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STEREOCHEMICAL ASPECTS OF CYTOSOLIC EPOXIDE HYDROLASE HYDRATION OF METHYL DIEPOXYSTEARATES

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Abstract: Hydration of methyl diepoxystearate (**2**) at high *cytosolic epoxide hydrolase* (CEH) concentration produces the corresponding tetraol, while at physiological CEH concentration four tetrahydrofuran diol products (**3-6**) are produced. These same four products are obtained in the acid-catalyzed hydration of **2**. Spectroscopic studies, primarily EI mass spectrometry and difference spectrum NOE, are reported which establish the regio- and stereoselectivity of **2** → **3-6** (MS differentiating **3/4** from **5/6** and NOE differentiating **3/5** from **4/6**). The observed *syn* arrangement of the two adjacent groups (i.e., substituents at C_2 and C_3) in the four THF-diols can only arise from an A_2 type opening of the first epoxide. The resulting epoxydiol intermediate then cyclizes by A_2 opening of the second *cis*-epoxide as established by synthesis of these hypothetical epoxydiol intermediates and their subsequent conversion to THF-diols **3-6**.

Introduction

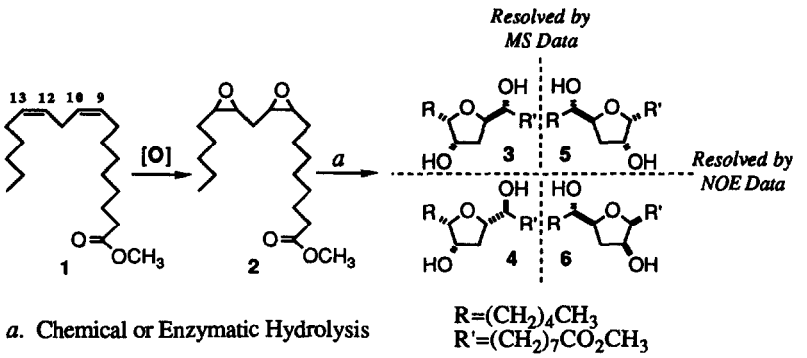
Recently, the epoxidation of endogenous compounds has received much attention for the generation of possible intermediates in metabolic pathways, chemical mediators and as toxic hazards.[1] Indeed, the covalent bonding of reactive epoxides to DNA, RNA, and proteins has been postulated to lead to the production of toxic and carcinogenic compounds with possible mutagenic effects.[2,3] On the other hand, squalene oxide and leukotriene A_4 are examples of important biosynthetic intermediates[3] and the oxidation of arachidonic acid leads to the eventual production of prostaglandins, leukotrienes, and lipoxins.[4] Epoxy derivatives of arachidonic acid have also been identified as potent stimulator of prolactin within the pituitary cells [5] Unsaturated fatty acids and their esters have been implicated as likely targets for epoxidation due to their proximity to areas where active oxygen species are generated.[6]

A family of enzymes, *epoxide hydrolases* (EH), are responsible for hydration of epoxides and are thought to protect the cell against the cytotoxic and genotoxic effects of endogenous epoxides.[7,8] For example, Halarnkar *et al* have shown that *cytosolic epoxide hydrolase* (CEH)

converts the mono epoxides derived from methyl oleate, methyl linoleate, and methyl arachidonate into their corresponding diols.[9]

Additionally, Capdevila *et al.* demonstrated conversion of the monoepoxides of arachidonic acid to diepoxides and epoxy-alcohols by cytochrome P-450 enzymes.[10] The biological activities of these oxidation products are unknown, yet their metabolic products are of interest since many arachidonic acid metabolites have been shown to be very active within living systems.[11]

Figure 1. Products of the Methyl Diepoxystearate Hydration



As an initial step in our investigation of EH catalyzed hydration of fatty acid diepoxides, methyl diepoxystearate (**2**) was chosen as a model compound for metabolic study since its synthesis from methyl linoleate (**1**) does not pose the regioselective and stoichiometric epoxidation problems faced with methyl arachidonate. Both, methyl diepoxystearate and mixtures of methyl arachidonate diepoxides are efficiently metabolized by CEH.[9] Herein, we report the synthesis and structural characterization of the four hydration products of methyl diepoxystearate. As described in detail below, structural characterization of these four products was based on a combination of mass spectrometry and NMR nuclear Overhauser effect (NOE) data (MS differentiating **3/4** from **5/6** and NOE differentiating **3/5** from **4/6**; see **Figure 1**)

Results and Discussion

As shown by Halarnkar *et al.*, methyl diepoxystearate (**2**; mixture of diastereoisomers) is converted to its corresponding tetraol at high CEH concentrations, while at physiological CEH concentration the tetraol is not observed[9]. Instead, two products (*vide infra*) with higher TLC R_f's (SiO_2,1:4::ethyl acetate:chloroform) and longer HPLC (reverse phase) retention times than the tetraol were produced and indicated that these products are less polar than the tetraol. Acidic hydrolysis [THF:H_2O:5% $HClO_4$, (3:1:1)] of methyl diepoxystearate led to the same two products as evidenced by comparative TLC, GC, and HPLC analysis. Characterization of these products was pursued on the chemically synthesized material.

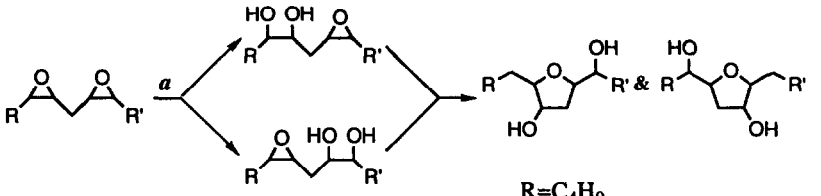
Structural Assignment by Mass Spectrometry

Acidic hydrolysis of diepoxide **2** proceeded to completion within 1 h at room temperature yielding two isolable fractions by flash chromatography. Initial GC/MS data for the two isolated

fractions (TMS-derivatized; fractions I and II) were identical (**Table I**), suggesting stereochemical differences between the two products. The *m/z* 488 peak was assigned as the molecular ion for the TMS-derivatized product; the molecular weight determined by FAB^+ for the free alcohol (GC/MS *m/z* 344) differs by two trimethylsilyl groups from the TMS-derivatized fraction, indicating that the products in fractions I and II are diols.

