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Cyclodextrin Ketones with the Catalytic Group at the Secondary Rim and Their Effectiveness in Enzyme-Like Epoxidation of Stilbenes

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Several new cyclodextrin ketones with a ketone attached to the secondary face of the cyclodextrin in the form of a 2,3-*O*-(2-oxopropane-1,3-diyl) or 2-oxo group are reported. These compounds and a selection of known cyclodextrin ketones having the ketone at the primary face were investigated as epoxidation catalysts for oxidation of stilbenes and styrene. A method for determination of k_{cat} in these epoxidations is presented, which was used to determine the rate accelerations for the cyclodextrin ketones relative to background reaction. The highest rate acceleration obtained for epoxidation of 4-methoxy-4′-nitro-*trans*-stilbene was 221. The highest enantioselectivity obtained was 76 % *ee* of (*S*)-styrene oxide.

Introduction

Artificial enzymes are molecules built to mimic an existing or perceived enzyme active-site as part of a bottom-up approach to understanding enzyme catalysis and/or to prepare new and selective catalysts.[1] Such chemzymes are typically made by modification of a supramolecular host capable of substrate binding aiming to turn it into a catalytic machine.[2] Artificial enzymes differ from other catalysts in that binding of the substrate by the catalyst before and during catalysis. This can result in rate-enhancements due to proximity effects that will not be observed with other, simpler catalysts. Cyclodextrins have been shown to be valuable supramolecular hosts in artificial enzyme models[3] and a number of different reactions are catalysed by cyclodextrin derivatives in aqueous solution in an enzymatic manner.[4] But while catalytic epoxidations, particularly asymmetric versions, are immensely popular in chemistry,[5] artificial enzymes that catalyse epoxidation have received comparatively little attention. Previously we prepared several cyclodextrin ketones with a ketone bridge spanning the primary face.[6,7] These compounds catalysed epoxidation of styrene and indene in water in the presence of oxone with an enantioselectivity up to 45 % *ee*, and inhibition experiments showed that the cyclodextrin cavity was involved in the catalysis. Turnover was also established though 30 mol-% or more of catalyst was required. On the other hand we were not able to demonstrate Michaelis–Mentene kinetics and determine the rate acceleration of these reactions. Subsequently Chan et al. reported that a cyclodextrin with pyruvate ester residues attached to the primary face catalysed epoxidation of 4-chlorostyrene with up to 40 % *ee* in the presence of oxone.[8] In this study stoichiometric amounts of ketone was used.

In neither of the above studies were enzyme kinetics demonstrated and which also made it impossible to determine if rate enhancements due to proximity effects where occurring. It was therefore a primary stimulus for this work to attempt to obtain Michaelis–Menten kinetic data for the cyclodextrin catalysed epoxidations. To reach this goal we decided to look at epoxidation of stilbenes since this reaction leads to a significant change in UV absorption. In the present work we have also prepared new cyclodextrin ketones 1–4 (Figure 1) with a 2,3-*O*-(1,3-acetone) group attached to the secondary face and investigated their catalysis of epoxidation of stilbenes. This is compared with the catalysis by a number of previously reported cyclodextrin ketones, primarily with the ketone attached to the primary

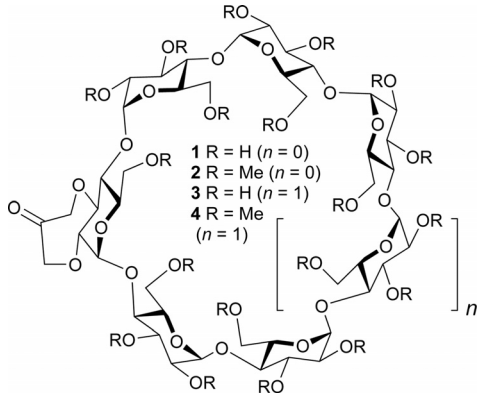

Figure 1. Structure of novel cyclodextrin ketones **1**–**4** having a propan-2-one moiety attached to O2 and O3.

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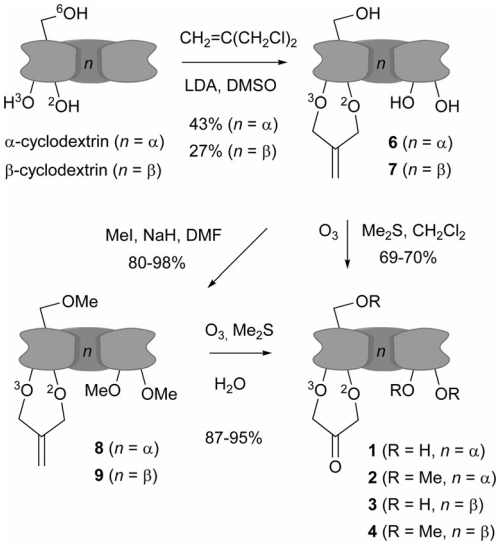
cyclodextrin face. We find many of the cyclodextrin ketones do display Michaelis–Menten catalysis of epoxidation, but the new secondary face ketones are the better catalysts giving a k_{cat}/k_{uncat} over 10^2. They are also more stereoselective than the previous epoxidation giving up to 76% *ee*.

Results

Epoxidation of stilbene was proposed to be a good reaction to follow by UV because the reaction results in disruption of the conjugated system and a significant change in the UV spectrum. Since the substrate is highly absorbing while the product is not it is however necessary to follow the reaction with a large excess of cyclodextrin ketone and a small amount of substrate making sure that saturation is obtained; under those conditions the k_{cat} is obtained directly from the rate.[9]

Synthesis

In order to catalyse epoxidation of stilbene, cyclodextrin derivatives with the ketone on the secondary rim are most likely to be effective since the secondary face is wider and a large substrate like stilbene will bind predominately from this face. Inspired by the work of Fernandez[10] who reported 2,3-alkylation of α,β- and γ-cyclodextrin with *o*-xylylene dibromide in 28–33% yield, we attempted a similar alkylation of α-cyclodextrin using methallyl dichloride and LDA in DMSO (Scheme 1). This reaction gave a 43% yield of 2,3-alkylated derivative **6**. The reaction could also be performed on β-cyclodextrin, but the product **7** was obtained in a lower yield (27%). The structure of **6** and **7** were determined by acidic hydrolysis of the compounds, acetylation and analysis of the acetalyted products using NMR and MS. This showed the presence of peracetylated glucose **7a** and the modified sugar **6a** (Figure 2) as the only products.



