Fluorescence Assay and Screening of Epoxide Opening by Nucleophiles

Fabrizio Badalassi,[a,b] Gérard Klein,[a] Paolo Crotti,[b] and Jean-Louis Reymond*[a]

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Terminal epoxides such as **1** react with nucleophiles (H_2O, Cl^-, Br^-, N_3^-, and CN^-) at the primary oxirane carbon atom to give mostly anti-Markovnikov-type regioisomers **5a–d**. The opening products of epoxide (*R*)-**1** with chloride (**5a**), bromide (**5b**) and azide (**5c**) are oxidized by horse liver alcohol dehydrogenase and NAD^+ to give the corresponding ketones **7a–c** and, subsequently, umbelliferone **4** by β-elimination, leading to a fluorescence increase at $\lambda_{em} = 460 \pm 20$ nm ($\lambda_{ex} = 360 \pm 20$ nm). The epoxide hydrolysis products give no signal. We used this enantio- and chemo-selective fluorogenic assay for epoxide opening to search for catalytic antibodies for nucleophilic epoxide opening that were raised against 1,2-azidoammonium hapten **8**, as a mimic for epoxide opening by azide, and against chloromethyl phosphonate hapten **9**, as a mimic for the transition state of chlorohydrin formation.

Introduction

Enzymes are versatile catalysts that are used in various areas, such as industrial processes, diagnostics, biomedical research and fine chemical synthesis. The development of new enzymes follows a discovery-oriented approach that consists of searching for the occurrence of catalysis in possible sources of a desired enzyme, particularly collections of microorganisms.[1] The design aspect of such experiments concentrates on the discovery techniques themselves rather than on the direct structural design of the catalyst. These aspects involve molecular biological methods, such as genome mining and gene shuffling, to define or generate possible targets of interest,[2] and chemical techniques of enzyme assays for detecting the desired catalytic activity as selectively and sensitively as possible under the operating conditions that are typical of enzymes, i.e., a buffered aqueous environment.[3] While enzyme assays are usually developed for reactions for which an enzyme already exists, it may be of interest to also provide assays for reaction of synthetic interest for which no enzymes are known yet. Such assays might then provide an opportunity for finding such enzymes. Herein, we report a fluorogenic assay that detects the chemoselective opening of epoxides by nucleophiles such as halides and azides, a process for which only a single enzyme has been described to date. The assay was implemented to search for catalysis in antibodies raised against two transition state analogs of epoxide opening reactions.

Epoxides are versatile chiral intermediates in a broad variety of syntheses. Their meaningful utilization depends on the availability of stereo- and regioselective opening reactions with nucleophiles; proper manipulation of reagents and reaction conditions allows the regioselective formation of β-halohydrins and amino- and azido alcohols by means of metal salt-catalyzed ring opening reactions with appropriate nucleophiles.[4] If an enantioselective catalyst is available, the opening reaction can be utilized for a kinetic resolution of racemic epoxides. This process is possible when using several metal catalysts having chiral ligands, some of which have been discovered by combinatorial ligand synthesis.[5] In addition to these chemical catalysts, epoxide hydrolases enzymes have been discovered that effect regio- and enantioselective hydrolyses of epoxides.[6] These enzymes, however, operate by nucleophilic attack on the epoxide by a carboxylate side chain, followed by hydrolysis, so that only diols can be obtained.

Catalytic antibodies can also catalyze enantioselective hydrolysis of epoxides, but the mechanism is different: there is a direct attack of a water nucleophile on the epoxide.[7] The mechanism of the reaction catalyzed by limonene epoxide hydrolase from *Rhodococcus erythropolis* has also been reported to involve direct attack by water as a nucleophile.[8] This different reaction mechanism suggests the possibility of obtaining the products of epoxide opening with nucleophiles other than water, such as halides or azides, using a biocatalyst. Indeed, there have been several reports of biocatalytic openings of epoxides with nucleophiles other than water.[9] Recently, a haloalkane dehalogenase enzyme was described that catalyzes the nucleophilic opening of epox-

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[a] Departement für Chemie und Biochemie, Universität Bern,
Freiestrasse 3, 3012 Bern, Switzerland,
Fax: (internat.) +41-31-6318057
E-mail: jean-louis.reymond@ioc.unibe.ch
[b] Dipartimento di Chimica Bioorganica e Biofarmacia,
Università di Pisa,
56126 Pisa, Italy
Fax: (internat.) +39-050-2219660

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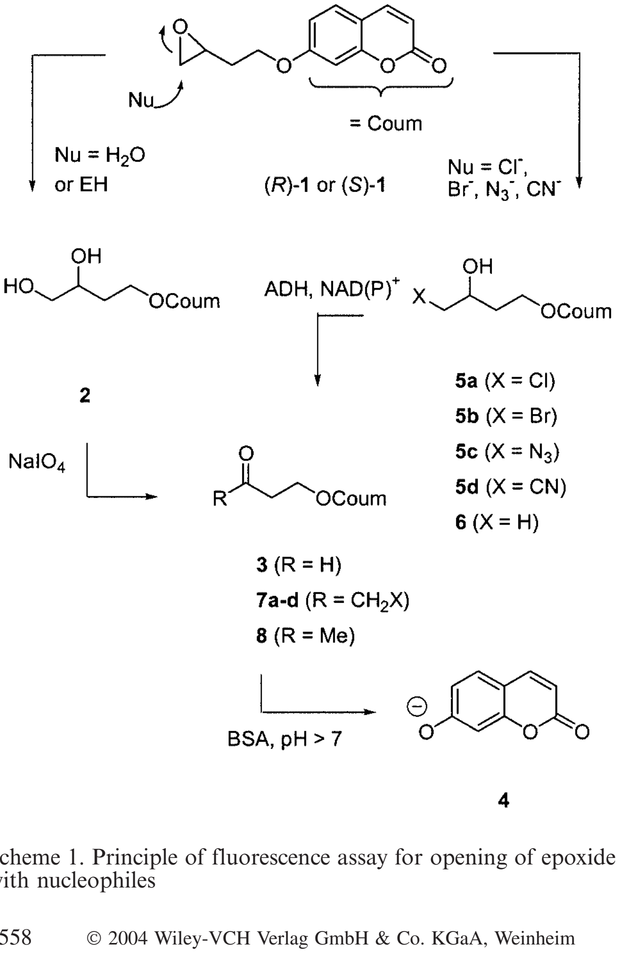
ides by azides.[10] We became interested in finding a generic assay for such reactions to facilitate the discovery of similar catalysts.

Results

Assay Design

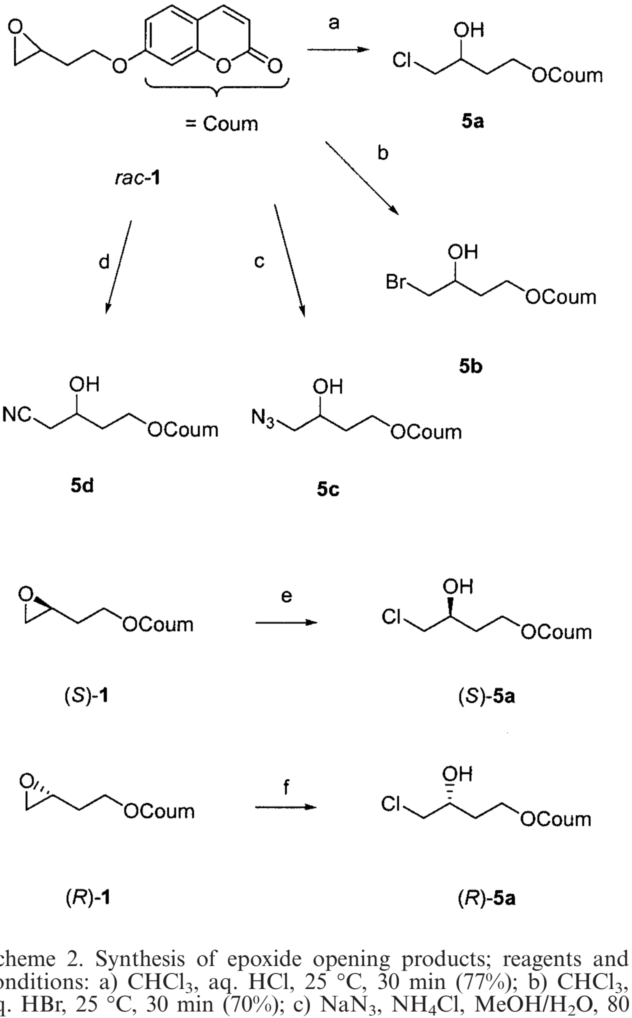
We recently reported an assay for epoxide hydrolysis reactions that is applicable to epoxide hydrolase enzymes.[11] The assay uses optically pure substrates, such as epoxide **1**. A fluorescent signal is produced upon formation of the hydrolysis product diol **2** in the presence of sodium periodate and BSA, which effect the oxidative cleavage of the diol to form aldehyde **3** and, subsequently, umbelliferone **4** by a β-elimination reaction (Scheme 1). This reaction sequence is suitable for a variety of enzyme assays, including lipases,[12] aldolases,[13] and proteases.[14] The assay was applied across a broad variety of epoxides in the context of enzyme fingerprinting experiments.[15] This assay would, however, not provide any signal if the epoxide was opened by a nucleophile other than water or ammonia, such as a halide, azide or cyanide, because the resulting β-hydroxy derivatives **5a−d** would not be oxidized by sodium periodate. Since we had shown previously that the secondary alcohol (*S*)-**6** was an excellent substrate for alcohol dehydrogenases,[16] we envisioned that β-hydroxy derivatives **5a−d** might be suitable substrates for alcohol dehydrogenases, thereby enabling the formation of the fluorescent product umbelliferone **4**, via ketones **7a−d**, as the assay signal for the epoxide opening. Such an assay would be chemoselective for nucleophilic opening of the epoxide by halide, azide, and cyanide nucleophiles against the formation of diol **2** because the latter would be oxidized by the alcohol dehydrogenases at the primary hydroxyl group, which would not lead to a fluorescence signal.[17] We decided, therefore, to explore the kinetics of formation and oxidation of β-hydroxy derivatives **5a−d** in an aqueous buffered environment using an alcohol dehydrogenase as a co-oxidant.



