Enzyme Fingerprints of Activity, and Stereo- and Enantioselectivity from Fluorogenic and Chromogenic Substrate Arrays

Denis Wahler,[a, c] Fabrizio Badalassi,[b, c] Paolo Crotti,[b] and Jean-Louis Reymond*[a]

Abstract: A series of stereochemically and structurally diverse fluorogenic and chromogenic substrates for hydrolytic enzymes has been synthesized and used to characterize enzyme activity profiles of esterases, lipases, proteases, peptidases, phosphatases, and epoxide hydrolases. The substrates used are particularly resilient to nonspecific reactions due to their mechanism of activation. The activities recorded with the individual substrates are therefore remarkably reproducible, and enable us to use the overall pattern of activity as a specific fingerprint for the enzyme sample. Fingerprints of activity, and enantio- and stereoselectivity are displayed as arrays of color-scale squares that are easily analyzed visually. Such fingerprints might be useful for quality control, enzyme discovery, and possibly for addressing the issue of functional convergence in enzymes.

Keywords: amidases · enzyme catalysis · enzymes · epoxide hydrolases · esterases · fluorogenic assays · high-throughput screening

Introduction

Enzymes are finding numerous uses as mild, environmentally benign and selective catalysts in a variety of settings, including consumer products, diagnostics, laboratory kits, and in the production of fine chemicals.[1] Potentially useful enzymes are isolated by screening samples isolated from the biosphere, and their properties are engineered by the techniques of directed evolution.[2] In this exercise, success depends critically on high-throughput screening assays for the desired biotransformation.[3] We have recently developed a broad class of fluorogenic enzyme substrates that enable to assay a variety of enzymatic activities, including alcohol dehydrogenases,[4] aldolases,[5] and hydrolases such as lipases and esterases, proteases, phosphatases, and epoxide hydrolases.[6] These substrates combine high sensitivity, selectivity, and the possibility to test enantioselectivity and stereoselectivity with a simple format suitable for high-throughput screening. Herein we present the synthesis of a large set of periodate-coupled fluorogenic and chromogenic substrates. We then show that these substrates can be assembled into arrays that can be used to record enzyme-specific activity fingerprints.[7] Visual recognition of the global activity fingerprint is presented as arrays of squares with a two-color scale combining stereoselectivity and activity.

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Results

Assay principle: Our enzyme assay is based on substrates that release diol **1** or amino alcohol **2** as reaction products. When the reaction is carried out in the presence of sodium periodate ($NaIO_4$) and bovine serum albumin (BSA) at slightly basic pH, the reaction product diol **1** or amino alcohol **2** undergoes an oxidative carbon–carbon bond cleavage, which releases aldehyde **3**. Aldehyde **3** then liberates the fluorescent product umbelliferone (**4**) through a β-elimination catalyzed by BSA (Scheme 1).[8]

Substrates such as acetates **5a/b**, epoxide **6**, amide **7** or phosphate **8**, which can be hydrolyzed to the oxidation sensitive products **1** and **2** by the corresponding enzyme, remain untouched by the oxidant. Therefore the enzymatic hydrolysis of these substrates induces a fluorescence increase, caused by the liberation of **4**, when the reaction is carried out in the presence of $NaIO_4$ and BSA. These reagents are found to be compatible with each other and with a variety of enzymes when used in aqueous buffer. Substrates **5**–**8** can be prepared in optically pure form of either enantiomers, which allows us to assay enzymes not only for catalysis, but also for enantioselectivity. Remarkably, the substrates are also chemically rather unreactive. Even ester **5a** and epoxide **6**, which are the chemically most reactive substrates, are stable at basic

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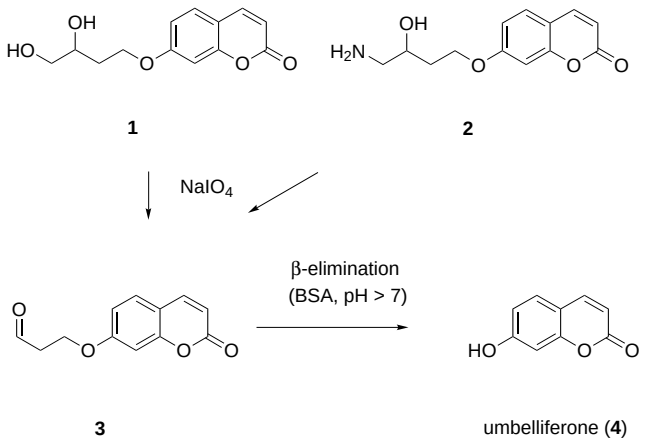
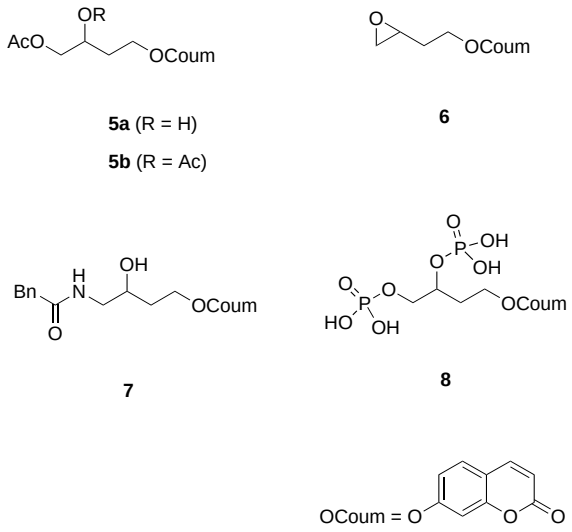
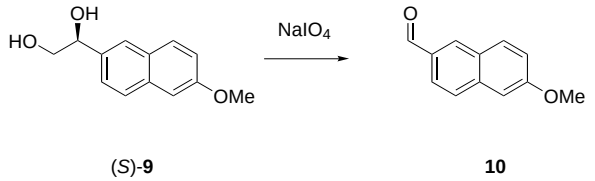


Scheme 1. Principle of periodate coupled fluorescence detection of diol and amino alcohol products **1** and **2**.



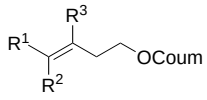
pH (pH 8.8) in the presence of BSA (2 mg mL^{-1}) for at least 12 hours, under which conditions simple enzyme substrates such as nitrophenyl butyrate or umbelliferyl butyrate have a half-life of less than one minute.

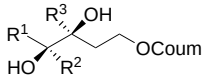
The periodate oxidation step involved in the assay is applicable to most diols or amino alcohols irrespective of the substitution pattern. A similar yet simpler revelation scheme can be devised if diol **9** is formed as the primary diol product, whose periodate oxidation releases the strongly fluorescent 6-methoxynaphthaldehyde **10** (Scheme 2).[9]



Scheme 2. Fluorogenic oxidation of 6-methoxynaphthalene derivatives.

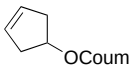
Synthesis: We used Sharpless's asymmetric dihydroxylation of olefins as a versatile entry into preparatively useful amounts of chiral diols as fluorogenic or chromogenic substrates. Procedures were in turn available to convert these into useful epoxide and amino alcohol functional groups to access further substrates. Simple commercially available homoallylic alcohols and bromides were chosen as starting materials, with the aim of generating a family of fluorogenic enzyme substrates displaying methyl, methylene, or ethyl groups in various arrangements close to the enzyme reactive groups. The homoallylic ethers **11**–**14** and **19** were obtained by ether formation between umbelliferone and the corresponding homoallylic tosylates or halides. Asymmetric dihydroxylation





	R^1	R^2	R^3
11	H	H	Me
12	Me	Me	H
13	Et	H	H
14	H	Et	H

	R^1	R^2	R^3
(*S*)-**15**	H	H	Me
(*S*)-**16**	Me	Me	H
(*S, S*)-**17**	Et	H	H
(*R*, S**)-**18**	H	Et	H



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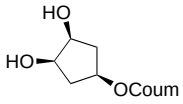

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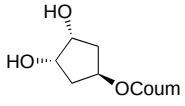

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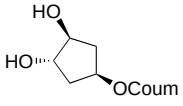
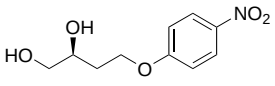


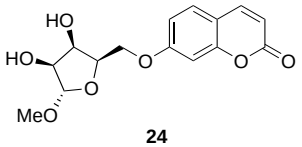
rac-**22**

with either AD-mix-α or AD-mix-β gave the optically active diols **15**–**17** in *R* and *S* configuration, respectively, except for dihydroxylation of the *Z*-olefin **14**, which gave racemic diol **18** with both reagents.[10] The corresponding racemic diols as well as the stereoisomeric *meso*-diols **20** and **21** were obtained by OsO_4-catalyzed dihydroxylation. The corresponding *trans*-diol **22** was obtained in racemic form by hydrolysis of the epoxide of olefin **19** (see below).



(*S*)-**23**

The fluorogenic naphthalene derived diols (*R*)- and (*S*)-**9** were also obtained by Sharpless asymmetric dihydroxylation. In addition, the nitrophenyl derivatives (*R*)- and (*S*)-**23** were prepared to provide a series of chromogenic substrates. Finally, cyclic diols **24** and **25** were prepared from D-lyxose and D-ribose, respectively.



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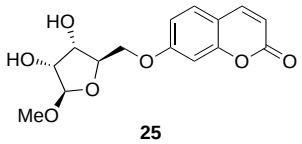

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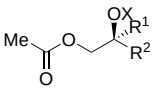
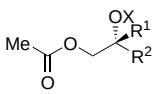
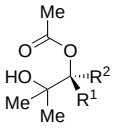
Partial or complete acetylation of diols **9**, **15**, **16**, and **20**–**23** gave mono- and diacetates **26**–**32** to expand the series of **5a/b** as potential substrates for lipases and esterases. Further candidate substrates for these enzymes were obtained in the form of fluorogenic carbonates substrates **33**–**38**, and chromogenic carbonate **39**, prepared by reaction of diols *rac*-**1**, *rac*-**15**, **20**, **21**, and **23**–**25** with carbonyl diimidazole.

To obtain substrates for epoxide hydrolases related to epoxide **6**, chiral diols **9**, **15**–**17**, and **23** were converted to epoxides **40**–**42**, **46** and **47** in both enantiomeric series by using Sharpless' one-pot procedure, which proceeds by double inversion at the less substituted hydroxyl function.[11] Racemic epoxide **43**, the *meso*-epoxides **44** and **45**, as well as the racemic forms of the other epoxides were





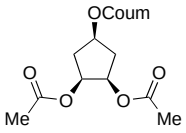
	X	R^1	R^2	
(*S*)-**5a**	H	H	CH_2CH_2OCoum	(*R*)-**5a**
(*S*)-**5b**	Ac	H	CH_2CH_2OCoum	(*R*)-**5b**
(*S*)-**26**	H	Me	CH_2CH_2OCoum	(*R*)-**26**
(*S*)-**30a**	H	H	NaphtOMe	(*R*)-**30a**
(*S*)-**30b**	Ac	H	NaphtOme	(*R*)-**30b**
(*S*)-**32a**	H	H	CH_2CH_2OpNP	(*R*)-**32a**
(*S*)-**32b**	Ac	H	CH_2CH_2OpNP	(*R*)-**32b**



R^1, R^2

(*S*)-**27** CH_2CH_2OCoum, H

(*R*)-**27** H, CH_2CH_2OCoum



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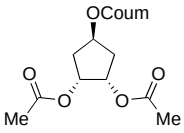

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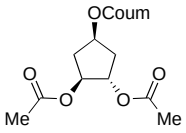

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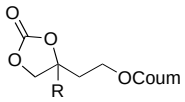
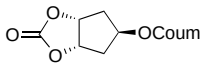


	R
33	H
34	Me



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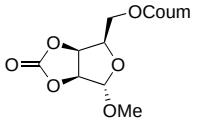

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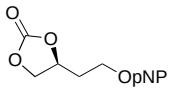
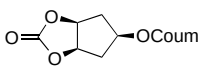


(*S*)-**39**



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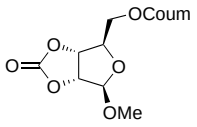

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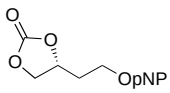
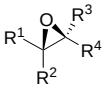
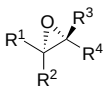
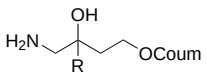


(*R*)-**39**



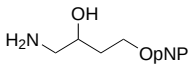


	R^1	R^2	R^3	R^4	
(*S*)-**6**	H	H	H	CH_2CH_2OCoum	(*R*)-**6**
(*S*)-**40**	H	H	Me	CH_2CH_2OCoum	(*R*)-**40**
(*S*)-**41**	Me	Me	H	CH_2CH_2OCoum	(*R*)-**41**
(*S,S*)-**42**	Et	H	H	CH_2CH_2OCoum	(*R,R*)-**42**
(*S*)-**46**	H	H	H	NaphtOMe	(*R*)-**46**
(*S*)-**47**	H	H	H	CH_2CH_2OpNP	(*R*)-**47**



2 (R = H)

48 (R = Me)



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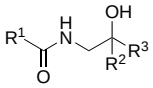
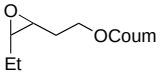
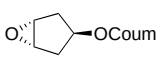


	R^1	R^2	R^3
7	Bn	H	CH_2CH_2OCoum
50	Bn	Me	CH_2CH_2OCoum
51	Me	H	CH_2CH_2OCoum
52	Me	Me	CH_2CH_2OCoum
53	Bn	H	CH_2CH_2OpNP
54	Me	H	CH_2CH_2OpNP



(*R*, S**)-**43**



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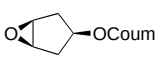

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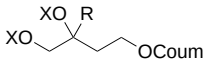
obtained by epoxidation of the corresponding olefins with *m*-CPBA.

Substrates for amidase enzymes similar to **7** were obtained by aminolysis of racemic epoxides **6**, **40**, and **47**, which proceeded chemoselectively at the primary carbon under catalysis by lanthanide salts.[12] The resulting primary amines (**2**, **48**, **49**) were then acylated to amides **50**–**54**.

The phosphorylation of several diols was investigated to access various phosphatase substrates related to bisphosphate **8**. Phosphorylation was carried out by phosphoramidite chemistry, giving first the protected phosphates **55**–**60**. Debenzylation by a brief hydrogenation, in a period too short to observe any reduction of the coumarine nucleus, and purification by preparative reverse-phase HPLC gave phosphates **61**–**65**.

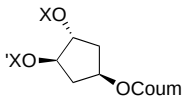


55 (X = $PO(OBn)_2$, R = H)

56 (X = $PO(OBn)_2$, R = Me)

8 (X = PO_3H_2, R = H)

61 (X = PO_3H_2, R = Me)

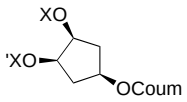


57 (X = X' = $PO(OBn)_2$)

58 (X = $PO(OBn)_2$, X' = H)

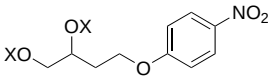
62 (X = X' = PO_3H_2)

63 (X = PO_3H_2, X' = H)



59 (X, X' = PO(OBn))

64 (X = PO_3H_2, X' = H)



60 (X = X' = $PO(OBn)_2$)

65 (X = X' = PO_3H_2)

Enzyme measurements: All substrates were used as 2 mM stock solutions in aqueous DMF or water. First, the maximum observable rate with each substrate type was determined by measuring the rate of product release from each diol or amino alcohol reaction product. All diols released the corresponding fluorescent or colored product upon reaction with sodium periodate and BSA both at neutral and at slightly basic pH (Table 1). The reaction rates of these diols and amino alcohols marks the maximum observable rate of fluorescence release in this assay.

The clean reactivity of $NaIO_4$ under these conditions was somewhat unexpected, since the working pH for this oxidant is usually reported to be acidic (pH 4.5–5.5). The compatibility of the oxidation reaction with a basic pH was welcome as the chromo/fluorogenic assay was impractical at acidic pH. Indeed an acidic pH makes the secondary β-elimination reaction catalyzed by BSA much slower and the fluorescence/absorbency differences between ethers and free umbelliferone/nitrophenol too weak to give a useful signal modulation.

The series of substrates were used to investigate the reactivity of commercially available hydrolytic enzymes, including lipases, esterases, acylases, proteases, and two epoxide hydrolases. The substrates were distributed in 96-well microtiter plates, and each enzyme was assayed simultaneously with the whole series of substrates. The fluorogenic substrates were distributed in 40 samples as a 5×8 array (two enzymes per plate), and the corresponding chromogenic substrates as a 4×3 array (eight enzymes per plate). In each case two positive controls were included with diols or amino alcohols reaction products (Figure 1).

Table 1. Rates for the conversion of diols/amino alcohols to colored/fluorescent products.[a]

diol/ amino alcohol	product	V [pM s^{-1}] pH 7.2	V [pM s^{-1}] pH 8.8
1	umbelliferone(**4**)	105000	155000
9	naphthaldehyde(**10**)	485000	340000
15	umbelliferone(**4**)	80000	135000
16	umbelliferone(**4**)	165000	170000
17	umbelliferone(**4**)	170000	50000
18	umbelliferone(**4**)	95000	35000
20	umbelliferone(**4**)	30000	70000
21	umbelliferone(**4**)	30000	115000
22	umbelliferone(**4**)	30000	30000
23	*p*-nitrophenol	220000	155000
24	umbelliferone(**4**)	10000	10000
25	umbelliferone(**4**)	10000	15000
2	umbelliferone(**4**)	70000	115000
48	umbelliferone(**4**)	15000	25000
49	*p*-nitrophenol	240000	430000
(*R*)-**46**[b]	naphthaldehyde(**10**)	–	20000

[a] Initial rate of product formation. Conditions: 0.1 mM diol/amino alcohol in 20 mM phosphate buffer pH 7.2, or 20 mM aq. borate buffer pH 8.8, 26 °C, containing 1 mM $NaIO_4$ and 2 mg mL^{-1} BSA. Fluorescence/absorbency values were converted to product concentration according to calibration curves with pure products in the corresponding buffers containing sodium periodate and BSA. The error margin on the rates given is ±10%. [b] Stereospecific reaction with BSA.

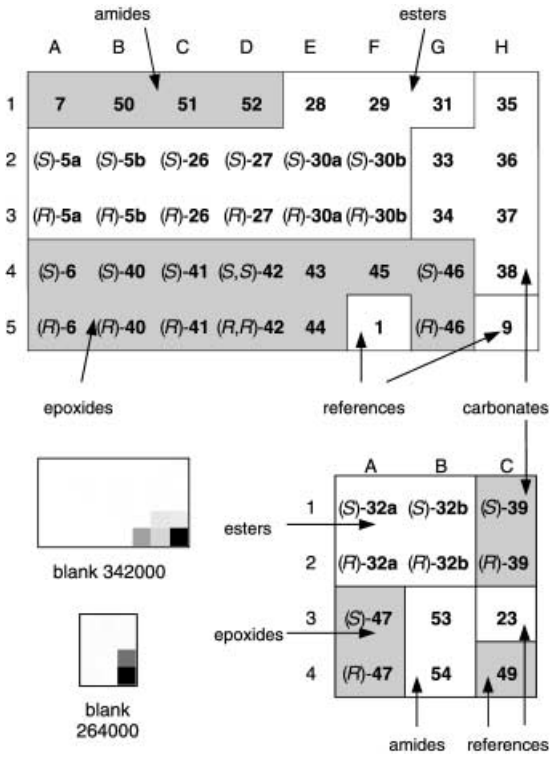


Figure 1. Array layout for activity measurements with chromogenic and fluorogenic substrates.

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All enzymes were used at a concentration of 0.1 mg mL^{-1} of the supplied solid or suspension, unless otherwise stated (Table 2). Reaction progress with each substrate was followed over two hours with a plate-reader instrument (Figure 2). The recorded signals (fluorescence or absorbency) were converted to product concentration by using a calibration curve. The steepest portion of each rate profile was then used to calculate the apparent reaction rate V_{app} in each well. The rates observed in the array without enzyme are very small except for the positive controls and the naphthalene epoxide (*R*)-**46**, which seems to undergo a stereospecific reaction with BSA. These rates were subtracted from the rates observed with enzymes, and the resulting net rates were used to compute the enzymatic activity profiles.

The fluorogenic phosphatase substrates, which were available only in small amounts due to the need for preparative HPLC purification, were tested separately against alkaline phosphatase and three different phytases, which are acidic phosphatase that hydrolyze phytic acid. Since these enzymes distinguish themselves mainly in their pH–activity profile, we applied a two-step procedure involving 1) an incubation at a given pH for a fixed time, and 2) a pH adjustment followed by

Table 2. Enzymes tested by using fluoro/chromogenic substrate arrays.

Code	Name[a]	Specific activity [U mg^{-1}]	Best substrate	Rate [pM s^{-1}]
PSBL	*Pseudomonas* sp. B lipoprotein lipase (F62336)	160[d]	**32a**	50500
PFL	*Pseudomonas fluorescens* lipase (F62321)	3500[e]	**30a**	31800
PSL1	*Pseudomonas* sp. lipoprotein lipase (SL-9656)	50000[d]	**32a**	27500
PSL2	*Pseudomonas* sp. lipoprotein lipase (F62335)	1500[e]	**32a**	17700
WGL	wheat germ lipase (F62306)	0.1[e]	**30b**	11300
CVL	*Chromobacterium visc.* lipoprotein lipase (F62333)	2500[e]	**32a**	11100
ANL1	*Aspergillus niger* lipase (A39,043-7)	–	**26**	10200
CAL	*Candida antarctica* lipase (F62299)	3[e]	**30a**	9600
CLL	*Candida lipolytica* lipase (F62303)	0.001[e]	**47**	7500
RNL	*Rhizopus niveus* lipase (F62310)	0.0025[e]	**39**	7400
RML	*Rhizomucor miehei* lipase (F62291)	0.5[e]	**30a**	6800
HPL	hog pancreatic lipase (F62300)	20[f]	**32a**	5800
PCL1	*Pseudomonas cepacia* lipase (F62312)	40[e]	**32a**	5600
MJL	*Mucor javanicus* lipase (F62304)	0.005[e]	**47**	2800
AOL	*Aspergillus oryzae* lipase (F62285)	50[e]	**32a**	2700
PCL2	*Pseudomonas cepacia* lipase (F62309)	50[e]	**53**	2600
CCL	*Candida cylindracea* lipase (F62316)	2[e]	**32a**	1700
MML	*Mucor miehei* lipase (F62298)	1[e]	**31**	1500
ANL2	*Aspergillus niger* lipase (F62294)	1[e]	**30a**	1100
PRL	*Penicillium roqueforti* lipase (F62308)	150[e]	**53**	720
RAL	*Rhizopus arrhizus* lipase (F62305)	0.002[e]	**6**	330
PLE	pig liver esterase (F46058)	220[g]	**30b**	228000
AChE	*Electrophorus electricus* acetylcholine esterase (F01023)	300[h]	**5a**	51400
BStE	*Bacillus stearothermophilus* esterase (F46051)	0.4[i]	**5a**	9900
BSE	*Bacillus* sp. esterase (F46062)	0.1[g]	**39**	7500
CLE	*Candida lipolytica* esterase (F46056)	0.1[g]	**54**	5200
BTE	*Bacillus thermoglucosidasius* esterase (F46054)	0.1[g]	**39**	3800
TBE	*Thermoanaerobium brockii* esterase (F46061)	0.002[g]	**36**	2600
HrLE	horse liver esterase (F46069)	0.7[j]	**5a**	2500
MME	*Mucor miehei* esterase (F46059)	1[g]	**53**	2500
SCE	*Saccharomyces cerevisiae* esterase (F46071)	0.002[g]	**32a**	1800
PGA	*Escherichia coli* penicillin G acylase (F76427)	30	**7**	152000
HKA	hog kidney acylase I (F01821)	15	**26**	4800
Chy	bovine pancreatic α-chymotrypsin (SC-4129)	37	**54**	3800
Clos	*Clostridium histolyticum* clostripain (SC-0888)	100	**39**	3500
Try	hog pancreatic trypsin (F93615)	1645	**39**	1700
Ela	porcine pancreatic elastase (Se20929)	180	**30a**	1500
Pep	porcine stomach mucosa pepsin (SP-6887)	4150[k]	**53**	870
Pap	*Papaya latex* papain (SR-4762)	30	**53**	850
AMA	*Aspergillus melleus* acylase I (F01818)	0.5	**5a**	700
The	*Bacillus thermoproteolyticus* thermolysin (BM161586)	35	**53**	530
AEH	*Aspergillus niger* epoxide hydrolase[b]	–	**47**	173000
REH	*Rhodotorula glutinis* epoxide hydrolase[c]	–	**47**	170000

[a] Product reference from each provider are given according the following abbreviations: F Fluka, A Aldrich, S Sigma, BM Boehringer Mannheim, Se Serva. [b] Sample provided by Prof. R. Furstoss and Dr. A. Archelas from the Faculté des Sciences de Luminy, Marseille (France). [c] Sample provided by Dr. C. Weijers at the Department of Food Technology and Nutrition Sciences, Wageningen University (The Netherlands). [d] 1 U corresponds to the amount of enzyme which liberates 1 μmol oleic acid per minute at pH 8.0 and 37 °C (cholesteryl oleat as substrate). [e] 1 U corresponds to the amount of enzyme which liberates 1 μmol oleic acid per minute at pH 8.0 and 40 °C (triolein as substrate). [f] 1 U corresponds to the amount of enzyme which liberates 1 μmol oleic acid per minute at pH 8.0 and 40 °C (triolein as substrate). [g] 1 U corresponds to the amount of enzyme which hydrolyzes 1 μmol ethyl *n*-valerate per minute at pH 8.0 and 25 °C. [h] 1 U corresponds to the amount of enzyme which hydrolyzes 1 μmol acetylcholine per minute at pH 8.0 and 37 °C. [i] 1 U corresponds to the amount of enzyme which liberates 1 μmol 4-nitrophenol per minute at pH 7 0 and 65 °C (4-nitrophenyl-*n*-caproate as substrate). [j] 1 U corresponds to the amount of enzyme which hydrolyzes 1 μmol ethyl *n*-butyrate per minute at pH 8.0 and 25 °C. [k] 1 U will produce a ΔA_{280} of 0.001 per minute at pH 2.0 and 37 °C, measured as TCA-soluble products using hemoglobin as substrate.