Scheme 1. Synthesis of ketones **1**–**4**.

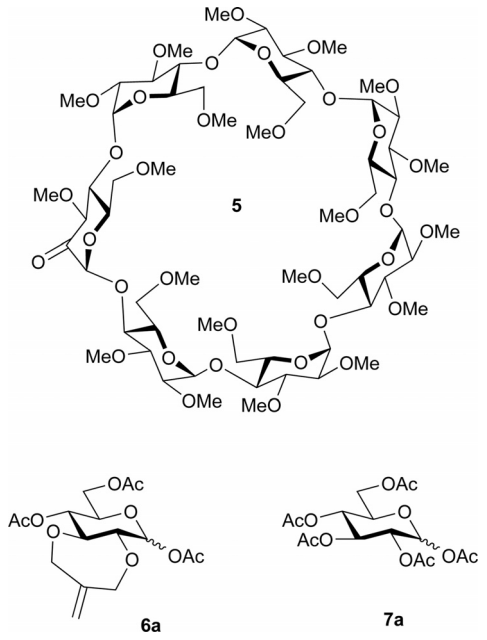
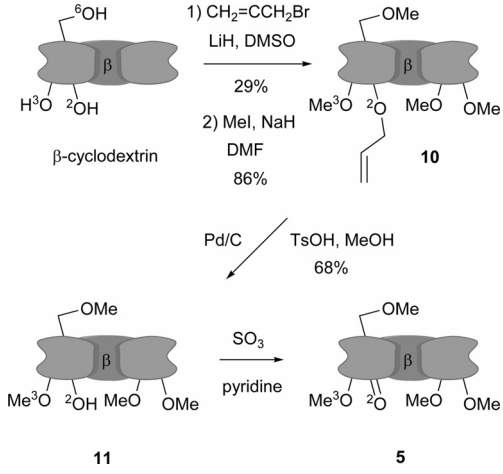


Figure 2. Structure of novel cyclodextrin ketone **5** where O2 has been oxidised.

The successful synthesis of **6** and **7** allowed preparation of the corresponding ketones: Either **6** or **7** were oxidised with ozone, which gave ketones **1** and **3**, respectively in 69–70% yield (Scheme 1). Alternatively per-*O*-methylation of **6** and **7** with MeI/NaH in DMF gave the per-*O*-methyl alkenes **8** and **9** in 80 and 98% yield, respectively. Ozonolysis of **8** and **9** gave the methylated ketones **2** and **4** in 87 and 95% yield, respectively (Scheme 1).

An alternative secondary rim ketone, **5**, was prepared (Figure 2) where the ketone is situated directly in the carbohydrate backbone through oxidation of the 2-OH. This compound was made as outlined in Scheme 2: monoallylation of β-cyclodextrin could be carried out in 29% yield (Scheme 2) and treatment with MeI and NaH gave the per-*O*-methyl 2-*O*-allyl derivative **10** in 86% yield. Deallylation with palladium on carbon, MeOH and TsOH gave the



Scheme 2. Synthesis of ketone **5**.

monool **11** in 68% yield (Scheme 2). Oxidation of this compound proved difficult. Swern or Jones oxidation did not give product and PDC only led to 25% conversion. $KMnO_4$ oxidation gave decomposition. We believe the difficulties here is caused by a strong hydrogen bond of the 2-OH to the 3-OMe of the neighboring glucose residue. The reaction was eventually achieved with pyridine/SO_3: When 20 equiv. of reagents were used 50% conversion was obtained, but ketone and alcohol were very difficult to seperate. With 40 equiv. of reagents full conversion was achieved but the yield of **5** was only 40%.

The new compounds **1**–**5** were supplemented with the known cyclodextrin ketones **12**, **13**,[7] **14**,[7] **15**,[6] **16**[11] and **17**.[11]

Compound **12** is formaly a new compound, but is nevertheless a simple analogue of Wong's ketone.[8] It was made by derivatisation of benzylated β-cyclodextrin diol[12] and hydrogenolysis.

Epoxidation Experiments

The ketones were tested for catalysis of epoxidation of *cis*- and *trans*-stilbene, 4-methoxy-4′-nitro-*trans*-stilbene and styrene. The experiments were conducted in 1:1 acetonitrile/H_2O at 25 °C with oxone 100 equiv. (2 mM), $NaHCO_3$ 500 equiv. (10 mM), a low concentration of substrate and a large excess of cyclodextrin. This setup has the advantages that 1) the substrate is dissolved despite its lipophilicity, 2) all substrate gets bound to the cyclodextrin which means that the rate for conversion of substrate equals V_{cat} and 3) the reaction can be followed by monitoring the disappearance of the UV-active substrate since its converted into less absorbing epoxide. The excess of cyclodextrin derivative was varied from 30–85 equiv. (0.6–1.7 mM) to ensure that saturation of binding was reached. This led to plots such as shown in Figure 3: The epoxidation rate catalysed by the cyclodextrin derivative as a function of cyclodextrin catalyst follows a curve (♦) that approaches a maximum rate when all substrate is bound in the cavity. For comparison the rate of epoxidation by a simple ketone, diacetoxyacetone, (▲) shows no saturation. From the rate at saturation k_{cat} was determined and is listed in Table 1 for each case. The rate of the uncatalyzed reaction was also determined for all substrates allowing k_{uncat} and thus the rate acceleration induced by binding to the cyclodextrin derivative (k_{cat}/k_{uncat}) to be determined. Depending on the catalyst rate-enhancements from 1–221 were obtained.

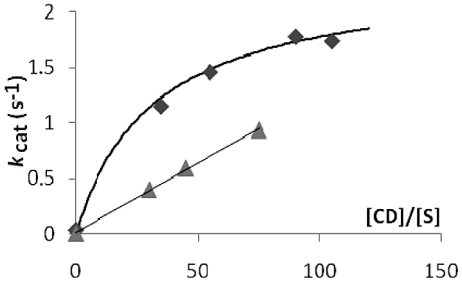


Figure 3. Plot of the epoxidation rate as a function of equivalents of catalyst either for catalyst **2** (♦) or diacetoxyacetone (▲). The substrate for **2** was 4-methoxy-4′-nitrostilbene (with a unit of k_{cat} of 10^{-6} s^{-1}), while it was *trans*-stilbene for diacetoxyacetone (with unit of 10^{-5} s^{-1}).

