 (d, $J = 7.0$ Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): $\delta = 209.5$, 162.1, 161.1, 155.9, 143.2, 128.7, 113.2, 112.7, 112.6, 101.6. 70.6, 67.3, 47.4, 37.7, 30.7, 10.9.

(3*S*,4*R*)-7-(3-Hydroxy-2-methyl-5-oxohex-1-yloxy)-2*H*-benzopyran-2-ones (10b): ^{1}H NMR ($CDCl_3$, 200 MHz): $\delta = 7.64$ (d, $J = 9.4$ Hz, 1H), 7.37 (d, $J = 9.3$ Hz, 1H), 6.84 (m, 2H), 6.25 (d, $J = 9.5$ Hz, 1H), 4.08 (m, 3H, H_2-C(1) + H-C(3)), 2.72 (m, 2H, H_2-C(4)), 2.22 (s, 3H, H_3-C(6)), 2.18 (m, 1H,

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H-C(2)), 1.08 (d, $J = 7.0$ Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): $\delta =$ 209.5, 162.0, 161.0, 155.6, 143.3, 128.6, 112.8, 112.4, 101.4, 70.0, 68.8, 47.3, 38.0, 30.6, 13.7.

7-(3-Hydroxy-4-methyl-5-oxohept-1-yloxy)-2*H*-benzopyran-2-one (11a–d): $BF_3 \cdot OEt_2$ (85 mg, 0.6 mmol) was added to a solution of the aldehyde **8** (110 mg, 0.5 mmol) in CH_2Cl_2 (1 mL) at $-78\,^\circ C$ under N_2. After 5 min the silyl enol ether (74 mg, 0.65 mmol) was added. After 1 h at $-78\,^\circ C$, water was added slowly and the mixture allowed to warm up to room temperature. The mixture was extracted with ether and the organic phases washed with aq. sat. $NaHCO_3$. Evaporation of the solvent gave a crude product (150 mg) containing $> 80\%$ of aldol **11** as judged by analytical RP-HPLC. The *syn* (**11a**+**d**) and *anti* (**11b**+**c**) stereoisomers were separated by preparative RP-HPLC followed by reseparation of the partially separated fraction on a semipreparative RP-HPLC column (Table 1). Small amounts (3–6 mg) of each enantiomer were obtained by separation on chiral-phase HPLC (Table 2). **11a**+**b**+**c**+**d**: IR ($CDCl_3$,): $\tilde{\nu} = 3502$, 2979, 2252, 1727, 1613, 1557, 1508, 1461, 1405, 1351, 1281, 1230, 1201, 1158, 1125, 917, 836 cm^{-1}; HRMS: calcd. for $C_{17}H_{20}O_5$ $[M]^+$: 304.1311, found: 304.1312. **11d**: $[\alpha]_D^{25} = +30 \pm 10$ ($c = 0.001$, $CHCl_3$). **11a**: $[\alpha]_D^{25} = -30 \pm 10$ ($c = 0.001$, $CHCl_3$).

(3*RS*,4*SR*)-7-(3-Hydroxy-4-methyl-5-oxohept-1-yloxy)-2*H*-benzopyran-2-one: **(11a+d)**: ^1H NMR ($CDCl_3$, 300 MHz): $\delta = 7.62$ (d, $J = 9.4$ Hz, 1H), 7.36 (d, $J = 8.5$ Hz, 1H), 6.83 (m, 2H), 6.23 (d, $J = 9.4$ Hz, 1H), 4.21 (m, 3H, H_2-C(1) + H-C(3)), 2.69–2.43 (m, 4H, H_2-C(6) + H-C(4) + OH), 1.87 (m, 2H, H_2-C(2)), 1.20 (d, $J = 7.3$ Hz, 3H, CH_3), 1.09 (t, $J = 7.3$ Hz, 3H, H_3-C(7)); ^{13}C NMR ($CDCl_3$, 50 MHz): $\delta = 216.2$, 162.0, 161.1, 155.8, 143.3, 128.7, 113.0, 112.6, 112.5, 101.6, 67.9, 65.6, 49.9, 35.0, 33.3, 10.2, 7.49.

(3*RS*,4*RS*)-7-(3-Hydroxy-4-methyl-5-oxohept-1-yloxy)-2*H*-benzopyran-2-one: (11b+c): ^1H NMR ($CDCl_3$, 300 MHz): $\delta = 7.63$ (d, $J = 9.6$ Hz, 1H), 7.37 (d, $J = 9.6$ Hz, 1H), 6.85 (m, 2H), 6.26 (d, $J = 9.5$ Hz, 1H), 4.22 (m, 2H, H_2-C(1)), 3.99 (m, 1H, H-C(3)), 2.77–2.43 (m, 3H, H-C(4) + H_2-C(6)), 2.05 (m, 1H) and 1.88 (m, 1H, H_2-C(2)), 1.70 (br s, 1H, OH), 1.21 (d, $J =$ 7.0 Hz, 3H, CH_3), 1.08 (t, $J = 7.1$ Hz, 3H, H_3-C(7)); ^{13}C NMR ($CDCl_3$, 50 MHz): $\delta = 216.6$, 162.2, 161.3, 156.1, 143.5, 129.0, 113.4, 112.9, 112.9, 101.9, 71.0, 65.8, 51.2, 36.1, 34.1, 14.4, 7.73.