Mass spectrometry fragmentation (EI) of *each* TMS-derivatized fraction produced signals at *m/z* 173\315 and 229\259 (**Table I**). These structurally discriminating fragmentations suggested that each diol fraction was in fact a mixture of *two* tetrahydrofurandiol (THF-diol) structural isomers formed from an epoxydiol intermediate (**Scheme I**). Moreover, these MS data clearly indicate that tetrahydropyrandiols (THP-diols) are not significant products of the hydrative cyclization of diepoxide **2**.

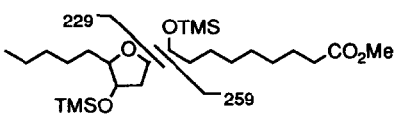
Scheme I



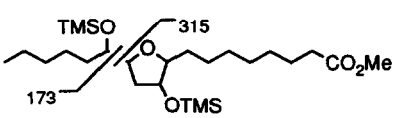
a. Chemical or Enzymatic Hydrolysis

The possibility that each chromatographically separated product is a combination of two structural isomers promted us to investigate a method for their separation. After extensive variation of chromatographic parameters, we discovered that utilization of a β-cyclodextrin capillary GC

Table I *EIMS data* for the products of methyl diepoxystearate hydrolysis*



TMS derivatives of **3\4**



TMS derivatives of **5\6**

m/z for Fractions TMS-I & TMS-II	*m/z* *TMS-3 & TMS-4*	*m/z* *TMS-5 & TMS-6*
473 *(13)*	398 *(20)*	389 *(29)*
345 *(50)*	259 *(58)*	345 *(28)*
315 *(70)*	244 *(16)*	315 *(32)*
259 *(99)*	229 *(10)*	173 *(75)*
225 *(12)*	129 *(18)*	129 *(14)*
173 *(80)*	113 *(52)*	113 *(13)*
113 *(85)*	73 *(99)*	73 *(99)*

**Numbers in the parentheses are MS relative intensities*

column[12] on either the free alcohols or their TMS-derivatives resolved each TLC fraction, I (low R_f) and II (high R_f), into two separate compounds and thus allowed us to probe each by GC/MS. The result of these GC/MS studies on the TMS-derivatized fractions are summarized in **Table I.**

GC/MS analysis of the first component of fraction I (3), produced the *m/z* 229 and 259 fragmentation (**Table I**). GC/MS analysis of **5**, the second component of fraction I, yielded only the 173\315 fragmentation (**Table I**). The two components of fraction II were also informative as **6**'s fragmentation produced the characteristic 173\315 tandem, while **4** yielded the 229\259 fragments. At this point it was clear that each fraction was a mixture of two THF-diol structural isomers and that **3** and **4** had identical mass spectra, as did **5** and **6**. However, **3\5** co-migrated with an R_f distinctly different from **4\6** . Therefore, a structural difference more pronounced than structural isomerism seemed to be dictating polarity.

Stereochemical Assignment by NOE

Assuming an epoxydiol intermediate and an A_2 epoxide opening mechanism, **Figure 1** depicts the only THF-diols possible from the acidic hydrolysis of methyl diepoxystearate. The spectral data precludes THP-diol isomers. It is of interest to note that diols **3** and **4** are structural isomers of **5** and **6**. However, diols **3** and **5** have the same relative stereochemistry about the furan ring, as do diols **4** and **6**.

All attempts to produce an X-ray crystallographic quality crystal of **3-6** (or various derivatives) failed. Therefore, we resorted to difference spectrum NOE (dsNOE) in an attempt to establish the relative stereochemistry of each THF-diol. 2D-COSY spectra along with selective ^{1}H-NMR irradiation experiments were performed to assign the ^{1}H-NMR spectra of fractions I and II.[13]

Numerous dsNOE experiments with different parameters were performed on the THF-diols, but all resulted in low quality NOE spectra because the resonance frequencies of protons H_a, H_b, H_c and H_d were too similar for clean selective irradiation leading to a significant noise to signal problem and incomprehensible data. To circumvent this, the dibenzoyl derivative of each fraction was synthesized (**3'-6'**; dibenzoyl derivative of **3-6**) so that H_a and H_d (**Figure 2**) would move down field by about 1 ppm and thus allow selective irradiation of H_a, H_b, H_c and H_d without energy dispersion to the hydrogens with close resonating frequency.

Figure 2. Stereochemistry of THF-diols (structural isomers not shown)

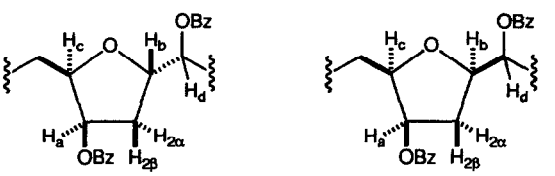


Figure 3a illustrates the dsNOE spectra (partial) obtained for benzoyl derivatized fraction I. H_a and H_c show a strong NOE effect while neither H_a or H_c cause any NOE enhancement of H_b. This suggests that H_a and H_c are *syn* while H_b is *anti* to both H_a and H_c. H_b has a small NOE effect on H_d. $H_{2\alpha}$ exhibits an NOE effect with H_a while $H_{2\alpha}$ and $H_{2\beta}$ are both enhanced by H_b. These data establish the stereochemistry about the furan ring of the two components of fraction I as that depicted in substructure **7**.

Figure 3a NOE of fraction I.