Table 1. Kinetic data for epoxidation of stilbenes by cyclodextrins. 4 M4′NSt = 4-methoxy-4′-nitro-*trans*-stilbene.% *ee* is the enantiomeric excess of the product as determined by HPLC (the letters in parentheses denotes which enantiomer is in excess); n.d.: not determined, *sel* is the selectivity for formation of one enantiomer over the other.

Cat.	Substrate	% *ee*	*sel*	k_{cat} ($\times 10^6$ s^{-1})	k_{cat}/k_{uncat}
1	styrene	n.d.		1.13 ± 0.09	12
1	(*Z*)-stilbene			0.70 ± 0.02	16
1	(*E*)-stilbene	56.8 (*R*,*R*)	3.6	1.73 ± 0.02	47
1	4M4′NSt	n.d.		1.20 ± 0.01	48
2	styrene	75.8 (*S*)	7.3	1.67 ± 0.09	17
2	(*Z*)-stilbene			0.91 ± 0.03	20
2	(*Z*)-stilbene	52.4 (*R*,*R*)	3.2	3.27 ± 0.06	74
2	4M4′NSt	n.d.		2.32 ± 0.02	93
3	styrene	n.d.		4.70 ± 0.04	49
3	(*Z*)-stilbene			3.87 ± 0.05	85
3	(*E*)-stilbene	70 (*R*,*R*)	5.7	3.34 ± 0.05	105
3	4M4′NSt	n.d.		2.71 ± 0.03	109
4	styrene	n.d.		0.28 ± 0.12	3
4	(*Z*)-stilbene			0.15 ± 0.001	3
4	(*E*)-stilbene	37.8 (*R*,*R*)	2.2	0.26 ± 0.01	7
4	4M4′NSt	n.d.		0.165 ±0.002	7
5	styrene	4.1 (*S*,*S*)	1.1	n.d.	n.d.
5	(*E*)-stilbene	6.5 (*S*,*S*)	1.1	3.46 ± 0.02	83
5	4M4′NSt	n.d.		2.94 ± 0.01	221
12	(*E*)-stilbene	n.d.		0.21 ±0.01	5
12	4M4′NSt	n.d.		0.086 ±0.002	3
13	(*E*)-stilbene	n.d.		0.29 ± 0.01	7
13	4M4′NSt	n.d.		0.36 ± 0.01	15
14	(*E*)-stilbene	n.d.		0.197 ±0.004	4
14	4M4′NSt	n.d.		0.23 ± 0.01	10
15	(*E*)-stilbene	n.d.		0.085 ±0.003	1.8
15	4M4′NSt	n.d.		0.030 ±0.001	1.2
16	4M4′NSt	–		no catalysis	–
17	4M4′NSt	–		no catalysis	–

For some of the best (*E*)-stilbene epoxidations and a styrene epoxidation, the epoxide was isolated upon completed reaction and the chirality of the product was determined by HPLC using a Kromasil AmyCoat column. The enantiomeric excess values for these reactions are listed in Table 1, as is the enantiomeric selectivity *sel* for each of these reactions.

Discussion

The highest rate acceleration is obtained with the new cyclodextrin ketones that have the ketone at the secondary rim. With ketones **1**–**3** rate acceleration up to about 100 was obtained with compound **3**, a β-cyclodextrin without methyl groups, being the best catalyst. The α-cyclodextrin

analogue **1** has about half the rate of catalysis, while methylation of α-cyclodextrin (**2**) improved catalysis somewhat. A common observation is that the (*E*)-stilbenes are by far the best substrates.

Stilbenes are large substrates and it is to be expected that β-cyclodextrin derivatives with their larger cavity might be better suited to these substrates. Indeed 2D NMR experiment show that stilbene bind to β-CD from the secondary face.[13] However the methylated β-cyclodextrin **4** is a remarkably poorer catalyst. Methylation influence the size and the dimensions of the cavity particularly its openings[14] it is possible this account for the lack of activity of **4** by a change in substrate binding towards unproductive binding.

The 2-ketocyclodextrin **5** gives the best rate accelerations with 83 for (*E*)-stilbene and 221 for the substituted (*E*)-stilbene. These accelrations are the highest ever observed for epoxidation by an enzyme model.

The cyclodextrins **12**–**17** (Figure 4) all gave modest or no rate-acceleration (Table 1). For **12**–**15**, that have the ketone on the primary rim, this is not surprising. The stilbenes are larger than the cyclodextrin cavity and are likely to bind so that one aromatic ring is inside, while the styryl group is pointing out through either face. Since the secondary face is larger it is more likely that the styryl is at this face predominantly (Scheme 3, center) so that a functional group at the other rim will be non-functional. Compounds **16** and **17** gave no catalysis and we believe that this may be because they are aldehydes[15] – there are no examples of aldehydes working as catalysts in this reaction in the literature.

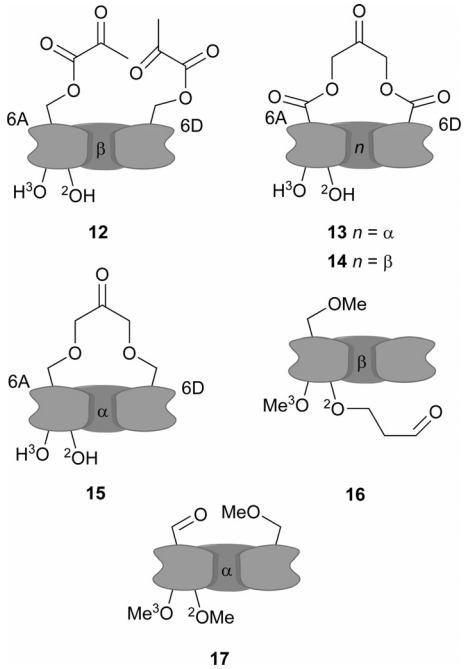
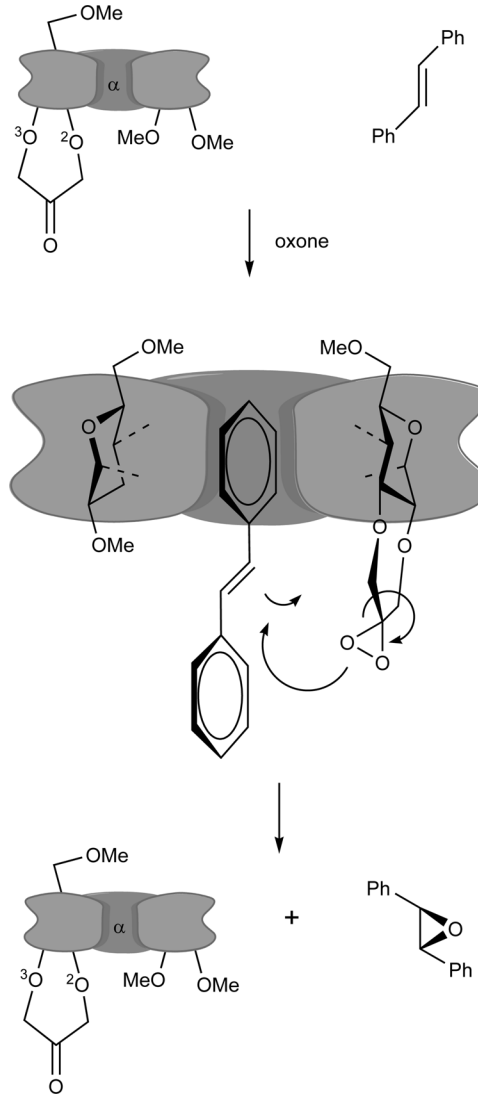