(*R*)-Mosher ester of 7-(3-hydroxy-4-methyl-5-oxoheptyloxy)-2*H*-benzopyran-2-one (12): A solution of aldol **11** (*syn*/*anti* = 80/10, 6 mg, 0.02 mmol) and DMAP (0.5 mg, 0.004 mmol) in acetonitrile (500 μL) was added under N_2 to a freshly flame-dried round-bottomed flask containing activated powdered 3 Å molecular sieves. After 30 min the solution was cooled to $0\,^\circ C$ and (*R*)-methoxytrifluoromethylphenylacetyl chloride (5 mg, 0.02 mmol) was added. Further Mosher acid chloride was added until no evolution of the reaction mixture was observed by TLC. The reaction mixture was then filtered and evaporated. Chromatography on silica gel gave Mosher ester **12** as colorless oil (5.3 mg, 52%). ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.64$ (d, $J = 9.5$ Hz, 1H); 7.49–7.27 (m, 7H), 6.80–6.63 (m, 2H), 6.27 (d, $J = 9.5$ Hz, 1H), 5.62 (m, 1H), 3.99–3.57 (m, 2H), 3.52 and 3.50 (2s, 3H), 2.90 (m, 1H), 2.57–2.41 (m, 2H), 2.21–2.10 (m, 2H), 1.18–1.11 (2d, $J = 7.0$ Hz, 3H), 1.09–0.99 (2t, $J = 7.3$ Hz, 3H); MS FAB + : 521; HR-LSMIS: calcd. for $C_{27}H_{28}O_7F_3$ $[M+H]^+$: 521.1787, found: 521.1772.

Similar reaction conditions were used to prepare small amount of the Mosher esters **12a** and **12d** starting with small samples (0.5–1 mg) of pure stereoisomers **11a** and **11d**, respectively. ^1H NMR (300 MHz, $CDCl_3$): **12a**: $\delta = 2.21$–2.15 (m, 2H, H_2-C(2)), 1.14–1.11 (d, $J = 7$ Hz, H_3C-C(4)); **12d**: $\delta = 2.17$–2.10 (m, 2H, H_2-C(2)), 1.18–1.15 (d, $J = 7$ Hz, H_3C-C(4)).

Equilibration of *anti* aldols 11b and 11c: A sample of pure aldol **11b** (30 μL of a 10 mM stock solution in 50% aq. acetonitrile) was diluted with aqueous morpholine buffer (2 M, pH 9.8 with HCl, 70 μL) and heated at $80\,^\circ C$ for 48 h. The sample was then cooled and injected onto an analytical RP-HPLC column (Table 1), showing 15% conversion of **11b** to a *syn* aldol, with 10% umbelliferone (**5**) formed as by-product. The peak corresponding to the *syn* aldol was collected and lyophilized. The residue was then reinjected onto an analytical chiralpak AS column (see Table 2), and found to consist exclusively of aldol **11a** ($t_R = 35.2$ min) with no detectable amount of its enantiomer **11d**. The same experiment starting with pure aldol **11c** gave exclusively the *syn* aldol **11d** ($t_R = 16.0$ min).

Kinetic measurements: All substrates were diluted from stock solutions in 50% aq. acetonitrile and stored at $-80\,^\circ C$ (the acetone aldols **9** and **10** proved particularly sensitive and required this low temperature for safe storage). Since the amounts of substrate weighed were small (1 mg), the stock solutions of the individual stereoisomers were standardized to the same concentration (10 mM) by comparison of their concentration by HPLC (integration of substrate peak at 325 nm, see Table 1). BSA (bovine serum albumin, crystalline 96% from Sigma) was diluted from a 40 $mg\,mL^{-1}$ stock solution in 20 mM aq. borate buffer (pH 8.8). Antibody 38C2 (Aldrich no. 47995-0) was diluted from 10 $mg\,mL^{-1}$ in PBS (10 mM phosphate, 160 mM NaCl, pH 7.4). Assays (50–100 μL) were carried out in individual wells of round-bottomed 96-well polypropylene plates (Corning-Costar) by following the fluorescence increase at $\lambda_{em} = 460 \pm 20$ nm ($\lambda_{ex} = 360 \pm 20$ nm) with a Cytofluor II instrument from Perseptive Biosystems. Fluorescence data was converted to umbelliferone concentration by means of a calibration curve. The rates indicated in the table are derived from the steepest linear portion in each curve.

X-ray crystallography: Suitable crystals of **3b** water solvate were grown from H_2O/CH_3CN as colorless plates. Intensity data were collected at 223 K on a Stoe Image Plate Diffraction system using $Mo_{K\alpha}$ graphite-monochromated radiation. Image plate distance 70 mm, ϕ oscillation scans 0–200°, step $\Delta\phi = 1^\circ$, 2θ range 3.27–52.1°, $d_{max} - d_{min} = 12.45 - 0.8$ 1 Å. The structure was solved by direct methods with the program SHELXS 97.[21] The refinement and all further calculations were carried out with SHELXL 97.[22] The water hydrogen atoms were located from difference maps and held fixed. The remaining H atoms were included in calculated positions and treated as riding atoms using SHELXL 97 default parameters. The non-hydrogen atoms were refined anisotropically with weighted full-matrix least-squares on F^2. The bond distances and angles are normal within experimental error. No attempt was made to determine the absolute structure of the molecule in the crystal. The molecular structure and crystallographic numbering scheme are illustrated in the ORTEP[23] drawing (Figure 1). Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-143509. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223 336-033; e-mail: deposit@ccdc.cam.ac.uk.

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