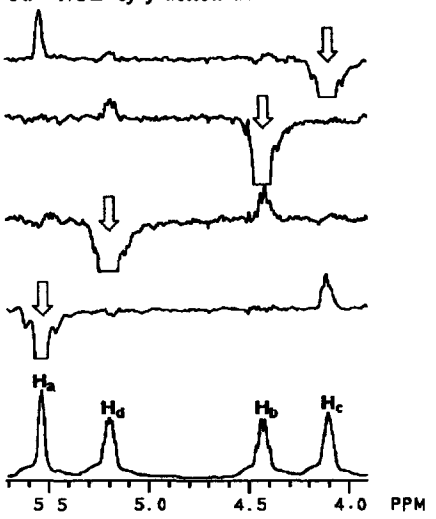


Figure 3b NOE of fraction II

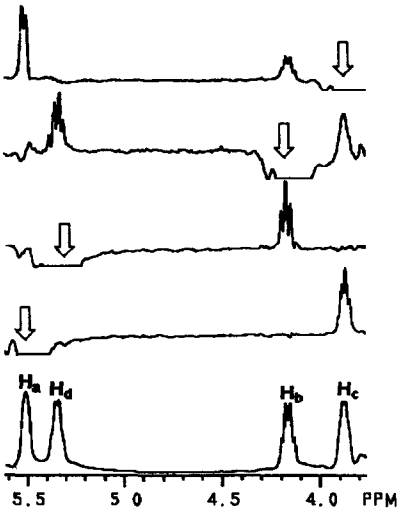
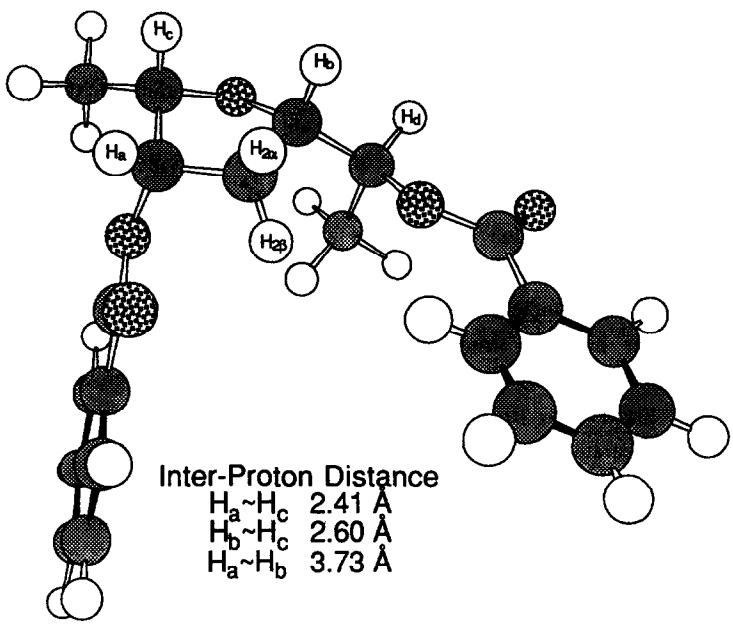


Figure 3b shows the dsNOE spectra (partial) obtained for fraction II. Again, H_a exhibits an NOE effect with H_c. H_b has an NOE with both H_c and H_d and, when H_c was irradiated, NOE's were observed for both H_a and H_b. These data suggest that H_a, H_b and H_c are *syn* Substructure **8** describes the stereochemistry about the tetrahydrofuran moiety of each component of fraction II As depicted in **Figure 4**, MM2 minimization[14] of a truncated model of **8** revealed a possible reason for the lack of an H_a-H_b NOE.

Figure 4 MM2 minimized truncated structure of fraction I I.



The H_a-H_c and H_b-H_c distances in this minimized structure were found to be 2.41 Å and 2.60 Å, respectively and positive NOE's were observed. In contrast, the H_a-H_b distance was calculated to be 3.73 Å. With the small energy used in each irradiation (5-10% suppression of irradiate peak), a 3 73 Å inter-proton distance is too great to observe an NOE enhancement. As further evidence

for the *syn* arrangement of H_a and H_b, $H_{2\alpha}$ exhibits a strong NOE effect on both H_a and H_b. $H_{2\beta}$ shows no NOE enhancement of either H_a or H_b, but does have a small NOE effect with H_d.

To support our MM2 minimized model, coupling constants for fraction II were experimentally determined by the method of selective irradiation. **Table II** lists the J values measured, along with the dihedral angles obtained from the minimized model. The measure of each dihedral angle closely matches the expected trend for the experimentally observed coupling constants. $H_{2\alpha}$'s coupling to H_a and H_b are the largest and so are the cosθ terms obtained for the calculated dihedral angles. $J_{a,2\beta}$ is smallest and these two protons have a 103.6° dihedral angle (i.e. cosθ is small). The remaining coupling constants also fall within the expected range.

Table II. *Coupling Constants for Fraction II*[a]

	H_c	H_d	$H_{2\alpha}$	$H_{2\beta}$
H_a	3.5 (40.4°)	n / a	5.1 (15.8°)	1.1 (103.6°)
H_b	n / a	3.8 (43.3°)	5.4 (11.5°)	4.2 (130.9°)

[a]*Values in parentheses are the dihedral angles from the MM2 model depicted if Figure 4*