Figure 4. Other ketones investigated in this work. For detailed structure see appropriate reference.

As it is seen in Table 1 ketones **1**–**4** gave an enantiomeric excess of the *R*,*R*-epoxide of 37–70%. This means these catalysts have a preference for formation of the *R*,*R*-isomer over the *S*,*S*-isomer of from 2:1 to 6:1 and overall stilbene



Scheme 3. Mechanism of epoxidation of stilbene catalyzed by **2** and oxone as the stoichiometric reagent.

appears to have a similar preferred binding and catalysis in all these reactions. On the other hand the ketone **5** gave very differently 7% *ee* of (*S*,*S*)-stilbene oxide, which reflect the different structure of this catalyst. This basically means that **5** can add oxygen to the stilbene equally well from both sides, while there is a preference for the *R*-face for **1**–**4**.

The epoxidation of alkenes by dioxiranes is a geometrically demanding reaction and the transition state is believed to be a spiro structure so that dioxirane and alkene are perpindicular.[16] It is possible that the ketone in **5** can better reach the required transition state for both enantiomers than is possible for **1**–**4**, and that this is causing the stereoselectivity in the latter case. Interestingly **5** is the better catalyst suggesting that the selectivity of **1**–**4** is obtained by reducing the reaction rate of formation of the (*S*,*S*)-stilbene oxide.

To get more insight into this question models of the transition states for epoxidation of stilbene from the *R*- and *S*-faces were made: the catalyst **1** was modelled in chem3D

based on crystal data of α-cyclodextrin, and stilbene was docked inside. MM2 calculation showed that the model of the *R*-transition state had a lower energy, which is in accordance with the observed. This model is shown in Figure 5. The model shows a snug fit of one phenyl group of stilbene into the cavity and that the remainder of stilbene is no longer in plane.

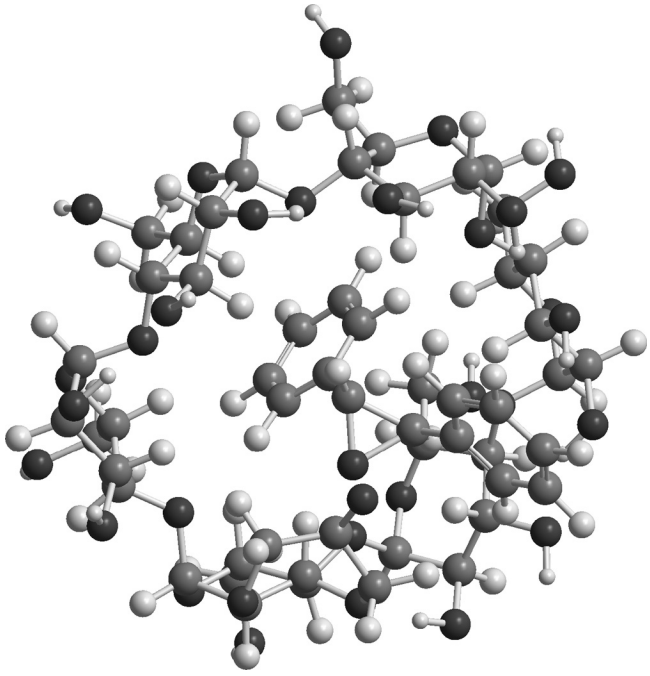


Figure 5. Model of the transition state of epoxidation of stilbene catalysed by **1**.

Conclusions

It has been shown that cyclodextrins with ketone at the secondary rim function as epoxidation catalysts in enzyme-like manner and giving rate enhancements up to 221-fold and enatioselectivities up to 75% *ee*. A phenyl group of the alkene is bound in the cavity of the cyclodextrin and the epoxidation proceed with preference for the *R*-face in stilbene.

Experimental Section

General: Solvents were distilled under anhydrous conditions. All reagents were used as purchased without further purification. Evaporation was carried out in a rotary evaporator. Glassware used for water-free reactions was dried for 2 h at 130 °C before use. Columns were packed with silica gel 60 (230–400 mesh) as the stationary phase. TLC plates (Merck, 60, F_{254}) were visualized by spraying with cerium sulfate (1%) and molybdic acid (1.5%) in 10% H_2SO_4 and heating until coloured spots appeared. ^{1}H-, ^{13}C NMR and COSY experiments were carried out with a Varian Mercury 300 instrument. Monoisotopic mass spectra (MALDI-TOF MS) were obtained on a Bruker Daltonics mass spectrometer using ditranol (1,8-dihydroxyanthron) as matrix. Spectra were calibrated using a peptide calibration standard solution.

2^A,3^A-Di-*O*-(prop-2-ene-1,3-diyl)-α-cyclodextrin (6): Dry α-CD (1.04 g, 1.07 mmol) was dissolved in DMSO (50 mL) and LDA (2 M, 0.54 mL, 1.07 mmol) was added at room temp. and the reaction was stirred overnight. 3-chloro-2-(chloromethyl)propene (0.12 mL, 1.07 mmol) was added dropwise and the mixture was left stirring overnight. DMSO was removed under reduced pressure and the residue was purified by flash chromatography, acetonitrile/H_2O/NH_4OH, 10:1:1 → 6:3:1 giving 0.47 g of the product in 43% yield. ^{1}H NMR ([D$_6$]DMSO, 400 MHz): δ = 5.52–5.45 (m, 6 H, 1-H), 5.01–4.97 (m, 2 H, C=CH_2), 4.78 (br. s, 3 H), 4.64–4.50 (br. s, 4 H), 4.47–4.28 (m, 4 H), 4.00–3.93 (m, 1 H), 3.85–3.43 (m, 33 H), 3.39–3.16 (m, 11 H) ppm. ^{13}C NMR ([D$_6$]DMSO, 100 MHz): δ = 147.0 (*C*=CH$_2$), 112.5 (C=*C*H$_2$), 102.2, 102.0, 100.6, 83.1, 82.9, 82.5, 82.3, 82.2, 82.1, 79.0, 73.8, 73.6, 72.7, 72.7, 72.5, 72.3, 72.3, 72.2, 72.2, 71.9, 60.2, 59.9 ppm. MALDI-TOF, *m*/*z* calcd. $C_{40}H_{64}NaO_{30}$: 1047.338; found 1046.997.