Scheme 1. Principle of fluorescence assay for opening of epoxide **1** with nucleophiles

Substrate Synthesis

Opening of the known racemic epoxide (±)-**1** with HCl/$CHCl_3$, HBr/$CHCl_3$, NaN_3/NH_4Cl/MeOH/H_2O, and KCN/$LiClO_4$/MeCN afforded the β-hydroxy derivatives **5a−d** (Scheme 2). Chlorohydrins (*R*)-**5a** and (*S*)-**5a** were prepared by the ring opening reactions of the known epoxides (*R*)-**1** and (*S*)-**1** with NH_4Cl/$LiClO_4$/MeCN.



Scheme 2. Synthesis of epoxide opening products; reagents and conditions: a) $CHCl_3$, aq. HCl, 25 °C, 30 min (77%); b) $CHCl_3$, aq. HBr, 25 °C, 30 min (70%); c) NaN_3, NH_4Cl, MeOH/H_2O, 80 °C, 18 h (67%); d) CH_3CN, KCN, $LiClO_4$, 70 °C, 18 h (50%); e) CH_3CN, NH_4Cl, $LiClO_4$, 65 °C, 18 h (66%); f) same as e (62%)

Fluorescence Assay

All epoxides and diols were assayed for their fluorogenic properties in aqueous buffer at 0.1 mM concentration in the

Table 1. Fluorogenic reactions of epoxides and alcohols with alcohol dehydrogenases

ADH, μg/mL, V_{app} [a]	**1**	(*R*)-**1**	(*S*)-**1**	**2**	**5a**	(*R*)-**5a**	(*S*)-**5a**	**5b**	**5c**	**5d**
Horse liver, 100[b]	2.9	3.8	1.2	16.5	175	502	8.2	1090	505	4.2
Yeast,100[b]	1.9	0.00	0.24	4.3	2.3	3.5	2.0	0.8	31	1.3
Therm. brockii, 50[c]	4.3	3.2	0.48	15	30	77	1.1	39	39	4.7

[a] Apparent rate of release of umbelliferone (pM s^{-1}). Conditions: 20 mM aq. borate buffer, pH 8.8, 2 mg/mL BSA, 100 μM substrate, enzyme and: see footnotes[b,c]. [b] 1 mM NAD^+. [c] 1 mM $NADP^+$, 30 °C. 200 μL assays were monitored in the individual wells of round-bottom polypropylene 96-well plates (Costar) using a Cytofluor II Fluorescence Plate Reader (Perseptive Biosystems, filters $\lambda_{ex} = 360 \pm 20$ nm, $\lambda_{em} = 460 \pm 20$ nm). Fluorescence (arbitrary units) was converted to umbelliferone concentration according to a calibration curve with pure **4** in the same buffer containing BSA.

presence of alcohol dehydrogenases and BSA (Table 1, Figure 1). The epoxides were found to be stable under these conditions and gave no fluorescence signal. As expected, diol **2** gave only a very weak fluorescence signal. Only horse liver alcohol dehydrogenase (HLDH) in the presence of NAD^+ proved a useful oxidation system, whereby only the products of epoxide opening with halide and azide nucleophiles (compounds **5a**−**c**) gave a strong fluorescence signal. The product of ring opening with cyanide (**5d**) gave only a very weak fluorescence signal. In the optically pure series, only the chlorohydrin (*R*)-**5a** gave a fluorescence signal. The weak fluorescence signal observed with its enantiomer (*S*)-**5a** is probably caused by the ca. 6% of the *R* enantiomer present in the sample.

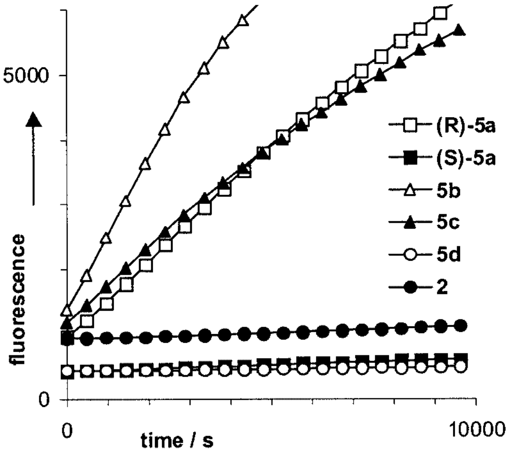


Figure 1. Fluorescence signals with epoxide opening products and alcohol dehydrogenase. Conditions: 100 μM substrate **2**, **5a**−**d** in 20 mM aqueous borate buffer, pH 8.8, 100 μg/mL horse liver alcohol dehydrogenase, 1 mM NAD^+, 2 mg/mL BSA, 30 °C; fluorescence signals (arbitrary units) were recorded with filters $\lambda_{ex} = 360 \pm 20$ nm, $\lambda_{em} = 460 \pm 20$ nm; see also table legend

The assay was validated by following the spontaneous formation of bromohydrin **5b** from epoxide **1** in the presence of bromide, the most nucleophilic halide in the series. The reactivities of epoxides (*R*)-**1** and (*S*)-**1** and of bromohydrin **5b** were assayed in the presence of HLDH and NAD^+ upon increasing the concentration of bromide from zero to 0.15 M (Figure 2). The activity of HLDH, as measured by a reference substrate, was affected only moderately by the addition of NaBr and decreased linearly by ca. 20% as the NaBr concentration increased from 0 and 0.15 M. A fluorogenic reaction that was proportionate to the bromide concentration was observed with epoxide (*R*)-**1**, which indicates the formation of bromohydrin (*R*)-**5b** under the reaction conditions.

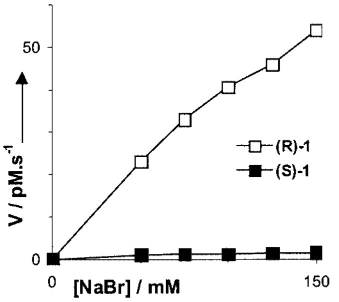
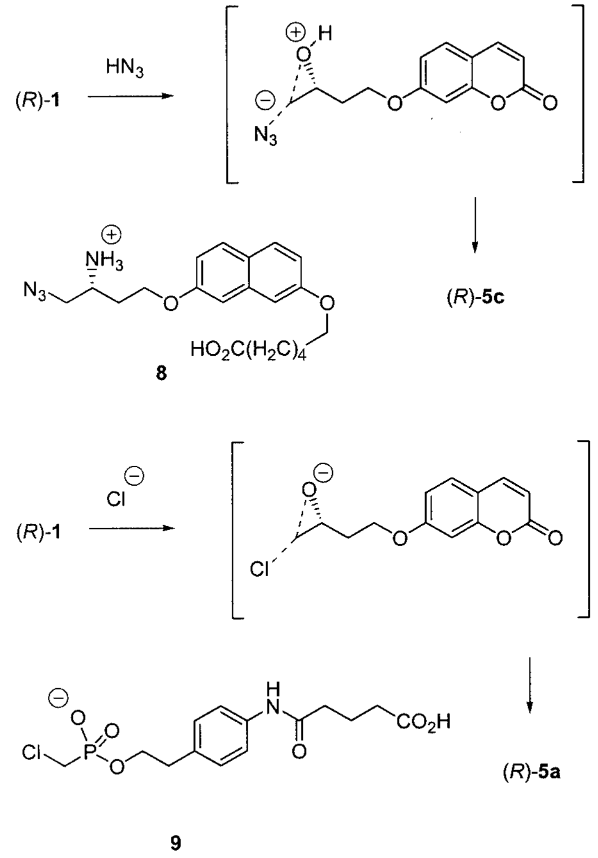


Figure 2. Fluorogenic reaction of epoxides (*R*)-**1** and (*S*)-**1** with NaBr, HLDH, and BSA; conditions: 100 μM epoxide (*R*)-**1** or (*S*)-**1** in 20 mM aqueous borate buffer, pH 8.8, 100 μg/mL horse liver alcohol dehydrogenase, 1 mM NAD^+, 2 mg/mL BSA, 30 °C; the rate of release of umbelliferone V (pM/s) was calculated from the fluorescence signal obtained; see also table legend

Hapten Design for Catalytic Antibodies

Next, we turned our attention to the application of this assay for screening antibodies for possible catalysis of the reaction.[18] We were interested in haptens tailored for epoxide opening of substrate **1** by nucleophiles, and aimed at two complementary designs. Our first attempt was directed at catalyzing the enantioselective azide opening of epoxide (*R*)-**1** to azido alcohol (*R*)-**5c**, because it would be detectable using our ADH-coupled assay (Scheme 3). We envisioned that the naphthalene-derived amino-azide **8** would be suitable as a transition state analog for this reaction. This hapten is essentially product-like, with the important difference that the positive charge in the ammonium group replaces the hydroxyl group in the product. The positive charge was expected to induce a catalytic acid−base functional group capable of activating the epoxide substrate by protonation. Furthermore, the positive charge in the hapten would help to form a combining site having reduced affinity for the neutral product, thereby relieving product inhibition.