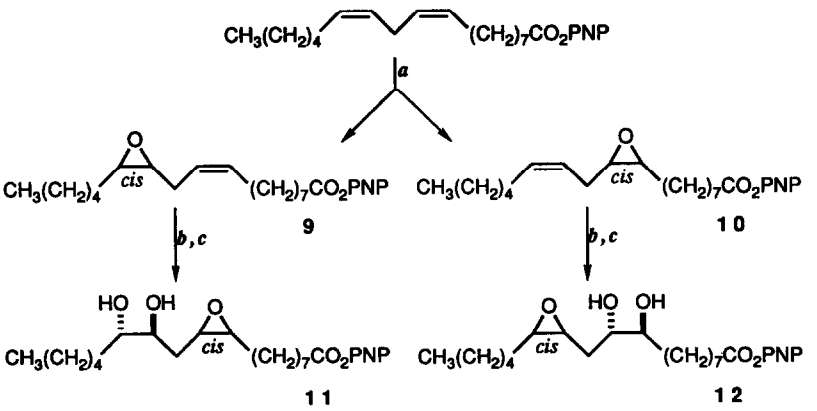
Mechanistic Implications

The mechanism of acidic epoxide hydrolysis has been a widely debated issue. Early reports suggested an A_1 mechanism which involves carbocation formation[15] and some investigators have favored A_1 due to the rate kinetics of the hydrolysis reaction.[16] Parker and Isaac describe the reaction as involving a pre-equilibrium protonation of the epoxide followed by modified S_n2 attack by water [17] In such a transition state, the electrophilic carbon bears a partial positive charge which rationalizes some of the observed S_n1 features. During the past 20 years, the A_1 versus A_2 mechanism has been well investigated in the aryloxide family Aryloxides which can stabilize a carbocation intermediate with electron donating groups or solvent effects undergo the A_1 mechanism.[18-20] In contrast, mechanistic studies of epoxide opening in the alkyloxide family is not as well studied. However, Hoye's work in acid catalyzed cascade reactions of optically active triepoxides provides strong evidence for an A_2 mechanism in such systems.[21,22]

The observed *syn* arrangement of the two adjacent groups (for example, substituents at C_2 and C_3 in **Figure 4**) in the four THF-diols produced by acidic hydrolysis of methyl *cis,cis*-diepoxystearate can only arise from an A_2 type opening of the first epoxide. The resulting epoxydiol intermediate then must cyclize by A_2 opening of the second *cis*-epoxide. An A_1 mechanism would form *both* the *syn* and the *anti* isomers of the two adjacent groups on the tetrahydrofuran ring.

We deemed it necessary to synthesize the hypothetical epoxydiol intermediate to determine if its hydrated products would be identical to the four THF-diols isolated. *p*-Nitrophenyl (PNP) esters were utilized for this study since their UV activity eased chromatographic analysis, especially with HPLC. The PNP-ester of 9,10-epoxy-12,13-dihydroxystearate (**11**) and 12,13-epoxy-9,10-dihydroxystearate (**12**) were synthesized from PNP-linoleate (**Scheme II**).

Scheme II



[a]MCPBA/CH_2Cl_2 [b]THF/H_2O/5% $HClO_4$/50°C leading to the corresponding diols 9a and 10a. [c]Dimethyl dioxirane/THF/Na_3PO_4/5 min.[23,24]

Acidic hydrolysis of PNP-9,10-epoxy-12,13-dihydroxystearate (**11**) and PNP-12,13-epoxy-9,10-dihydroxystearate (**12**) yielded two fractions based on TLC. Trans-esterification of the PNP-esters in each isolated fraction with a catalytic amount of KCN in methanol yielded the corresponding methyl ester furandiols and GC analysis of these products proved them to be identical to the four products characterized from the acidic hydrolysis of methyl diepoxystearate. The fact that the epoxydiol is the intermediate is very plausible. The latter data also suggest that the second intramolecular epoxide opening is also under A_2 mechanistic restraints.

In conclusion, these experiments establish that the same four tetrahydrofurandiol products are produced by both the acidic and the enzymatic hydrolysis of methyl diepoxystearate. Their structures were assigned by GC/MS and dsNOE data. The hydrative cyclization of these diepoxides is thought to proceed via an epoxydiol intermediate and both epoxide openings are under A_2 mechanistic constraints.

Experimental

Elemental analyses were performed at the MidWest Microlab, Indianapolis. High resolution mass spectra were obtained with a VG TRIO2 with VG-11-250 data system and FAB were determined with a VG ZAB-HS-2F (VG Analytical, Wythenshawe, UK) analytical instrument by Dr. Dan Jones at the Facility for Advanced Instrumentation, University of California, Davis. Magnetic resonance spectra including dsNOE's were obtained with a General Electric QE-300 (300 MHz) spectrometer using the solvent as internal standard Multiplicities are abbreviated as follows: s, singlet; d, doublet; t, triplet; q, quartet; p, pentet, bs, broad singlet, m, multiplet; c, complex Infrared spectra were recorded with an IBM FTIR-32 with IBM 9000 data system. High pressure liquid chromatography (HPLC) analysis of the PNP-esters employed a Spectra Physics system consisting of an SP 8700 delivery system, a model 8750 pump system, and a Rheodyne injector, 25 µL injection loop, and UV-absorbance was monitored at 272 nm using a Spectroflow 757 detector.

A C_8 column was employed and products were eluted with 3:7 acetonitrile:water at a flow of 1mL/min. GC analysis was performed on a Hewlett Packard 5890A gas chromatograph equipped with flame ionization detector. Prior to GC analysis, the sample was dried under a stream of nitrogen, 200 μL of 1:1 MSTFA (N-methyl-N-trimethylsilyl-trifluoroacetamide):acetonitrile was added and incubated at 60° C for 30 min. The solvent was removed under a stream of nitrogen, the residue was taken up in 50 μL of hexane and 1-2 μL of the sample injected into the GC. With the β-cyclodextrin column, the analyses were performed under isothermal conditions. Operating parameters were as follows: injector temperature, 250° C; column temperature, 210° C (as TMS ether), 240° C (as underivatized alcohol).