Hydrolysis and Acetylation of Compound 6: Compound **6** (146 mg, 0.14 mmol) was dissolved in 5 mL H_2O and amberlite IR120H (35 mL) was added, the mixture was heated to 100 °C, for 48 h, until TLC (acetonitrile/H_2O/NH_4OH, 6:3:1) showed no starting material. The mixture was filtered and washed with water (15 mL), and the pH adjusted to 7 by $NaHCO_3$ before evaporation. The residue was dissolved in pyridine (12 mL) and acetic anhydride (12 mL) and left stirring overnight. The mixture was evaporated and the compounds purified by flash chromatography EtOAc/toluene, 1:3 giving 124 mg of the acetylated sugar residues. R_f = 0.52 and 0.47, EtOAc/toluene, 1:2. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 6.32 (d, *J* = 3.7 Hz, 5 H, 1-H), 6.25 (d, *J* = 3.7 Hz, 1 H, 1-H), 5.71 (d, *J* = 8.3 Hz, 6 H, 1-H), 5.59 (d, *J* = 8.0 Hz, 1 H, 1-H), 5.46 (d, *J* = 19.8 Hz, 4 H), 5.24 (t, *J* = 9.4 Hz, 6 H), 5.16–5.06 (m, 21 H), 5.03 (dd, *J* = 9.8, 5.9 Hz, 4 H), 4.98 (d, *J* = 4.8 Hz, 2 H), 4.50–4.37 (m, 4 H), 4.31–4.22 (m, 16 H), 4.14–4.05 (m, 18 H), 4.02 (dd, *J* = 11.9, 1.9 Hz, 2 H), 3.95 (ddd, *J* = 10.2, 4.3, 2.1 Hz, 1 H), 3.83 (ddd, *J* = 10.0, 4.5, 2.3 Hz, 7 H), 3.75–3.66 (m, 2 H), 3.56 (dd, *J* = 9.2, 3.8 Hz, 1 H), 3.43 (dt, *J* = 16.7, 8.7 Hz, 2 H), 2.17 (s, 13 H, CH_3), 2.15 (s, 3 H, CH_3), 2.14 (s, 3 H, CH_3), 2.10 (s, 19 H, CH_3), 2.09 (s, 4 H, CH_3), 2.08 (s, 14 H, CH_3), 2.08 (s, 19 H, CH_3), 2.06 (s, 3 H, CH_3), 2.06 (s, 4 H, CH_3), 2.03 (s, 13 H, CH_3), 2.02–2.02 (m, 33 H, CH_3), 2.02 (s, 10 H, CH_3), 2.01 (s, 14 H, CH_3), 2.00 (s, 18 H, CH_3) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = C:170.5, 170.4, 170.0, 169.9, 169.5, 169.4, 169.3, 169.2, 169.1, 169.0, 168.8, 168.6 (CO), 146.7, 146.3 (*C*=CH$_2$), 113.8, 112.4 (C=*C*H$_2$), 92.0, 91.6, 90.3, 88.9, (C-1) 84.0, 81.5, 81.3, 80.4, 73.3, 73.2, 73.0, 72.7, 72.7, 72.6, 70.1, 69.7, 69.6, 69.1, 67.8, 67.6, 61.8, 61.7, 61.3 (C-2,3,4,5,6), 20.9, 20.7, 20.6, 20.5, 20.4, 20.3 ($COCH_3$) ppm.

2^A,3^A-Di-*O*-(prop-2-ene-1,3-diyl)-β-cyclodextrin (7): Dry β-CD (1.83 g, 1.61 mmol) was dissolved in DMSO (96 mL) and LDA (2 M, 0.81 mL, 1.61 mmol) was added at room temp. and the reaction was stirred overnight. 3-chloro-2-(chloromethyl)propen (0.19 mL, 1.61 mmol) was added dropwise and the mixture was left stirring overnight. DMSO was removed under reduced pressure and the residue was purified by flash chromatography, acetonitrile/H_2O/NH_4OH, 10:1:1 → 6:3:1 giving 0.51 g of the product in 27% yield. ^{1}H NMR ([D$_6$]DMSO, 300 MHz): δ = 5.82–5.61 (m, 7 H, 1-H), 5.01 (m, 2 H, C=CH_2), 4.79 (br. s, 4 H), 4.72–4.26 (m, 9 H), 4.15–4.06 (d, *J* = 10.0 Hz, 1 H), 3.77–3.15 (m, 51 H) ppm. ^{13}C NMR ([D$_6$]DMSO, 100 MHz): δ = 148.1 (*C*=CH$_2$), 116.7 (C=*C*H$_2$), 104.5, 104.4, 104.3. 104.2, 103.1 (C-1), 85.5, 84.6, 84.3, 84.0, 83.9, 83.8, 83.7, 80.8, 80.7, 76.1, 76.0, 75.9, 75.8, 75.7, 75.5, 75.4, 75.0, 74.9, 74.8, 74.6, 74.4, 74.3, 74.2, 62.6, 62.4, 62.3 ppm. MALDI-TOF, *m*/*z* calcd. $C_{46}H_{74}NaO_{35}$: 1209.391; found 1209.533.