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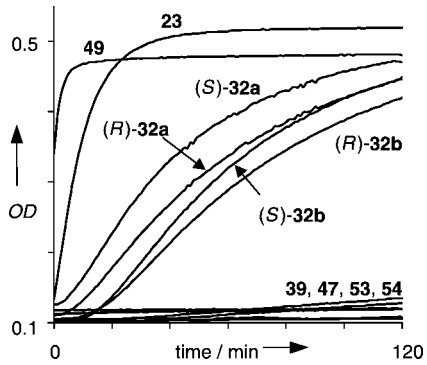

Figure 2. Time course of optical density (OD, at $\lambda = 405$ nm) observed for the reaction of *Pseudomonas fluorescens* lipase with the chromogenic substrates array. Conditions: 0.1 mg mL^{-1} enzyme, 100 μM substrate, 20 mM aq. borate pH 8.8, 2 mg. mL^{-1} BSA, 1 mM $NaIO_4$. The rates derived from these curves for the different substrates are as follows: **49** 20900 pM s^{-1}, **23** 130800 pM s^{-1}, (*S*)-**32a** 31800 pM s^{-1}, (*S*)-**32b** 25500 pM s^{-1}, (*R*)-**32a** 25000 pM s^{-1}, (*R*)-**32b** 22000 pM s^{-1}, **54** 4000 pM s^{-1}, (*R*)-**39** 2600 pM s^{-1}, (*S*)-**39** 2300 pM s^{-1}, **53** 1600 pM s^{-1}, (*S*)-**47** 1400 pM s^{-1}, (*R*)-**47** 1300 pM s^{-1}. See also text and Experimental Section.

treatment with periodate and BSA to reveal the amount of free diol formed. There was no measurable background hydrolysis of any of the phosphatase substrates under the reaction conditions; this is not unexpected due to the known stability of aliphatic phosphates towards hydrolysis.

Activity arrays (gray scale): The reaction rates observed with the different substrates can be displayed graphically as an array of gray-scale squares corresponding to the microtiter-plate layout, as we have shown previously.[7] These displays are generated by simple conversion of the rate-data to a portable-gray-map (.pgm) file format, which is recognized by common photographic software (see Experimental Section). The measurements for the phosphatase substrates are displayed similarly by combining the measurements done at the different pH values. The gray-scale pattern is proportional to the maximum concentration of product formed with each enzyme at any pH, set as full black (Figure 3). To our surprise, our measurements showed that alkaline phosphatase reacts quite well with our diphosphates at pH 5.5, so that our substrates do not allow us to distinguish well between the acidic phytases and alcaline phosphatases.

Selectivity arrays (color): Due to the importance of stereo- and enantioselectivity as property of enzymes in synthetic applications, we propose here a colored array display that combines the intensity scale as above with a color scale of green to red for stereoselectivity. This display is generated by converting the data to the portable-pixel-map (.ppm) format (see Experimental Section). In this display, enantiomeric and stereoisomeric pairs of substrates are combined in one position. Selectivity arrays were generated by combining the rate data from both the chromogenic and the fluorogenic substrate arrays. Racemic or achiral substrates are also reported in simple gray scale, such that these selectivity arrays show the entire set of measurements done. Although the grid does not correspond to an actual microtiter-plate layout, the information on the activity and the selectivity of each enzyme is rendered more clearly by this display (Figure 4).

Discussion

Fingerprints: We have found that all measurements with our substrate arrays are reproducible within 10% of each rate. The patterns observed consistently appear indistinguishable from one measurement to the next. Therefore one may consider that the measurement delivers a specific fingerprint for the enzyme sample. This is to be attributed mainly to the resilience of our periodate-coupled enzyme substrates to nonspecific reactions, which makes them particularly selective probes for true enzyme activities. A similar pattern analysis based on qualitative tests of enzyme activities by using nineteen different enzyme substrates is used to identify microorganisms and is known to microbiologists under the name APIZYM.[13]

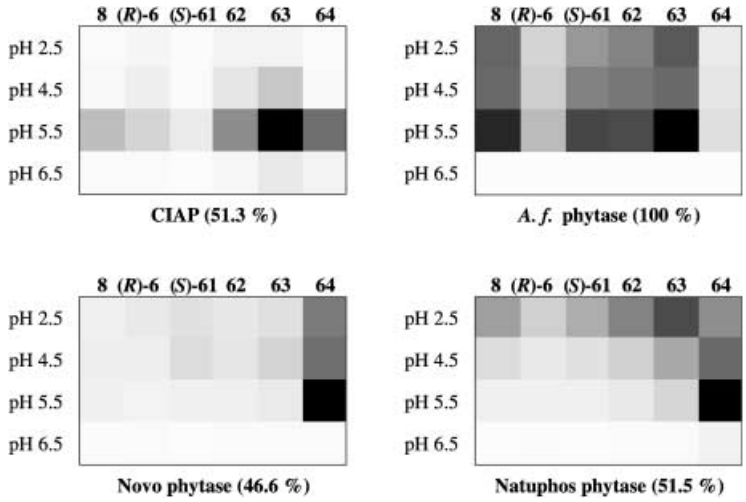

Figure 3. Activity-pH profile as array for phosphatase substrates. Below each array is indicated the enzyme name (CIAP: calf intestinal alkaline phosphatase, *A.f.* phytase: *Aspergillus ficuum* phytase), together with the percentage of substrate conversion as measured in the conditions of the test. Conditions: 0.1 mg mL^{-1} enzyme, 100 μM substrate, 10 mM aq. citrate, acetate or phosphate with 1 mM $CaCl_2$, 55 °C, 2 mg mL^{-1} BSA, 1 mM $NaIO_4$. Enzymes and substrates are incubated 1 hour at the desired pH and 55 °C, and the pH is then adjusted to 7.2 with 0.5 M aq. dibasic phosphate. Sodium periodate and BSA are added, and the assays incubated for a further 60 minutes before fluorescence reading.

The fingerprint recorded with our substrates for a sample could generally be either sample-specific or enzyme-specific depending on purity. Indeed, as for any enzyme assay, the reactivities observed may stem either from the listed enzyme, or from other enzyme impurities. The commercial preparations used here are not highly purified, and the fingerprints ob-

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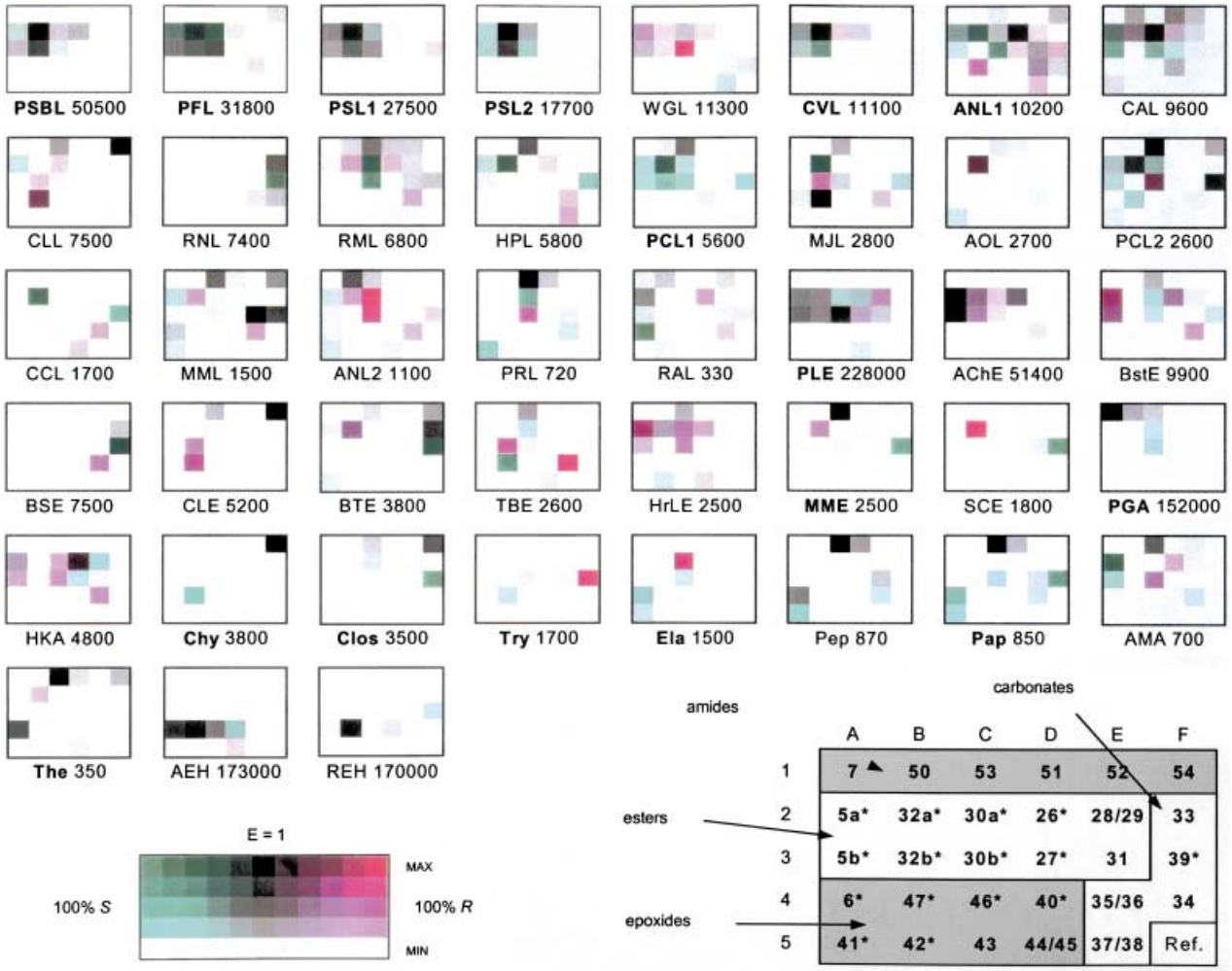

Figure 4. Selectivity arrays for enzyme activities. Substrates marked * in the layout (bottom right) are enantiomeric pairs. Below each array: Enzyme code, and apparent maximum rate in pM s^{-1}, which appears as the darkest (gray scale or color) square in each array. Enzymes marked in bold appear homogeneous (> 90%) by SDS-PAGE analysis (key for enzymes in Table 2). Conditions: 0.1 mg mL^{-1} enzyme, 100 μM substrate, 20 mM aq. borate pH 8.8 with 2.5% v/v DMF, 26 °C, 2 mg mL^{-1} BSA, 1 mM $NaIO_4$. To view this figure at a better resolution, please access the html data on Wiley Interscience (www.interscience.wiley.com).

served in this study are most probably batch-specific, as suggested, for example, by the different fingerprints observed with two different samples of the lipoprotein lipase of *Pseudomonas species* sold under two different product numbers by Fluka (PSL1 and PSL2).

Enzyme purity and substrate reactivities: Gel electrophoresis (SDS-PAGE) shows a strong, main band by commassie-blue staining for fifteen of the 43 samples tested, while most others contains only traces of detectable protein. These fifteen samples are presumably pure, or at least contain a large amount of a single protein which is presumably the listed enzyme (code in bold in Table 2 and Figure 4). There is no correlation between sample purity and the level of activity as defined either by the best array substrate or by the manufacturers data; this is not unexpected since enzymes have very different activity levels. In several cases unexpected functional groups occur as excellent substrates of certain enzymes, such as monoacetate (*R*)-**30a** for elastase, carbonate (*R*)-**39** for trypsin. Acetamide **54** reacts with *Candida lipolytica* esterase (CLE) and *Candida lipolytica* lipase (CLL), which is accompanied in both cases by a similarly weaker activity with the corresponding nitrophenol-derived phenylacetamide **53**. This pattern might show a protease impurity from this microorganism with an activity related to chymotrypsin (Chy), which reacts similarly with these two amide substrates. The weak, yet significant reactivites observed with the papain sample with phenylacetamide **53** is difficult to explain, since this thiol protease should be inactivated by oxidation in the presence of sodium periodate.

As mentionned above, epoxide (*R*)-**46** reacts in the reference plate without enzyme by a stereospecific reaction with BSA, which could be either hydrolysis or an alkylation of lysine residues, which both give periodate-sensitive products. The measurement with this substrate showed poor reproducibility and serveral artifacts; activities within 30% of the reference rate (Table 1) were ignored. Several other reactions of epoxide substrates are observed, such as the reaction of both enantiomers of **47**, which are the strongest reactivity observed with the lipase of *Mucor javanicus* (MJL). Epoxide reactivities occur at overall low activity levels and very probably reflect hydrolysis or alkylation reactions with either lysine side-chains of the proteins or other nucleophiles. Specific alkylation of proteins can be targeted to enzyme

active sites by using covalent inhibitors,[14] but may also occur generally and can used as a labeling strategy in the context of activity-based proteome analysis.[15]

Enzyme activities: Enzyme activities are classically defined and measured in Units (U), which corresponds to a micromole of a given reaction product produced per minute. Units are usually given per milligram of enzyme preparation ($U\,mg^{-1}$), as measured under a defined set of conditions, including a particular substrate at saturating concentration, buffer, pH, and temperature. Whether the substrate used is actually a good substrate for the enzyme, or the fact that some of the activity observed in a given test may be induced by non-enzymic impurities present in the enzyme sample being tested (typically when using nitrophenyl esters for lipases), is not being considered.

Here we have addressed the issue of measuring an enzyme's activity from a different viewpoint that encompasses a broader set of parameters. Using the periodate-coupled fluorogenic and chromogenic substrates solves the problem of nonspecific reactions, and guarantees that actual enzyme activities are being measured. By scanning a broad variety of substrates, we also address the issue of finding the best substrate for any given enzyme (Table 2). The series of substrates used here is very limited and was selected to provide stereochemical and structural diversity in a well-defined structure space that is quite remote from the optimum for many enzymes. Triglycerides of long-chain acids are the best substrates for lipases,[16] and none of these is included in our arrays. In the present series the monoacetates **30a** and **32a** are consistently the best substrates for lipases. The phenylacetamides **7**, **50**, and **53** possess the optimal acyl group for the enzyme penicillin G acylase, and react strongly with this enzyme. However we do not have any peptide substrates, which are the natural best substrates for proteases and can be used in combinatorial format for activity profiling of these enzymes.[17] The linear epoxides **6** and **47** react stronly with the epoxide hydrolases and are certainly excellent reporter substrates for these enzymes, in accordance with the fact that epoxide hydrolases react well with a broad variety of terminal epoxides.[18] Our measurement uses dilute substrates to enable to read stereoselectivities (see below), and, therefore, a direct comparison with Units is not possible. Nevertheless, our fingerprint measurement delivers a qualitatively much more complete assessment of enzyme activity than a single determination of Units.[19]

Stereoselectivity: In terms of synthetic applications, stereoselectivity is the most important property of enzymes beside their catalytic activity. Measuring isolated stereoisomers of a substrate separately reflects stereoselectivity in the sense of a kinetic resolution if the substrate concentrations used are below the Michaelis–Menten dissociation constant K_M of the substrates.[4][20] This condition is fulfilled in most cases with our assay, which uses a concentration of 100 μM for all substrates. This high dilution is possible due to the high sensitivity of detection of the end product, nitrophenol and umbelliferone, formed after the oxidative decomposition of the primary hydrolysis products. The stereoselectivities observed on our limited substrate series does not allow to predict the general utility of an enzyme for stereoselective reactions. In the present context, we find that probing enzyme activity with separated stereoisomers is a good way to differentiate between enzymes in the sense of producing selective fingerprints.

Array display: When dealing with large sets of measurements, the method used to display the results is quite important in making those results readable and interpretable. The two-color array display proposed here renders simultaneously activity and stereoselectivity on a large number of substrates and enzymes in compact manner, and, therefore, enables a manageable overview. It must be pointed out that the calculation and data formatting involved is minimal, extremely easy, and does not require any special software. This display might prove useful to show selectivity tables for other types of catalysts and substrates. The only drawback of the two-color display is of course its inacessibility to color-blind readers.

Conclusion

We have shown that periodate-coupled fluorogenic and chromogenic enzyme substrates can be assembled into arrays that allow to record specific activity and stereoselectivity fingerprints of enzymes. The most important aspect of these substrates is their resilience to nonspecific degradation, and the fact that our measurements are highly reproducible certainly depends on this property. Although the number of substrates used is small, it is well possible that such a limited substrate array will be sufficient to differentiate between thousands of enzymes. The key to a differentiating array is indeed not necessarily the absolute number of substrates used, but whether these show differential reactivities with different enzymes. We have installed structural and stereochemical variations very close to the reacting functional group of our substrates, and this probably explains the excellent differentiation observed here.

We are now enlarging and optimizing the structural and functional types to be integrated and the array to address all enzyme classes simultaneously. Specialized substrate arrays concentrated on one or two functional types might also prove useful to address the problem of functional convergence in enzymes. The interesting question to ask with such arrays will be whether two enzymes reacting identically on a given set of substrates, say 100 substrates, will also show the same activity for their reaction with the 101st substrate. If this is indeed the case, then classifying enzymes according to their fingerprints will be useful to pre-select enzymes for testing in synthetic applications. Activity analysis using substrate arrays might also allow to characterize and better understand the results of enzyme evolution experiments.

Experimental Section

General: All reactions were followed by TLC on Alugram SIL G/UV_{254} silica gel sheets (Macherey-Nagel) with detection by UV or with 0.5%

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phosphomolybdic acid solution in 95% EtOH. Silica gel 60 (Macherey-Nagel 230–400 mesh) was used for flash chromatography. Elution conditions given correspond to a retention factor $R_f = 0.3$ unless otherwise stated. Preparative TLC were performed on 0.5 or 2.0 mm Macherey-Nagel DC-Fertigplatten UV_{254} silica gel plates. Melting points were determined on a Kofler apparatus or with a Büchi 510 apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer 241 digital polarimeter with a 1 dm cell. ^{1}H NMR and ^{13}C NMR spectra were recorded with Bruker AC-200, AC-300 or Varian Gemini 200 spectrometers. Infra-red spectroscopy was performed on a Perkin–Elmer 1600 or Perkin–Elmer Spectrum One series FTIR apparatus. HPLC was done on a Waters 600 cntroller with a Waters 996 photodiode array detector, by using three different solvents: A (0.1% TFA in H_2O), B (H_2O/CH_3CN 50/50) and C (H_2O). Preparative HPLC was performed on a Waters Prep LC and Delta Prep 4000 with a Waters 486 tunable absorbance detector. The *ee* of all the epoxides were directly determined by analysis on a chiral HPLC column OD-H (Daicel: 25 cm × 0.46 cm inner diameter) with hexane/*i*PrOH as the eluent. Only in the case of epoxides (*R*)-, (*S*)-**46** and (*R*)-, (*S*)-**47** were the *ee* derived from those obtained in the same way for the corresponding precursors, the diols (*R*)-, (*S*)-**9**, and (*R*)-, (*S*)-**23**, respectively, by admitting that in the cyclization process[11] no change in the *ee* should occur (Table 3). The following compounds were prepared as previously described: 3-cyclopentenol,[21] the mixture (3:1) of 4-methyl-3-penten-1-ol and 4-methyl-4-penten-1-ol,[22] 3-butenyl-1-tosylate,[23a] (*E*)-3-hexenyl-1-tosylate,[23a] (*Z*)-3-hexenyl-1-tosylate,[23b] 3-methyl-3-butenyl-1-tosylate,[23c] 6-methoxy-2-naphtaldeyde,[24] methyl-2,3-*O*-isopropylidene-5-*O*-*p*-toluenesulfonyl-α-L-lyxofuranoside,[25] and methyl-2,3-*O*-isopropylidene-5-*O*-*p*-toluenesulfonyl-β-D-ribofuranoside.[26]

Table 3. Chiral HPLC data for epoxides and diols.

Compound	*ee* [%]	Conditions[a]	t_R [min]
(*R*)-**6**	96	a	36.4
(*S*)-**6**	87		40.1
(*R*)-**40**	94	b	38.1
(S)-**40**	92		34.8
(*R*)-**41**	99	b	38.0
(*S*)-**41**	70		33.8
(*R*,*R*)-**42**	> 99	b	36.5
(*S*,*S*)-**42**	> 99		32.5
(*R*)-**9**	> 99	c	26.1
(*S*)-**9**	> 99		32.4
(*R*)-**23**	96	c	41.8
(*S*)-**23**	96		45.5

[a] Injection condition analysis on chiral HPLC column OD-H (Daicel: 25 cm × 0.46 i.d); a: 8:2 hexane/*i*PrOH, 0.5 mL min^{-1}, $\lambda = 320$ nm; b: 8.5:1.5 hexane/*i*PrOH, 0.5 mL min^{-1}, $\lambda = 320$ nm; c: 9:1 hexane/*i*PrOH, 0.5 mL min^{-1}, $\lambda = 230$ nm.