CEH Enzymatic Hydrolysis. CEH was prepared and purified to apparent homogeneity by affinity chromatography as described by Halarnkar *et al.*[9] CEH enzyme activity was determined by partition assay with tritiated *trans*-stilbene oxide as described in detail by Wixtrom and Hammock.[25] Enzymatic hydrolysis of methyl diepoxystearate at high CEH concentration was performed with 100μg of CEH/mL, while 2μg of CEH/mL was employed to mimic physiological concentration. CEH was diluted with 100mM sodium phosphate buffer at pH of 7 4, containing 0.1mM ethylenediaminetetraacetic acid and 100μg of bovine serum albumin/mL. Enzyme solution (100 μL) was preincubated in a test tube for 1 min at 37°C at which time methyl diepoxystearate (1 μL of 5mM; or PNP-diepoxystearate for HPLC analysis) was added. The sample was incubated at 37°C for 10 min and, after partitioning with ethyl acetate (300 μL), the solvent was removed under a stream of nitrogen. The residue was redissolved in isopropanol (100 μL) for HPLC analysis or derivatized (neat) with MSTFA (20 μL) as described above for GC analysis.

Methyl [9*R(2β,4α,5α)]-(±)- and Methyl [9*S**(2α,4α,5α)]-(±)-9-Tetrahydro-9-hydroxy-9-(4-hydroxy-5-pentylfuran-2-yl)nonanoate (3 and 4) and Methyl 8[(2α,3α,5β (1*S**))]-(±)- and Methyl 8[(2β,3β,5β(1*S**))]-(±)-8-[Tetrahyro-3-hydroxy-5-(1-hydroxy)hexylfuran-2-yl]octanoate (5 and 6).** Dimethyl diepoxystearate (2; 4.30 g, 12.5 mmol), obtained from the oxidation of methyl linoleate with excess *m*CPBA, was dissolved in THF:H_2O (4:1, 80 mL) and 5% aqueous $HClO_4$ (20 mL) was added at room temperature. As evidenced by TLC, reaction was complete in 1h, yielding two products (fraction I and II). The aqueous layer was extracted with ethyl acetate (3x), and the combined organic layer was washed with 5% aqueous $NaHCO_3$ and saturated NaCl, dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The products were separated into two fractions on a silica gel column using hexane:ethyl acetate (5·3) as eluant. The products in order of elution, were clear oily liquids consisting of fraction II (4, 6: 1.68 g, 4.87 mmol, 39.0% yield) [FTIR (Neat) 3396, 2929, 2856, 1739, 1457, 1437, 1253, 1197, 1076, 1034 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300MHz) δ 0.86 (t, *J*=6.0 Hz, 3H), 1 28 (c, 14H), 1.58 (c, 6H), 1.83 (dd, *J*=13.2, 4 1 Hz, 1H), 2.27 (t, *J*=7.8 Hz, 2H), 2.36 (m, 1H), 3.00 (bs, 2H, D_2O exchangeable), 3.43 (m, 1H), 3.63 (s, 3H), 3.91 (m, 1H), 4.00 (m, 1H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 14.5, 23.1, 25 3, 25.4, 26.2, 26.4, 26.6, 29.2, 29.2, 29.5, 29.6, 29.8, 30.0, 32.2, 32.5, 34.5, 34.7, 39.1, 51.9, 72.0, 74.2, 74.3, 79.6, 84.6, 84.7, 174.9; HRMS (EI) calcd for $C_{19}H_{36}O_5$ 344.2563, found 344.2652] and fraction I (3, 5. 1.55 g, 4.50 mmol, 36% yield) [FTIR (Neat) 3470, 2931, 2858, 1740, 1457, 1437, 1252, 1120, 1070, 1026 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 0.85 (t, *J*=6.0 Hz, 3H), 1.28 (c, 14H), 1.58 (c, 6H), 1.82 (m, 1H), 1.96 (dd, *J*=12 4, 7 1 Hz, 1H), 2.26 (t,

J=7.7 Hz, 2H), 2.43 (bs, 2H, D_2O exchangeable), 3.33 (m, 1H), 3.63 (s, 3H), 3.70 (m, 1H), 4.00 (q, J=8.8 Hz, 1H), 4.20 (m, 1H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 14.5, 23.0, 25.3, 25.4, 25.7, 26.0, 26.4, 26.6, 29.3, 29.4, 29.5, 29.6, 29.9, 30.0, 32.3, 32.4, 33.5, 33.6, 34.5, 38.4, 51.9, 73.7, 74.5, 74.6, 80.7, 82.9, 83.0, 174.9; HRMS (EI) calcd for $C_{19}H_{36}O_5$ 344.2563, found 344.2652].

Methyl [9*R(2β,4α,5α)]-(±)-9-Benzoxy-9-[4-benzoxytetrahydro-5-pentylfuran-2-yl]-nonanoate (3') and Methyl 8[(2α,3α,5β(1*S**))]-(±)-8-[3-Benzoxy-5-(1-benzoxy)-hexyltetrahydrofuran-2-yl]octanoate (5').** THF-diols 3 and 5 (fraction I, 300 mg, 0.925 mmol) and DMAP (6 mg, 0.463 mmol) were dissolved in pyridine (5 mL) and benzoyl chloride (286 mg, 2.03 mmol) was added at room temperature. The reaction was quenched after 12 h with ice-water and extracted with CH_2Cl_2 (30 mL, 3x). The combined organics where washed with 1% aqueous H_2SO_4 (20 mLs, 3x), 4% aqueous $NaHCO_3$ (20 mL, 3x) and saturated NaCl (20 mL). After drying with anhydrous $MgSO_4$, the solvent was removed under reduced pressure and the product was applied to a 2mm preparative TLC plate and developed with acetone:hexane (1:4). The product isolated by extraction was a clear oily liquid (448 mg, 0.811 mmol, 88% yield) [FTIR ($CDCl_3$) 3063, 2929, 2856, 2255, 1715, 1602, 1451, 1273, 1111, 911 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 0.83 (dt, J=13.4, 6.7 Hz, 3H), 1.28 (c, 10H), 1.38 (c, 4H), 1.67 (c, 6H), 2.13 (m, 1H), 2.24 (m, 3H), 3.64 (s, 3H), 4.08 (m, 1H), 4.42 (q, J=6.4 Hz, 1H), 5.16 (m, 1H), 5.52 (m, 1H), 7.44 (m, 4H), 7.56 (t, J=7.6 Hz, 2H), 8.06 (t, J=7.6 Hz, 4H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 14.4, 22.9, 25.3, 25.6, 25.9, 26.4, 26.6, 29.4, 29 5, 29.5, 29.6, 29.8, 29.8, 31.3, 31.4, 32.1, 32.2, 34.5, 36.3, 51.9, 76.0, 76.1, 76.3, 78.3, 78.4, 82.0, 82.1, 128.8, 128.9, 130.1, 130.2, 133.4, 133.6, 166.3, 167.1, 174.7; HRMS (EI) calcd for $C_{33}H_{44}O_7$ 552.3087, found 552.3167].