Our second hapten was designed for catalysis of the epoxide opening of (*R*)-**1** by chloride to form the fluorogenic chlorohydrin (*R*)-**5a**. We selected the chloromethyl phos-

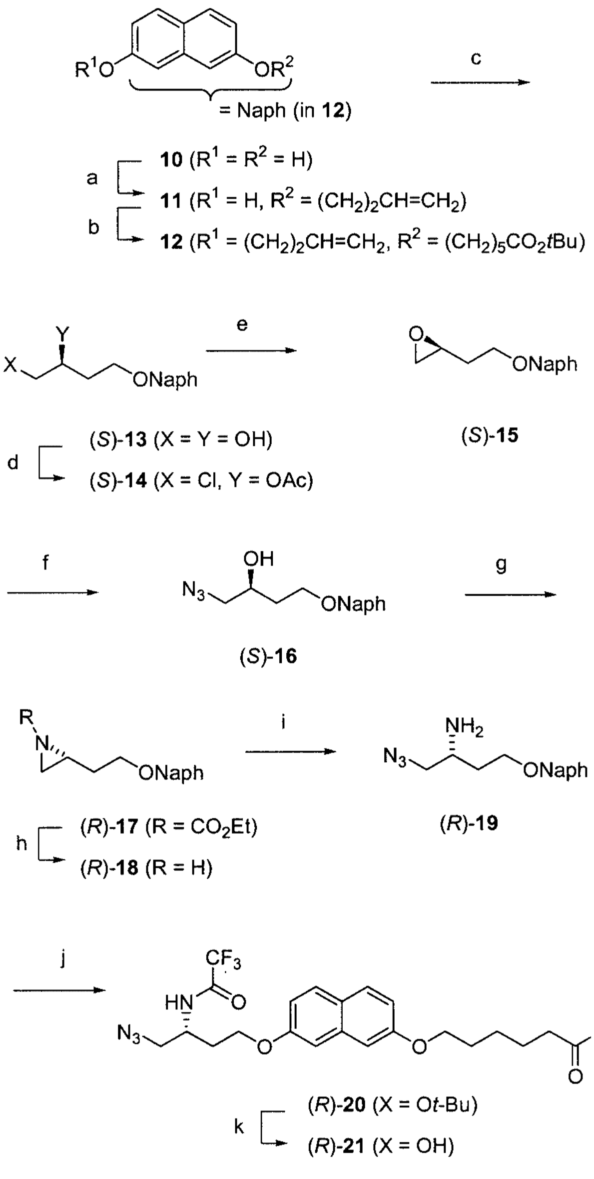


Scheme 3. Transition state analog design of haptens for epoxide opening reactions

phonate **9** as a suitable anionic, product-like transition state analog. The chloromethyl group acts as a mimic of the incoming chloride ion, while the negatively charged phosphonate represents the initially formed alkoxide. The phosphonate function would also help induce an acid−base catalytic group in the antibody combining site.

Hapten Synthesis

The azido amine hapten **8** was prepared from 2,7-dihydroxynaphthalene **10** (Scheme 4). *O*-Alkylation with 4-bromo-1-butene gave mono-ether **11** in low yield (18%), in spite of the several modifications we attempted. A second alkylation with *tert*-butyl 4-bromohexanoate gave the 2,7-di-*O*-substituted naphthalene **12**. Asymmetric Sharpless dihydroxylation with AD mix α afforded the diol (*S*)-**13**, which was transformed into the corresponding epoxide (*S*)-**15** via the chloroacetate (*S*)-**14**.[19] Regioselective opening of the epoxide at the terminal carbon atom using NaN_3 in DMF gave azido alcohol (*S*)-**16**. Reaction of azido alcohol (*S*)-**16** with PPh_3 in MeCN at 80 °C, followed by derivatization with ethyl chloroformate, gave the aziridine carbamate (*R*)-**17**. Basic deprotection with *t*BuOK/*t*BuOH afforded pure aziridine (*R*)-**18**. Treatment with NaN_3/NH_4Cl gave azido amine (*R*)-**19**, which was acylated to give the trifluoroacetamide (*R*)-**20**. The *tert*-butyl ester was removed by treatment with trifluoroacetic acid to give acid (*R*)-**21**, which was converted into the *N*-hydroxysuccinimide ester and conjugated to carrier proteins keyhole lympet hemocyanin (KLH) and bovine serum albumin (BSA). Both protein conjugates were finally exposed at pH 14 for 16 h to afford the corresponding conjugated hapten **8** bearing a primary amine functionality.



Scheme 4. Synthesis of azidoamine hapten **8** as the trifluoroacetamide **21**; reagents and conditions: a) NaH, DMF, $BrCH_2CH_2CH{=}CH_2$, 25 °C, 48 h (18%); b) NaH, DMF, $Br(CH_2)_5CO_2$*t*Bu, 25 °C, 20 h (83%); c) AD mix α, *t*BuOH/H_2O, 0 °C, 48 h (83%, 94% *ee*); d) $MeC(OMe)_3$, CH_2Cl_2, PPTS cat., 25 °C, 1.5 h, then TMSCl, 25 °C, 18 h (99%); e) C_6H_6, *t*BuOK, 25 °C, 20 h (81%); f) NaN_3, DMF, 80 °C, 22 h (78%); g) CH_3CN, PPh_3, 25 °C 1 h, then 80 °C, 15 h, then Et_3N, $ClCO_2Et$, Et_2O, 0 °C, 3 h (42%); h) *t*BuOK, *t*BuOH, 25 °C, 32 h (98%); i) NaN_3, *t*BuOH/H_2O, NH_4Cl, 60 °C, 20 h (83%); j) CH_2Cl_2, TFAA, Et_3N, 0 °C, 2 h (83%); k) CF_3CO_2H/CH_2Cl_2, 25 °C, 1.5 h (100%)

Hapten **9** was prepared in three steps from nitrophenethyl alcohol by esterification with chloromethyl phosphonyl dichloride, reduction of the nitro group, and acylation with glutaric anhydride. Details of the synthesis have been re-

Table 2. Data for immunization with haptens **9** and **8** and catalysis HTS data [the following reactions were assayed: (*R*)-**1** with azide (hapten **8**) or chloride (hapten **9**) and HLDH/NAD$^+$/BSA; pivalate ester hydrolysis with **22**]

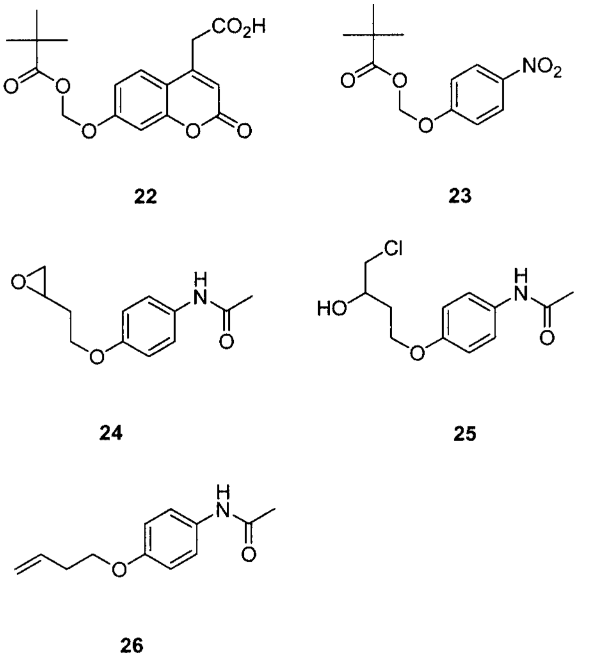
Hapten		Mouse L[a]	Mouse R[a]	Mouse LL[a]	Mouse LR[a]
9	Serum titer[b]	12800	19200	25600	25600
	Nr. of binders[c]	36	45	160	50
	Monoclonals[d]	0	0	16	1
	Catalytic[e]	0	0	11	0
8	Serum titer[b]	1600	1600	1600	800
	Nr. of binders[c]	6	0	7	0
	Monoclonals[d]	0	0	0	0
	Catalytic[e]	0	0	0	0