(±)-7-(3,4-Dihydroxybutyloxy)-2*H*-1-benzopyran-2-one (1): *The following procedure is typical.* A solution of **69** (0.432 g, 2.0 mmol) in an acetone/water mixture (2.5:1; 15 mL) was treated at 25 °C under stirring with *N*-methylmorpholine-*N*-oxide (0.281 g, 1.2 equiv) and 2.5% solution of OsO_4 in *t*BuOH (0.1 mL) and stirred at 20 °C for 18 h. 10% aqueous Na_2SO_3 (2 mL) was added and stirring was prolonged for 30 min. The product was extracted with EtOAc (3 × 20 mL), washed (saturated aqueous NaCl), and purified by flash chromatography (7:3 CH_2Cl_2/acetone) to give **1** (0.440 g, 88%) as a solid. M.p. 97–99 °C; IR (KBr): $\tilde{\nu} = 3300$ (br s), 2934 (w), 2876 (w), 1724 (s), 1611 cm^{-1} (s); ^{1}H NMR (200 MHz, CD_3OD): $\delta = 7.83$ (d, $J =$ 9.8 Hz, 1H), 7.47 (d, $J = 8.3$ Hz, 1H), 6.92–6.85 (m, 2H), 6.21 (d, $J =$ 9.3 Hz, 1H), 4.23–4.16 (m, 2H), 3.93–3.81 (m, 1H), 3.54 (d, $J = 6.3$ Hz, 2H), 2.13–1.97 (m, 1H), 1.91–1.74 (m, 1H); ^{13}C NMR (50 MHz, CD_3OD): $\delta = 163.51$, 162.94, 156.64, 145.32, 129.99, 113.76, 113.51, 112.86, 101.83, 69.58, 67.03, 66.14, 33.54; elemental analysis calcd (%) for $C_{13}H_{14}O_5$: C 62.39, H 5.64; found: C 62.24, H 5.87.

(*S*)-7-(3,4-Dihydroxybutyloxy)-2*H*-1-benzopyran-2-one (1): *The following procedure is typical.*[27] A solution of AD-mix-α (4.2 g) in 1:1 *t*BuOH/water (30 mL) was stirred at 25 °C until both phases were clear[28] and then was cooled at 0 °C. Olefin **69** (0.648 g, 3.0 mmol) was added and the slurry was stirred at 0 °C for 18 h, following the reaction by TLC. Solid $Na_2S_2O_5$ (4.5 g) was added at 0 °C, and the resulting reaction mixture was warmed to room temperature and stirred at this temperature for 1 h. Extraction with EtOAc, evaporation, and flash chromatography (7:3 CH_2Cl_2/acetone) gave (*S*)-**1** (0.60 g, 80%), as a solid. M.p. 92–93 °C; $[\alpha]_D^{20} = -22.4$ ($c = 0.46$, MeOH); elemental analysis calcd (%) for $C_{13}H_{14}O_5$: C 62.39, H 5.64; found: C 62.11, H 5.87.

(*R*)-7-(3,4-Dihydroxybutyloxy)-2*H*-1-benzopyran-2-one (1): When the same reaction on **69** (0.648 g, 3.0 mmol) was repeated by the use of AD-mix-β, pure (*R*)-**1** (0.645 g, 86%) was obtained as a solid. M.p. 94–96 °C; $[\alpha]_D^{20} = +17.9$ ($c = 0.55$, MeOH); elemental analysis calcd (%) for $C_{13}H_{14}O_5$: C 62.39, H 5.64; found: C 62.12, H 5.41.

(±)-7-(4-Amino-3-hydroxybutyloxy)-2*H*-1-benzopyran-2-one (2): *The following procedure is typical.* A solution of epoxide **6** (0.464 g, 2.00 mmol) in EtOH saturated with NH_3 (16 mL) in a pressure tube was treated with $Yb(OTf)_3$[12] (10 mol%, 0.2 mmol, 0.124 g). The tube was sealed and the reaction was stirred at 25 °C for 1 h and then at 70 °C for 5 h (TLC). After cooling, the solution was filtered and evaporated to dryness to give pure **2** (0.448 g, 90%) as a yellow syrup. IR (KBr): $\tilde{\nu} = 3419$ (br s), 2954 (w), 1708 (s), 1615 (s), 1284 (s), 1236 (s), 1161 (s), 1132 (s), 1031 cm^{-1} (s); ^{1}H NMR (200 MHz, CD_3OD): $\delta = 7.83$ (d, $J = 9.8$ Hz, 1H), 7.48 (d, $J = 8.3$ Hz, 1H), 7.00–6.73 (m, 2H), 6.21 (d, $J = 9.3$ Hz, 1H), 4.19 (t, $J = 5.6$ Hz, 2H), 4.00–3.76 (m, 1H), 2.90 (dd, $J = 13.2$, 3.4 Hz, 1H), 2.73 (dd, $J = 12.7$, 8.3 Hz, 1H), 2.10–1.70 (m, 2H); ^{13}C NMR (50 MHz, CD_3OD): $\delta = 163.77$, 163.34, 157.01, 145.75, 130.45, 114.10, 113.35, 102.29, 68.71, 66.25, 47.65, 35.27; elemental analysis calcd (%) for $C_{13}H_{15}NO_4$: C 62.64, H 6.07, N 5.62; found: C 62.46, H 6.39, N 5.49.

(±)-7-(4-Acetoxy-3-hydroxybutyloxy)-2*H*-1-benzopyran-2-one (5a): *The following procedure is typical.* AcCl (0.36 mmol, 25 μL) was added to a solution of diol **1** (0.090 mg, 0.36 mmol) in dry CH_2Cl_2 (8.0 mL) and Et_3N (0.72 mmol, 0.1 mL) at 0 °C, under an argon atmosphere, and the reaction mixture was stirred at 0 °C for 1 h (TLC). Evaporation of the washed (brine) organic solution and preparative TLC (EtOAc/hexane 6:4) gave **5a** (0.063 g, 60%) as a semisolid. IR (neat): $\tilde{\nu} = 3448$ (br s), 3088 (w), 2954 (w), 1733 (br, s), 1615 (s), 1403 (m), 1352 (m), 1282 (m), 1232 (s), 1129 (s), 1048 cm^{-1} (m); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 7.61$ (d, $J = 9.8$ Hz, 1H), 7.33 (d, $J = 7.8$ Hz, 1H), 6.83–6.78 (m, 2H), 6.21 (d, $J = 8.8$ Hz, 1H), 4.29–4.01 (m, 5H), 2.09 (s, 3H), 2.00–1.91 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 171.82$, 162.58, 161.86, 156.52, 144.07, 129.49, 113.87, 113.49, 113.33, 102.17, 69.22, 67.72, 65.70, 33.27, 21.52; elemental analysis calcd (%) for $C_{15}H_{16}O_6$: C 61.64, H 5.52; found: C 61.47, H 5.23.

(*R*)-7-(4-Acetoxy-3-hydroxybutyloxy)-2*H*-1-benzopyran-2-one (5a): Following the same procedure, diol (*R*)-**1** (0.080 g, 0.32 mmol) yielded pure (*R*)-**5** (0.053 g, 57%, 90% *ee*) as a semisolid: $[\alpha]_D^{20} = +9.3$ ($c = 0.30$, $CHCl_3$); elemental analysis calcd (%) for $C_{15}H_{16}O_6$: C 61.64, H 5.52; found: C 61.91, H 5.79.

(*S*)-7-(4-Acetoxy-3-hydroxybutyloxy)-2*H*-1-benzopyran-2-one (5a): Following the same procedure, diol (*S*)-**1** (0.090 g, 0.36 mmol) yielded pure (*S*)-**5** (0.065 g, 62%, 95% *ee*), as a semisolid. $[\alpha]_D^{20} = -9.6$ ($c = 0.31$, $CHCl_3$); elemental analysis calcd (%) for $C_{15}H_{16}O_6$: C 61.64, H 5.52; found: C 61.86, H 5.77.

(±)-7-(3,4-Diacetoxybutyloxy)-2*H*-1-benzopyran-2-one (5b): *The following procedure is typical*. Ac_2O (1 mL) was added to a solution of diol (±)-**1** (0.040 g, 0.16 mmol) in dry pyridine (2 mL) at 0 °C. The reaction was left at 0 °C for 18 h. Co-evaporation of excess reagents with toluene followed by flash chromatography (CH_2Cl_2/acetone 9:1) gave **5b** (0.051 g, 96%) as a white solid. M.p. 80–82 °C; IR (KBr): $\tilde{\nu} = 3088$ (w), 3057 (w), 2996 (w), 2954 (w), 1742 (s), 1722 (s), 1711 (s), 1623 (s), 1357 (m), 1286 (m), 1234 (br s), 1132 (m), 1031 cm^{-1} (m); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 7.61$ (d, $J = 9.8$ Hz, 1H), 7.34 (d, $J = 8.8$ Hz, 1H), 6.82–6.74 (m, 2H), 6.22 (d, $J =$ 9.3 Hz, 1H), 5.35–5.24 (m, 1H), 4.32 (dd, $J = 11.7$, 3.4 Hz, 1H), 4.15–3.97 (m, 2H), 4.10 (dd, $J = 11.7$, 5.9 Hz, 1H), 2.16–2.05 (m, 2H), 2.05 (s, 6H); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 171.25$, 170.99, 162.29, 161.69, 156.40, 143.99, 129.46, 113.84, 113.55, 113.35, 101.88, 69.42, 65.47, 65.03, 30.96, 21.60, 21.37; elemental analysis calcd (%) for $C_{17}H_{18}O_7$: C 61.07, H 5.43; found: C 61.29, H 5.68.

(*R*)-7-(3,4-Diacetoxybutyloxy)-2*H*-1-benzopyran-2-one (5b): Following the procedure for (±)-**5b**, diol (*R*)-**1** (0.100 g, 0.40 mmol) afforded pure (*R*)-**5b** (0.120 g, 90%, 90% *ee*) as a white solid. M.p. 76–78 °C; $[\alpha]_D^{20} = +9.6$ ($c = 0.65$, $CHCl_3$); elemental analysis calcd (%) for $C_{17}H_{18}O_7$: C 61.07, H 5.43; found: C 60.84, H 5.21.

(*S*)-7-(3,4-Diacetoxybutyloxy)-2*H*-1-benzopyran-2-one (5b): Following the procedure for (±)-**5b**, diol (*S*)-**1** (0.100 g, 0.40 mmol) afforded pure (*S*)-**5b** (0.123 g, 92%, 95% *ee*) as a white solid. M.p. 74–76 °C; $[\alpha]_D^{20} = -9.8$ ($c =$ 0.50, $CHCl_3$); elemental analysis calcd (%) for $C_{17}H_{18}O_7$: C 61.07, H 5.43; found: C 61.15, H 5.69.

(±)-7-(3,4-Epoxybutyloxy)-2*H*-1-benzopyran-2-one (6): *The following procedure is typical.* A solution of **69** (0.75 g, 3.47 mmol) in CH_2Cl_2 (15 mL) was treated at 0 °C with 70% *m*-CPBA (1.111 g, 4.51 mmol). After 18 h at 0 °C, the solution was washed (10% aqueous Na_2SO_3, 5% aqueous NaOH, and water), evaporated, and purified by flash chromatography (6:4 mixture of hexane/EtOAc) to give **6** (0.708 g, 88%) as a solid. M.p. 67–69 °C; IR (KBr): $\tilde{\nu} = 3084$ (w), 3051 (w), 2992 (w), 2961 (w) 2932 (w), 1714 (s), 1614 (s), 1295 (m), 1241 (m), 1136 (s), 1119 (s), 1020 cm^{-1} (m); ^1H NMR (200 MHz, $CDCl_3$): $\delta = 7.58$ (d, 1H, $J = 9.8$ Hz), 7.31 (d, 1H, $J = 8.3$ Hz), 6.81–6.75 (m, 2H), 6.18 (d, 1H, $J = 9.8$ Hz), 4.19–4.03 (m, 2H), 3.14–3.05 (m, 1H), 2.78 (t, 1H, $J = 4.9$ Hz), 2.53 (dd, 1H, $J = 4.9$, 2.4 Hz), 2.20–2.04 (m, 1H), 1.97–1.79 (m, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 162.53$, 161.84, 156.44, 144.08, 129.46, 113.78, 113.41, 113.29, 102.06, 65.93, 50.08, 47.80, 32.81; elemental analysis calcd (%) for $C_{13}H_{12}O_4$: C 67.23, H 5.21; found: C 67.02, H 5.00.

(*S*)-7-(3,4-Epoxybutyloxy)-2*H*-1-benzopyran-2-one (6): *The following procedure is typical*.[29] Trimethyl orthoacetate (0.1 mL, 0.78 mmol) and PPTS (1 mg) were added at room temperature to a stirred solution of (*S*)-**1** (0.150 g, 0.60 mmol) in dry CH_2Cl_2 (1 mL) under argon atmosphere. After 40 min (the disappearance of the diol was monitored by TLC) evaporation of the volatile compounds afforded a crude residue, which was taken up with CH_2Cl_2 (1 mL) and treated with trimethylsilyl chloride (0.78 mmol, 0.1 mL). After 1 h stirring, the organic solvent was evaporated. The residue was dissolved in MeOH (1 mL). K_2CO_3 (0.204 g, 1.48 mmol) was added, and the resulting suspension was vigorously stirred for 2 h at 25 °C, then filtered and evaporated. The residue was purified by flash chromatography (6:4 hexane/EtOAc) to give (*S*)-**6** (0.097 g, 70%, 87% *ee*) as a solid. M.p. 61–64 °C; $[\alpha]_D^{20} = -23.0$ ($c = 0.30$, $CHCl_3$); elemental analysis calcd (%) for $C_{13}H_{12}O_4$: C 67.23, H 5.21; found: C 66.97, H 5.08.

(*R*)-7-(3,4-Epoxybutyloxy)-2*H*-1-benzopyran-2-one (6): Following the procedure for (*S*)-**6**, diol (*R*)-**1** (0.150 g, 0.60 mmol) yielded pure (*R*)-**6** (0.104 g, 75%, 96% *ee*) as a solid. M.p. 60–63 °C; $[\alpha]_D^{20} = +21.8$ ($c = 0.45$, $CHCl_3$); elemental analysis calcd (%) for $C_{13}H_{12}O_4$: C 67.23, H 5.21; found: C 67.39, H 5.52.

(±)-7-(4-Phenylacetamido-3-hydroxybutyloxy)-2*H*-1-benzopyran-2-one (7): *The following procedure is typical*. A solution of amino alcohol **2** (0.075 g, 0.3 mmol) in dry CH_2Cl_2 (7.0 mL) and Et_3N (0.1 mL) was treated at 0 °C with phenylacetyl chloride (0.3 mmol, 40 µL). After 2 h at 0 °C (TLC), aqueous workup (CH_2Cl_2/saturated aqueous NaCl) and flash chromatography (EtOAc) gave **7** (0.044 g, 40%) as a semisolid. IR (KBr): $\tilde{\nu} = 3426$ (brs), 3088 (w), 2931 (w), 1709 (s), 1642 (s), 1614 (s), 1556 (m), 1385 (m), 1283 (m), 1232 (m), 1129 cm^{-1} (s); ^1H NMR (200 MHz, $CHCl_3$): $\delta = 7.56$ (d, $J = 9.8$ Hz, 1H), 7.46–7.00 (m, 6H), 6.74–6.57 (m, 2H), 6.17 (d, $J = 9.8$ Hz, 1H), 4.16–3.07 (m, 2H), 3.97–3.77 (m, 1H), 3.54 (s, 2H), 3.40 (ddd, $J = 13.9$, 6.1, 3.2 Hz, 1H), 3.24–3.05 (m, 1H), 1.90–1.62 (m, 2H); ^{13}C NMR (75 MHz, CD_3OD): $\delta = 174.46$, 163.93, 157.14, 145.78, 136.93, 130.42, 130.13, 129.59, 127.92, 114.22, 114.01, 113.32, 102.28, 68.10, 66.34, 46.70, 43.86, 35.08; elemental analysis calcd (%) for $C_{21}H_{21}NO_5$: C 68.65, H 5.76, N 3.81; found: C 68.34, H 5.93, N 3.69.

(±)-7-(3,4-Dihydroxybutyloxy)-2*H*-1-benzopyran-2-one-3,4-bis-(dihydrogenphosphate) (8): *The following procedure is typical.* A solution of **55** (0.035 g, 0.045 mmol) in a mixture ethanol/water (4:1, 10 mL) is treated with Pd on charcoal (0.003 g, 0.1 equiv) at 25 °C under 1 atmosphere H_2 and stirred vigorously for 3 h. Filtration over Celite and concentration in vacuo gave pure **8** (0.020 g, quant.) as a syrup. IR (KBr): $\tilde{\nu} = 3480$ (brs), 1706 (m), 1620 (s), 1557 (w), 1509 (w), 1402 (w), 1385 (m), 1285 (m), 1136 (m), 1081 cm^{-1} (m); ^1H NMR (300 MHz, D_2O): $\delta = 7.88$ (d, $J = 9.2$ Hz, 1H), 7.50 (d, $J = 8.5$ Hz, 1H), 6.96–6.91 (m, 2H), 6.23 (d, $J = 9.2$ Hz, 1H), 4.53 (m, 1H), 4.20 (m, 2H), 4.00 (m, 2H), 2.12 (m, 2H); ^{13}C NMR (75 MHz, D_2O/[D_6]Ac): $\delta = 164.65$, 163.20, 156.27, 146.78, 130.77, 114.58, 113.87, 112.85, 102.49, 65.98, 60.82, 58.22, 32.17; ^{31}P NMR (81 MHz, D_2O): $\delta = 4.1$ (d, $J =$ 16.2 Hz), 3.3 (d, $J = 16.2$ Hz); HRMS: calcd for $C_{13}H_{17}O_{11}P_2^+$: 411.0246; observed: 411.0250.

(±)-2-(6-Methoxy-2-naphthyl)-1,2-ethanediol (9): Application of the procedure for (±)-**1** to **71** (0.300 g, 1.63 mmol), and flash chromatography (7:3 CH_2Cl_2/acetone) gave **9** (0.231 g, 65%) as a solid. M.p. 148–150 °C; IR (KBr): $\tilde{\nu} = 3339$ (brs), 3060 (w), 3011 (w), 2924 (w), 2888 (w), 2839 (w), 1637 (m), 1608 (S), 1487 (s), 1463 (S), 1261 (s), 1214 (s), 1165 (s), 1080 (s), 1030 cm^{-1} (s); ^1H NMR (200 MHz, CD_3OD): $\delta = 7.84–7.70$ (m, 3H), 7.48 (dd, $J = 8.6$, 1.5 Hz, 1H), 7.24 (d, $J = 2.5$ Hz, 1H), 7.14 (dd, $J = 9.0$, 2.6 Hz, 1H), 4.84 (t, $J = 6.1$ Hz, 1H), 3.93 (s, 3H), 3.73 (d, $J = 6.3$ Hz, 2H); ^{13}C NMR (75 MHz, CD_3OD): $\delta = 159.13$, 138.40, 135.70, 130.33, 130.19, 127.88, 126.08, 126.02, 119.79, 106.61, 76.04, 68.65, 55.69; elemental analysis calcd (%) for $C_{13}H_{14}O_3$: C 71.54, H 6.47; found: C 71.25, H 6.11.

(*S*)-2-(6-Methoxy-2-naphthyl)-1,2-ethanediol (9): Application of the procedure for (*S*)-**1** to **71** (0.368 g, 2.0 mmol) by using AD-mix-α followed by flash chromatography (7:3 EtOAc/hexane) gave (*S*)-**9** (0.222 g, 51%, >99% *ee*), as a solid. M.p. 163–164 °C; $[\alpha]_D^{20} = +36.2$ ($c = 0.40$, MeOH); elemental analysis calcd (%) for $C_{13}H_{14}O_3$: C 71.54, H 6.47; found: C 71.63, H 6.21.

(*R*)-2-(6-Methoxy-2-naphthyl)-1,2-ethanediol (9): Application of the procedure for (*R*)-**1** to **71** (0.368 g, 2.0 mmol) with AD-mix-β gave (*R*)-**9** (0.240 g, 55%, >99% *ee*) as a solid. M.p. 162–163 °C; $[\alpha]_D^{20} = -36.0$ ($c =$ 0.42, MeOH); elemental analysis calcd (%) for $C_{13}H_{14}O_3$: C 71.54, H 6.47; found: C 71.42, H 6.15.

7-(3-Methyl-3-butenyloxy)-2*H*-1-benzopyran-2-one (11): Application of the procedure for **69** to 3-methyl-3-butenyl-1-tosylate[23c] (1.20 g, 5.0 mmol) afforded **11** (0.87 g, 76%). Flash chromatography (hexane/EtOAc 7:3) of an analytical sample gave pure **11**, as a solid. M.p. 50–52 °C; IR (KBr): $\tilde{\nu} =$ 3080 (w), 3054 (w), 2937 (w), 2878 (w), 1724 (s), 1612 cm^{-1} (s); ^1H NMR (200 MHz, $CDCl_3$): $\delta = 7.64$ (d, $J = 9.3$ Hz, 1H), 7.37 (d, $J = 8.8$ Hz, 1H), 6.89–6.78 (m, 2H), 6.25 (d, $J = 9.3$ Hz, 1H), 4.87 (s, 1H), 4.81 (s, 1H), 4.14 (t, $J = 6.8$ Hz, 2H), 2.54 (t, $J = 6.8$ Hz, 2H), 1.82 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 162.85$, 161.87, 156.61, 144.08, 142.26, 129.44, 113.71, 113.67, 113.24, 113.12, 102.15, 67.76, 37.55, 23.43; elemental analysis calcd (%) for $C_{14}H_{14}O_3$: C 73.03, H 6.13; found: C 73.31, H 5.92.

7-(4-Methyl-3-pentenyloxy)-2*H*-1-benzopyran-2-one (12) and 7-(4-methyl-4-pentenyloxy)-2*H*-1-benzopyran-2-one (74): Application of the procedure for **69** to the 3:1 mixture of **67** and **68** (1.60 g, 6.30 mmol) afforded a corresponding mixture of **12** and **74** (1.18 g, 77%) which were not separated, but directly used in the next step. Compound **12**: ^1H NMR (200 MHz, $CDCl_3$): $\delta = 7.60$ (d, $J = 9.3$ Hz, 1H), 7.33 (d, $J = 8.3$ Hz, 1H), 6.86–6.70 (m, 2H), 6.19 (d, $J = 9.3$ Hz, 1H), 5.20–5.10 (m, 1H), 3.94 (t, $J =$ 7.0 Hz, 2H), 2.52–2.43 (m, 2H), 1.70 (s, 3H), 1.63 (s, 3H). Compound **74**: ^1H NMR (200 MHz, $CDCl_3$): $\delta = 4.72$ (s, 1H), 4.68 (s, 1H), 4.20–3.94 (m, 2H), 2.30–1.85 (m, 4H).

(*E*)-7-(3-Hexenyloxy)-2*H*-1-benzopyran-2-one (13): The procedure for **69** applied to (*E*)-3-hexenyl-1-tosylate[23a] (2.50 g, 9.84 mmol) afforded a crude liquid product consisting of practically pure **13** (1.80 g, 75%). Flash chromatography (hexane/EtOAc 8:2) of an analytical sample gave pure **13**, as a liquid. ^1H NMR (200 MHz, $CDCl_3$): $\delta = 7.60$ (d, 1H, $J = 9.3$ Hz), 7.33 (d, 1H, $J = 8.8$ Hz), 6.85–6.72 (m, 2H), 6.20 (d, $J = 9.3$ Hz) , 1H, 5.70–5.53 (m, 1H), 5.52–5.34 (m, 1H), 3.98 (t, $J = 6.8$ Hz, 2H), 2.47 (q, $J = 6.8$ Hz, 2H), 2.01 (quintet, $J = 7.0$ Hz, 2H), 0.95 (t, $J = 7.3$ Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 162.89$, 161.82, 156.48, 144.06, 136.00, 129.36, 124.42, 113.54, 113.23, 113.07, 102.04, 69.04, 32.82, 26.24, 14.31; elemental analysis calcd (%) for $C_{15}H_{16}O_3$: C 73.75, H 6.60; found: C 73.49, H 6.84.