Hydrolysis and Acetylation of 7: Compound **7** (136 mg, 0.11 mmol) was dissolved in 2 mL H_2O and amberlite IR120H (30 mL) was added, the mixture was heated to 100 °C, for 48 h, until TLC (acetonitrile/H_2O/NH_4OH, 6:3:1) showed no starting material. The mixture was filtered and washed with water (15 mL), and the pH adjusted to 7 by $NaHCO_3$ before evaporation. The residue was dissolved in pyridine (10 mL) and acetic anhydride (10 mL) and left stirring overnight. The mixture was evaporated and the compounds purified by flash chromatography EtOAc/toluene, 1:3 giving 124 mg of the acetylated sugar residues. R_f = 0.52 and 0.47, EtOAc/toluene, 1:2. ^{1}H NMR ([D$_6$]DMSO, 300 MHz): δ = 6.27 (d, J = 3.6 Hz, 6 H, 1-H), 6.19 (d, J = 3.6 Hz, 1 H, 1-H), 5.67 (d, J = 8.1 Hz, 9 H, 1-H), 5.54 (d, J = 8.0 Hz, 1 H, 1-H), 5.41 (t, J = 9.9 Hz, 6 H), 5.25–5.15 (m, 9 H), 5.13–5.01 (m, 29 H), 5.01–4.91 (m, 8 H), 4.46–4.33 (m, 5 H), 4.20 (dq, J = 9.8, 5.5 Hz, 23 H), 4.04 (tt, J = 13.3, 6.8 Hz, 25 H), 3.80 (dd, J = 9.9, 2.2 Hz, 9 H), 3.71–3.62 (m, 3 H), 3.52 (dd, J = 9.2, 3.7 Hz, 1 H), 3.47–3.28 (m, 2 H), 2.14–2.12 (m, 18 H, CH_3), 2.11–2.08 (m, 9 H, CH_3), 2.07–2.05 (m, 29 H, CH_3), 2.04–2.02 (m, 53 H, CH_3), 2.02–1.99 (m, 13 H, CH_3), 1.99–1.98 (m, 19 H, CH_3), 1.98 (m, 61 H, CH_3), 1.96 (s, 48 H, CH_3), 1.89 (s, 6 H, CH_3) ppm. ^{13}C NMR ([D$_6$]DMSO, 75 MHz): δ = 170.9, 170.8, 170.7, 170.4, 170.2, 169.8, 169.7, 169.6, 169.5, 169.4, 169.2, 169.1, 168.9, (CO) 147.0, 146.6 (C=CH$_2$), 114.11, 112.74 (C=CH$_2$), 94.0, 92.3, 91.8, 90.6, 89.2, 84.2, 81.7, 81.6, 80.7, 73.6, 73.5, 73.3, 73.0, 72.9, 72.8, 70.4, 70.0, 69.9, 69.3, 68.0, 67.9, 62.4, 61.9, 61.6 (C-2,3,4,5,6), 21.2, 21.0, 20.9, 20.8, 20.7, 20.6 ($COCH_3$) ppm.

2^A,3^A-Di-*O*-(prop-2-ene-1,3-diyl)-2$^{B–F}$,3$^{B–F}$,6$^{A–F}$-hexadecakis-*O*-methyl-α-cyclodextrin (8): Compound **6** (0.51 g, 0.50 mmol) was dissolved in DMSO (35 mL) and NaH (60%, 1.59 g, 39.7 mmol) was added and the reaction was stirred for 30 min under N_2. The mixture was cooled to 0 °C and iodomethane (2.47 mL, 39.7 mL) was slowly added and the reaction was stirred for 24 h. Water (40 mL) was added and the mixture was extracted with $CHCl_3$ (4 × 50 mL) and the combined org. phases were washed with brine (40 mL). Concentration and purification by flash chromatography toluene/acetone, 1:1 (R_f = 0.26), afforded the desired product in 0.61 g, 98% yield. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 4.98–4.95 (m, 5 H, 1-H), 4.94 (d, J = 3.54 Hz, 1 H, 1-H), 4.89 (d, J = 23.5 Hz, 2 H, C=CH$_2$), 4.45 (d, J = 15.3 Hz, 1 H, CHH), 4.29 (d, J = 13.2 Hz, 1 H, CHH), 4.24 (d, J = 15.4 Hz, 1 H, CHH), 4.18 (d, J = 13.3 Hz, 1 H, CHH), 3.85–3.76 (m, 4 H), 3.74–3.68 (m, 7 H), 3.64–3.55 (m, 22 H), 3.54–3.45 (m, 12 H), 3.44–3.39 (m, 16 H), 3.35–3.26 (m, 18 H), 3.12–3.06 (m, 5 H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 148.4 (C=CH$_2$), 111.8 (C=CH$_2$), 101.7, 100.6, 100.4, 100.3, 100.2, 99.9 (C-1), 83.7, 82.7, 82.5, 82.5, 82.4, 82.4, 82.3, 82.1, 81.8, 81.5, 81.4, 81.3, 81.2, 73.3, 73.0, 71.8, 71.7, 71.5, 71.4, 71.3, 71.3, 70.7, 62.2, 62.1, 62.0, 61.9, 59.2, 59.1, 59.1, 58.4, 58.3, 58.0, 58.0, 57.9 ppm. MALDI-TOF, m/z calcd. $C_{56}H_{96}NaO_{30}$: 1271.588; found 1271.541.

2^A,3^A-Di-*O*-(2-oxopropane-1,3-diyl)-2$^{B–F}$,3$^{B–F}$,6$^{A–F}$-hexadecakis-*O*-methyl-α-cyclodextrin (2): Compound **8** (612 mg, 0.49 mmol) was dissolved in 200 mL CH_2Cl_2 and cooled to –78 °C, O_3 was bubbled through for 10 min, the solution turned blue, then O_2 was bubbled through for additional 5 min, $S(CH_3)_2$ (3 mL) was added and the reaction was left stirring at room temp. overnight. The solvent was evaporated and the product purified by chromatography toluene/acetone, 1:1, to afford 582 mg of the desired compound, 95% yield. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.06 (d, J = 3.49 Hz, 1 H, 1-H) 5.02 (m, 5 H, 1-H), 4.33 (d, J = 17.6 Hz, 1 H, CHH), 4.30 (d, J = 15.3 Hz, 1 H, CHH), 4.24 (d, J = 15.3 Hz, 1 H, CHH), 4.18 (d, J = 17.6 Hz, 1 H, CHH), 3.92–3.82 (m, 4 H), 3.80–3.74 (m, 7 H), 3.73–3.59 (m, 22 H), 3.57–3.49 (m, 12 H), 3.48–3.44 (m, 16), 3.41–3.35 (m, 18), 3.17–3.11 (m, 5 H) ppm. ^{13}C NMR (CDC_{13}, 100 MHz): δ = 210.8 (C=O), 100.9, 100.2, 100.1, 100.0, 99.9, 99.6 (C-1), 86.0, 83.7, 82.4, 82.3, 82.2, 82.1, 81.9, 81.9, 81.2, 81.1, 81.1, 80.1, 77.4, 76.8, 71.4, 71.3, 71.2, 71.1, 71.1, 71.0, 71.0, 70.9, 70.7, 61.7, 61.7, 61.6, 58.8, 58.8, 58.2, 58.0, 57.8, 57.7 ppm. MALDI-TOF, m/z calcd. $C_{55}H_{94}NaO_{31}$: 1273.568; found 1273.147.