[a] Mouse code according to ear marks. [b] Dilution factor of blood serum (after immunization with hapten-KLH conjugates) for 50% reduction of ELISA signal against the hapten-BSA conjugate. A high number indicates strong immune response. [c] Number of hybridoma cell lines testing positive for binding by ELISA against the hapten-BSA conjugate after fusion and that were grown up to 5 mL for catalysis testing. [d] Number of fully subcloned and stabilized hybridoma. The purified antibodies were also assayed by RP-HPLC for hydrolysis and chlorohydrin formation with **24** and epoxide formation with **25** and for hydrolysis of *rac*-**1** with NaIO$_4$/BSA. [e] Number of fully subcloned hybridoma producing catalytic antibodies. Only activities for the hydrolysis of pivalate esters **22** and **23** were detected.[20]

ported in relation to the esterolytic activity of antibodies against this hapten.[20]

Antibody Generation and Screening

The KLH-conjugates of the haptens were used for immunization following strandard protocols.[21] Hybridoma cell lines were generated by fusing spleen cells of the immunized animals with myeloma. The azido-amine hapten **8** was only poorly immunogenic. Because of the low serum titers observed, only 13 hapten-binding hybridoma cell lines were obtained. By contrast, the chloromethyl phosphonate hapten **9** proved strongly immunogenic and a total of 291 initial clones were obtained after fusion (Table 2). Cell lines producing hapten-specific antibodies, as judged by ELISA against the BSA-conjugates, were initially assayed for epoxide opening reactions with either chloride (0.15 M) or azide (0.15 M, buffered at pH 8.8) using substrate (*R*)-**1** and the dehydrogenase-coupled assay described above (15 h incubation at 25 °C). There was no observable catalysis using any of the samples tested. The cell lines derived from hapten **9** were also tested for hydrolysis of the fluorogenic pivalate **22**, which led to the identification and successful subcloning of sixteen stable cell lines, nine of which produced catalytic esterolytic antibodies ($k_{cat}/k_{uncat} \approx 10^3$ for the hydrolysis of **22** and **23**).[20] Purified antibodies from these sixteen cell lines were also tested for hydrolysis of the epoxide *rac*-**1** using the periodate-coupled procedure. These antibodies were also tested by HPLC for chlorohydrin formation from epoxide **24** and epoxide formation from chlorohydrin **25**, because these substrates represent exact structural analogs of hapten **9** (Scheme 5). None of these reactions were catalyzed by the antibodies.



Scheme 5. Additional substrates tested with antibodies against hapten **9**; the pivalates **22** and **23** are substrates for various *anti*-**9** antibodies ($k_{cat}/k_{uncat} = 10^3$), but **24** and **25** are not

Discussion

The fluorescence assay for epoxide hydrolysis based on substrate **1**, which has been used by us and others to measure the activity of epoxide hydrolases, has established beyond doubt that this substrate is well suitable as a probe of enzymatic activity. Similarly, the reactivity of the various β-hydroxy derivatives (*R*)-**5a**−**d** with horse liver alcohol dehydrogenase demonstrate that the primary opening products of epoxide **1** are well suited for enzyme reactions. Although no direct enzyme reaction was observed for forming a halohydrin from epoxide **1**, the chemical reaction observed in the presence of bromide in turn establishes the validity of the high-throughput screening fluorescence assay for epoxide opening based on epoxide (*R*)-**1**. Other formats have been reported for assaying epoxide hydrolysis in HTS.[11,22] In contrast, the enzyme-coupled assay reported here is unprecedented for detecting epoxide opening reactions with nucleophiles other than water.

Interestingly, the epoxide−halohydrin equilibrium can be readily manipulated in aqueous buffer by adjusting the halide concentration. An assay for epoxide closure from the halohydrin might be possible based on the detection of chloride ions, but such an assay might be difficult to implement because chloride ions are present in large concentrations in most enzyme preparations as buffer or culture medium components. Here we used a direct HPLC detection system in the context of analyzing the reactivity of a few catalytic antibodies against hapten **9** for epoxide formation from halohydrin **25**. Given that an epoxide hydrolase from *Aspergillus niger* is now commerically available — it converts both enantiomers of epoxide **1** rapidly to the corresponding diol **2**[11,23] — an enzyme/periodate/BSA-coupled procedure should be possible to test epoxide formation from both enantiomers of halohydrins **5a/b**. A corresponding back-titration protocol should allow us to extend the assay to any number of halohydrins as long as the corresponding epoxides are hydrolyzed by the epoxide hydrolase.

Our attempts to discover epoxide-reactive catalytic antibodies by immunization against tailored haptens were not successful. The azido-amine **8** was clearly too weakly immunogenic to generate a sufficient number of hybridoma, which precludes any discussion of whether this was an accurate transition state analog of the targeted reaction or not. The low immunogenicity of the KLH-conjugates of **8** is not due to the trifluoroacetamide deprotection strategy we chose, because a similar strategy has been used successfully in preparing two other unrelated haptens.[24] In the case of the chloromethyl phosphonate hapten **9**, we did isolate several antibodies that catalyze the hydrolysis of pivalates, such as **22** and **23**, which clearly establishes that the hapten was able to induce a catalytic binding pocket.[20] Also, one might have expected that an epoxide opening reaction could be triggered by the same factors that are necessary to induce ester hydrolysis. In the present series, the catalytic antibodies against hapten **9** were not irreversibly inhibited by incubation with epoxide **1** or hapten **9** itself, which implies that a reactive nucleophilic side-chain was not present in the antibody combining sites. To date, an intramolecular hydroxy-epoxide cyclization reported by the Janda group[25] and an epoxide hydrolysis reaction from our group[7] remain the only successful examples of catalytic antibodies for epoxide opening reactions.

Conclusion

In summary, the opening of epoxide **1** with nucleophiles is a fluorogenic reaction when coupled with ADH-catalyzed alcohol oxidation and BSA-catalyzed β-elimination. The assay is enantioselective in the sense that only opening products from enantiomer epoxide (*R*)-**1** give rise to a fluorescence signal, which represents a limitation of the assay. Most importantly, the assay is chemoselective because the simple hydrolysis product, the diol **2**, does not yield a fluorescent product, and only the products of opening with chloride, bromide, and azide (**5a−c**) are detected, which, therefore, allows chemoselective screening. The assay was implemented to search for high-throughput screening of hybridoma cell culture supernatant with the goal of discovering catalytic antibodies, but, unfortunately, without success. This assay should be generally useful to assay catalysts capable of opening epoxides with nucleophiles.

Experimental Section

General: IR spectra were obtained using a Mattson 3000 FTIR spectrophotometer. ^{1}H and ^{13}C NMR spectra were determined using a Bruker AC-200 spectrometer from $CDCl_3$ solutions using Me_4Si as the internal standard. Optical rotations were measured using a Perkin−Elmer 241 digital polarimeter and a 1-dm cell. Direct-phase HPLC analyses were carried out on a Waters 600 E System Controller equipped with a Waters 990 Photodiode Array Detector. Reverse-phase HPLC was performed on a Waters 660 Controller equipped with a Waters 996 Photodiode Array Detector and using three different solvents: A (0.1% TFA in H_2O), B (H_2O/MeCN, 1:1), and C (H_2O). The *ee* of epoxides (*R*)- and (*S*)-**1** were determined directly by analysis on a chiral HPLC column OD-H [Daicel: 25 cm × 0.46 cm (i.d)] using hexane/*i*PrOH (8:2) as the eluent: the chlorohydrins (*R*)- and (*S*)-**5a** did not separate under these conditions. Values of *ee* for the diol (*S*)-**13** and azido amine (*R*)-**19** were determined by HPLC on a Chiracel OD-H chiral column [25 cm × 0.46 cm (i.d.)], using hexane/*i*PrOH mixtures. All reactions were followed by TLC on Alugram SIL G/UV254 silica gel sheets (Macherey−Nagel) with detection by UV and/or spraying with 20% phosphomolybdic acid in EtOH. Benzene and CH_2Cl_2 were distilled under nitrogen from sodium/benzophenone ketyl and CaH_2, respectively.

(±)-7-(4-Chloro-3-hydroxybutyloxy)-2*H*-1-benzopyran-2-one (5a): A solution of epoxide (±)-**1**[15] (0.10 g, 0.43 mmol) in $CHCl_3$ (3.0 mL) was treated with 36% aqueous HCl (3.0 mL) and the reaction mixture was vigorously stirred for 30 min at room temperature. Evaporation of the washed (saturated aqueous $NaHCO_3$ and water) organic solution afforded a crude reaction product that was subjected to flash chromatography (hexane/EtOAc, 6:4; R_f = 0.19) to give pure (±)-**5a** (0.089 g, 77% yield) as a solid. M.p. 59−61 °C. IR (nujol): $\tilde{\nu}$ = 3403 (br. s, OH), 1692 (s, C=O) cm^{-1}. ^{1}H NMR: δ = 7.63 (d, *J* = 9.8 Hz, 1 H), 7.36 (d, *J* = 8.8 Hz, 1 H), 6.86−6.79 (m, 2 H), 6.24 (d, *J* = 9.3 Hz, 1 H), 4.31−4.07 (m, 3 H), 3.72 (dd, *J* = 11.2, 3.9 Hz, 1 H), 3.60 (dd, *J* = 11.2, 6.8 Hz, 1 H), 2.21−1.95 (m, 2 H) ppm. ^{13}C NMR: δ = 162.50, 161.98, 156.35, 144.19, 129.46, 113.67, 113.44, 113.26, 102.06, 69.02, 65.62, 50.74, 34.14 ppm. $C_{13}H_{13}ClO_4$ (268.7): calcd. C 58.11, H 4.88; found C 58.27, H 5.19.