(*Z*)-7-(3-Hexenyloxy)-2*H*-1-benzopyran-2-one (14): Application of the procedure for **69** to (*Z*)-3-hexenyl-1-tosylate[23b] (2.50 g, 9.84 mmol) yielded a crude product consisting of practically pure **14** (1.90 g, 79%). An analytical sample was purified by flash chromatography (hexane/EtOAc 8:2) to give pure **14** as a semisolid. IR (Nujol): $\tilde{\nu} = 3084$ (w), 1730 (s), 1612 (s), 1276 (m), 1224 (m), 1121 cm^{-1} (m); ^1H NMR (200 MHz, $CDCl_3$): $\delta =$ 7.63 (d, $J = 9.3$ Hz, 1H), 7.32 (d, $J = 8.3$ Hz, 1H), 6.72–6.90 (m, 2H), 6.24 (d, $J = 9.8$ Hz, 1H), 5.29–5.64 (m, 2H), 3.97 (t, $J = 6.8$ Hz, 2H), 2.52 (q, $J =$ 6.8 Hz, 2H), 2.06 (quintet, $J = 7.0$ Hz, 2H), 0.96 (t, $J = 7.3$ Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 162.80$, 161.73, 156.45, 144.00, 135.40, 129.33, 124.08, 113.52, 113.45, 113.05, 101.99, 68.72, 27.63, 21.27, 14.78; elemental analysis calcd (%) for $C_{15}H_{16}O_3$: C 73.75, H 6.60; found: C 73.51, H 6.89.

(±)-7-(3,4-Dihydroxy-3-methylbutyloxy)-2*H*-1-benzopyran-2-one (15): *The following procedure is typical*. A solution of epoxide (±)-**40** (0.096 g, 0.391 mmol) in THF (4 mL) was treated with 0.2 N aqueous H_2SO_4 (4 mL) and the reaction mixture was stirred at 25 °C for 2 h. Aqueous workup (EtOAc/saturated aqueous $NaHCO_3$) and flash chromatography (7:3

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CH_2Cl_2/acetone) gave **15** (0.085 g, 83%) as a solid. M.p. 104–106 °C; IR (KBr): $\tilde{\nu}$ = 3463 (brs), 3425 (brs), 3076 (w), 2934 (w), 2884 (w), 1700 (s), 1613 cm^{-1} (S); ^1H NMR (200 MHz, CD_3OD): δ = 7.88 (d, J = 9.8 Hz, 1H), 7.53 (d, J = 9.3 Hz, 1H), 6.99–6.87 (m, 2H), 6.24 (d, J = 9.8 Hz, 1H), 4.24 (t, J = 7.1 Hz, 2H), 3.43 (s, 2H), 2.02 (t, J = 6.8 Hz, 2H), 1.24 (s, 3H); ^{13}C NMR (50 MHz, CD_3OD): δ = 163.36, 162.86, 156.57, 145.25, 129.89, 113.72, 113.43, 112.76, 101.76, 72.33, 70.17, 65.75, 37.85, 23.96; elemental analysis calcd (%) for $C_{14}H_{16}O_5$: C 63.63, H 6.10; found: C 63.37, H 6.41.

(*S*)-7-(3,4-Dihydroxy-3-methylbutyloxy)-2*H*-1-benzopyran-2-one (15): Application of the procedure for (*S*)-**1** to olefin **11** (0.40 g, 1.74 mmol) gave (*S*)-**15** (0.32 g, 70%), as a solid. M.p. 100–102 °C; $[\alpha]_D^{20} = -8.3$ (c = 0.62, MeOH); elemental analysis calcd (%) for $C_{14}H_{16}O_5$: C 63.63, H 6.10; found: C 63.29, H 5.81.

(*R*)-7-(3,4-Dihydroxy-3-methylbutyloxy)-2*H*-1-benzopyran-2-one (15): Application of the procedure for (*R*)-**1** to olefin **11** (0.40 g, 1.74 mmol) gave (*R*)-**15** (0.31 g, 67%) as a solid. M.p. 98–100 °C; $[\alpha]_D^{20} = +6.1$ (c = 1.06, MeOH); elemental analysis calcd (%) for $C_{14}H_{16}O_5$: C 63.63, H 6.10; found: C 63.82, H 5.92.

(±)-7-(3,4-Dihydroxy-4-methylpentyloxy)-2*H*-1-benzopyran-2-one (16): Application of the procedure for (±)-**1** to the 3:1 mixture of **12** and **74** (0.51 g, 2.09 mmol) and flash chromatography (7:3 CH_2Cl_2/acetone) gave **16** (0.34 g, 58%) as a solid. M.p. 92–94 °C; IR (KBr): $\tilde{\nu}$ = 3391 (brs), 3097 (w), 2974 (w), 2964 (w), 2946 (w), 1736 (s), 1615 (S), 1283 (m), 1229 (m), 1164 (m), 1128 cm^{-1} (m); ^1H NMR (200 MHz, CD_3OD): δ = 7.87 (d, J = 9.3 Hz, 1H), 7.52 (d, J = 8.3 Hz, 1H), 7.00–6.85 (m, 2H), 6.23 (d, J = 9.1 Hz, 1H), 4.23 (dd, J = 7.8, 4.9 Hz, 2H), 3.56 (dd, J = 10.7, 1.7 Hz, 1H), 2.27–2.05 (m, 1H), 1.85–1.62 (m, 1H), 1.21 (s, 3H), 1.18 (s, 3H); ^{13}C NMR (50 MHz, CD_3OD): δ = 163.91, 163.28, 156.92, 145.66, 130.30, 114.10, 113.78, 113.17, 102.17, 75.63, 73.50, 67.37, 31.91, 25.79, 24.95; elemental analysis calcd (%) for $C_{15}H_{18}O_5$: C 64.74, H 6.52; found: C 64.96, H 6.31.

(*S*)-7-(3,4-Dihydroxy-4-methylpentyloxy)-2*H*-1-benzopyran-2-one (16): Application of the procedure for (*S*)-**1** to the 3:1 mixture of **12** and **74** (2.0 g, 8.20 mmol) and flash chromatography (7:3 CH_2Cl_2/acetone) gave (*S*)-**16** (40% *ee*).[21] The product was recrystallized from diisopropyl ether to give pure (*S*)-**16** as a solid (70% *ee*). M.p. 104–105 °C; $[\alpha]_D^{20} = -32.5$ (c = 0.33, MeOH); elemental analysis calcd (%) for $C_{15}H_{18}O_5$: C 64.74, H 6.52; found: C 64.89, H 6.23.

(*R*)-7-(3,4-Dihydroxy-4-methylpentyloxy)-2*H*-1-benzopyran-2-one (16): Application of the procedure for (*R*)-**1** to the mixture of **12** and **74** (2.0 g, 8.20 mmol) gave (*R*)-**16** as a solid (61% *ee*),[30] which was recrystallized from diisopropyl ether to give pure (*R*)-**16** as a solid (99% *ee*). M.p. 105–106 °C; $[\alpha]_D^{20} = +31.0$ (c = 0.12, MeOH); elemental analysis calcd (%) for $C_{15}H_{18}O_5$: C 64.74, H 6.52; found: C 64.50, H 6.31.

(*S*,*S*)-7-(3,4-Dihydroxyhexyloxy)-2*H*-1-benzopyran-2-one (17): Application of the procedure for (*S*)-**1** to olefin **13** (0.430 g, 1.762 mmol) gave (*S*,*S*)-**17** (0.33 g, 67%) as a solid. M.p. 134–136 °C; $[\alpha]_D^{20} = 27.1$ (c = 0.38, MeOH); IR (KBr): $\tilde{\nu}$ = 3410 (brs), 3308 (brs), 3060 (w), 2954 (w), 2920 (w), 2886 (w), 1713 (s), 1615 (S), 1283 (m), 1235 (m), 1129 cm^{-1} (m); ^1H NMR (200 MHz, CD_3OD): δ = 7.87 (d, J = 9.3 Hz, 1H), 7.52 (d, J = 7.8 Hz, 1H), 6.99–6.86 (m, 2H), 6.23 (d, J = 9.8 Hz, 1H), 4.28–4.14 (m, 2H), 3.79–3.64 (m, 1H), 3.42–3.27 (m, 1H), 2.14–1.80 (m, 2H), 1.73–1.38 (m, 2H), 0.99 (t, J = 7.3 Hz, 3H); ^{13}C NMR (50 MHz, CD_3OD): δ = 163.99, 163.36, 157.03, 145.71, 130.38, 114.15, 113.89, 113.22, 102.22, 76.90, 71.41, 66.93, 33.67, 26.74, 10.74; elemental analysis calcd (%) for $C_{15}H_{18}O_5$: C 64.74, H 6.52; found: C 64.51, H 6.17.

(*R*,*R*)-7-(3,4-Dihydroxyhexyloxy)-2*H*-1-benzopyran-2-one (17): Application of the procedure for (*R*)-**1** to olefin **13** (0.430 g, 1.762 mmol) gave (*R*,*R*)-**17** (0.348 g, 71%) as a solid. M.p. 130–132 °C; $[\alpha]_D^{20} = +22.1$ (c = 0.83, MeOH); elemental analysis calcd (%) for $C_{15}H_{18}O_5$: C 64.74, H 6.52; found: C 64.48, H 6.27.

(3*R,4*S**)-7-(3,4-Dihydroxyhexyloxy)-2*H*-1-benzopyran-2-one (18)**: Application of the procedure for (±)-**1** to **14** (0.300 g, 1.23 mmol) and flash chromatography (7:3 CH_2Cl_2/acetone) gave (3*R**,4*S**)-**18** (0.270 g, 79%) as a white solid. M.p. 112–114 °C; IR (KBr): $\tilde{\nu}$ = 3259 (brs), 2966 (w), 2933 (w), 2877 (w), 1733 (s), 1623 (s), 1555 (m), 1406 (m), 1295 (s), 1235 (s), 1141 (s), 1090 (m), 1051 cm^{-1} (m); ^1H NMR (200 MHz, CD_3OD): δ = 7.83 (d, J = 9.3 Hz, 1H), 7.48 (d, J = 8.3 Hz, 1H), 6.93–6.81 (m, 2H), 6.19 (d, J = 9.8 Hz, 1H), 4.29–4.13 (m, 2H), 3.74–3.55 (m, 1H), 3.42–3.22 (m, 1H), 2.29–2.06 (m, 1H), 1.90–1.55 (m, 2H), 1.53–1.22 (m, 1H), 0.97 (t, J = 7.3 Hz, 3H); ^{13}C NMR (50 MHz, CD_3OD): δ = 164.03, 163.36, 157.07, 145.75, 130.36, 114.18, 113.90, 113.20, 102.26, 77.51, 72.20, 66.94, 33.07, 26.71, 10.63; elemental analysis for $C_{15}H_{18}O_5$: C 64.74, H 6.52; found: C 64.85, H 6.33.

7-(3-Cyclopentenyloxy)-2*H*-1-benzopyran-2-one (19): Application of the procedure for **69** to **66** (2.38 g, 10.0 mmol) afforded practically pure **19** (1.892 g, 83%). An analytical sample was recrystallized from petroleum ether (b.p. 80–100 °C) to give **19** as a solid. M.p. 112–114 °C; IR (KBr): $\tilde{\nu}$ = 3063 (w), 3043 (w), 2957 (w), 2927 (w) 2908 (w), 1718 (s), 1610 (s), 1233 (m), 1127 cm^{-1} (m); ^1H NMR (200 MHz, $CDCl_3$): δ = 7.62 (d, J = 9.3 Hz, 1H), 7.34 (d, J = 8.3 Hz, 1H), 6.86–6.70 (m, 2H), 6.21 (d, J = 9.8 Hz, 1H), 5.75 (s, 2H), 5.09–4.93 (m, 1H), 2.85 (dd, J = 17.0, 6.8 Hz, 2H), 2.55 (d, J = 18.1 Hz, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ = 161.91, 161.70, 156.39, 144.15, 129.42, 128.93, 114.32, 113.45, 112.90, 102.68, 77.99, 40.48; elemental analysis calcd (%) for $C_{14}H_{12}O_3$: C 73.67, H 5.30; found: C 73.40, H 5.04.

(1r,3*R*,4*S*)-7-(3,4-Dihydroxycyclopentyloxy)-2*H*-1-benzopyran-2-one (20) and (1s,3*R*,4*S*)-7-(3,4-dihydroxycyclopentyloxy)-2*H*-1-benzopyran-2-one (21): Application of the procedure for (±)-**1** to olefin **19** (0.35 g, 1.53 mmol) afforded a crude solid product consisting of a 2:1 mixture (^1H NMR) of diols **20** and **21**. Flash chromatography (silica gel 60 RP-18, elution with 1:1 MeOH/H_2O) gave diol **20** (0.187 g, 47%) and **21** (0.067 g, 17%). **20**: white solid. M.p. 171–173 °C; IR (KBr): $\tilde{\nu}$ = 3385 (brs), 3077 (w), 2942 (w), 1708 (s), 1619 (s), 1350 (m), 1282 (m), 1235 (m), 1123 (m), 1111 (m), 1099 cm^{-1} (m); ^1H NMR (200 MHz, CD_3OD): δ = 7.87 (d, J = 9.8 Hz, 1H), 7.51 (d, J = 8.8 Hz, 1H), 6.97–6.77 (m, 2H), 6.23 (d, J = 9.3 Hz, 1H), 4.97–4.73 (m, 1H), 4.07–3.87 (m, 2H), 2.50–2.30 (m, 2H), 2.00–1.80 (m, 2H); ^{13}C NMR (50 MHz, CD_3OD): δ = 163.46, 163.06, 157.14, 145.82, 130.45, 115.00, 113.91, 113.24, 103.14, 76.89, 73.62, 39.00; elemental analysis calcd (%) for $C_{14}H_{14}0_5$: C 64.12, H 5.38; found: C 64.33, H 5.61. Compound **21**: white solid; m.p. 148–150 °C; IR (KBr): $\tilde{\nu}$ = 3431 (s), 3344 (s), 3081 (w), 2910 (w), 1704 (s), 1621 (s), 1554 (m), 1287 (m), 1240 (m), 1137 (s), 1015 cm^{-1} (m); ^1H NMR (200 MHz, CD_3OD): δ = 7.86 (d, J = 9.8 Hz, 1H), 7.51 (d, J = 8.8 Hz, 1H), 6.91–6.74 (m, 2H), 6.23 (d, J = 9.8 Hz, 1H), 5.03–4.91 (m, 1H), 4.19 (t, J = 4.4 Hz, 2H), 2.34–2.08 (m, 2H), 2.07–1.88 (m, 2H); ^{13}C NMR (50 MHz, CD_3OD): δ = 162.34, 162,79, 157.10, 145.73, 130.51, 114.71, 113.96, 113.35, 103.19, 77.60, 73.65, 39.34; elemental analysis calcd (%) for $C_{14}H_{14}O_5$: C 64.12, H 5.38; found: C 63.91, H 5.59.

(3*R,4*R**)-7-(3,4-Dihydroxycyclopentyloxy)-2*H*-1-benzopyran-2-one (22)**: Application of the procedure for **15** to the 1:2 mixture of epoxides **45** and **44** (0.20 g, 0.82 mmol) and flash chromatography (1:1 CH_2Cl_2/acetone) gave **22** (0.142 g, 66%) as a solid. M.p. 122–124 °C; IR (KBr): $\tilde{\nu}$ = 3374 (brs), 3075 (w), 2935 (w), 1732 (s), 1612 (S), 1352 (m), 1280 (m), 1233 (m), 1122 cm^{-1} (s); ^1H NMR (200 MHz, CD_3OD): δ = 7.78 (d, J = 9.3 Hz, 1H), 7.42 (d, J = 8.3 Hz, 1H), 6.84–6.72 (m, 2H), 6.17 (d, J = 9.3 Hz, 1H), 4.98–4.87 (m, 1H), 4.14 (quartet, J = 5.2 Hz, 1H), 4.07–3.91 (m, 1H), 2.63 (dt, J = 14.5, 7.2 Hz, 1H), 2.25–2.00 (m, 2H), 1.74 (dt, J = 14.6, 3.9 Hz, 1H); ^{13}C NMR (50 MHz, CD_3OD): δ = 163.24, 162.69, 156.89, 145.60, 130.35, 114.73, 113.77, 113.20, 103.06, 78.17, 78.09, 77.59, 39.73; elemental analysis calcd (%) for $C_{14}H_{14}O_5$: C 64.12, H 5.38; found: C 64.01, H 5.10.

(±)-4-(*p*-Nitrophenoxy)-1,2-butanediol (23): Application of the procedure for (±)-**1** to **70** (0.080 g, 0.414 mmol) and flash chromatography (1:1 CH_2Cl_2/acetone) gave **23** (0.070 g, 74%) as a solid. M.p. 98–100 °C; IR (KBr): $\tilde{\nu}$ = 3380 (brs), 3275 (brs), 3081 (w), 2964 (w), 2926 (w), 2880 (w), 1610 (s), 1597 (s), 1513 (s), 1499 (s), 1389 (m), 1333 (s), 1303 (m), 1265 (s), 1243 (m), 1176 (m), 1124 (m), 1105 (m), 1067 (m), 1046 cm^{-1} (m); ^1H NMR (200 MHz, CD_3OD): δ = 8.2 (d, J = 9.3 Hz, 2H), 7.1 (d, J = 9.3 Hz, 2H), 4.37–4.14 (m, 2H), 3.93–3.76 (m, 1H), 3.53 (d, J = 5.9 Hz, 2H), 2.19–1.97 (m, 1H), 1.93–1.72 (m, 1H); ^{13}C NMR (75 MHz, CD_3OD): δ = 165.68, 142.71, 126.79, 115.75, 69.92, 67.41, 66.80, 33.93; elemental analysis calcd (%) for $C_{10}H_{13}NO_5$: C 52.86, H 5.77, N 6.16; found: C 52.55, H 5.91, N 5.90.

(*S*)-4-(*p*-Nitrophenoxy)-1,2-butanediol (23): Application of the procedure for (*S*)-**1** to **70** (0.193 g, 1.0 mmol) and flash chromatography (1:1 CH_2Cl_2/acetone) gave (*S*)-**23** (0.181 g, 80%, 96% *ee*), as a solid. M.p. 121–122 °C; $[\alpha]_D^{20} = -31.1$ (c = 0.37, MeOH); elemental analysis calcd (%) for $C_{10}H_{13}NO_5$: C 52.86, H 5.77, N 6.16; found: C 52.98, H 5.53, N 5.96.

(*R*)-4-(*p*-Nitrophenoxy)-1,2-butanediol (23): Application of the procedure for (*R*)-**1** to **70** (0.193 g, 1.0 mmol) gave (*R*)-**23** (0.188 g, 83%, 96% *ee*) as a solid. M.p. 120–121 °C; $[\alpha]_D^{20} = +31.3$ (c = 0.37, MeOH); elemental analysis calcd (%) for $C_{10}H_{13}NO_5$: C 52.86, H 5.77, N 6.16; found: C 52.64, H 5.45, N 5.91.

7-[5-(Methyl-α-L-lyxofuranosyloxy)]-2*H*-1-benzopyran-2-one (24): Compound **72** (0.039 g, 0.11 mmol) was suspended in methanol (10 mL), and 2 mL of a 50% aqueous TFA solution were added. Overnight reflux, aqueous workup (50 mL EtOAc, wash with 3 × 10 mL 1M NaOH, dry over $MgSO_4$), and chromatography of the residue (EtOAc/hexane 8:2) gave **24** as a white solid. M.p. 168–169 °C; $[\alpha]_D^{20} = -7.0$ ($c = 0.2$, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD$): $\delta = 7.80$ (d, $J = 9.5$ Hz, 1H), 7.46 (d, $J = 8.5$ Hz, 1H), 6.92 (m, 2H), 6.23 (d, $J = 9.5$ Hz, 1H), 4.88 (d, $J = 2.2$ Hz, 1H), 4.30 (m, 4H), 4.05 (m, 1H), 3.39 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3/CD_3OD$): $\delta = 162.4$, 144.6, 129.2, 113.6, 113.3, 112.5, 108.8, 101.6, 78.9, 76.0, 71.0, 68.3, 55.2; EI-MS: m/z: 308 [M^+], 276 [$M^+ - CH_3OH$], 258 [$M^+ - CH_3OH - H_2O$], 162 [umbelliferyl$^+$], 134 [umbelliferyl$^+ - CO$].

7-[5-(Methyl-β-D-ribofuranosyloxy)]-2*H*-1-benzopyran-2-one (25): Application of the procedure for **24** to **73** (0.3 g, 0.86 mmol) gave **25** as a white solid. M.p. 141–142 °C; $[\alpha]_D^{20} = +20.9$ ($c = 0.1$, CH_3OH); IR (KBr): $\tilde{\nu} =$ 3486 (s), 3370 (s), 3096 (w), 2927 (m), 1704 (s), 1623 (s), 1611 (s), 1556 (m), 1385 (m), 1300 (s), 1240 (m), 1151 (s), 1106 (m), 1047 cm^{-1} (m); ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD$): $\delta = 7.79$ (d, $J = 9.5$ Hz, 1H), 7.46 (d, $J = 8.5$ Hz, 1H), 6.92 (m, 2H), 6.23 (d, $J = 9.5$ Hz, 1H), 4.82 (s, 1H), 4.28 (m, 3H), 4.11 (m, 1H), 3.95 (m, 1H), 3.32 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3/CD_3OD$): $\delta = 163.0$, 156.3, 150.6, 145.1, 129.8, 113.9, 113.6, 113.1, 109.3, 102.2, 81.5, 75.2, 72.1, 70.6, 55.4; elemental analysis calcd (%) for $C_{15}H_{16}O_7$: C 58.44, H 5.23; found: C 58.42, H 5.13.

(±)-7-(4-Acetoxy-3-hydroxy-3-methylbutyloxy)-2*H*-1-benzopyran-2-one (26): Application of the procedure for **5a** to diol **15** (0.088 g, 0.33 mmol) and flash chromatography (8:2 CH_2Cl_2/acetone) gave **26** (0.076 g, 75%) as a solid. M.p. 76–78 °C; IR (nujol): $\tilde{\nu} = 3461$ (m), 1710 (s), 1622 (s), 1465 (m), 1278 (m), 1139 (m), 1035 cm^{-1} (m); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta =$ 7.64 (d, $J = 9.8$ Hz, 1H), 7.37 (d, $J = 9.3$ Hz, 1H), 6.86–6.78 (m, 2H), 6.25 (d, $J = 9.8$ Hz, 1H), 4.24 (t, $J = 6.3$ Hz, 2H), 4.07 (s, 2H), 2.11 (s, 3H), 2.11–2.00 (m, 2H), 1.32 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 171.55$, 162.31, 161.73, 156.33, 144.00, 129.39, 113.62, 113.42, 113.19, 101.93, 71.58, 71.36, 65.25, 37.93, 25.08, 21.39; elemental analysis calcd (%) for $C_{16}H_{18}O_6$: C 62.74, H 5.92; found: C 62.95, H 6.12.

(*R*)-7-(4-Acetoxy-3-hydroxybutyloxy)-2*H*-1-benzopyran-2-one (26): Following the same procedure, diol (*R*)-**15** (0.065 g, 0.25 mmol) gave (*R*)-**26** (0.074 g, 98%, 95% *ee*) as a solid. M.p. 87–89 °C; $[\alpha]_D^{20} = +4.8$ ($c = 0.88$, MeOH); elemental analysis calcd (%) for $C_{16}H_{18}O_6$: C 62.74, H 5.92; found: C 62.58, H 6.21.