2^A,3^A-Di-*O*-(prop-2-ene-1,3-diyl)-2$^{B–G}$,3$^{B–G}$,6$^{A–G}$-nonadecakis-*O*-methyl-β-cyclodextrin (9): Compound **7** (288 mg, 0.24 mmol) was dissolved in DMSO (20 mL), NaH (60%, 914 mg, 22.9 mmol) was added and the reaction was stirred for 30 min under N_2. The mixture was cooled to 0 °C and iodomethane (1.41 mL, 22.9 mL) was slowly added and the reaction was stirred for 24 h. Water (20 mL) was added and the mixture was extracted with $CHCl_3$ (4 × 30 mL) and the combined org. phases were washed with brine (30 mL). Concentration and purification by flash chromatography toluene/acetone, 1:1 (R_f = 0.31), afforded the desired product in 283 mg, 80% yield. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 5.20 (d, J = 3.8 Hz, 1 H, 1-H), 5.12 (d, J = 3.7 Hz, 1 H, 1-H), 5.10 (d, J = 3.6 Hz, 1 H, 1-H), 5.08 (d, J = 3.5 Hz, 1 H, 1-H), 5.06 (d, J = 3.5 Hz, 1 H, 1-H), 5.05 (d, J = 3.5 Hz, 1 H, 1-H), 5.00 (d, J = 3.6 Hz, 1 H, 1-H), 4.90 (d, J = 21.6 Hz, 2 H, C=CH$_2$), 4.45 (d, J = 15.0 Hz, 1 H, CHH, 4 H), 4.38 (d, J = 13.7 Hz, 1 H, CHH), 4.29 (d, J = 15.1 Hz, 1 H, CHH), 4.21 (d, J = 13.7 Hz, 1 H, CHH), 3.91–3.83 (m, 5 H), 3.80–3.68 (m, 9 H), 3.66–3.40 (m, 57 H), 3.35–3.29 (m, 22 H), 3.17–3.13 (m, 6 H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 148.2 (C=CH$_2$), 110.9 (C=CH$_2$), 102.2, 100.3, 99.0, 98.9, 98.8, 98.6, 98.4 (C-1), 84.0, 82.2, 82.0, 81.9, 81.7, 81.6, 81.5, 81.4, 80.7, 80.3, 80.2, 79.8, 77.7, 72.9, 72.9, 71.7, 71.4, 71.3, 71.0, 70.9, 70.9, 70.8, 70.7, 70.6, 70.0, 61.5, 61.4, 61.3, 61.2, 61.0, 58.8, 58.7, 58.6, 58.5, 58.3, 58.2, 58.0 ppm. MALDI-TOF, m/z calcd. $C_{65}H_{112}NaO_{35}$: 1475.688; found 1475.401.

2^A,3^A-Di-*O*-(2-oxopropane-1,3-diyl)-2$^{B–G}$,3$^{B–G}$,6$^{A–G}$-nonadecakis-*O*-methyl-β-cyclodextrin (4): Compound **2** (207 mg, 0.14 mmol) was dissolved in 200 mL CH_2Cl_2 and cooled to –78 °C, O_3 was bubbled through for 10 min, the solution turned blue, then O_2 was bubbled through for additional 5 min, $S(CH_3)_2$ (2 mL) was added and the reaction was left stirring at room temp. overnight. The solvent was evaporated and the product purified by chromatography toluene/acetone, 1:1, to afford 180 mg of the desired compound, 87% yield. ^{1}H NMR (CDC_{13}, 400 MHz): δ = 5.14 (d, J = 3.7 Hz, 1 H, 1-H), 5.11 (m, 3 H, 1-H), 5.06 (m, 3 H, 1-H), 4.34–4.22 (m, 4 H), 3.94–3.32 (m, 93 H), 3.20–3.12 (m, 6 H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 210.8 (C=O), 99.9, 99.1, 99.1, 98.9, 98.9, 98.8, 98.5 (C-1), 86.2, 84.1, 82.0, 81.9, 81.8, 81.7, 81.6, 81.6, 81.5, 81.4, 80.7, 80.5, 80.4, 80.3, 80.2, 80.1, 77.4, 71.4, 71.1, 71.0, 70.9, 70.8, 70.7, 70.4, 61.5, 61.4, 61.2, 61.1, 59.1, 58.9, 58.8, 58.6, 58.4, 58.3, 58.1 ppm. MALDI-TOF, m/z calcd. $C_{64}H_{110}NaO_{36}$: 1477.667; found 1477.506.

2^A,3^A-Di-*O*-(2-oxopropane-1,3-diyl)-β-cyclodextrin (3): Compound **7** (229 mg, 0.14 mmol) was dissolved in 200 mL H_2O and cooled to 0 °C, O_3 was bubbled through for 7 min, the solution turned blue, then O_2 was bubbled through for additional 5 min, $S(CH_3)_2$ (2 mL) was added and the reaction mixture was left stirring at room temp. overnight. The solvent was evaporated and the product purified by chromatography acetonitrile/H_2O/NH_4OH, 10:1:1 → 6:3:1, to afford 162 mg of the desired compound, 70% yield. ^{1}H NMR ([D$_6$]DMSO, 400 MHz): δ = 5.78–5.64 (m, 9 H), 5.09 (d, J = 3.6 Hz, 1 H, 1-H) 4.86 (d, J = 3.4 Hz, 1 H, 1-H), 4.82 (d, J = 3.0 Hz, 5 H, 1-H), 4.70 (br. s, 1 H), 4.59–4.44 (m, 9 H), 4.35 (s, 1 H), 4.19 (d, J = 5.6 Hz, 1 H), 4.15 (d, J = 5.3 Hz, 1 H), 4.05 (d, J = 10.3 Hz, 1 H), 3.97–3.92 (m, 1 H), 3.72–3.19 (m, 41 H) ppm. ^{13}C NMR ([D$_6$]DMSO, 100 MHz): δ = 210.3 (C=O), 102.2, 102.1,

102.0, 101.9, 101.8, 101.6, 99.6 (C-1), 85.3, 83.7, 81.9, 81.7, 81.6, 81.5, 81.1, 77.4, 76.9, 76.6, 73.4, 73.3, 73.2 72.7, 72.6, 72.5, 72.4, 72.3, 72.0, 60.3, 60.2, 60.0, 59.9 ppm. MALDI-TOF, *m*/*z* calcd. $C_{45}H_{72}NaO_{36}$: 1211.370; found 1211.758.