(*R*)-7-(4-Chloro-3-hydroxybutyloxy)-2*H*-1-benzopyran-2-one (5a): A solution of epoxide (*R*)-**1** (0.050 g, 0.21 mmol) in CH_3CN (0.2 mL) containing NH_4Cl (0.017 g, 0.31 mmol) and $LiClO_4$ (0.033 g, 0.31 mmol) was stirred at 65 °C for 18 h. After cooling, dilution with diethyl ether, and evaporation of the washed (saturated aqueous NaCl) organic solution afforded a crude reaction product that was purified by flash chromatography (hexane/EtOAc, 6:4; R_f = 0.19) to give pure (*R*)-**5a** (0.037 g, 66% yield, 96% *ee*[26]) as a semisolid. $[\alpha]_D^{20}$ = +8.9 (*c* = 0.55, MeOH). $C_{13}H_{13}ClO_4$ (268.7): calcd. C 58.11, H 4.88; found C 58.20, H 5.15.

(*S*)-7-(4-Chloro-3-hydroxybutyloxy)-2*H*-1-benzopyran-2-one (5a): Proceeding as described above for the preparation of (*R*)-**5a**, epoxide (*S*)-**1** (0.050 g, 0.21 mmol) afforded a crude reaction product that was purified by flash chromatography (hexane/EtOAc, 6:4) to give pure (*S*)-**5a** (0.035 g, 62% yield, 87% *ee*) as a semisolid. $[\alpha]_D^{20} = -8.6$ ($c = 0.86$, MeOH). $C_{13}H_{13}ClO_4$ (268.7): calcd. C 58.11, H 4.88; found C 58.05, H 4.71.

(±)-7-(4-Bromo-3-hydroxybutyloxy)-2*H*-1-benzopyran-2-one (5b): Proceeding as described above for the preparation of (±)-**5a**, the reaction of epoxide (±)-**1** (0.116 g, 0.50 mmol) in $CHCl_3$ (3.0 mL) with 48% aqueous HBr (2.0 mL) afforded a crude reaction product that was subjected to flash chromatography (hexane/EtOAc, 6:4; $R_f = 0.19$) to give pure (±)-**5b** (0.109 g, 70% yield) as a solid. M.p. 73−75 °C. IR (nujol): $\tilde{\nu} = 3410$ (br. s, OH), 1691 (s, C=O) cm^{-1}. ^1H NMR: $\delta = 7.63$ (d, $J = 9.4$ Hz, 1 H), 7.37 (d, $J = 9.3$ Hz, 1 H), 6.87−6.80 (m, 2 H), 6.25 (d, $J = 9.5$ Hz, 1 H), 4.32−4.02 (m, 3 H), 3.61 (dd, $J = 10.4$, 3.7 Hz, 1 H), 3.47 (dd, $J = 10.3$, 6.8 Hz, 1 H), 2.22−1.90 (m, 2 H) ppm. ^{13}C NMR: $\delta = 162.5$, 161.9, 156.4, 144.1, 129.5, 113.7, 113.4, 113.3, 102.1, 68.6, 65.7, 40.5, 35.0 ppm. $C_{13}H_{13}BrO_4$ (313.1): calcd. C 49.86, H 4.18; found C 50.09, H 4.51.

(±)-7-(4-Azido-3-hydroxybutyloxy)-2*H*-1-benzopyran-2-one (5c): A solution of epoxide (±)-**1** (0.464 g, 2.0 mmol) in a $MeOH/H_2O$ mixture (8:1, 9.0 mL) was treated with NaN_3 (0.60 g, 9.2 mmol) and NH_4Cl (0.214 g, 4.0 mmol) and then the reaction mixture was stirred at 80 °C for 18 h.[19,27] After cooling, dilution with diethyl ether and evaporation of the washed (water) organic solution afforded a solid residue that was purified by flash chromatography (hexane/EtOAc, 6:4; $R_f = 0.17$) to give pure (±)-**5c** (0.37 g, 67% yield) as a solid. M.p. 58−60 °C. IR (nujol): $\tilde{\nu} = 3297$ (br. m, OH), 2093 (m, $-N_3$), 1697 (s, C=O) cm^{-1}. ^1H NMR: $\delta = 7.63$ (d, $J = 9.3$ Hz, 1 H), 7.35 (d, $J = 8.8$ Hz, 1 H), 6.71−6.89 (m, 2 H), 6.23 (d, $J = 9.8$ Hz, 1 H), 4.21−4.00 (m, 3 H), 3.47 (dd, $J = 12.4$, 4.1 Hz, 1 H), 3.37 (dd, $J = 12.2$, 6.8 Hz, 1 H), 2.15−1.86 (m, 2 H) ppm. ^{13}C NMR: $\delta = 162.5$, 162.0, 156.3, 144.2, 129.5, 113.6, 113.5, 113.2, 102.0, 68.5, 65.7, 57.7, 34.0 ppm. $C_{13}H_{13}N_3O_4$ (275.3): calcd. C 56.73, H 4.76, N 15.27; found C 56.34, H 4.40, N 15.04.

(±)-7-(4-Cyano-3-hydroxybutyloxy)-2*H*-1-benzopyran-2-one (5d): A solution of epoxide (±)-**1** (0.116 g, 0.50 mmol) in CH_3CN (0.5 mL) was treated with KCN (0.049 g, 0.75 mmol) and $LiClO_4$ (0.080 g, 0.75 mmol) and then the reaction mixture was stirred at 70 °C for 18 h.[28] After cooling, dilution with diethyl ether and evaporation of the washed (saturated aqueous NaCl) organic solution afforded a solid residue that was subjected to preparative TLC (EtOAc/hexane, 7:3; $R_f = 0.29$). Extraction of the most-intense band afforded pure (±)-**5d** (0.064 g, 50% yield) as a solid. M.p. 95−97 °C. IR (nujol): $\tilde{\nu} = 3435$ (br. m, OH), 2251 (w, CN), 1687 (s, C=O) cm^{-1}. ^1H NMR: $\delta = 7.59$ (d, $J = 9.5$ Hz, 1 H), 7.31 (d, $J = 8.5$ Hz, 1 H), 6.86−6.65 (m, 2 H), 6.18 (d, $J = 9.4$ Hz, 1 H), 4.36−4.02 (m, 3 H), 2.64 (dd, $J = 5.7$, 2.2 Hz, 2 H), 2.28−1.90 (m, 2 H) ppm. ^{13}C NMR: $\delta = 162.3$, 162.0, 156.3, 144.2, 129.5, 118.2, 113.7, 113.5, 113.3, 102.0, 65.5, 65.4, 36.1, 27.0 ppm. $C_{14}H_{13}NO_4$ (259.3): calcd. C 64.86, H 5.05, N 5.40; found C 64.71, H 5.26, N 5.49.

Determination of Optical Purities: The *ee* of the diols (*R*)- and (*S*)-**2** and epoxides **1** were determined by analyses on a chiral HPLC column OD-H (Daicel) [25 cm × 0.46 cm (i.d.)] using hexane/*i*PrOH (8:2): the *R* isomers were the first-eluting ones in each case. Chlorohydrins (*R*)- and (*S*)-**5a** did not separate under these conditions.[26] The absolute configurations of the diols (*R*)- and (*S*)-**2** were assigned on the basis of the Sharpless mnemonic device applied to the olefin.[19] The configuration of the diols (*R*)- and (*S*)-**2** is maintained in the corresponding epoxides (*R*)- and (*S*)-**1**. The β-chlorohydrins (*R*)- and (*S*)-**5a** have the same absolute configuration as the starting epoxide.

7-*O*-(3-Butenyl)-2-hydroxynaphthalene (11): A suspension of NaH (2.08 g of a 60% dispersion in mineral oil, 52.0 mmol) in anhydrous DMF (80 mL) was treated with 2,7-dihydroxynaphthalene (**10**; 6.40 g, 40.0 mmol) and then the reaction mixture was stirred at room temp. for 1 h. 4-Bromo-1-butene (8.10 g, 60.0 mmol) was added and stirring was prolonged for 48 h. Dilution with water, extraction with Et_2O, and evaporation of the washed (saturated aqueous NaCl) diethyl ether extracts afforded a crude reaction product that was subjected to flash chromatography. Elution with hexane/EtOAc (7:3) afforded pure compound **11** (1.53 g, 18% yield) as a solid. M.p. 54−56 °C. ^1H NMR ($CDCl_3$): $\delta = 7.66$ (d, $J = 8.8$ Hz, 2 H), 7.16−6.66 (m, 4 H), 6.11−5.80 (m, 1 H), 5.30−5.00 (m, 2 H), 4.11 (t, $J = 6.8$ Hz, 2 H), 2.61 (q, $J = 6.8$ Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$): $\delta = 158.2$, 154.6, 136.7, 130.2, 130.1, 129.9, 125.1, 117.7, 117.2, 115.9, 109.5, 106.4, 67.9, 34.3 ppm. $C_{14}H_{14}O_2$ (214.3): calcd. C 78.48, H 6.59; found C 78.21, H 6.69.