(*S*)-7-(4-Acetoxy-3-hydroxybutyloxy)-2*H*-1-benzopyran-2-one (26): Following the same procedure, diol (*S*)-**15** (0.075 g, 0.28 mmol) gave (*S*)-**26** (0.078 g, 91%, 92% *ee*) as a solid. M.p. 85–87 °C; $[\alpha]_D^{20} = -5.7$ ($c = 0.11$, MeOH); elemental analysis calcd (%) for $C_{16}H_{18}O_6$: C 62.74, H 5.92; found: C 62.89, H 6.00.

(±)-7-(3-Acetoxy-4-hydroxy-4-methylpentyloxy)-2*H*-1-benzopyran-2-one (27): Ac_2O (25 μL) was added to a solution of diol **16** (0.050 g, 0.18 mmol) in pyridine at 0 °C, and the reaction mixture was left at 0 °C for 18 h. Coevaporation of excess reagents with toluene followed by flash chromatography (hexane/EtOAc 8:2) gave **27** (0.043 g, 75%) as a white solid. M.p. 55–57 °C; IR (KBr): $\tilde{\nu} = 3416$ (br s), 3054 (w), 2981 (w), 2940 (w), 1720 (s), 1712 (s), 1620 (s), 1398 (m), 1385 (m), 1352 (m), 1286 (m), 1237 (s), 1132 (m), 1041 cm^{-1} (m); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 7.62$ (d, $J = 9.3$ Hz, 1H), 7.34 (d, $J = 8.9$ Hz, 1H), 6.85–6.75 (m, 2H), 6.23 (d, $J = 9.3$ Hz, 1H), 5.04 (dd, $J = 9.8$, 2.9 Hz, 1H), 4.14–3.93 (m, 2H), 2.33–1.98 (2H), 2.09 (s, 3H), 1.25 (s, 6H); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 171.48$, 162.56, 161.81, 156.46, 144.05, 129.43, 113.72, 113.52, 113.29, 102.06, 77.31, 72.75, 66.22, 29.89, 26.89, 26.05, 21.60; elemental analysis calcd (%) for $C_{17}H_{20}O_6$: C 63.74, H 6.29; found: C 63.91, H 5.98.

(*R*)-7-(3-Acetoxy-4-hydroxy-4-methylpentyloxy)-2*H*-1-benzopyran-2-one (27): Following the same procedure, diol (*R*)-**16** (0.050 g, 0.18 mmol) afforded pure (*R*)-**27** (0.042 g, 73%, 99% *ee*) as a syrup. $[\alpha]_D^{20} = +21.2$ ($c =$ 0.25, MeOH); elemental analysis calcd (%) for $C_{17}H_{20}O_6$: C 63.74, H 6.29; found: C 63.57, H 6.07.

(*S*)-7-(3-Acetoxy-4-hydroxy-4-methylpentyloxy)-2*H*-1-benzopyran-2-one (27): Following the same procedure, diol (*S*)-**16** (0.050 g, 0.18 mmol) afforded pure (*S*)-**27** (0.045 g, 79%, 83% *ee*) as a syrup. $[\alpha]_D^{20} = -23.3$ ($c =$ 0.23, MeOH); elemental analysis calcd (%) for $C_{17}H_{20}O_6$: C 63.74, H 6.29; found: C 63.55, H 6.44.

(1r,3*R*,4*S*)-7-(3,4-Diacetoxycyclopentyloxy)-2*H*-1-benzopyran-2-one (28): The typical procedure for **27** with diol **20** (0.050 g, 0.19 mmol) followed by flash chromatography (4:6 hexane/EtOAc) gave (3*R**,4*S**)-**28** (0.058 g, 89%) as a white solid. M.p. 135–137 °C; IR (KBr): $\tilde{\nu} = 3050$ (w), 2929 (w), 1743 (s), 1726 (s), 1621 (s), 1385 (m), 1255 (s), 1239 (s), 1137 (s), 1036 cm^{-1} (m); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 7.61$ (d, $J = 9.3$ Hz, 1H), 7.35 (d, $J =$ 8.8 Hz, 1H), 6.81–6.65 (m, 2H), 6.22 (d, $J = 9.8$ Hz, 1H), 5.24–5.07 (m, 2H), 4.83–4.68 (m, 1H), 2.65–2.41 (m, 2H), 2.20–1.94 (m, 2H). 2.04 (s, 6H); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 170.55$, 161.28, 160.96, 156.08, 143.58, 129.23, 113.55, 112.97, 102.74, 74.59, 72.57, 36.10, 21.14; elemental analysis calcd (%) for $C_{18}H_{18}O_7$: C 62.42, H 5.24; found: C 62.21, H 5.01.

(1s,3*R*,4*S*)-7-(3,4-Diacetoxycyclopentyloxy)-2*H*-1-benzopyran-2-one (29): Following the typical procedure for **27** on diol **21** (0.040 g, 0.15 mmol) and flash chromatography (4:6 hexane/EtOAc) gave (3*R**,4*S**)-**29** (0.045 g, 86%) as a white solid. M.p. 125–127 °C; IR (KBr): $\tilde{\nu} = 3053$ (w), 2994 (w), 2952 (w), 1743 (s), 1727 (s), 1619 (s), 1266 (s), 1238 (s), 1132 (m), 1088 cm^{-1} (m); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 7.64$ (d, $J = 9.3$ Hz, 1H), 7.37 (d, $J =$ 8.3 Hz, 1H), 6.83–6.68 (m, 2H), 6.26 (d, $J = 9.3$ Hz, 1H), 5.50–5.33 (m, 2H), 5.03–4.87 (m, 1H), 2.47–2.13 (m, 4H), 2.07 (s, 6H); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 170.49$, 161.39, 160.87, 156.22, 143.63, 129.31, 114.09, 113.74, 113.11, 102.37, 74.99, 72.88, 36.55, 21.19; elemental analysis calcd (%) for $C_{18}H_{18}O_7$: C 62.42, H 5.24; found: C 62.16, H 4.92.

(±)-2-(6-Methoxy-2-naphthyl)-1-acetoxy-2-hydroxyethane (30a): The procedure for **5a** with diol **9** (0.087 g, 0.40 mmol) and flash chromatography (6:4 hexane/EtOAc) gave **30a** (0.060 g, 58%) as a solid. M.p. 90–92 °C; IR (KBr): $\tilde{\nu} = 3450$ (br s), 3062 (w), 3009 (w), 2962 (w), 2939 (w), 1733 (s), 1609 (m), 1384 (m), 1267 (s), 1218 (s), 1166 (m), 1030 cm^{-1} (s); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 7.77–7.69$ (m, 3H), 7.46–7.42 (m, 1H), 7.18–7.12 (m, 2H), 5.12–5.04 (m, 1H) 4.35 (dd, $J = 11.5$, 3.7 Hz, 1H), 4.23 (dd, $J =$ 11.7, 8.3 Hz, 1H), 3.91 (s, 3H), 2.11 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 171.53$, 158.27, 135.28, 134.79, 129.83, 129.05, 127.57, 125.44, 124.83, 119.52, 106.06, 72.88, 69.67, 55.67, 21.35; elemental analysis calcd (%) for $C_{15}H_{16}O_4$: C 69.22, H 6.20; found: C 69.47, H 6.03.

(*R*)-2-(6-Methoxy-2-naphthyl)-1-acetoxy-2-hydroxyethane (30a): Following the same procedure, diol (*R*)-**9** (0.090 g, 0.41 mmol) afforded pure (*R*)-**30a** (0.055 g, 51%, 99% *ee*) as a solid. M.p. 101–103 °C; $[\alpha]_D^{20} = -40.7$ ($c =$ 0.21, $CHCl_3$); elemental analysis calcd (%) for $C_{15}H_{16}O_4$: C 69.22, H 6.20; found: C 69.02, H 5.95.

(*S*)-2-(6-Methoxy-2-naphthyl)-1-acetoxy-2-hydroxyethane (30a): Following the same procedure, diol (*S*)-**9** (0.090 g, 0.41 mmol) afforded pure (*S*)-**30a** (0.050 g, 47%, 99% *ee*) as a solid. M.p. 102–104 °C; $[\alpha]_D^{20} = +38.0$ ($c =$ 0.20, $CHCl_3$); elemental analysis calcd (%) for $C_{15}H_{16}O_4$: C 69.22, H 6.20; found: C 69.39, H 6.09.

(±)-2-(6-Methoxy-2-naphthyl)-1,2-diacetoxyethane (30b): Application of the procedure for **27** to diol **9** (0.050 g, 0.23 mmol) and flash chromatography (7:3 hexane/EtOAc) gave **30b** (0.061 g, 88%) as a white solid. M.p. 65–66 °C; IR (KBr): $\tilde{\nu} = 3014$ (w), 2964 (w), 2951 (w), 2941 (w), 1739 (br s), 1633 (m), 1610 (m), 1487 (m), 1392 (m), 1379 (m), 1273 (s), 1248 (s), 1223 (s). 1062 (s), 1049 (s), 1027 cm^{-1} (s); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 7.83–7.65$ (m, 3H), 7.50–7.33 (m, 1H), 7.22–7.04 (m, 2H), 6.15 (t, $J = 6.1$ Hz, 1H), 4.40 (d, $J = 5.9$ Hz, 2H), 3.92 (s, 3H), 2.14 (s, 3H), 2.07(s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 169.02$, 168.47, 156.54, 132.98, 129.94, 127.92, 126.97, 125.70, 124.43, 123.19, 117.70, 104.07, 71.87, 64.45, 53.71, 19.52, 19.14; elemental analysis calcd (%) for $C_{17}H_{18}O_5$: C 67.54, H 6.00; found: C 67.72, H 5.76.

(*R*)-2-(6-Methoxy-2-naphthyl)-1,2-diacetoxyethane (30b): Following the same procedure, diol (*R*)-**9** (0.040 g, 0.18 mmol) afforded pure (*R*)-**30b** (0.049 g, 91%, 99% *ee*) as a white waxy solid. $[\alpha]_D^{20} = -76.5$ ($c = 0.16$, $CHCl_3$); elemental analysis calcd (%) for $C_{17}H_{18}O_5$: C 67.54, H 6.00; found: C 67.29, H 6.17.

(*S*)-2-(6-Methoxy-2-naphthyl)-1,2-diacetoxyethane (30b): Following the same procedure, diol (*S*)-**9** (0.040 g, 0.18 mmol) afforded pure (*S*)-**30b** (0.047 g, 87%, 99% *ee*) as a white waxy solid. $[\alpha]_D^{20} = +73.9$ ($c = 0.14$, $CHCl_3$); elemental analysis calcd (%) for $C_{17}H_{18}O_5$: C 67.54, H 6.00; found: C 67.42, H 5.86.

(3*R,4*R**)-7-(3,4-Diacetoxycyclopentyloxy)-2*H*-1-benzopyran-2-one (31)**: The typical procedure for **27** was applied with diol **22** (0.050 g, 0.19 mmol). Purification by flash chromatography (4:6 hexane/EtOAc) gave (3*R**,4*R**)-**31** (0.040 g, 61%) as a white solid. M.p. 103–105 °C; IR (KBr): $\tilde{\nu} = 2968$ (w), 1733 (s), 1623 (s), 1242 (br s), 1142 (m), 1043 cm^{-1} (m); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 7.63$ (d, $J = 9.8$ Hz, 1H), 7.36 (d, $J = 8.3$ Hz, 1H), 6.84–6.69 (m, 2H), 6.24 (d, $J = 9.8$ Hz, 1H), 5.42–5.27 (m, 1H), 5.19–5.04

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(m, 1H), 4.95–4.80 (m, 1H), 2.80–2.58 (m, 1H), 2.54–2.34 (m, 1H), 2.27–1.84 (m, 2H), 2.07 (s, 3H), 2.05 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ = 170.71, 170.52, 161.44, 161.01, 156.27, 143.71, 129.36, 113.94, 113.82, 113.19, 102.79, 77.55, 76.51, 37.56, 37.27, 21.41; elemental analysis calcd (%) for $C_{18}H_{18}O_7$: C 62.42, H 5.24; found: C 62.31, H 5.10.

(±)-4-(*p*-Nitrophenoxy)-1-acetoxy-2-hydroxybutane (32a): The procedure for **5a** was applied on diol **23** (0.100 g, 0.44 mmol). Flash chromatography (1:1 hexane/EtOAc) gave **32a** (0.078 g, 66%) as a pale yellow waxy solid. IR (neat): $\tilde{\nu}$ = 3457 (brm), 3117 (w), 3087 (w), 2953 (w), 1738 (s), 1608 (s), 1595 (s), 1513 (s), 1343 (s), 1263 (brs), 1112 cm^{-1} (s); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.20 (d, J = 9.2 Hz, 2H), 6.96 (d, J = 9.2 Hz, 2H), 4.32–4.23 (m, 3H), 4.21–4.11 (m, 1H), 4.06 (dd, J = 10.8, 6.8 Hz, 1H), 2.11 (s, 3H), 2.05–1.89 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.15, 163.69, 141.62, 125.93, 114.43, 68.56, 66.99, 65.10, 32.53, 20.82; elemental analysis calcd (%) for $C_{12}H_{15}NO_6$: C.53.53, H 5.62, N 5.20; found: C 53.46, H 5.68, N 5.10.

(*S*)-4-(*p*-Nitrophenoxy)-1-acetoxy-2-hydroxybutane (32a): Following the same procedure, diol (*S*)-**23** (0.028 g, 0.12 mmol) afforded (*S*)-**32a** (0.024 g, 72%, 96% *ee*) as a pale yellow waxy solid. $[\alpha]_D^{20} = -10.2$ (c = 0.46, $CHCl_3$); elemental analysis calcd (%) for $C_{12}H_{15}NO_6$: C.53.53, H 5.62, N 5.20; found: C 53.63, H 5.71, N 5.27.

(*R*)-4-(*p*-Nitrophenoxy)-1-acetoxy-2-hydroxybutane (32a): Following the same procedure, diol (*R*)-**23** (0.028 g, 0.12 mmol) afforded (*R*)-**32a** (0.023 g, 70%, 96% *ee*) as a pale yellow waxy solid. $[\alpha]_D^{20} = +10.1$ (c = 0.67, $CHCl_3$); EI-MS: m/z: 269 [M^+], 196 [$M^+ - CH_3C(O)OCH_2$], 152 [$M^+ - CH_3C(O)OCH_2CH(OH)CH_2$], 139 [*p*-nitrophenyl$^+$+1], 131 [$M^+$ – *p*-nitrophenyl].

(±)-4-(*p*-Nitrophenoxy)-1,2-diacetoxybutane (32b): The procedure for **27** was applied to diol **23** (0.045 g, 0.20 mmol). Flash chromatography (7:3 hexane/EtOAc) gave **32b** (0.059 g, 95%) as a pale yellow solid. M.p. 62–63 °C; IR (neat): $\tilde{\nu}$ = 3117 (w), 3088 (w), 2952 (w), 1742 (s), 1609 (m), 1595 (s), 1514 (s), 1500 (s), 1372 (m), 1343 (s), 1244 (brs), 1175 (m), 1112 (m), 1049 (m), 1020 cm^{-1} (m); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.19 (d, J = 9.2 Hz, 2H), 6.93 (d, J = 9.2 Hz, 2H), 5.34–5.26 (m, 1H), 4.33 (dd, J = 11.9, 3.5 Hz, 1H), 4.16–4.05 (m, 2H), 4.13 (dd, J = 11.8, 6.1 Hz, 1H), 2.17–2.11 (m, 2H), 2.07 (s, 3H), 2.06 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.29, 171.02, 164.17, 142.45, 126.62, 115.12, 69.47, 65.50, 65.38, 31.11, 21.63, 21.39; elemental analysis calcd (%) for $C_{14}H_{17}NO_7$: C 54.02, H 5.50, N 4.50; found: C 54.04, H 5.40, N 4.49.

(*S*)-4-(*p*-Nitrophenoxy)-1,2-diacetoxybutane (32b): Following the same procedure, diol (*S*)-**23** (0.015 g, 0.066 mmol) afforded pure (*S*)-**32b** (0.019 g, 93%, 95% *ee*) as a pale yellow oil. $[\alpha]_D^{20} = -22.4$ (c = 0.38, $CHCl_3$); elemental analysis calcd (%) for $C_{14}H_{17}NO_7$: C 54.02, H 5.50, N 4.50; found: C 54.10, H 5.45, N 4.58.

(*R*)-4-(*p*-Nitrophenoxy)-1,2-diacetoxybutane (32b): Following the same procedure, diol (*R*)-**23** (0.015 g, 0.066 mmol) afforded pure (*R*)-**32b** (0.020 g, 98%, 90% *ee*) as a pale yellow oil. $[\alpha]_D^{20} = +22.1$ (c = 0.52, $CHCl_3$); elemental analysis calcd (%) for $C_{14}H_{17}NO_7$: C 54.02, H 5.50, N 4.50; found: C 54.15, H 5.56, N 4.45.

(±)-7-[(3,4-Carbonyldioxy)-butyloxy]-2*H*-1-benzopyran-2-one (33): *The following procedure is typical.* Carbonyldiimidazole (0.085 g, 0.52 mmol) in dry CH_2Cl_2 (1 mL) was added to a stirred solution of diol **1** (0.10 g, 0.40 mmol) in dry CH_2Cl_2 (2 mL) at 25 °C under nitrogen. After 18 h at 25 °C, aqueous workup (brine/CH_2Cl_2) and flash chromatography gave **33** (0.045 g, 41%) as a white solid. M.p. 114–115 °C; IR (KBr): $\tilde{\nu}$ = 3096 (w), 3043 (w), 2997 (w), 2952 (w), 2887 (w), 1821 (s), 1729 (s), 1704 (m), 1629 (s), 1557 (m), 1509 (m), 1409 (m), 1370 (m), 1298 (s), 1245 (m), 1179 (s), 1141 (m), 1070 cm^{-1} (s); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.63 (d, J = 9.6 Hz, 1H), 7.39 (d, J = 8.5 Hz, 1H), 6.82 (m, 2H), 6.27 (d, J = 9.6 Hz, 1H), 5.01 (dt, J = 6.2, 7.4 Hz, 1H), 4.65, 4.26 (2dd, J = 7.7, 8.5 Hz, 2H), 4.23 (t, J = 5.5 Hz, 2H), 2.29 (q, J = 6.6 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 161.9, 161.6, 156.5, 155.2, 143.9, 129.7, 114.3, 113.8, 113.1, 102.3, 77.3, 74.8, 70.1, 64.5, 34.1; elemental analysis calcd (%) for $C_{14}H_{12}O_6$: C 60.87, H 4.38; found: C 60.72, H 4.40.

(±)-7-[(3,4-Carbonyldioxy-4-methyl)-butyloxy]-2*H*-1-benzopyran-2-one (34): The procedure for **33** was applied with diol **15** (0.020 g, 0.076 mmol). Flash chromatography (9:1 CH_2Cl_2/acetone) gave **34** (0.020 g, 91%) as a white solid. M.p. 152–153 °C; IR (KBr): $\tilde{\nu}$ = 2930 (w), 1814 (s), 1728 (s), 1710 (m), 1626 (s), 1614 (s), 1394 (m), 1385 (m), 1295 (m), 1245 (m), 1199 (m), 1139 (m), 1109 (m), 1069 cm^{-1} (m); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.64 (d, J = 9.5 Hz, 1H), 7.39 (d, J = 8.5 Hz, 1H), 6.81 (m, 2H), 6.28 (d, J = 9.5 Hz, 1H), 4.47, 4.21 (2d, J = 8.8 Hz, 2H), 4.20 (m, 2H), 2.31 (m, 2H), 1.60 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 161.7, 161.6, 156.5, 154.9, 143.9, 129.7, 114.4, 113.8, 113.2, 102.2, 83.0, 75.4, 64.4, 38.4, 25.3. EI-MS: m/z: 290 [M^+], 262 [$M^+ - CO$], 162 [umbelliferyl$^+$], 134 [umbelliferyl$^+ - CO$].

(1s,3*R*,4*S*)-7-(3,4-Carbonyldioxycyclopentyloxy)-2*H*-1-benzopyran-2-one (35): The procedure for **33** was applied to diol **21** (0.015 g, 0.057 mmol). Flash chromatography (99:1 CH_2Cl_2/acetone) gave **35** (0.011 g, 67%) as a white solid. M.p. 155–157 °C; IR (KBr): $\tilde{\nu}$ = 2933 (w), 1793 (s), 1723 (s), 1620 (s), 1558 (m), 1510 (m), 1409 (m), 1385 (m), 1370 (m), 1294 (m), 1240 (m), 1177 (m), 1140 (m), 1108 (m), 1040 cm^{-1} (m); ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD): δ = 7.63 (d, J = 9.6 Hz, H-C(4′)), 7.37 (d, J = 9.2 Hz, H-C(5′)), 6.78 (m, H-C(6′), H-C(8′)), 6.24 (d, J = 9.6 Hz, H-C(3′)), 5.22 (m, H-C(3), H-C(4)), 5.01 (quintet, J = 6.0 Hz, H-C(1)), 2.54 (dd, J = 14.9, 5.4 Hz, H_{Re}-C(2), H_{Si}-C(5)), 2.25 (m, H_{Si}-C(2), H_{Re}-C(5)); NOE observed between H-C(3), H-C(4) and H_{Si}-C(2), H_{Re}-C(5), and H-C(1) and H_{Re}-C(2), H_{Si}-C(5); ^{13}C NMR (75 MHz, $CDCl_3$/CD_3OD): δ = 165.7, 165.5, 156.6, 156.2, 144.1, 129.8, 114.2, 113.4, 103.4, 80.2, 76.5, 39.0; elemental analysis calcd (%) for $C_{15}H_{12}O_6$: C 62.50, H 4.20; found: C 62.64, H 4.31.

(1r,3*R*,4*S*)-7-(3,4-Carbonyldioxycyclopentyloxy)-2*H*-1-benzopyran-2-one (36): The procedure for **33** was applied with diol **20** (0.020 g, 0.076 mmol). Flash chromatography (99:1 CH_2Cl_2/acetone) gave **36** (0.014 g, 64%) as a white solid. M.p. 240–241 °C; IR (KBr): $\tilde{\nu}$ = 3104 (w), 3070 (w), 2962 (w), 2938 (w), 1789 (s), 1725 (s), 1616 (s), 1406 (m), 1382 (m), 1354 (m), 1280 (m), 1230 (m), 1213 (m), 1170 (s), 1124 (s), 1095 (s), 1051 (s), 1002 cm^{-1} (m); ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD): δ = 7.58 (d, J = 9.6 Hz, 1H), 7.27 (d, 1H), 6.69 (m, 2H), 6.10 (d, J = 9.6 Hz, 1H), 5.14 (m, 2H), 4.94 (m, 1H), 2.44, 1.95 (2m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$/CD_3OD): δ = 164.3, 161.5, 155.4, 155.2, 144.6, 129.8, 115.2, 113.4, 102.6, 81.7, 78.5, 38.7; elemental analysis calcd (%) for $C_{15}H_{12}O_6$: C 62.50, H 4.20; found: C 61.73, H 4.38.