2^A,3^A-Di-*O*-(2-oxopropane-1,3-diyl)-α-cyclodextrin (1): Compound **6** (612 mg, 0.14 mmol) was dissolved in 200 mL H_2O and cooled to 0 °C, O_3 was bubbled through for 7 min, the solution turned blue, then O_2 was bubbled through for additional 5 min, $S(CH_3)_2$ (2 mL) was added and the reaction mixture was left stirring at room temp. overnight. The solvent was evaporated and the product purified by flash chromatography acetonitrile/H_2O/NH_4OH, 10:1:1 → 6:3:1, to afford 423 mg of the desired compound, 69% yield. ^{1}H NMR ([D$_6$]DMSO, 300 MHz): δ = 5.52 (br. s, 7 H), 5.03 (s, 1 H, 1-H), 4.79 (s, 5 H, 1-H), 4.68–4.44 (m, 9 H), 4.25–4.20 (m, 2 H), 4.15 (d, J = 16.5 Hz, 2 H, CH_2), 4.06 (d, 2H CH_2), 3.85–3.13 (m) ppm. ^{13}C NMR ([D$_6$]DMSO, 75 MHz): δ = 210.1 (C=O), 102.2, 101.9, 100.2 (C-1), 84.4, 83.8, 83.1, 82.3, 82.2, 82.0, 78.8, 76.6, 76.5, 73.6, 73.5, 72.8, 72.4, 72.2, 72.0, 60.5, 60.2, 59.8 ppm. MALDI-TOF, *m*/*z* calcd. $C_{39}H_{62}NaO_{31}$: 1049.317; found 1049.200.

2^A-Oxo-$2^{B–G}$,$3^{A–G}$,$6^{A–G}$-Icosakis-*O*-methyl-β-cyclodextrin (5): Py-SO_3 (234 mg, 1.41 mmol) was dissolved in DMSO (0.5 mL), $2^{B–G}$,$3^{A–G}$,$6^{A–G}$-*O*-methyl-β-CD (**11**) (51 mg, 0.04 mmol) was dissolved in DMSO (0.5 mL), the two solutions were mixed and stirred for 30 min. Et_3N (0.26 mL, 1.85 mmol) was added and the mixture was stirred for 1 h. Brine (1 mL) and water (1 mL) were added, and the mixture was extracted with CH_2Cl_2 (4 × 3 mL), the organic extracts were evaporated and purified by flash chromatography (Et_2O/MeOH, 20:1), giving 21 mg of the desired product as a colourless foam in 40% yield. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 5.85 (d, J = 1.9 Hz, 1 H), 5.65 (d, J = 3.6 Hz, 1 H), 5.60–5.49 (m, 4 H), 5.35 (d, J = 3.5 Hz, 1 H), 5.15 (d, J = 3.6 Hz, 1 H), 5.13–5.09 (m, 3 H), 5.07 (d, J = 3.6 Hz, 1 H), 5.04 (d, J = 3.1 Hz, 1 H), 4.87–4.77 (m, 1 H), 4.62 (d, J = 7.6 Hz, 1 H), 4.28 (d, J = 8.9 Hz, 1 H), 4.07–3.99 (m, 1 H), 3.96–3.69 (m, 12 H), 3.69–3.44 (m, 51 H), 3.44–3.32 (m, 19 H), 3.32–3.12 (m, 8 H), 3.02 (dd, J = 9.0, 7.7 Hz, 1 H) ppm. MALDI-TOF, *m*/*z* calcd: $C_{62}H_{108}NaO_{35}$, 1435.657; found 1435.441.

Determination of Epoxidation Rates: The epoxidations were monitored on a spectrophotometer Spetronic Genesys 5 by Milton Roy. For each epoxidation assay 6 samples of 2 mL were made. Four stock solutions were made, substrate (1 mM, 1:1 acetonitrile:water), Oxone (10 mM, 32 mg in 5 mL 1:1 acetonitrile:water), $NaHCO_3$ (100 mM, 58 mg in 7 mL 1:1 acetonitrile:water), enzyme (6.8 mM, 1:1 acetonitrile:water). Each sample contained the following: 20 μL substrate (20 μM), 230 μL Oxone (2.4 mM), 95 μL $NaHCO_3$ (9.4 mM), 0 or 90–260 μL enzyme (0.6–1.7 mM), 655 or 395–565 μL solvent (1:1 acetonitrile:water). Four samples contained an increasing concentration of enzyme, and two with none as control. The reactions were monitored at the following wavelengths: 4-methoxy-4′-nitro-*trans*-stilbene: 377 nm ε = 24360 ± 149 $M^{-1}cm^{-1}$; *trans*-stilbene: 308 nm, ε = 25631 ± 781 $M^{-1}cm^{-1}$; *cis*-stilbene: 276 nm, ε = 85283 ± 5023 $M^{-1}cm^{-1}$; styrene: 248 nm ε = 12191 ± 197 $M^{-1}cm^{-1}$. The rate constant was calculated as for the slope of the rate expression $\ln([A]/[A_0]) = -kt$. k_{cat} was determined as the maximum k obtained at saturation. The *ee* was calculated using the area of the first isomer eluting 1 and the last isomer eluting 2; *ee* = (1–2)/(1+2).

Determination of Enantiomeric Excess in Epoxides by HPLC Analysis: The epoxide products formed in epoxidation of stilbene and styrene were analysed by HPLC. The HPLC column used was a Kromasil® 5-AmyCoat, 4.6 × 2500 mm, mounted on a UFLC Shimadzu HPLC, with degasser DGU-20A, Pump LC-20AD, UV/Vis detector SPD-20A, and Communication Unit CMB-20A. The solvent used for elution was *n*-heptane/2-propanol, 90:10, with a flow rate of 0.5 mL/ min, and a pressure of 34 bar. For sample preparation the cuvettes were extracted with EtOAc and concentrated prior to inlet. The enantiomeric excess of the epoxidations were determined by integration of the elution profile of the sample.

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