7-*O*-(3-Butenyl)-2-*O*-(*tert*-butoxycarbonylpentyl)naphthalene (12): A solution of compound **11** (1.20 g, 5.60 mmol) in anhydrous DMF (5 mL) was added dropwise under argon at 0 °C to a stirred suspension of NaH (0.224 g of a 60% dispersion in mineral oil, 5.60 mmol) in anhydrous DMF (5.0 mL). After stirring for 1 h at room temp., *tert*-butyl 6-bromohexanoate (1.40 g, 5.6 mmol) was added and the resulting reaction mixture was stirred at the same temperature for 20 h. Dilution with Et_2O (40 mL) and evaporation of the washed (saturated aqueous NaCl) diethyl ether extracts afforded a crude product (1.98 g) that was subjected to flash chromatography. Elution with hexane/EtOAc (8:2; $R_f = 0.53$) afforded pure ester **12** (1.79 g, 83% yield) as a yellow liquid. IR: $\tilde{\nu} = 1726$ cm^{-1} (C=O). ^1H NMR ($CDCl_3$): $\delta = 7.64$ (d, $J = 8.8$ Hz, 2 H), 7.05−6.93 (m, 4 H), 6.06−5.86 (m, 1 H), 5.30−5.12 (m, 2 H), 4.16−4.03 (m, 4 H), 2.61 (q, $J = 6.8$ Hz, 2 H), 2.28 (t, $J = 7.3$ Hz, 2 H), 1.97−1.74 (m, 2 H), 1.74−1.61 (m, 2 H), 1.61−1.47 (m, 2 H), 1.46 (s, 9 H) ppm. ^{13}C NMR ($CDCl_3$): $\delta = 173.7$, 158.3, 158.2, 136.7, 135.21, 129.8, 125.0, 117.7, 116.9, 107.0, 106.8, 80.7, 68.4, 67.9, 36.2, 34.3, 29.7, 28.9, 26.4, 25.6 ppm. $C_{24}H_{32}O_4$ (384.5): calcd. C 74.97, H 8.39; found C 74.63; H 8.31.

(*S*)-2-*O*-(*tert*-Butoxycarbonylpentyl)-7-*O*-(3,4-dihydroxybutyl)-naphthalene (13): AD Mix α (5.46 g) was added at room temp. to a stirred $tBuOH/H_2O$ mixture (1:1, 40 mL). When a clear biphasic system was obtained, the reaction mixture was cooled to 0 °C and treated with a solution of olefin **12** (1.50 g, 3.9 mmol) in *t*BuOH (1 mL). After stirring for 48 h at the same temperature, the starting material had completely reacted (TLC). Solid $Na_2S_2O_5$ (5.85 g) was added in three portions at 0 °C, and stirring was prolonged for 1 h at room temp. Extraction with CH_2Cl_2 and evaporation of the collected organic phases afforded a crude solid (1.61 g) that was subjected to flash chromatography. Elution with CH_2Cl_2/acetone (7:3; $R_f = 0.46$) afforded pure diol (*S*)-**13** (1.35 g, 83% yield, 94% *ee*) as a white solid, m.p. 51−53 °C. $[\alpha]_D^{20} = -1.86$ ($c = 1.17$, $CHCl_3$). IR (nujol): $\tilde{\nu} = 3321$ (OH), 1726 cm^{-1} (C=O). ^1H NMR ($CDCl_3$): $\delta = 7.63$ (d, $J = 8.8$ Hz, 2 H), 7.12−6.93 (m, 4 H), 4.34−4.14 (m, 2 H), 4.12−3.94 (m, 3 H), 3.74 (dd, $J = 11.0$, 3.2 Hz, 1 H), 3.56 (dd, $J = 10.5$, 6.6 Hz, 1 H), 2.26 (t, $J = 6.6$ Hz, 2 H), 1.99 (q, $J = 5.9$ Hz, 2 H), 1.91−1.75 (m, 2 H), 1.75−1.59 (m, 2 H), 1.59−1.47 (m, 2 H), 1.44 (s, 9 H) ppm. ^{13}C NMR ($CDCl_3$): $\delta = 173.9$, 158.4, 157.8, 136.6, 129.8, 125.1, 117.3, 116.7, 107.1, 106.8, 80.8, 70.96, 68.4, 67.5, 65.9, 36.3, 33.3, 29.7, 28.9, 26.4, 25.6 ppm. $C_{24}H_{34}O_6$ (418.5): calcd. C 68.87, H 8.19; found C 69.01, H 8.39.

(*S*)-2-*O*-(*tert*-Butoxycarbonylpentyl)-7-*O*-(4-chloro-3-methoxycarbonyloxybutyl)naphthalene (14): A stirred solution of diol (*S*)-**13** (0.63 g, 1.51 mmol) in anhydrous CH_2Cl_2 (2.5 mL) was treated under argon with $MeC(OMe)_3$ (0.25 mL, 1.96 mmol) and PPTS (5 mg) and the resulting reaction mixture was stirred at room temp. for 1.5 h. Evaporation of the solvent afforded a crude product, containing the corresponding intermediate cyclic orthoester,[19] which was dissolved in anhydrous CH_2Cl_2 (2.5 mL) and treated with Me_3SiCl (0.25 mL, 1.97 mmol). After stirring for 18 h at room temp., evaporation of the solvent afforded a crude reaction product consisting of chloroacetate (*S*)-**14** (0.71 g, 99% yield), practically pure as a solid (m.p. 39−42 °C), which was used directly in the next step without any further purification. IR $\tilde{\nu}$ = 1739 (O−C=O), 1728 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$): δ = 7.64 (d, *J* = 8.8 Hz, 2 H), 7.11−6.86 (m, 4 H), 5.42−5.21 (m, 1 H), 4.23−3.92 (m, 4 H), 3.82 (dd, *J* = 11.7, 4.4 Hz, 1 H), 3.69 (dd, *J* = 11.7, 4.9 Hz, 1 H), 2.30−2.12 (m, 4 H), 2.10 (s, 3 H), 1.95−1.74 (m, 2 H), 1.74−1.62 (m, 2 H), 1.62−1.47 (m, 2 H), 1.45 (s, 9 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 173.8, 171.1, 158.4, 157.8, 136.6, 129.9, 129.8, 125.1, 117.2, 116.7, 106.8, 106.8, 80.8, 71.2, 68.4, 64.4, 46.5, 36.2, 32.1, 29.7, 28.9, 26.4, 25.6, 21.7 ppm.

(*S*)-2-*O*-(*tert*-Butoxycarbonylpentyl)-7-*O*-(3,4-epoxybutyl)naphthalene (15): A stirred solution of chloroacetate (*S*)-**14** (1.36 g, 2.85 mmol) in anhydrous benzene (80 mL) was treated with two portions of *t*BuOK (0.48 g × 2, 4.28 mmol × 2), 3 h apart, and then stirring was prolonged for 20 h. Evaporation of the filtered solution afforded crude epoxide (*S*)-**15** that was filtered through a short silica gel column. Elution with hexane/EtOAc (8:2) afforded epoxide (*S*)-**15** (0.93 g, 81% yield) as a liquid that was sufficiently pure to be utilized in the next step. An analytical sample of crude (*S*)-**15** was purifed by flash chromatography. Elution with hexane/EtOAc (8:2; R_f = 0.29) afforded pure epoxide (*S*)-**15** as a colourless liquid. $[\alpha]_D^{20}$ = −7.63 (*c* = 1.52, $CHCl_3$). IR (film): $\tilde{\nu}$ = 1728 cm^{-1} (C=O). ^1H NMR ($CDCl_3$): δ = 7.65 (d, *J* = 8.8 Hz, 2 H), 7.10−6.87 (m, 4 H), 4.22 (t, *J* = 6.3 Hz, 2 H), 4.06 (t, *J* = 6.3 Hz, 2 H), 3.27−3.11 (m, 1 H), 2.85 (t, *J* = 4.6 Hz, 1 H), 2.61 (dd, *J* = 5.1, 2.7 Hz, 1 H), 2.27 (t, *J* = 7.3 Hz, 2 H), 2.21−2.08 (m, 1 H), 2.08−1.97 (m, 1 H), 1.97−1.74 (m, 2 H), 1.74−1.63 (m, 2 H), 1.63−1.48 (m, 2 H), 1.45 (s, 9 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 173.7, 158.3, 157.9, 136.6, 129.8, 129.7, 125.0, 117.1, 116.7, 106.8, 106.7, 80.8, 68.4, 65.3, 50.5, 47.8, 36.2, 33.2, 29.7, 28.8, 26.3, 25.6 ppm. $C_{22}H_{28}O_5$ (372.5): calcd. C 70.94, H 7.58; found C 70.76; H 7.72.