7-[5-(Methyl-(2,3-carbonyldioxy)-α-L-lyxofuranosyloxy)]-2*H*-1-benzopyran-2-one (37): The procedure for **33** was applied with diol **24** (0.007 g, 0.023 mmol). Flash chromatography (97:3 CH_2Cl_2/acetone) gave **37** (0.003 g, 39%) as a white solid. M.p. 172–173 °C; IR (neat): $\tilde{\nu}$ = 3022 (m), 2940 (w), 1812 (s), 1729 (s), 1672 (m), 1617 (s), 1216 (s), 1160 (m), 1095 cm^{-1} (m); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.64 (d, J = 9.6 Hz, 1H), 7.41 (d, J = 9.2 Hz, 1H), 6.88 (m, 2H), 6.29 (d, J = 9.6 Hz, 1H), 5.27 (m, 1H), 5.17 (s, 1H), 5.00 (m, 1H), 4.50 (m, 1H), 4.35 (m, 2H), 3.43 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 161.9, 161.5, 153.7, 153.2, 143.9, 129.7, 114.5, 112.9, 106.4, 102.9, 83.3, 79.5, 77.3, 65.7, 55.9; elemental analysis calcd (%) for $C_{16}H_{14}O_8$: C 57.49, H 4.22; found: C 57.55, H 4.25.

7-[5-(Methyl-(2,3-carbonyldioxy)-β-D-ribofuranosyloxy)]-2*H*-1-benzopyran-2-one (38): The procedure for **33** was applied to diol **25** (0.020 g, 0.065 mmol). Flash chromatography (98:2 CH_2Cl_2/acetone) gave **38** (0.020 g, 92%) as a yellow solid: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.59 (d, J = 9.5 Hz, 1H), 7.30 (d, J = 8.5 Hz, 1H), 6.72 (m, 2H), 6.11 (d, J = 9.5 Hz, 1H), 5.15 (d, J = 6.6 Hz, 1H), 5.05 (s, 1H), 4.94 (d, J = 7.0 Hz, 1H), 4.59 (m, 1H), 3.94 (m, 2H), 3.24 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 162.3, 161.6, 156.0, 155.7, 144.4, 113.6, 113.4, 108.3, 101.9, 84.0, 83.7, 81.4, 68.0, 55.8; elemental analysis calcd (%) for $C_{16}H_{14}O_8$: C 57.49, H 4.22; found: C 57.59, H 4.36.

(±)-4-[(*p*-Nitrophenoxy)-ethyl]-1,3-dioxolan-2-one (39): The procedure for **33** was applied to **23** (0.088 g, 0.33 mmol). Flash chromatography (8:2 CH_2Cl_2/acetone) gave **39** (0.071 g, 75%) as a yellow solid. M.p. 59–60 °C; IR (KBr): $\tilde{\nu}$ = 3118 (m), 3091 (m), 2968 (w), 2946 (m), 2889 (w), 1798 (brs), 1612 (m), 1596 (s), 1510 (s), 1471 (m), 1403 (m), 1342 (s), 1305 (m), 1267 (s), 1177 (s), 1111 (s), 1064 cm^{-1} (s); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.20 (d, J = 9.2 Hz, 1H), 6.95 (d, J = 9.2 Hz, 2H), 5.01 (dt, J = 14.7, 6.6 Hz, 1H), 4.65 (dd, J = 8.8, 8.1 Hz, 1H), 4.25 (m, 3H), 2.29 (dt, J = 6.2, 5.9 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.7, 155.2, 142.6, 130.5, 126.7, 115.1, 74.6, 70.1, 64.6, 34.1; elemental analysis calcd (%) for $C_{11}H_{11}NO_6$: C 52.18, H 4.38, N 5.53; found: C 52.28, H 4.47, N 5.43.

(*S*)-4-[(*p*-Nitrophenoxy)-ethyl]-1,3-dioxolan-2-one (39): The same reaction applied to (*S*)-**23** (0.40 g, 1.74 mmol) gave (*S*)-**39** (0.32 g, 70%) as a yellow solid. M.p. 67–68 °C; $[\alpha]_D^{20} = -54.6$ (c = 0.2, $CHCl_3$); elemental analysis calcd (%) for $C_{11}H_{11}NO_6$: C 52.18, H 4.38, N 5.53; found: C 52.12, H 4.54, N 5.48.

(*R*)-4-[(*p*-Nitrophenoxy)-ethyl]-1,3-dioxolan-2-one (39): The same reaction applied on diol (*R*)-**23** (0.40 g, 1.74 mmol) afforded pure (*R*)-**39** (0.31 g, 67%) as a yellow solid. M.p. 67–68 °C; $[\alpha]_D^{20} = +55.8$ (c = 1.06, MeOH);

elemental analysis calcd (%) for $C_{11}H_{11}NO_6$: C 52.18, H 4.38, N 5.53; found: C 52.22, H 4.40, N 5.50.

(±)-7-(3,4-Epoxy-3-methylbutyloxy)-2*H*-1-benzopyran-2-one (40): The reaction for (±)-**6** was applied to olefin **11** (0.42 g, 1.83 mmol). Flash chromatography (hexane/EtOAc 55:45) gave **40** (0.337 g, 75%) as a solid. M.p. 63–65 °C; IR (KBr): $\tilde{\nu}$ = 3077 (w), 2990 (w), 2968 (w), 2936 (w) 2889 (w), 1729 (s), 1619 (s), 1398 (m), 1354 (m), 1286 (m), 1233 (m), 1209 (m), 1161 (m), 1127 (s), 1022 cm^{-1} (m); ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.63 (d, J = 9.8 Hz, 1H), 7.37 (d, J = 8.3 Hz, 1H), 6.93–6.78 (m, 2H), 6.25 (d, J = 9.8 Hz, 1H), 4.11 (t, J = 5.4 Hz, 2H), 2.73 (d, J = 4.4 Hz, 1H), 2.65 (d, J = 4.4 Hz, 1H), 2.10 (t, J = 5.8 Hz, 2H), 1.41 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ = 162.41, 161.66, 156.40, 143.98, 129.43, 113.69, 113.29, 113.22, 102.03, 65.49, 55.53, 54.46, 36.24, 22.15; elemental analysis calcd (%) for $C_{14}H_{14}O_4$: C 68.28, H 5.73; found: C 68.14, H 5.54.

(*S*)-7-(3,4-Epoxy-3-methylbutyloxy)-2*H*-1-benzopyran-2-one (40): The procedure for (*S*)-**6** was applied to diol (*S*)-**15** (0.079 g, 0.30 mmol). Fash chromatography (9:1 CH_2Cl_2/acetone) gave (*S*)-**40** (0.044 g, 60%, 92% *ee*) as a solid. M.p. 86–88 °C; $[\alpha]_D^{20}$ = +7.7 (c = 0.79, $CHCl_3$); elemental analysis calcd (%) for $C_{14}H_{14}O_4$: C 68.28, H 5.73; found: C 67.95, H 6.05.

(*R*)-7-(3,4-Epoxy-3-methylbutyloxy)-2*H*-1-benzopyran-2-one (40): The procedure for (*S*)-**6** was applied to diol (*R*)-**15** (0.074 g, 0.28 mmol) to give pure (*R*)-**40** (0.038 g, 55%, 95% *ee*) as a solid. M.p. 85–87 °C; $[\alpha]_D^{20}$ = −8.3 (c = 0.60, $CHCl_3$); elemental analysis calcd (%) for $C_{14}H_{14}O_4$: C 68.28, H 5.73; found: C 68.01, H 5.93.

(±)-7-(3,4-Epoxy-4-methylpentyloxy)-2*H*-1-benzopyran-2-one (41): The procedure for (*S*)-**6** was applied to diol **16** (0.086 g, 0.31 mmol). Flash chromatography (9:1 CH_2Cl_2/acetone) gave **41** (0.016 g, 20%) as a semisolid. IR (Nujol): $\tilde{\nu}$ = 3057 (w), 1733 (s), 1612 (s), 1279 (m), 1273 (m), 1221 (m), 1124 cm^{-1} (m); ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.63 (d, J = 9.8 Hz, 1H), 7.37 (d, J = 8.3 Hz, 1H), 6.88–6.79 (m, 2H), 6.24 (d, J = 9.3 Hz, 1H), 4.18 (dd, J = 7.3, 5.4 Hz, 2H), 2.95 (dd, J = 7.3, 5.4 Hz, 1H), 2.21–2.05 (m, 1H), 2.00–1.81 (m, 1H), 1.34 (s, 3H), 1.32 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ = 162.73, 161.83, 156.58, 144.05, 129.49, 113.93, 113.45, 113.36, 102.20, 66.57, 61.86, 59.15, 29.63, 25.45, 19.61; elemental analysis calcd (%) for $C_{15}H_{16}O_4$: C 69.22, H 6.20; found: C 68.91, H 5.89.

(*R*)-7-(3,4-Epoxy-4-methylpentyloxy)-2*H*-1-benzopyran-2-one (41): The procedure for (*S*)-**6** was applied to diol (*R*)-**16** (0.050 g, 0.18 mmol) and yielded pure (*R*)-**41** (0.012 g, 25%, 99% *ee*) as a semisolid. $[\alpha]_D^{20}$ = +11.8 (c = 0.11, $CHCl_3$); elemental analysis calcd (%) for $C_{15}H_{16}O_4$: C 69.22, H 6.20; found: C 69.05, H 6.49.

(*S*)-7-(3,4-Epoxy-4-methylpentyloxy)-2*H*-1-benzopyran-2-one (41): The procedure for (*S*)-**6** was applied to diol (*S*)-**16** (0.050 g, 0.18 mmol) and yielded pure (*S*)-**41** (0.010 g, 21%, 70% *ee*) as a semisolid. $[\alpha]_D^{20}$ = −13.4 (c = 0.48, $CHCl_3$); elemental analysis calcd (%) for $C_{15}H_{16}O_4$: C 69.22, H 6.20; found: C 69.37, H 6.11.

(3*R,4*R**)-7-(3,4-Epoxyhexyloxy)-2*H*-1-benzopyran-2-one (42)**: The procedure for (±)-**6** was applied to olefin **13** (0.90 g, 3.69 mmol). The product was recrystallized from hexane to give pure **42** (0.67 g, 70%) as a solid. M.p. 53–54 °C; IR (KBr): $\tilde{\nu}$ = 3070 (w), 2997 (w), 2963 (w), 2925 (w) 2875 (w), 1731 (s), 1614 (s), 1355 (m), 1282 (s), 1235 (s), 1202 (m), 1119 (s), 1018 cm^{-1} (m); ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.65 (d, J = 9.3 Hz, 1H), 7.39 (d, J = 8.3 Hz, 1H), 6.86–6.75 (m, 2H), 6.27 (d, J = 9.3 Hz, 1H), 4.16 (t, J = 6.3 Hz, 2H), 2.96–2.87 (m, 1H), 2.82–2.72 (m, 1H), 2.24–2.06 (m, 1H), 2.02–1.84 (m, 1H), 1.69–1.51 (m, 2H), 1.01 (t, J = 7.3 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ = 162.66, 161.85, 156.56, 144.06, 129.50, 113.88, 113.42, 113.00, 102.19, 66.09, 60.72, 56.07, 32.53, 25.72, 10.53; elemental analysis calcd (%) for $C_{15}H_{16}O_4$: C 69.22, H 6.20; found: C 69.60, H 5.98.

(*S*,*S*)-7-(3,4-Epoxyhexyloxy)-2*H*-1-benzopyran-2-one (42): The procedure for (*S*)-**6** was applied to diol (*S*,*S*)-**17** (0.078 g, 0.28 mmol). Flash chromatography (9:1 CH_2Cl_2/acetone) gave (*S*,*S*)-**42** (0.051 g, 70%, 99% *ee*) as a solid. M.p. 54–55 °C; $[\alpha]_D^{20}$ = −26.1 (c = 0.68, $CHCl_3$); elemental analysis calcd (%) for $C_{15}H_{16}O_4$: C 69.22, H 6.20; found: C 69.34, H 6.10.

(*R*,*R*)-7-(3,4-Epoxyhexyloxy)-2*H*-1-benzopyran-2-one (42): The procedure for (*S*)-**6** was applied to diol (*R*,*R*)-**17** (0.10 g, 0.36 mmol) and yielded pure (*R*,*R*)-**42** (0.068 g, 73%, 99% *ee*) as a solid. M.p. 54–56 °C; $[\alpha]_D^{20}$ = +26.3 (c = 1.15, $CHCl_3$); elemental analysis calcd (%) for $C_{15}H_{16}O_4$: C 69.22, H 6.20; found: C 69.01, H 6.13.

(3*R,4*S**)-7-(3,4-Epoxyhexyloxy)-2*H*-1-benzopyran-2-one (43)**: The procedure for (±)-**6** was applied to olefin **14** (0.90 g, 3.69 mmol). Recrystallization from hexane gave pure **43** (0.60 g, 63%) as a solid. M.p. 77–79 °C; IR (KBr): $\tilde{\nu}$ = 3073 (w), 2963 (w), 2939 (w), 2876 (w), 1733 (s), 1610 (s), 1355 (m), 1284 (m), 1230 (m), 1204 (m), 1124 cm^{-1} (s); ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.64 (d, J = 9.3 Hz, 1H), 7.38 (d, J = 9.3 Hz, 1H), 6.89–6.81 (m, 2H), 6.26 (d, J = 9.3 Hz, 1H), 4.23–4.15 (m, 2H), 3.20–3.05 (m, 1H), 3.00–2.84 (m, 1H), 2.23–2.00 (m, 1H), 2.00–1.80 (m, 1H), 1.67–1.48 (m, 2H), 1.08 (t, J = 7.3 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ = 162.64, 161.77, 156.52, 144.01, 129.49, 113.86, 113.49, 113.33, 102.17, 66.59, 58.94, 54.90, 28.47, 21.89, 11.18; elemental analysis calcd (%) for $C_{15}H_{16}O_4$: C 69.22, H 6.20; found: C 69.38, H 6.49.

(1r,3*R*,4*S*)-7-(3,4-Epoxycyclopentyloxy)-2*H*-1-benzopyran-2-one (45) and (1s,3*R*,4*S*)-7-(3,4-epoxycyclopentyloxy)-2*H*-1-benzopyran-2-one (44): The procedure for (±)-**6** applied to olefin **19** (1.0 g, 4.38 mmol) gave a 1:2 mixture (^{1}H NMR) of epoxides **45** and **44**. Flash chromatography (6:4 hexane/EtOAc) gave **45** (minor R_f, 0.314 g, 29%) and **44** (major R_f, 0.618 g, 58%). Compound **45**: white solid. M.p. 147–148 °C; IR (KBr): $\tilde{\nu}$ = 3070 (w), 3024 (w), 2938 (w), 1706 (s), 1603 (s), 1279 (m), 1231 (m), 1202 (m), 1127 (s), 1074 (m), 998 cm^{-1} (m); ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.63 (d, J = 9.8 Hz, 1H), 7.34 (d, J = 8.8 Hz, 1H), 6.80 (dd, J = 8.5, 2.2 Hz, 1H), 6.68 (d, J = 2.4 Hz, 1H), 6.24 (d, J = 9.3 Hz, 1H), 4.86 (t, J = 6.3 Hz, 1H), 3.62 (s, 2H), 2.41–2.17 (m, 4H); ^{13}C NMR (50 MHz, $CDCl_3$): δ = 161.88, 161.50, 156.32, 144.14, 129.42, 114.69, 113.54, 112.95, 102.30, 77.45, 58.14, 35.82; elemental analysis calcd (%) for $C_{14}H_{12}O_4$: C 68.85, H 4.95; found: C 69.13, H 4.57. Compound **44**: white solid. M.p. 123–124 °C; IR (KBr): $\tilde{\nu}$ = 3069 (w), 3046 (w), 2964 (w), 2926 (w), 1730 (s), 1624 (s), 1552 (m), 1301 (s), 1274 (m), 1237 (s), 1160 (m), 1141 (s), 1018 cm^{-1} (m); ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.63 (d, J = 9.8 Hz, H-C(4′)), 7.36 (d, J = 7.8 Hz, H-C(5′)), 6.83–6.73 (m, H-C(6′), H-C(8′)), 6.25 (d, J = 9.8 Hz, H-C(3′)), 4.61 (quintet, J = 6.6 Hz, H-C(1)), 3.62 (s, H-C(4) , H-C(3)), 2.75 (dd, J = 14.2, 7.3 Hz, H_{Si}-C(2), H_{Re}-C(5)), 1.88 (dd, J = 14.6, 6.3 Hz, H_{Si}-C(2), H_{Re}-C(5)); NOE observed between H-C(3), H-C(4) and H_{Si}-C(2), H_{Re}-C(5), and H-C(1) and H_{Re}-C(2), H_{Si}-C(5); ^{13}C NMR (50 MHz, $CDCl_3$): δ = 161.73, 161.58, 156.33, 143.94, 129.45, 113.77, 113.59, 113.20, 102.94, 75.74, 56.36, 34.97; elemental analysis calcd (%) for $C_{14}H_{12}O_4$: C 68.85, H 4.95; found: C 68.99, H 5.16.

(±)-2-(6-Methoxy-2-naphthyl)-1,2-epoxyethane (46): The procedure for (*S*)-**6** was applied to diol **9** (0.065 g, 0.30 mmol). Flash chromatography (6:4:0.1 hexane/EtOAc/Et_3N) gave **46** (0.035 g, 59%) as a solid. M.p. 81–83 °C; IR (KBr): $\tilde{\nu}$ = 3060 (w), 2965 (w), 2927 (w), 2844 (w), 1633 (m), 1608 (s), 1491 (m), 1388 (m), 1258 (s), 1233 (s), 1197 (m), 1179 (m), 1164 (m), 1031 cm^{-1} (s); ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.75–7.70 (m, 3H,), 7.31–7.26 (m, 1H), 7.19–7.13 (m, 2H), 4.00 (dd, J = 4.1, 2.4 Hz, 1H), 3.92 (s, 3H), 3.22 (dd, J = 5.4, 4.0 Hz, 1H), 2.91 (dd, J = 5.4, 2.4 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 160.77, 137.43, 135.57, 132.15, 131.59, 130.13, 127.98, 126.18, 122.08, 108.69, 58.21, 55.57, 54.05; elemental analysis calcd (%) for $C_{13}H_{12}O_2$: C 77.98, H 6.04; found: C 77.75, H 6.18.

(*S*)-2-(6-Methoxy-2-naphthyl)-1,2-epoxyethane (46): Following the same procedure, diol (*S*)-**9** (0.065 g, 0.30 mmol) yielded pure (*S*)-**46** (0.035 g, 58%, 99% *ee*) as a solid. M.p. 81–83 °C; $[\alpha]_D^{20}$ = +6.1 (c = 0.43, $CHCl_3$); elemental analysis calcd (%) for $C_{13}H_{12}O_2$: C 77.98, H 6.04; found: C 77.64, H 5.79.

(*R*)-2-(6-Methoxy-2-naphthyl)-1,2-epoxyethane (46): Following the same procedure, diol (*R*)-**9** (0.065 g, 0.30 mmol) yielded pure (*R*)-**46** (0.030 g, 50%, 99% *ee*) as a solid. M.p. 82–84 °C; $[\alpha]_D^{20}$ = −7.1 (c = 0.31, $CHCl_3$); elemental analysis calcd (%) for $C_{13}H_{12}O_2$: C 77.98, H 6.04; found: C 78.10, H 6.25.

(±)-4-(*p*-Nitrophenoxy)-1,2-epoxybutane (47): The procedure for (±)-**6** was applied to **59** (0.70 g, 3.63 mmol). Flash chromatography (hexane/EtOAc 6:4) gave **47** (0.635 g, 84%) as a solid. M.p. 35–36 °C; IR (KBr): $\tilde{\nu}$ = 3116 (w), 3086 (w), 2998 (w), 2932 (w), 1608 (s), 1595 (s), 1513 (s), 1343 (s), 1301 (m), 1264 (s), 1176 (m), 1112 (m), 1023 cm^{-1} (m); ^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.18 (d, J = 9.3 Hz, 2H), 6.95 (d, J = 9.3 Hz, 2H), 4.32–4.09 (m, 2H), 3.13 (m, 1H), 2.84 (t, J = 4.4 Hz, 1H), 2.58 (dd, J = 4.9, 2.4 Hz, 1H), 2.31–2.07 (m, 1H), 2.03–1.78 (m, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ = 164.42, 142.24, 126.59, 115.09, 66.23, 50.00, 47.78, 32.85; elemental analysis calcd (%) for $C_{10}H_{11}NO_4$: C 57.41, H 5.30, N 6.70; found: C 57.29, H 5.45, N 6.93.

(*S*)-4-(*p*-Nitrophenoxy)-1,2-epoxybutane (47): The procedure for (*S*)-**6** was applied to diol (*S*)-**23** (0.079 g, 0.35 mmol). Flash chromatography (6:4 hexane/EtOAc) gave (*S*)-**47** (0.038 g, 52%, 96% *ee*) as a solid. M.p. 44 °C;

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$[\alpha]_D^{20} = -30.3$ ($c = 0.50$, $CHCl_3$); elemental analysis calcd (%) for $C_{10}H_{11}NO_4$: C 57.41, H 5.30, N 6.70; found: C 57.32, H 5.11, N 6.39.

(*R*)-4-(*p*-Nitrophenoxy)-1,2-epoxybutane (47): Following the same procedure, diol (*R*)-**23** (0.079 g, 0.35 mmol) afforded pure (*R*)-**47** (0.030 g, 41%, 96% *ee*) as a solid. M.p. 43–44 °C; $[\alpha]_D^{20} = +28.8$ ($c = 0.46$, $CHCl_3$); elemental analysis calcd (%) for $C_{10}H_{11}NO_4$: C 57.41, H 5.30, N 6.70; found: C 57.69, H 5.02, N 6.95.

(±)-7-(4-Amino-3-methyl-3-hydroxybutyloxy)-2*H*-1-benzopyran-2-one (48): Following the procedure for **2**, epoxide **40** (0.492 g, 2.00 mmol) afforded pure **48** (0.458 g, 87%) as a yellow syrup; IR (KBr): $\tilde{\nu} = 3416$ (br s), 2970 (w), 1709 (s), 1616 (s), 1283 (s), 1256 (s), 1233 (s), 1161 (s), 1132 (s), 1032 cm^{-1} (s); ^1H NMR (200 MHz, CD_3OD): $\delta = 7.81$ (d, $J = 9.3$ Hz, 1H), 7.46 (d, $J = 8.8$ Hz, 1H), 7.00–6.71 (m, 2H), 6.19 (d, $J = 9.8$ Hz, 1H), 4.20 (t, $J = 6.1$ Hz, 2H), 2.91–2.60 (m, 2H), 2.03 (t, $J = 6.1$ Hz, 2H), 1.29 (s, 3H); ^{13}C NMR (50 MHz, CD_3OD): $\delta = 163.53$, 163.27, 156.95, 145.69, 130.44, 114.04, 113.34, 102.25, 71.50, 65.98, 51.40, 39.36, 24.92; elemental analysis calcd (%) for $C_{14}H_{17}NO_4$: C 63.87, H 6.51, N 5.32; found: C 64.04, H 6.56, N 5.13.

(±)-4-(*p*-Nitrophenoxy)-1-amino-2-hydroxybutane (49): Application of the procedure for **2** to epoxide **47** (0.522 g, 2.5 mmol) gave **49** (0.486 g, 86%) as a pale yellow solid. M.p. 88–91 °C; IR (KBr): $\tilde{\nu} = 3373$ (br s), 3114 (w), 3080 (w), 2952 (w), 1609 (s), 1596 (s), 1502 (s), 1336 (s), 1265 (s), 1180 (s), 1112 (s), 1030 cm^{-1} (s); ^1H NMR (200 MHz, CD_3OD): $\delta = 8.20$ (d, $J = 8.8$ Hz, 2H), 7.08 (d, $J = 8.8$ Hz, 2H), 4.34–4.10 (m, 2H), 4.00–3.74 (m, 1H), 2.86 (dd, $J = 12.9$, 3.7 Hz, 1H), 2.70 (dd, $J = 12.9$, 8.1 Hz, 1H), 2.14–1.67 (m, 2H); ^{13}C NMR (50 MHz, CD_3OD): $\delta = 165.57$, 142.76, 126.82, 115.76, 69.01, 66.55, 47.87, 35.22; elemental analysis calcd (%) for $C_{10}H_{14}N_2O_4$: C 53.09, H 6.24, N 12.38; found: C 53.34, H 6.03, N 12.04.