Methyl [9*S(2α,4α,5α)]-(±)-9-Benzoxy-9-[4-benzoxytetrahydro-5-pentylfuran-2-yl]-octanoate (4') and Methyl 8[(2β,3β,5β(1*S**))]-(±)-8-[3-Benzoxy-5-(1-benzoxy)-hexyltetrahydrofuran-2-yl]octanoate (6').** THF-diols 4 and 6 (fraction II, 300 mg, 0.925 mmol) and DMAP (6 mg, 0.463 mmol) were dissolved in pyridine (5 mL) and benzoyl chloride (286 mg, 2.03 mmol) was added at room temperature. The reaction was quenched after 12 h with ice-water and extracted with CH_2Cl_2 (30 mL, 3x). The combined organics where washed with 1% aqueous H_2SO_4 (20 mLs, 3x), 4% aqueous $NaHCO_3$ (20 mL, 3x) and saturated NaCl (20 mL). After drying with anhydrous $MgSO_4$, the solvent was removed under reduced pressure and the product was applied to a 2mm preparative TLC plate and developed with acetone:hexane (1:4). The product isolated by extraction was a clear oily liquid (403 mg, 0.729 mmol, 79% yield) [FTIR ($CDCl_3$) 3068, 2932, 2860, 2256, 1720, 1603, 1452,1275, 1113, 912 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 0.80 (m, 3H), 1.23 (c, 14H), 1.55 (c, 2H), 1.72 (c, 4H), 2.00 (dd, J=14 6, 6.4 Hz, 1H), 2.24 (q, J=5 1 Hz, 2H), 2.52 (p, J=7.6 Hz, 1H), 3.64 (s, 3H), 3.86 (m,1H), 4.14 (q, J=8.0 Hz, 1H), 5.32 (m, 1H), 5.51 (m, 1H), 7.41 (m, 6H), 8.08 (d, J=7.4 Hz, 4H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 14.4, 22.8, 22.9, 25.3, 25.6, 25.9, 26.3, 26.6, 29.4, 29.5, 29.7, 29.9, 31.1, 32.1, 32.3, 34.5, 35.7, 51.9, 75.3, 75.4, 75.5, 78.5, 78.6, 82.5, 82.6, 128.7, 130.1, 133.2, 133.5, 134.1, 166.5, 167.4, 174.7; HRMS (EI) calcd for $C_{33}H_{44}O_7$ 552.3087, found 552.3202].

4-Nitrophenyl (9*Z*,12*R,13*S**)-(±)-12,13-Epoxyoctadec-9-enoate (9) and 4-Nitrophenyl (9*R**,10*S**,12*Z*)-(±)-9,10-Epoxyoctadec-12-enoate (10).** PNP-linoleate (1.30 g, 3.24 mmol) was dissolved in CH_2Cl_2 (20 mL) and *m*CPBA (0.70 g, 4.06 mmol) was added in small amounts over a 30 min period The reaction mixture was stirred at room temperature for 5 h after which time the

organic layer was washed with 5% aqueous $NaHCO_3$ (30 mL) and saturated NaCl (30 mL) and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure. Silica gel flash chromatography of the product with ethyl acetate:hexane (1:20) resulted in the separation of monoepoxides yellow oily liquids with an elution order of **9** (558 mg, 1.34 mmol, 41.3% yield) [FTIR ($CDCl_3$) 3084, 3010, 2929, 2857, 1768, 1594, 1523, 1347, 1211, 1112, cm^{-1}; ^1H-NMR ($CDCl_3$, 300 MHz) δ 0.90 (t, *J*=6.0 Hz, 3H), 1.35 (c, 14H), 1.53 (c, 2H), 1.76 (m, 2H), 2.05 (q, *J*=7.5 Hz, 2H), 2.22 (m, 1H), 2.35 (m, 1H), 2.60 (t, *J*=7.7 Hz, 2H), 2.93 (t, *J*=4.2 Hz, 2H), 5.45 (m, 1H), 5.51 (m, 1H), 7.27 (d, *J*=9.0 Hz, 2H), 8.26 (d, *J*=9.0 Hz, 2H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 14.4, 23.0, 25.1, 26.7, 27.8, 28.2, 29.4, 29.5, 29.6, 29.9, 32.2, 34.7, 56.9, 57.6, 122.9, 124.5, 125.6, 132.9, 145.7, 156.0, 171.6]; HRMS (EI) calcd for $C_{24}H_{35}NO_5$ 417.2515, found 417.2606; and **10** [(700 mg, 1.68 mmol, 51.8% yield), FTIR ($CDCl_3$) 3085, 3011, 2929, 2857, 1769, 1616, 1526, 1347, 1211, 1111, cm^{-1}; ^1H-NMR ($CDCl_3$, 300 MHz) δ 0.88 (t, *J*=6.0 Hz, 3H), 1.40 (c, 14H), 1.54 (c, 2H), 1.76 (m, 2H), 2.02 (q, *J*=7.6 Hz, 2H), 2.20 (m, 1H), 2.35 (m, 1H), 2.60 (t, *J*=7.7 Hz, 2H), 2.93 (t, *J*=4.3 Hz, 2H), 5.42 (m, 1H), 5.50 (m, 1H), 7.27 (d, *J*=9.0 Hz, 2H), 8.26 (d, *J*=9.0 Hz, 2H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 14.5, 23.0, 25.1, 26.7, 27.1, 27.9, 28.2, 29.4, 29 .5, 29.6, 29.7, 29.8, 32.0, 34.8, 57.1, 57.7, 122.9, 124.2, 125.6, 133.3, 145.7, 155.8, 171.7; HRMS (EI) calcd for $C_{24}H_{35}NO_5$ 417.2515, found 417.2578]. The regiochemistry of **9** and **10** was determined by analysis of the fragmentation pattern of the corresponding TMS-derivatized diols (**9a** and **10a**). GC/MS analysis of the hydrolysis/TMS-derivatived first fraction (i.e., **9** → **9a**; see below) produced *m/z* fragmentations of 173 and 406 as a result of C-C bond cleavage of the C12:C13 disilyloxy moiety. Fragmentation of the hydrolysis/TMS-derivatived second fraction (i.e., **10** → **10a**; see below) resulted in diagnostic *m/z* fragments of 213 and 366 which places the two silyloxy moieties at C8 and C9.