(*S*)-7-*O*-(4-Azidobutyl-3-hydroxy)-2-*O*-(*tert*-butoxycarbonylpentyl)naphthalene (16): A solution of epoxide (*S*)-**15** (0.99 g, 2.48 mmol) in anhydrous DMF (11 mL) was treated with NaN_3 (0.647 g, 9.95 mmol) and then the reaction mixture was stirred at 80 °C for 22 h. After cooling, dilution with Et_2O (50 mL) and evaporation of the washed (water) organic solution afforded azido alcohol (*S*)-**16** (0.85 g, 78% yield), practically pure as a liquid, which was directly used in the next step without any further purification. IR (film): $\tilde{\nu}$ = 2100 (N_3), 1726 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$): δ = 7.57 (d, *J* = 8.8 Hz, 2 H), 7.03−6.81 (m, 4 H), 4.31−4.09 (m, 2 H), 4.09−4.37 (m, 3 H), 3.40 (dd, *J* = 12.7, 3.9 Hz, 1 H), 3.30 (dd, *J* = 12.2, 6.8 Hz, 1 H), 2.20 (t, *J* = 7.3 Hz, 2 H), 1.95 (q, *J* = 5.9 Hz, 2 H), 1.87−1.70 (m, 2 H), 1.70−1.55 (m, 2 H), 1.55−1.43 (m, 2 H), 1.38 (s, 9 H) ppm.

(*R*)-2-*O*-(*tert*-Butoxycarbonylpentyl)-7-*O*-(3,4-*N*-ethoxycarbonylaziridinobutyl)naphthalene (17): A solution of azido alcohol (*S*)-**16** (0.90 g, 1.92 mmol) in anhydrous MeCN (3.0 mL) was treated with Ph_3P (0.503 g, 1.92 mmol); the reaction mixture was stirred at room temp. until N_2 developed (0.5−1 h) and then for 15 h at 80 °C. After cooling, evaporation of the solvent afforded a crude product that was dissolved in Et_2O (9.0 mL) and treated, whilst stirring at 0 °C, with NEt_3 (0.67 mL, 4.8 mmol) and EtOCOCl (0.26 g, 2.4 mmol). After stirring for 3 h at the same temperature, evaporation of the filtered solution afforded a crude product that was subjected to flash chromatography. Elution with hexane/EtOAc (7:3) afforded pure *N*-protected aziridine (*R*)-**17** (0.38 g, 42% yield) as a liquid. ^1H NMR ($CDCl_3$): δ = 7.64 (d, *J* = 8.8 Hz, 2 H), 7.09−6.88 (m, 4 H), 4.33−4.15 (m, 2 H), 4.15−3.94 (m, 2 H), 4.14 (q, *J* = 6.8 Hz, 2 H), 2.80−2.62 (m, 1 H), 2.41 (d, *J* = 6.3 Hz, 1 H), 2.27 (t, *J* = 7.3 Hz, 2 H), 2.11 (d, *J* = 3.9 Hz, 1 H), 2.06−1.92 (m, 2 H), 1.92−1.77 (m, 2 H), 1.77−1.61 (m, 2 H), 1.61−1.47 (m, 2 H), 1.45 (s, 9 H), 1.20 (t, *J* = 6.8 Hz, 3 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 173.9, 163.7, 157.8, 157.5, 136.1, 129.2, 124.5, 116.6, 116.3, 106.4, 106.3, 80.2, 67.9, 65.5, 62.7, 49.9, 35.8, 32.3, 31.9, 29.2, 28.3, 25.8, 25.1, 14.4 ppm. $C_{27}H_{37}NO_5$ (455.6): calcd. C 71.18, H 8.19, N 3.07; found C 71.44, H 7.91, N 3.18.

(*R*)-7-*O*-(3,4-Aziridinobutyl)-2-*O*-(*tert*-butoxycarbonylpentyl)naphthalene (18): A stirred solution of *N*-protected aziridine (*R*)-**17** (0.136 g, 0.29 mmol) in *t*BuOH (3.0 mL) was treated with two portions of 0.1 M *t*BuOK in *t*BuOH (1.5 mL × 2), 16 h apart, and then stirring was prolonged for 32 h at room temp. After dilution with Et_2O (40 mL), evaporation of the washed (water) diethyl ether solution afforded a crude product, consisting of pure aziridine (*R*)-**18** (0.114 g, 98% yield), as a liquid that was used directly in the next step without any further purification. IR (film): $\tilde{\nu}$ = 1726 cm^{-1} (C=O). ^1H NMR ($CDCl_3$): δ = 7.63 (d, *J* = 8.8 Hz, 2 H), 7.09−6.81 (m, 4 H), 4.20 (t, *J* = 6.3 Hz, 2 H), 4.05 (t, *J* = 6.3 Hz, 2 H), 2.37−2.12 (m, 3 H), 2.09−1.87 (m, 1 H), 1.87−1.34 (m, 7 H), 1.85 (t, *J* = 6.6 Hz, 2 H), 1.44 (s, 9 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 173.20, 157.8, 157.6, 136.1, 129.2, 124.5, 116.5, 116.3, 106.3, 80.2, 67.9, 66.3, 49.5, 35.7, 34.2, 29.2, 28.3, 27.7, 25.8, 25.1 ppm.

(*R*)-7-*O*-(3-Amino-4-azidobutyl)-2-*O*-(*tert*-butoxycarbonylpentyl)naphthalene (19): A solution of aziridine (*R*)-**18** (0.105 g, 0.264 mmol) in *t*BuOH/H_2O (8:1, 2.3 mL) was treated with NaN_3 (0.069 g, 1.056 mmol) and NH_4Cl (0.056 g, 1.056 mmol) and then the resulting reaction mixture was stirred at 60 °C for 20 h. After cooling, dilution with CH_2Cl_2 and evaporation of the washed (saturated aqueous NaCl) organic phase afforded amino azide (*R*)-**19** (0.097 g, 83% yield, 91% *ee*), practically pure, as a liquid that was used directly in the next step without any further purification. IR (film): $\tilde{\nu}$ = 2100 (N_3), 1726 cm^{-1} (C=O). ^1H NMR ($CDCl_3$): δ = 7.64 (d, *J* = 8.8 Hz, 2 H), 7.16−6.81 (m, 4 H), 4.19 (t, *J* = 5.8 Hz, 2 H), 4.05 (t, *J* = 6.3 Hz, 2 H), 3.59−3.37 (m, 1 H), 3.37−3.16 (m, 1 H), 2.27 (t, *J* = 7.3 Hz, 2 H), 2.12−1.91 (m, 1 H), 1.91−1.72 (m, 4 H), 1.72−1.47 (m, 4 H), 1.45 (s, 9 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 173.3, 157.9, 157.4, 136.1, 129.3, 124.6, 116.7, 116.2, 106.3, 80.3, 67.9, 65.2, 58.7, 49.2, 35.7, 34.6, 29.2, 28.4, 25.9, 25.1 ppm.

(*R*)-7-*O*-(4-Azidobutyl-3-*N*-trifluoroacetylamino)-2-*O*-(*tert*-butoxycarbonylpentyl)naphthalene (20): A solution of amino azide (*R*)-**19** (0.075 g, 0.169 mmol) in CH_2Cl_2 (3 mL), containing NEt_3 (0.045 mL), was treated at 0 °C with trifluoroacetic anhydride (0.038 g, 0.0254 mL, 0.18 mmol) and then the reaction mixture was stirred at the same temperature for 2 h. CH_2Cl_2 and ice-water were added. Evaporation of the washed (water) organic phase afforded a crude product that was subjected to flash chromatography. Elution with hexane/EtOAc (7:3) afforded pure *N*-protected amino azide (*R*)-**20** (0.075 g, 83% yield) as a liquid. $[\alpha]_D^{20}$ = +11.2 (*c* = 0.34, $CHCl_3$). IR (film): $\tilde{\nu}$ = 2104 (N_3), 1724 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$): δ = 7.66 (d, *J* = 8.8 Hz, 2 H), 7.09−6.83 (m, 4 H), 4.47−4.24 (m, 1 H), 4.24−4.09 (m, 2 H), 4.09−3.92 (m, 2 H), 3.74−3.50 (m, 2

H), 2.36−2.07 (m, 4 H), 1.95−1.72 (m, 2 H), 1.72−1.47 (m, 4 H), 1.45 (s, 9 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 173.8, 158.5, 157.2, 136.5, 130.1, 129.8, 125.2, 119.3, 117.5, 116.2, 106.9, 106.8, 80.8, 68.4, 65.3, 53.4, 49.3, 36.2, 31.1, 29.6, 28.8, 26.3, 25.5, 14.8 ppm. $C_{26}H_{33}F_3N_4O_5$ (538.6): calcd. C 57.98, H 6.18, N 10.40; found C 57.71, H 5.93, N 10.27.