(±)-7-(4-Phenylacetamido-3-methyl-3-hydroxybutyloxy)-2*H*-1-benzopyran-2-one (50): Application of the procedure for **7** to amino alcohol **48** (0.053 g, 0.20 mmol) and flash chromatography (99.5:0.5 EtOAc/MeOH) gave **50** (0.036 g, 48%) as a white semisolid. IR (KBr): $\tilde{\nu} = 3413$ (br s), 3085 (w), 2971 (w), 1709 (s), 1638 (s), 1615 (s), 1555 (m), 1282 (m), 1232 (m), 1128 cm^{-1} (m); ^1H NMR (200 MHz, $CHCl_3$): $\delta = 7.56$ (d, $J = 9.3$ Hz, 1H), 7.34–7.10 (m, 6H), 6.74–6.57 (m, 2H), 6.19 (d, $J = 9.8$ Hz, 1H), 4.21–3.95 (m, 2H), 3.56 (s, 2H), 3.25 (d, $J = 5.9$ Hz, 2H), 1.91–1.77 (m, 2H), 1.13 (s, 3H); ^{13}C NMR (50 MHz, $CHCl_3$): $\delta = 173.34$, 162.25, 161.85, 156.53, 144.06, 135.37, 130.14, 129.85, 129.56, 128.26, 114.06, 113.51, 113.31, 102.36, 72.93, 65.77, 50.41, 44.43, 38.95, 25.98; elemental analysis calcd (%) for $C_{22}H_{23}NO_5$: C 69.28, H 6.08, N 3.67; found: C 69.10, H 5.87, N 3.32.

(±)-7-(4-Acetamido-3-hydroxybutyloxy)-2*H*-1-benzopyran-2-one (51): *The following procedure is typical.* A solution of amino alcohol **2** (0.075 g, 0.3 mmol) in dry CH_2Cl_2 (7.0 mL) and Et_3N (0.6 mmol, 84 μL) was treated at 0 °C with AcCl (0.3 mmol, 21 μL). The reaction was stirred at 0 °C for 2 h. Aqueous workup (CH_2Cl_2/saturated aqueous NaCl) and flash chromatography (99.5:0.5 EtOAc/MeOH) gave **51** (0.030 g, 34%) as a syrup; IR (KBr): $\tilde{\nu} = 3335$ (br s), 3090 (w), 2935 (w), 1710 (br s), 1645 (s), 1615 (s), 1557 (s), 1283 (m), 1233 (m), 1129 cm^{-1} (m); ^1H NMR (200 MHz, $CHCl_3$): $\delta = 7.62$ (d, $J = 9.9$ Hz, 1H), 7.35 (d, $J = 8.3$ Hz, 1H), 6.91–6.71 (m, 2H), 6.23 (d, $J = 9.8$ Hz, 1H), 4.31–4.09 (m, 2H), 4.09–3.91 (m, 1H), 3.60–3.41 (m, 1H), 3.38–3.14 (m, 1H), 2.07–1.81 (m, 2H), 2.03 (s, 3H); ^{13}C NMR (50 MHz, $CHCl_3$): $\delta = 172.38$, 162.65, 161.99, 156.53, 144.17, 129.56, 113.85, 113.47, 113.37, 102.30, 69.40, 66.26, 46.85, 34.66, 23.83; elemental analysis calcd (%) for $C_{15}H_{17}NO_5$: C 61.85, H 5.88, N 4.81; found: C 61.54, H 5.67, N 4.59.

(±)-7-(4-Acetamido-3-methyl-3-hydroxybutyloxy)-2*H*-1-benzopyran-2-one (52): Application of the procedure for **51** to **48** (0.079 g, 0.30 mmol) and flash chromatography (9:1 CH_2Cl_2/acetone) gave **52** (0.060 g, 66%) as a white semisolid. IR (KBr): $\tilde{\nu} = 3348$ (br s), 3087 (w), 2965 (w), 2935 (w), 1704 (br s), 1640 (s), 1613 (s), 1557 (s), 1385 (m), 1286 (m), 1233 (m), 1131 (s), 1019 cm^{-1} (m); ^1H NMR (200 MHz, $CHCl_3$): $\delta = 7.63$ (d, $J = 9.3$ Hz, 1H), 7.36 (d, $J = 8.3$ Hz, 1H), 6.91–6.74 (m, 2H), 6.24 (d, $J = 9.3$ Hz, 1H), 4.34–4.07 (m, 2H), 3.36 (d, $J = 5.4$ Hz, 2H), 2.14–1.81 (m, 2H), 2.05 (s, 3H), 1.27 (s, 3H); ^{13}C NMR (50 MHz, $CHCl_3$): $\delta = 172.47$, 162.42, 161.96, 156.47, 144.20, 129.59, 113.85, 113.42, 102.27, 72.73, 65.77, 50.52, 38.88, 25.83, 23.81; elemental analysis calcd (%) for $C_{16}H_{19}NO_5$: C 62.94, H 6.27, N 4.59; found: C 63.16, H 5.97, N 4.22.

(±)-4-(*p*-Nitrophenoxy)-1-phenylacetamido-2-hydroxybutane (53): Application of the procedure for **7** to amino alcohol **49** (0.079 g, 0.35 mmol) and flash chromatography (9:1 EtOAc/hexane) gave **53** (0.060 g, 51%) as a pale yellow solid. M.p. 109–111 °C; IR (KBr): $\tilde{\nu} = 3415$ (br s), 3087 (w), 2947 (w), 1639 (s), 1617 (s), 1594 (s), 1502 (s), 1346 (s), 1263 (s), 1115 cm^{-1} (m); ^1H NMR (200 MHz, $CHCl_3$): $\delta = 8.10$ (d, $J = 9.3$ Hz, 2H), 7.38–7.07 (m, 5H), 6.83 (d, $J = 9.3$ Hz, 2H), 4.24–4.00 (m, 2H), 3.95–3.77 (m, 1H), 3.55 (s, 2H), 3.38 (ddd, $J = 14.3$, 5.9, 2.6 Hz, 1H), 3.27–3.07 (m, 1H), 1.95–1.64 (m, 2H); ^{13}C NMR (50 MHz, $CHCl_3$): $\delta = 173.55$, 164.46, 142.28, 135.26, 130.12, 129.83, 128.27, 126.63, 115.16, 69.33, 66.30, 46.98, 44.33, 34.62; elemental analysis calcd (%) for $C_{18}H_{20}N_2O_5$: C 62.78, H 5.85, N 8.13; found: C 62.45, H 5.91, N 7.87.

(±)-4-(*p*-Nitrophenoxy)-1-acetamido-2-hydroxybutane (54): Application of the procedure for **51** to amino alcohol **49** (0.068 g, 0.30 mmol) and flash chromatography (99.5:0.5 EtOAc/MeOH) gave **54** (0.045 g, 56%) as a pale yellow solid. M.p. 134–136 °C; IR (KBr): $\tilde{\nu} = 3395$ (br s), 2927 (w), 1641 (s), 1618 (s), 1595 (s), 1338 (s), 1267 (s), 1125 (m), 1113 (m), 1082 cm^{-1} (m); ^1H NMR (200 MHz, $CHCl_3$): $\delta = 8.19$ (d, $J = 9.0$ Hz, 2H), 6.95 (d, $J = 8.8$ Hz, 2H), 4.32–4.11 (m, 2H), 4.07–3.89 (m, 1H), 3.50 (ddd, $J = 14.2$, 6.34, 2.9 Hz, 1H), 3.36–3.18 (m, 1H), 2.04 (s, 3H), 2.00–1.82 (m, 2H); ^{13}C NMR (50 MHz, $CHCl_3$): $\delta = 172.45$, 164.27, 142.15, 126.50, 115.01, 69.21, 66.24, 46.83, 34.53, 23.67; elemental analysis calcd (%) for $C_{12}H_{16}N_2O_5$: C 53.73, H 6.01, N 10.44; found: C 53.41, H 5.73, N 10.39.

(±)-7-(3,4-Dihydroxybutyloxy)-2*H*-1-benzopyran-2-one-3,4-bis(dibenzylphosphate) (55): *The following procedure is typical.* A solution of **1** (0.020 g, 0.08 mmol) and 1*H*-tetrazole (0.034 g, 6 equiv) in anhydrous dichloromethane (5 mL) was treated at 25 °C under stirring with dibenzyl-*N*,*N*-diisopropylphosphoramidite (0.055 mL, 2 equiv) and stirred at 25 °C for 2 h. The solution was cooled to −78 °C, *m*-CPBA (0.055 g, 4 equiv) was added and stirring was prolonged for 45 min at 0 °C. A solution of saturated aqueous $NaHCO_3$ (1 mL) was added, and the mixture concentrated in vacuo. The residue was taken with CH_2Cl_2, washed (saturated aqueous $NaHCO_3$, water and brine) and purified by flash chromatography (9:1 CH_2Cl_2/acetone) to give **55** (0.035 g, 57%) as a yellow syrup; ^1H NMR (300 MHz, [D$_6$]Ac): $\delta = 7.87$ (d, $J = 9.5$ Hz, 1H), 7.53 (d, $J = 8.5$ Hz, 1H), 7.41–7.30 (m, 20H), 6.89–6.82 (m, 2H), 6.21 (d, $J = 9.5$ Hz, 1H), 5.11–5.00 (m, 9H), 4.34–4.18 (m, 4H), 2.19–2.17 (m, 2H); ^{13}C NMR (75 MHz, [D$_6$]Ac): $\delta = 163.46$, 161.58, 157.64, 145.20, 137.94, 130.80, 130.03, 129.97, 129.88, 129.78, 129.51, 129.42, 129.30, 114.39, 114.19, 102.79, 75.89, 70.57, 70.50, 70.10, 65.66, 32.57; ^{31}P NMR (81 MHz, [D$_6$]Ac): $\delta = 5.85$ (d, $J = 40.5$ Hz), 5.05 (d, $J = 40.5$ Hz).

(*R*)-7-(3,4-Dihydroxy-4-methylbutyloxy)-2*H*-1-benzopyran-2-one-3,4-bis-(dibenzylphosphate) (56): Application of the procedure for **55** to diol (*R*)-**15** (0.020 g, 0.076 mmol) gave (*R*)-**56** (0.011 g, 18%, 94.5% ee) as a yellow syrup. ^1H NMR (300 MHz, $CDCl_3$): $\delta = 8.04$ (d, $J = 9.5$ Hz, 1H), 7.63 (d, $J = 9.6$ Hz, 1H), 7.38–7.33 (m, 22H), 6.26 (d, $J = 9.5$ Hz, 1H), 5.19–4.99 (m, 8H), 4.41–4.03 (m, 4H), 2.33–2.21 (m, 2H), 1.54 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 162.25$, 161.76, 156.46, 143.94, 129.63, 129.39, 129.11, 128.72, 128.64, 128.14, 114.21, 113.07, 102.53, 76.15, 71.15, 68.04, 64.58, 64.41, 39.21, 25.48; ^{31}P NMR (81 MHz, $CDCl_3$): $\delta = 0.1$, −4.4.

(*S*)-7-(3,4-Dihydroxy-4-methylbutyloxy)-2*H*-1-benzopyran-2-one-3,4-bis-(dibenzylphosphate) (56): Application of the procedure for **55** to diol (*S*)-**15** (0.100 g, 0.38 mmol) gave (*S*)-**56** (0.163 g, 55%, 92.1% ee) as a colorless syrup; IR (neat): $\tilde{\nu} = 3475$ (m), 3035 (m), 1732 (s), 1615 (s), 1558 (m), 1509 (m), 1499 (m), 1457 (s), 1399 (m), 1352 (m), 1279 (s), 1124 (s), 1015 (s), 892 cm^{-1} (s); ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.57$ (d, $J = 9.2$ Hz, 1H), 7.25–7.21 (m, 21H), 6.71–6.62 (m, 2H), 6.20 (d, $J = 9.5$ Hz, 1H), 5.00–4.92 (m, 8H), 4.11–3.94 (m, 4H), 2.19–2.12 (m, 2H), 1.49 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 162.25$, 161.76, 156.46, 143.99, 136.33, 129.42, 129.27, 129.23, 129.18, 128.64, 128.56, 113.92, 113.47, 113.31, 102.07, 83.79, 72.10, 70.21, 69.96, 64.38, 37.04, 23.48; ^{31}P NMR (81 MHz, $CDCl_3$): $\delta = 0.1$, −4.4; HRMS: calcd for $C_{42}H_{43}O_{11}P_2$: 785.2281, obs 785.2285.

(3*R,4*R**)-7-(3,4-Dihydroxycyclopentyloxy)-2*H*-1-benzopyran-2-one-3,4-bis(dibenzylphosphate) (57) and (3*R**,4*R**)-7-(3,4-dihydroxycyclopentyloxy)-2*H*-1-benzopyran-2-one-3-dibenzylphosphate (58)**: Application of the procedure for **55** to diol **22** (0.040 g, 0.15 mmol) afforded a crude mixture consisting of phosphates derivatives **57** and **58**. Flash chromatography (8:2 CH_2Cl_2/acetone) gave compound **57** (0.019 g, 16%) and **58** (0.026 g, 33%). Compound **57**: yellow syrup; IR (neat): $\tilde{\nu} = 3438$ (s), 1731 (m), 1614 (m), 1557 (w), 1499 (w), 1457 (w), 1402 (w), 1278 (m), 1124 (w), 1000 cm^{-1} (m); ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.62$ (d, $J = 9.6$ Hz, 1H), 7.34–7.28 (m, 21H), 6.69–6.59 (m, 2H), 6.26 (d, $J = 9.2$ Hz, 1H), 5.04–4.96 (m, 9H), 4.79–4.70 (m, 2H), 2.48–2.39 (m, 1H), 2.25–2.22 (m, 1H), 2.02–1.97 (m,

1H), 1.28–1.25 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 161.69$, 161.22, 156.47, 143.93, 136.30, 136.21, 129.54, 129.41, 129.34, 129.27, 128.77, 128.66, 128.59, 114.10, 113.38, 102.90, 76.33, 70.27, 38.22, 37.71; ^{31}P NMR (81 MHz, $CDCl_3$): $\delta = -1.1$; HRMS: calcd for $C_{42}H_{41}O_{11}P_2$: 783.2124; observed: 783.2119. Compound **58**: colorless syrup; IR (neat): $\tilde{\nu} = 3420$ (s), 2955 (w), 1732 (s), 1615 (s), 1558 (w), 1507 (w), 1457 (w), 1403 (w), 1352 (w), 1280 (m), 1233 (m), 1125 (m), 1015 cm^{-1} (s); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta =$ 7.62 (d, $J = 9.2$ Hz, 1H), 7.37–7.32 (m, 11H), 6.76–6.67 (m, 2H), 6.25 (d, $J = 9.2$ Hz, 1H), 5.09–4.98 (m, 4H), 4.74–4.64 (m, 2H), 4.22–4.16 (m, 1H), 2.61–2.51 (m, 1H), 2.22–2.07 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 161.77$, 161.11, 156.46, 143.99, 129.55, 129.48, 129.38, 129.35, 128.82, 128.76, 128.58, 114.27, 113.99, 113.34, 102.79, 84.40, 76.64, 75.52, 70.53, 38.65, 38.21; ^{31}P NMR (81 MHz, $CDCl_3$): $\delta = 0.9$; HRMS: calcd for $C_{28}H_{27}O_8P$: 523.1521; observed: 523.1508.

(1*R*,5*S*,7r)-7-(3-benzyloxy-3-oxido-2,4-dioxa-3-phospha-bicyclo[3.3.0]octyl)-2*H*-1-benzopyran-2-one (59): Following the procedure for **55**, diol **20** (0.060 g, 0.23 mmol) afforded pure **59** (0.021 g, 22%) as a white solid. M.p. 93–95 °C; IR (neat): $\tilde{\nu} = 3423$ (s), 2925 (m), 2854 (w), 1652 (m), 1459 (w), 1072 cm^{-1} (m); ^{1}H NMR (300 MHz, CD_3OD): $\delta = 7.74$ (d, $J = 9.6$ Hz, 1H), 7.46–7.41 (m, 1H), 7.36–7.32 (m, 5H), 7.07–6.89 (m, 2H), 6.22 (d, $J =$ 9.6 Hz, 1H), 5.12–5.00 (m, 5H), 2.15–2.08 (m, 2H), 1.24–1.21 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$/CD_3OD): $\delta = 162.76$, 160.91, 155.98, 144.89, 134.38, 129.68, 129.12, 129.06, 128.26, 115.75, 113.43, 113.05, 102.58, 82.41, 78.45, 70.37, 39.30; ^{31}P NMR (81 MHz, CD_3OD): $\delta = 22.1$; HRMS: calcd for $C_{21}H_{19}O_7P$: 415.0947; observed: 415.0947.

(±)-4-(*p*-Nitrophenoxy)-1,2-butanediol-1,2-bis(dibenzylphosphate) (60): Following the procedure for **55**, diol **23** (0.150 g, 0.66 mmol) afforded pure **60** (0.193 g, 39%) as a yellow oil. IR (neat): $\tilde{\nu} = 3036$ (w), 2955 (w), 1717 (w), 1594 (m), 1514 (m), 1498 (m), 1342 (s), 1264 (s), 1000 (s), 881 cm^{-1} (m); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.03$ (d, $J = 9.2$ Hz, 2H), 7.24–7.16 (m, 20H), 6.71 (d, $J = 9.2$ Hz, 2H), 5.00–4.87 (m, 8H), 4.71–4.68 (m, 1H), 4.16–3.94 (m, 4H), 2.03–1.97 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta =$ 129.34, 129.31, 129.28, 129.27, 129.25, 128.70, 128.61, 128.52, 126.52, 115.06, 70.31, 70.24, 64.71; ^{31}P NMR (81 MHz, $CDCl_3$): $\delta = -0.03$, -0.53; HRMS: calcd for $C_{38}H_{40}NO_{11}P_2$: 748.2077; observed: 748.2080.

(*R*)-7-(3,4-Dihydroxy-3-methylbutyloxy)-2*H*-1-benzopyran-2-one-3,4-bis-(dihydrogen phosphate) (61): Following the procedure for **8**, hydrogenation of bis-dibenzylphosphate (*R*)-**56** (0.011 g, 0.014 mmol) afforded pure (*R*)-**61** (0.002 g, 25%) as a colorless syrup; ^{1}H NMR (300 MHz, CD_3OD): $\delta = 7.89$ (d, $J = 9.6$ Hz, 1H), 7.53 (d, $J = 8.1$ Hz, 1H), 6.97–6.94 (m, 2H), 6.24 (d, $J = 9.6$ Hz, 1H), 4.29–4.24 (m, 2H), 3.85–3.83 (m, 2H), 2.10–2.05 (m, 2H), 1.30 (s, 3H); ^{31}P NMR (81 MHz, D_2O/CD_3OD): $\delta = 3.1$, -1.4.

(*S*)-7-(3,4-Dihydroxy-3-methylbutyloxy)-2*H*-1-benzopyran-2-one-3,4-bis-(dihydrogen phosphate) (61): Following the procedure for **8**, hydrogenation of bis-dibenzylphosphate (*S*)-**56** (0.10 g, 0.13 mmol) afforded pure (*S*)-**61** (0.044 g, 80%) as a yellow syrup; IR (KBr): $\tilde{\nu} = 3439$ (br s), 1725 (s), 1631 (s), 1556 (m), 1510 (m), 1408 (m), 1387 (m), 994 cm^{-1} (s); ^{1}H NMR (300 MHz, D_2O/CD_3CN): $\delta = 7.82$ (d, $J = 9.6$ Hz, 1H), 7.47 (d, $J = 7.7$ Hz, 1H), 6.93–6.87 (m, 2H), 6.20 (d, $J = 9.2$ Hz, 1H), 4.03–3.96 (m, 4H), 2.16–2.15 (m, 2H), 1.48 (s, 3H); ^{13}C NMR (75 MHz, D_2O/CD_3CN): $\delta =$ 164.12, 162.80, 156.21, 146.16, 130.44, 114.12, 113.69, 112.94, 102.24, 82.53, 71.41, 65.31, 37.14, 25.49; ^{31}P NMR (81 MHz, D_2O/CD_3CN): $\delta = 3.1$, -1.4.

(3*R,4*R**)-7-(3,4-Dihydroxycyclopentyloxy)-2*H*-1-benzopyran-2-one-3,4-bis-(dihydrogen phosphate) (62)**: Following the procedure for **8**, hydrogenation of bis-dibenzylphosphate **57** (0.015 g, 0.019 mmol) afforded pure **62** (0.009 g, quant.) as a yellow syrup; IR (KBr): $\tilde{\nu} = 3438$ (s), 2927 (w), 1705 (m), 1620 (s), 1557 (w), 1509 (w), 1385 (m), 1165 (m), 1089 cm^{-1} (s); ^{1}H NMR (300 MHz, D_2O/CD_3OD): $\delta = 7.99$ (d, $J = 9.5$ Hz, 1H), 7.61 (d, $J =$ 8.5 Hz, 1H), 7.00–6.98 (m, 2H), 6.32 (d, $J = 9.5$ Hz, 1H), 5.06 (m, 2H), 2.78 (m, 1H), 2.34 (m, 2H), 1.98 (m, 1H); ^{13}C NMR (75 MHz, D_2O/CD_3CN): $\delta = 164.34$, 161.92, 156.24, 146.29, 130.62, 114.86, 113.82, 113.02, 103.24, 77.41, 65.83, 64.97, 38.32, 38.22; ^{31}P NMR (81 MHz, D_2O/CD_3CN): $\delta = 4.4$.

(3*R,4*R**)-7-(3,4-dihydroxycyclopentyloxy)-2*H*-1-benzopyran-2-one-3-dihydrogen phosphate (63)**: Following the procedure for **8**, hydrogenation of dibenzylphosphate **58** (0.020 g, 0.038 mmol) afforded pure **63** (0.012 g, 92%) as a yellow syrup; IR (KBr): $\tilde{\nu} = 3424$ (s), 2931 (w), 1706 (s), 1619 (s), 1557 (w), 1508 (w), 1385 (m), 1284 (m), 1089 cm^{-1} (s); ^{1}H NMR (300 MHz, D_2O/CD_3CN): $\delta = 7.84$ (d, $J = 9.5$ Hz, 1H), 7.49 (d, $J = 8.5$ Hz, 1H), 6.89–6.83 (m, 2H), 6.21 (d, $J = 9.5$ Hz, 1H), 4.90 (m, 1H), 4.44 (m, 1H), 4.12 (m, 1H), 2.61–2.51 (m, 1H), 2.21–2.16 (m, 2H) 1.69–1.61 (m, 1H); ^{13}C NMR (75 MHz, D_2O/CD_3CN): $\delta = 164.09$, 161.97, 156.28, 146.14, 130.53, 114.76, 113.66, 113.01, 103.08, 81.48, 77.11, 76.45, 38.40, 38.30; ^{31}P NMR (81 MHz, D_2O/CD_3CN): $\delta = 3.2$.