4-Nitrophenyl (9*Z*,12*R,13*R**)-(±)-12,13-Dihydroxyoctadec-9-enoate (9a).** PNP-ester **9** (105 mg, 0.252 mmol) was dissolved in H_2O:THF (1:3, 4 mL), 5% aqueous $HClO_4$ (1 mL) was added and the reaction was heated to 50° C. As evidenced by TLC, the reaction was complete within 1 h at which time the reaction was extracted with ethyl acetate (20 mL, 3x) and the combined organic was washed with saturated NaCl and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to yield PNP-12,13-dihydroxyoleate as a clear yellow oily liquid (107 mg, 0.246 mmol, 98% yield) [FTIR ($CDCl_3$) 3413, 3084, 3010, 2929, 2857, 1768, 1594, 1526, 1347, 1211, 1113, cm^{-1}; ^1H-NMR ($CDCl_3$, 300 MHz) δ 0.88 (t, *J*=6.0 Hz, 3H), 1.34 (c, 14H), 1.48 (c, 2H), 1.75 (m, 2H), 2.04 (m, 2H), 2.28 (m, 1H), 2.59 (t & bs, *J*=7.9 Hz, 4H, 2H's exchangeable with D_2O), 3.46 (bs, 2H), 5.44 (m, 1H), 5.53 (m, 1H), 7.27 (d, *J*=9.0 Hz, 2H), 8.26 (d, *J*=9.0 Hz, 2H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 14.5, 23.0, 25.1, 25.8, 27.8, 29.4, 29.5, 29.9, 32.1, 32.3, 34.1, 34.7, 74.3, 74.4, 122.9, 125.3, 125.6, 133.7, 145.7, 155.9, 171.7; HRMS (EI) calcd for $C_{24}H_{37}N0_6$ 436.2621, found 436.2675].

4-Nitrophenyl (9*R,10*R**,12*Z*)-(±)-9,10-Dihydroxyoctadec-12-enoate (10a).** PNP-ester **10** (62.4 mg, 0.150 mmol) was dissolved in H_2O:THF (1:3, 2 mL), 5% aqueous $HClO_4$ (0.5 mL) was added and the reaction was heated to 50° C for 1 h after which time the reaction was complete (TLC). The reaction was extracted with ethyl acetate (10 mL, 3x) and the combined organic was washed with saturated NaCl (20 mL) and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to yield PNP-9,10-dihydroxyoleate as a clear yellow oily liquid: 54.0 mg, 0.124 mmol, 83% yield) [FTIR ($CDCl_3$) 3380, 3087, 3010, 2928, 2857, 1757, 1594, 1526, 1347, 1208, 1111,

cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 0.88 (t, *J*=6.0 Hz, 3H), 1.35 (c, 14H), 1.50 (c, 4H), 1.75 (m, 2H), 2.04 (m, 2H), 2.29 (m, 1H), 2.59 (t, *J*=7.8 Hz, 2H), 3.47 (bs, 2H), 5.43 (m, 1H), 5.58 (m, 1H), 7.27 (d, *J*=9.0 Hz, 2H), 8.26 (d, *J*=9.0 Hz, 2H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 14.5, 23.0, 25.1, 26.0, 27.8, 29.4, 29.5, 29.6, 29.7, 29.9, 30.0, 32.0, 32.2, 34.0, 34.8, 74.4, 122.9, 124.9, 125.6, 134.4, 145.7, 155.9, 171.7; HRMS (EI) calcd for $C_{24}H_{37}NO_6$ 435.2621, found 435.2703].