Synthesis of Acid (*R*)-21: Amide (*R*)-**20** (0.054 g, 0.10 mmol) was treated with CF_3COOH/CH_2Cl_2 (1:1, 0.3 mL) and then the reaction mixture was left at r.t. for 1.5 h. Evaporation of the solvent afforded acid (*R*)-**21** (0.048 g, 100% yield), practically pure as a solid. m.p. 108−110 °C. ^{1}H NMR ($CDCl_3$): δ = 7.66 (d, *J* = 8.8 Hz, 2 H), 7.10−6.82 (m, 4 H), 4.45−4.26 (m, 1 H), 4.26−4.10 (m, 2 H), 4.06 (t, *J* = 6.3 Hz, 2 H), 3.72−3.50 (m, 2 H), 2.42 (t, *J* = 7.1 Hz, 2 H), 2.21 (q, *J* = 5.0 Hz, 2 H), 2.00−1.79 (m, 2 H), 1.79−1.63 (m, 2 H), 1.63−1.47 (m, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 179.3, 158.5, 157.1, 136.5, 130.2, 129.9, 125.3, 117.5, 116.2, 106.9, 106.9, 68.3, 65.4, 53.4, 49.4, 34.4, 31.1, 29.6, 26.4, 25.2 ppm.

Conjugation of Acid (*R*)-21 with KLH and BSA Carrier Proteins: A solution of 1.5 M aqueous *N*-[3-(dimethylamino)propyl]-*N′*-ethylcarbodiimide·HCl (EDC) (40 μL) and 1.6 M aqueous *N*-hydroxysulfosuccinimide sodic salt (Sulfo-NHS) (39 μL) were added in succession to a solution of acid (*R*)-**21** (0.010 g, 0.020 mmol) in DMF (100 μL). After incubation for 24 h at room temp. (the reaction was monitored by reverse-phase HPLC), a portion (45 μL) of the solution containing the activated hapten was added to a solution of Keyhole-limpet hemocyanin (KHL, 5 mg/mL) in 50 mM phosphate buffer (pH 7.4; 500 μL); a second portion (90 μL) of the same solution was added to a solution of bovine serum albumine (BSA, 5 mg/mL) in 50 mM phosphate buffer (pH 7.4; 1 mL). The two solutions, so obtained, were maintained at 4 °C for 24 h (saturated aqueous $NaHCO_3$ was added, if necessary, to obtain pH 7.4) and then kept at −18 °C. The conjugates were used in the next step without any further purification.

Deprotection of Trifluoroacetamide in Protein Conjugates: The solutions containing the BSA and KLH conjugates of (*R*)-**21**, obtained as described above, were treated independently with 5 N aqueous NaOH (11 and 18 μL, respectively) at pH 14, and then the resulting reaction mixtures were stirred at room temp. for 16 h (hydrolysis of the *N*-trifluoroacetyl group was monitored by TLC, using ninydrine assay). Aqueous 5 N HCl was added to both solutions (11 and 18μL, respectively) in obtain pH 7.4 once again. The solution of the KLH-conjugate was partitioned in four fractions that were subsequently used for immunization. The solution of the BSA-conjugate was diluted with PBS and then partitioned into fractions that were used for the ELISA assay.

4-(Butenyloxy)acetamidobenzene (26): *p*-Acetamidophenol (3.0 g, 19.9 mmol) was added to a suspension of NaH (0.80 g of a 60% dispersion in mineral oil, 19.9 mmol) in anhydrous DMF (18 mL) and then the reaction mixture was stirred at room temp. for 1 h. 4-Bromo-1-butene (2.2 mL, 21.89 mmol) was added and stirring was prolonged for 48 h. Dilution with water, extraction with EtOAc, and evaporation of the washed (aqueous 1 N NaOH) organic phase afforded a crude reaction product consisting of practically pure olefin **26** (0.66 g, 16% yield) that was used in the next step without any further purification. IR (film): $\tilde{\nu}$ = 1670 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ = 7.37 (d, *J* = 9.0 Hz, 2 H), 6.82 (d, *J* = 9.0 Hz, 2 H), 5.96−5.82 (m, 1 H), 5.20−5.08 (m, 2 H), 3.98 (t, *J* = 6.8 Hz, 2 H), 2.56−2.49 (m, 2 H), 2.12 (s, 3 H) ppm.

(±)-4-(*p*-Acetamidophenoxy)-1,2-epoxybutane (24): A solution of olefin **26** (0.50 g, 2.44 mmol) in CH_2Cl_2 was treated at 0 °C with 70% MCPBA (0.67 g, 2.70 mmol) and then the reaction mixture was stirred at the same temperature for 7 h. A new portion of MCPBA (0.30 g, 1.22 mmol) was added and stirring was prolonged for 12 h at 0 °C. Dilution with CH_2Cl_2 and evaporation of the washed (5% aqueous $Na_2S_2O_3$, saturated aqueous $NaHCO_3$, and saturated aqueous NaCl) organic solution afforded a crude product that was subjected to flash chromatography. Elution with hexane/EtOAc/CH_2Cl_2 (4:4:2) afforded pure epoxide **24** (0.25 g, 46% yield) as a colorless solid. ^{1}H NMR ($CDCl_3$): δ = 7.37 (d, *J* = 9.0 Hz, 2 H), 6.84 (d, *J* = 9.0 Hz, 2 H), 4.17−4.02 (m, 2 H), 3.21−3.09 (m, 1 H), 2.83 (t, *J* = 4.6 Hz, 1 H), 2.58 (q, *J* = 2.6 Hz, 1 H), 2.15 (s, 3 H), 2.13−2.02 (m, 1 H), 2.00−1.86 (m, 1 H) ppm.

(±)-4-(*p*-Acetamidophenoxy)-1-chloro-2-butanol (25): A solution of epoxide **24** (0.10 g, 0.45 mmol) in CH_2Cl_2 (3.0 mL) was treated with aqueous 36% HCl (3.0 mL) and then the reaction mixture was stirred at room temp. for 40 min. Dilution with CH_2Cl_2 and evaporation of the washed (saturated aqueous $NaHCO_3$) organic solution afforded a crude product that was subjected to flash chromatography. Elution with hexane/EtOAc (8:2) afforded pure chlorohydrin **25** (0.080 g, 70% yield) as a colorless solid. ^{1}H NMR ($CDCl_3$): δ = 7.37 (d, *J* = 9.0 Hz, 2 H), 6.84 (d, *J* = 9.0 Hz, 2 H), 4.12−3.96 (m, 3 H), 3.68 (dd, *J* = 11.2, 4.2 Hz, 1 H), 3.57 (dd, *J* = 11.0, 6.6 Hz, 1 H), 2.04 (s, 3 H), 2.05−1.92 (m, 1 H), 1.94−1.80 (m, 1 H) ppm.

Assays with Fluorogenic Epoxide 1: A. Screening of hybridoma cell culture supernatants. Antibody cell culture supernatant (50 μL) was added to 20 mM aq. borate buffer (50 μL) containing substrate (*R*)-**1** (200 μM), NAD$^+$ (1 mM), HLDH (200 μg/mL), BSA (4 mg/mL), and 0.2 M of either NaCl (291 samples from fusion with hapten **9**) or NaN_3 (13 samples from fusions with hapten **8**) in the wells of 96-well polypropylene microtiter plates (Costar). A portion of this solution (50 μL) was immediately transferred to another well containing a 2 mM hapten stock solution (0.5 μL). B. Assay for epoxide hydrolysis on purified anti-**9** antibodies: Solutions containing purified anti-**9** antibodies (0.2 mg/mL), epoxide *rac*-**1** (100 mM), $NaIO_4$ (1 mM), and BSA (2 mg/mL) were monitored at 28 °C in the individual wells of 96-well polypropylene microtiter plates by fluorescence using a Cytofluor II Fluorescence Plate Reader (Perseptive Biosystems, filters λ_{ex} = 360 ± 20 nm, λ_{em} = 460 ± 20 nm).

HPLC-Assay with 24 and 25: Antibody solution (> 50 μg/mL) was obtained by purification of cell culture supernatants as described previously.[20] Chlorohydrin formation from epoxide **24**. A 10 mM stock solution (2 μL) of epoxide **24** in DMF was added to cell culture supernatant in PBS (160 mM NaCl, 10 mM phosphate, pH 7.4; 100 μL) in the individual wells of a 96-well polypropylene microtiter plate (Costar). A portion of this solution (50 μL) was transferred to another well containing a 2 mM stock solution (1 μL) of the corresponding hapten in PBS. The reactions were incubated overnight at 25 °C and assayed by RP-HPLC [Vydac C18 218TP54, 0.5 × 22 cm; 1.5 mL min^{-1}; eluent: 80% H_2O/20% CH_3CN; t_R(**24**) = 4.97 min, t_R(**25**) = 5.65 min], showing, on average, 2.5% conversion into epoxide. The conversion was the same with or without the hapten. Epoxide formation from chlorohydrin **25**: A 10 mM stock solution (2 μL) of chlorohydrin **25** in DMF was added to cell culture supernatant in PBS (160 mM NaCl, 10 mM phosphate, pH 7.4; 94.5 μL) in the individual wells of a 96-well polypropylene microtiter plate (Costar). 1 N HCl (4.5 μL) was added to adjust the pH to ≈ 6. A portion of this solution (50 μL) was transferred to another well containing a 2 mM stock solution of the corresponding hapten in PBS (1 μL). The reactions were incubated overnight at 25 °C and assayed, as above, by RP-HPLC. There was, on average, 2.5% conversion into the chlorohydrin and 3.5% conversion into the corresponding 1,2-diol (t_R = 2.64 min). The conversion was the same with or without the hapten.

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