(1r,3*R*,4*S*)-7-(3-Phosphonoxy-4-hydroxycyclopentyloxy)-2*H*-1-benzopyran-2-one (64): Following the procedure for **8**, hydrogenation of cyclic monobenzylphosphate **59** (0.020 g, 0.048 mmol) afforded pure **64** (0.011 g, 67%) as a yellow syrup; IR (KBr): $\tilde{\nu} = 3439$ (s), 2930 (w), 1705 (m), 1620 (s), 1509 (w), 1404 (w), 1385 (m), 1235 (m), 1085 cm^{-1} (s); ^{1}H NMR (300 MHz, D_2O/CD_3CN): $\delta = 7.87$ (d, $J = 9.5$ Hz, 1H), 7.51 (d, $J = 8.4$ Hz, 1H), 6.89–6.83 (m, 2H), 6.22 (d, $J = 9.2$ Hz, 1H), 4.82–4.74 (m, 1H), 4.47 (m, 1H), 4.11–4.06 (m, 1H), 2.56–2.33 (m, 2H), 2.03–1.78 (m, 2H); ^{13}C NMR (75 MHz, D_2O/CD_3CN): $\delta = 164.77$, 161.92, 156.13, 146.54, 130.61, 114.99, 113.76, 112.84, 103.03, 77.42, 76.16, 72.32, 37.76, 37.13; ^{31}P NMR (81 MHz, D_2O/CD_3CN): $\delta = 2.9$.

(±)-4-(*p*-Nitrophenoxy)-1,2-butanediol-1,2-bis-(dihydrogen phosphate) (65): A stirred solution of **60** (0.100 g, 0.13 mmol) in dry dichloromethane (5 mL) was treated at 0 °C under nitrogen with trimethylsilyl bromide, and the mixture stirred for 45 minutes more at 25 °C. Addition of water (2 mL) and aqueous work-up afforded crude **65** which was purified by preparative HPLC. Pure **65** (0.041 g, 81%) was recovered as a brown syrup; IR (KBr): $\tilde{\nu} = 3421$ (m), 3118 (m), 2934 (m), 2351 (w), 1609 (s), 1595 (s), 1510 (s), 1346 (s), 1267 (s), 1015 cm^{-1} (s); ^{1}H NMR (300 MHz, CD_3OD): $\delta = 8.20$ (d, $J =$ 9.2 Hz, 2H), 7.10 (d, $J = 9.2$ Hz, 2H), 4.67 (m, 1H), 4.31–4.27 (m, 2H), 4.15 (m, 2H), 2.24–2.11 (m, 2H); ^{13}C NMR (75 MHz, CD_3OD): $\delta = 165.46$, 142.82, 126.74, 115.88, 74.22, 68.97, 65.90, 32.76; ^{31}P NMR (81 MHz, CD_3OD): $\delta = 4.3$, 4.0.

3-Cyclopentenyl-1-tosylate (66): A solution of 3-cyclopentenol[21] (2.0 g, 0.024 mol) in anhydrous pyridine (30 mL) was treated with TsCl (5.147 g, 0.027 mol), and the reaction mixture was left at 5 °C for 18 h. Dilution with Et_2O, aqueous washing (10% aqueous HCl, saturated aqueous $NaHCO_3$ and water), and evaporation gave pure **66** (4.0 g, 70%): ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 7.79$ (d, $J = 8.3$ Hz, 2H), 7.34 (d, $J = 8.3$ Hz, 2H), 5.65 (s, 2H), 5.23–5.09 (m, 1H), 2.63–2.51 (m, 4H), 2.45 (s, 3H).

4-Methyl-3-pentenyl-1-tosylate (67) and 4-methyl-4-pentenyl-1-tosylate (68): Tosylation of a 3:1 mixture of 4-methyl-3-penten-1-ol and 4-methyl-4-penten-1-ol[22] (7.2 g, 0.072 mol) as above gave the corresponding tosylates (13.83 g, 76%), which were used as mixture in the next step. Compound **67**: ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 7.83–7.44$ (m, 2H), 7.38–7.29 (m, 2H), 5.01–4.89 (m, 1H), 3.96 (t, $J = 7.4$ Hz, 2H), 2.45 (s, 3H), 2.32 (quartet, $J = 7.1$ Hz, 2H), 1.64 (s, 3H), 1.55 (s, 3H). Compound **68**: ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 4.69$ (s, 1H), 4.58 (s, 1H), 4.18–3.98 (m, 2H), 2.10–1.75 (m, 4H).

7-(3-Butenyloxy)-2*H*-1-benzopyran-2-one (69): *The following procedure is typical*. A solution of 3-butenyl-1-tosylate[23a] (2.94 g, 13.0 mmol) in DMF (32 mL) was treated with the sodium salt of **4** (2.39 g, 13.0 mmol), and the reaction mixture was stirred at 80 °C for 16 h. Aqueous workup (diethyl ether/water) and evaporation of the residue gave **69** (2.19 g, 78%), which was used in the next step without further purification. Flash chromatography of an analytical sample (8:2 hexane/EtOAc) gave pure **69** as a solid. M.p. 46–47 °C; IR (KBr): $\tilde{\nu} = 3084$ (w), 3051 (w), 2943 (w), 1722 (s), 1612 (s), 1283 (m), 1235 (m), 1122 cm^{-1} (m); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta =$ 7.57 (d, $J = 9.8$ Hz, 1H), 7.30 (d, $J = 8.3$ Hz, 1H), 6.88–6.64 (m, 2H), 6.17 (d, $J = 9.8$ Hz, 1H), 5.96–5.68 (m, 1H), 5.20–4.98 (m, 2H), 3.99 (t, $J = 6.6$ Hz, 2H), 2.50 (q, $J = 6.7$ Hz, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 162.83$, 161.97, 156.54, 144.15, 134.47, 129.42, 118.20, 113.68, 113.19, 112.97, 102.06, 68.43, 33.98; elemental analysis calcd (%) for $C_{13}H_{12}O_3$: C 72.21, H 5.59; found: C 72.01, H, 5.37.

1-(*p*-Nitrophenoxy)-3-butene (70): Application of the procedure for **69** to 3-butenyl-1-tosylate (2.26 g, 10.0 mmol) and the sodium salt of *p*-nitrophenol (1.61 g, 10.0 mmol) gave **70** (1.428 g, 74%), which was directly used in the next step. Flash chromatography (hexane/EtOAc 8:2) of an analytical sample gave pure **70** as a liquid; IR (Nujol): $\tilde{\nu} = 3120$ (w), 3081 (w), 1616 (s), 1597 (s), 1519 (s), 1500 (s), 1371 (s), 1290 (s), 1120 cm^{-1} (m); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 8.18$ (d, $J = 9.3$ Hz, 2H), 6.94 (d, $J =$ 9.3 Hz, 2H), 6.01–5.75 (m, 1H), 5.25–5.08 (m, 2H), 4.10 (t, $J = 6.6$ Hz, 2H), 2.58 (q, $J = 6.7$ Hz, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 164.69$, 142.16, 134.31, 126.57, 118.34, 115.16, 68.70, 33.99; elemental analysis calcd (%) for $C_{10}H_{11}NO_3$: C 62.17, H 5.74, N 7.25; found: C 62.53, H 6.11, N 6.88.

1-(6-Methoxy-2-naphthyl)-ethene (71):[31a] A 1.6 M solution in hexane of BuLi (3.25 mL, 5.21 mmol) was added, at -20 °C under an argon

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atmosphere, to a stirred slurry of $(Ph_3PCH_3)Br$ (1.897 g, 5.31 mmol) in anhydrous THF (15 mL). After 1.5 h stirring (during this period the temperature was allowed to raise to 0 °C) the reaction was cooled again at −20 °C and a solution of 6-methoxy-2-naphthaldeyde[24] (0.94 g, 5.05 mmol) in anhydrous THF (40 mL) was added dropwise. The reaction mixture was stirred for 2 h at 25 °C and saturated aqueous NH_4Cl was added (10 mL). Aqueous workup (Et_2O/water) and flash chromatography (9:1 hexane/EtOAc) gave pure **71** (0.726 g, 78 %) as a solid.[31b]

7-[5-(Methyl-2,3-*O*-isopropylidene-*α*-L-lyxofuranosyloxy)]-2*H*-1-benzopyran-2-one (72): A solution of methyl-2,3-*O*-isopropylidene-5-*O*-*p*-toluenesulfonyl-*α*-L-lyxofuranoside[25] (1.2 g, 3.35 mmol) in dry DMF (20 mL) was cooled to 0 °C and NaH (0.12 g, 5 mmol) and **4** (0.81 g, 5 mmol) were added under nitrogen. The reaction mixture was stirred at 80 °C for 18 h. Aqueous workup (50 mL EtOAc, 3 × 20 mL 1M NaOH), drying over $MgSO_4$ and concentrated in vacuo gave **72**, which was directly used for the preparation of **24**, without any further purification. ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.64$ (d, $J = 9.6$ Hz, 1H), 7.38 (d, $J = 9.6$ Hz, 1H), 6.91 (m, 2H), 6.27 (d, $J = 9.6$ Hz, 1H), 4.98 (m, 1H), 4.84 (m, 1H), 4.62 (m, 1H), 4.34 (m, 2H), 4.25 (m, 1H), 3.39 (s, 3H), 1.49 (s, 3H), 1.34 (s, 3H).

7-[5-(Methyl-2,3-*O*-isopropylidene-*β*-D-ribofuranosyloxy)]-2*H*-1-benzopyran-2-one (73): Application of the procedure for **72** to methyl-2,3-*O*-isopropylidene-5-*O*-*p*-toluenesulfonyl-*β*-D-ribofuranoside[26](1.2 g, 3.35 mmol) gave **73**, which was used for the preparation of **25** without further purification. ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.64$ (d, $J = 9.5$ Hz, 1H), 7.38 (d, $J = 8.8$ Hz, 1H), 6.87 (m, 2H), 6.28 (d, $J = 9.5$ Hz, 1H), 5.04 (s, 1H), 4.80 (d, $J = 5.9$ Hz, 1H), 4.65 (d, $J = 5.9$ Hz, 1H), 4.56 (m, 1H), 4.06 (m, 2H), 3.35 (s, 3H), 1.53 (s, 3H), 1.36 (s, 3H).

Kinetic measurements: All substrates were diluted from stock solutions in 50 % aqueous DMF, and stored at +4 °C. Stock solutions were adjusted according to the integration of substrate peak as analyzed by RP-HPLC (peak at 254 or 325 nm, see Table 4). For each enzyme measurement set, a stock solution of enzyme (lyophilized powder, 100 or 10 μg mL^{-1}), BSA (2 mg mL^{-1}), and $NaIO_4$ (1 mM) was prepared in advance in 20 mM aqueous borate at pH 8.8, and mixed to the stock solution of substrate just before the measurement. Assays (0.1 mL) were followed in individual wells of round-bottom polypropylene 96-well plates (Costar) by using a Cytofluor II fluorescence plate reader (Perseptive Biosystems, filters $\lambda_{ex} = 360 \pm 20$ nm, $\lambda_{em} = 460 \pm 20$ nm), or of polystyrene 96-well plates (Costar) by using a Spectramax 250 microplate spectrophotometer (Molecular Devices). Fluorescence data were converted to umbelliferone or 6-methoxy-2-naphthaldehyde concentration by means of a calibration curve, whereas optical density at 405 nm was converted to 4-nitrophenol concentration. The rates indicated in the tables are derived from the steepest linear portion in each curve. Commercial enzyme preparations were purchased from Fluka, Aldrich, Sigma, Boehringer Mannheim, or Serva.

Table 4. Analytical RP-HPLC conditions for selected compounds.[a]

Compound	% A	% B	t_R [min]
1	60	40	3.9
2	60	40	2.8
5a	50	50	6.2
5b	50	50	19.6
6	80	20	10.7
7	60	40	28.4
9	60	40	9.2
15	60	40	5.1
16	60	40	7.4
17	60	40	8.6
18	60	40	7.6
20	60	40	3.7
21	60	40	4.4
22	60	40	3.5
25	50	50	3.0
26	50	50	8.4
41	80	20	36.5
42	80	20	11.6
44	60	40	4.0
45	60	40	8.4
46	50	50	5.5
47	95	5	2.3

[a] Isocratic elution at 1.5 mL min^{-1}, detection by UV at 254 or 325 nm, A = 0.1 % TFA in H_2O, B = 50/50 CH_3CN/H_2O, analytical column: Vydac 218TP-54 (C18, pore size 300 Å), 0.45 × 22 cm.

Data treatment for gray scale activity arrays: The file format used to generate the gray scale arrays is the portable-gray-map (.pgm) format. Each grid position is first assigned a whole number between 0 (full black, maximum activity) and 255 (white, no activity), which is done by simple calculation from the reaction rates determined, typically in a Excel file. The grid of numbers is then saved as comma-separated value (.csv) file. This file is then opened in a text editor (wordpad, notepad, simpletext), and the following three (or four) lines are inserted at the top of the file:

P2
(optional line with identifier)
X Y
255

where *X* is the number of columns in the array and *Y* the number of lines in the array. The file is then saved from the text editor in the portable-gray-map format by simply adding ".pgm" to the filename. This file is opened by a photo-software such as Photoshop, Paintshop, Coreldraw, etc., resized to a width of approximately 200 pixels, and saved in bitmap file format (.bmp).

Data treatment for colored selectivity arrays: The file format used to generate the colored selectivity arrays is the portable-pixel-map (.ppm) format. Each grid position is first assigned three whole numbers corresponding to the RGB color-code between 0 (zero intensity, maximum activity) and 255 (maximum intensity, no activity) as follows: the first number is set according to the activity observed with the (*R*)-enantiomer (or the first of two given stereoisomers), the second number according to the activity observed with the (*S*)-enantiomer (or second stereoisomer), and the third number is simply the mathematical average of the first two numbers. Thus a grid with *X* columns and *Y* lines is coded with 3*X* columns and *Y* lines of whole numbers between 0 and 255. The grid of numbers is saved as comma-separated-value (.csv) file. This file is then opened in a text editor as above and the following three (or four) lines are inserted at the top of the file:

P3
(optional line with identifier)
X Y
255

where *X* is the number of columns in the array and *Y* the number of lines in the array. The file is then saved from the text editor in the portable-pixel-map format by simply adding ".ppm" to the filename. This file is opened by a photo-software as above and resized to a width of approximately 200 pixels, and saved as bitmap file format (.bmp).

In this format it is possible to permutate the order of the three columns corresponding to the RGB code, and obtain also an orange-to-blue and a green-to-blue scale.

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[1] A. T. Bull, A. W. Bunch, G. K. Robinson, *Curr. Opin. Microbiol.* **1999**, *2*, 246.

[2] a) B. Marrs, S. Delagrave, D. Murphy, *Curr. Opin. Microbiol.* **1999**, *2*, 241; b) W. P. C. Stemmer, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 10747; c) W. P. C. Stemmer, *Nature* **1994**, *370*, 389; d) L. You, F. H. Arnold,

Protein Eng. **1996**, *9*, 77; e) M. T. Reetz, K.-E. Jaeger, *Chem. Eur. J.* **2000**, *6*, 407; f) S. V. Taylor, P. Kast, D. Hilvert, *Angew. Chem.* **2001**, *113*, 3408; *Angew. Chem. Int. Ed.* **2001**, *40*, 3310.

[3] a) M. T. Reetz, *Angew. Chem.* **2001**, *113*, 292; *Angew. Chem. Int. Ed.* **2001**, *40*, 284; b) D. Wahler, J.-L. Reymond, *Curr. Opin. Chem. Biol.* **2001**, *5*, 152; c) F. Beisson, A. Tiss, C. Rivière, R. Verger, *Eur. J. Lipid Sci. Technol.* **2000**, 133; d) D. Wahler, J.-L. Reymond, *Curr. Opin. Biotechnol.* **2001**, *12*, 535.

[4] G. Klein, J.-L. Reymond, *Helv. Chim. Acta* **1999**, *82*, 400.

[5] R. Pérez Carlòn, N. Jourdain, J.-L. Reymond, *Chem. Eur. J.* **2000**, *6*, 4154.

[6] F. Badalassi, D. Wahler, G. Klein, P. Crotti, J.-L. Reymond, *Angew. Chem.* **2000**, *112*, 4233; *Angew. Chem. Int. Ed.* **2000**, *39*, 4067.

[7] For preliminary communication of this work, see: D. Wahler, F. Badalassi, P. Crotti, J.-L. Reymond, *Angew. Chem.* **2001**, *113*, 4589, *Angew. Chem. Int. Ed.* **2001**, *40*, 4457.

[8] a) K. Kikuchi, S. N. Thorn, S. N., D. Hilvert *J. Am. Chem. Soc.* **1996**, *118*, 8184; b) F. Hollfelder, A. J. Kirby, D. S. Tawfik, *Nature* **1996**, *383*, 60.

[9] a) B. L. Vallee, US Patent 5162203, **1992**; b) B. List, C. F. Barbas, R. A. Lerner, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 15351.

[10] H. C. Kolb, M. S. VanNieuwenhze, K. B. Sharpless, *Chem. Rev.* **1994**, *94*, 2483.

[11] H. C. Kolb, K. B. Sharpless, *Tetrahedron* **1992**, *48*, 10515.

[12] M. Chini, P. Crotti, L. Favero, F. Macchia, M. Pineschi, *Tetrahedron Letters* **1994**, *35*, 433.

[13] a) J. Bussière, A. Foucart, L. Colobert, *C. R. Séances Acad. Sci. Ser. D* **1967**, *264*, 415; b) D. Monget, P. Nardon, French patent FR 2341865, Paris, **1976**; US patent 4277561, **1981**; c) M. W. Humble, A. King, I. Philipps, *J. Clin. Pathol.* **1977**, *30*, 275; d) E. Gruner, A. Von Graevenitz, M. Altwegg, *J. Microbiol. Methods* **1992**, *16*, 101; e) P. Garcia-Martos, P. Marin, J. M. Hernandez-Molina, L. Garcia-Agudo, S. Aoufi, J. Mira, *Mycopathologia* **2001**, *150*, 1; f) http://www.biomerieux.com

[14] a) K. Brocklehurst, D. Kowlessur, M. O'Driscoll, G. Patel, S. Quenby, E. Salih, W. Templeton, E. W. Thomas, F. Willenbrock, *Biochem. J.* **1987**, *244*, 173; b) Y. Liu, M. P. Patricelli, B. F. Cravatt, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 14694; c) D. Kidd, Y. Liu, B. F. Cravatt, *Biochemistry* **2001**, *40*, 4005; d) M. Bogyo, S. Verhelst, V. Bellingard-Dubouchaud, S. Toba, D. Greenbaum, *Chem. Biol.* **2000**, *7*, 27; e) D. Greenbaum, K. F. Medzihradszky, A. Burlingame, M. Bogyo, *Chem. Biol.* **2000**, *7*, 569; f) T. Nazif, M. Bogyo, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 2967.

[15] G. C. Adam, B. F. Cravatt, E. J. Sorensen, *Chem. Biol.* **2001**, *8*, 81.

[16] Natural vegetal oils and other natural substrates of hydrolytic enzymes can be assayed directly with lipases and esterases by using a back-titration scheme relying on adrenaline as a chromogenic indicator for sodium periodate: D. Wahler, J.-L. Reymond, *Angew. Chem.* **2002**, 114, 1277; *Angew. Chem. Int. Ed.* **2002**, *41*, 1229.

[17] a) K. Breddam, M. Meldal, *Eur. J. Biochem.* **1992**, *206*, 103; b) M. Meldal, I. Svendsen, K. Breddam, F. I. Auzanneau, *Proc. Natl. Acad. Sci.USA* **1994**, *91*, 3314; c) T. A. Rano, T. Timkey, E. P. Peterson, J. Rotonda, D. W. Nicholson, J. W. Becker, K. T. Chapman, N. A. Thornberry, *Chem. Biol.* **1997**, *4*, 149; d) P. M. St. Hilaire, M. Willert, M. A. Juliano, L. Juliano, M. Meldal, *J. Comb. Chem.* **1999**, *1*, 509; e) B. J. Backes, J. L. Harris, F. Leonetti, C. S. Craik, J. A. Ellman, *Nature Biotechnol.* **2000**, *18*, 187; f) J. H. Harris, B. J. Brackes, F. Leonetti, S. Mahrus, J. A. Ellmann, C. S. Craik, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 7754; g) J. E. Sheppeck II, H. Kar, L. Gosink, J. B. Wheatley, E. Gjerstad, S. M. Loftus, A. R. Zubiria, J. W. Janc, *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2639; h) D. J. Maly, L. Huang, J. A. Ellmann, *ChemBioChem* **2002**, *3*, 16.

[18] A. Archelas, R. Furstoss, *Curr. Opin. Chem. Biol.* **2001**, *5*, 112.

[19] D. Wahler, J.-L. Reymond, *ChemBioChem* **2002**, in press.

[20] For measuring E-values in high-thoughput, see also: a) L. E. Janes, R. J. Kazlauskas, *J. Org. Chem.* **1997**, *62*, 4560; b) L. E. Janes, A. C. Löwendahl, R. J. Kazlauskas, *Chem. Eur. J.* **1998**, *4*, 2324; c) A. M. F. Liu, N. A. Somers, R. J. Kazlauskas, T. S. Brush, F. Zocher, M. M. Enzelberger, U. T. Bornscheuer, G. P. Horsman, A. Mezzetti, C. Schmidt-Dannert, R. D. Schmid, *Tetrahedron: Asymmetry* **2001**, *12*, 545.

[21] J. K. Crandall, D. B. Banks, R. A. Colyer, R. J. Watkins, J. P. Arrington, *J. Org. Chem.* **1968**, *33*, 423.

[22] The mixture of 4-methyl-3-penten-1-ol and 4-methyl-4-penten-1-ol was prepared by addition of MeMgI to α-butyrolactone, as previously described: B. K. Sarmah, N. C. Barua, *Tetrahedron* **1993**, *49*, 2253. The alcohols regioselectivity (3:1) obtained by us is different from the corresponding result (95:5) reported by the original authors.

[23] a) X. F. Ren, E. Turos, C. H. Lake, M. R. Churchill, *J. Org. Chem.* **1995**, *60*, 6468; b) M. C. Musatto, D. Savoia, C. Trombini, A. Umani-Ronchi, *J. Org. Chem.* **1980**, *45*, 4002; c) V. J. Davisson, A. B. Woodside, T. R. Neal, K. E. Stremler, M. Muehlbacher, C. D. Poulter, *J. Org. Chem.* **1986**, *51*, 4768.

[24] R. G. Harvey, C. Cortez, T. W Sawyer, J. DiGiovanni, *J. Med. Chem.* **1988**, *31*, 1308.

[25] R. K. Hulyalkar, M. B. Perry, *Can. J. Chem.* **1965**, *43*, 3241.

[26] E. J. Reist, R. R. Spencer, M. E. Wain, I. G. Junga, L. Goodman, B. R. Baker, *J. Org. Chem.* **1961**, *26*, 2821.

[27] The configuration of the diols were assigned on the basis of the Sharpless mnemonic device applied to the parent olefin.

[28] In the case of olefins **11**, **13** and of the mixture of olefin **12** and 7-(4-methyl-4-pentenyloxy)-2*H*-1-benzopyran-2-one, $MeSO_2NH_2$ (1 equiv, based on olefin) was added to the reaction mixture. In these cases, the organic extracts were washed also with aqueous 1N NaOH.

[29] As a consequence of the cyclization procedure adopted, the configuration of the diol is maintained in the corresponding epoxide.

[30] The reported *ee* value of diols (*R*)- and (*S*)-**16** were derived from those of the corresponding epoxides (*R*)- and (*S*)-**41**.

[31] a) For a similar preparation of 1-(6-methoxy-2-naphthyl)-ethene see: A. L. Casalnuovo, T. V. RajanBabu, T. A. Ayers, T. H. Warren, *J. Am. Chem. Soc.* **1994**, *116*, 9869; b) for a full characterization of 1-(6-methoxy-2-naphthyl)-ethene see: W. Nugent, R. J. McKinney, *J. Org. Chem.* **1985**, *50*, 5370.

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