4-Nitrophenyl (9*R,10*S**,12*R**,13*R**)-(±)- and 4-Nitrophenyl (9*R**,10*S**,12*S**, 13*S**)-(±)-12,13-Dihydroxy-9,10-epoxyoctadecanoate (11).** PNP-12,13-dihydroxyoleate (53 mg, 0.122 mmol) and Na_3PO_4 (10 mg) were dissolved in CH_2Cl_2 (4 mL) and epoxidized with an excess of freshly prepared dimethyl dioxirane[23] (3 mole equivalents). The dimethyl dioxirane was prepared prior to reaction. H_2O (15 mL), acetone (10 mL) and $NaHCO_3$ (9 g) were placed in a diazomethane generator and vigorously stirred. Oxone® ($2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$; 18 g) was added slowly over a 30 min period. After the addition of Oxone® (15 min) the apparatus was attached to an aspirator and the dimethyl dioxirane/acetone reagent was distilled into a dry ice/acetone cooled receiving vessel. The concentration of the dimethyl dioxirane was determined to be between 70-90 mmolar by method described by Murray *et al*[21] The reaction of olefin with dimethyl dioxirane was monitored by TLC and judged complete with in 10 min. at room temperature. The reaction mixture was extracted with saturated NaCl (10 mL), dried over anhydrous Na_2SO_4 the solvent removed under reduced pressure to yield **11** as a clear oily liquid. The product unstable and, in the presence of moisture, cyclized to the THF-diols (56 mg, 0.124 mmol, 102% yield) [FTIR (CH_2Cl_2) 3453, 2933, 2860, 2253, 1762, 1734, 1594, 1527, 1348, 1211 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 0.88 (t, *J*=6.0 Hz, 3H), 1.10-1.95 (c, 24H), 2.58 (t & bs, *J*=7.7 Hz, 4H, 2H's exchangeable with D_2O), 2.94 (m, 1H), 3.14 (m, 1H), 3.46 (m, 1H), 3.71(dq, *J*=22.4, 6.4 Hz, 1H), 7.27 (d, *J*=9.0 Hz, 2H), 8.26 (d, *J*=9.0 Hz, 2H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 14.5, 23.1, 25.1,25.2, 25.8, 26.9, 28.3, 28.4, 29.4, 29.6, 29.7, 32.0, 32.3, 32.5, 33.9, 34.0, 34.7, 55.0, 55.5, 57.1, 57.9, 72.8, 73.5, 73.6, 74.7, 122.9, 125.5, 125.6, 125.7, 145.7, 155.9, 171.7; HRMS (EI) calcd for $C_{24}H_{37}NO_7$ 451.2571, found 451.2644].

4-Nitrophenyl (9*R,10*R**,12*S**,13*R**)-(±)- and 4-Nitrophenyl (9*R**,10*R**,12*R**,13*S**)-(±)-9,10-Dihydroxy-12,13-epoxyoctadecanoate (12).** PNP-9,10-dihydroxyoleate (30.0 mg, 0.0690 mmol) was oxidized by dimethyl dioxirane as described above, yielding **12** as a clear oily liquid (29 1 mg, 0.0644 mmol, 93% yield) [FTIR (CH_2Cl_2) 3423, 2931, 2860, 2252, 1762, 1734, 1594, 1527, 1348, 1208 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 0.88 (t, *J*=6.0 Hz, 3H), 1.10-1.95 (c, 24H), 2.54 (t & bs, *J*=7.5 Hz, 4H, 2H's exchangeable with D_2O), 2.92 (m, 1H), 3.11 (m, 1H), 3.46 (m, 1H), 3.73(dq, *J*=20.2, 6.0 Hz, 1H), 7.27 (d, *J*=9.0 Hz, 2H), 8.26 (d, *J*=9.0 Hz, 2H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 14.5, 23.0, 25.1, 25.8, 26.9, 28.4, 29.4, 29.6, 32.0, 32.2, 32.5, 33.9, 34.0, 34.7, 55.0, 55.5, 57.1, 57.9, 72.8, 73.5, 73.6, 74.7, 122.9, 125.5, 125.7, 145.7, 155.8, 171.7; HRMS (EI) calcd for $C_{24}H_{37}NO_7$ 451.2571, found 452.2671].

Products of acidic hydrolysis of (11) and (12). Epoxy-diols **11** and **12** yielded identical products upon acidic hydrolysis with THF:H_2O:5% aqueous $HClO_4$ (4:1:1). Subsequent transesterification with methanol/cat. KCN yielded the four methyl ester THF-diols **3-6** as evidenced by comparative TLC, GC and HPLC. The reactions were quantitative in all scales.

THF-diol PNP Ester (Fraction I) [FTIR (KBr Pellet) 3447, 3121, 2928, 2854, 1755, 1622, 1536, 1351, 1203, 1166, 858 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300MHz) δ 0.88 (t, *J*=6.0 Hz, 3H), 1 36 (c, 14H), 1.59 (c, 4H), 1.74

(t, *J*=7.1 Hz, 2H), 1.85 (m, 1H), 1.99 (dd, *J*=12.7, 6.4 Hz, 1H), 2.41 (bs, 2H, D_2O exchangeable), 2.58 (t, *J*=7.4 Hz, 2H), 3.36 (m, 1H), 3.73 (dt, *J*=6.5, 2.5 Hz, 1H), 3.99 (q, *J*=7.4 Hz, 1H), 4.23 (m, 1H), 7.24 (d, *J*=9.0 Hz, 2H), 8.25 (d, *J*=9.0 Hz, 2H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 14.5, 23.0, 23.1, 25.1, 25.7, 26.0, 26.4, 26.7, 29.3, 29.4, 29.5, 29.6, 29.9, 30.0, 32.3, 32.4, 33.6, 34.7, 38.3, 38.4, 73.8, 73.9, 74.5, 74.6, 80.6, 80.7, 82.9, 83.0, 122.9, 125.6, 171.7; HRMS (EI) calcd for $C_{24}H_{37}NO_7$ 451.2570, found 451.2659]. Anal. Calcd for $C_{24}H_{37}NO_7$: C, 63.84; H, 8.26; N, 3.10. Found: C, 63.94; H, 8.48; N, 3.14.

THF-diol PNP Ester (Fraction II) [FTIR (KBr Pellet) 3273, 2930, 2855, 1752, 1622, 1538, 1350, 1201, 1151, 856 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300MHz) δ 0.88 (t, *J*= 6.0 Hz, 3H), 1.34 (c, 16H), 1.63 (c, 4H), 1.74 (t, 2H), 1.87 (dt, *J*=12.4, 1.4 Hz, 1H), 2.38 (m, 1H), 2.59 (t, *J*=7.4 Hz, 2H), 2.75 (bs, 2H, D_2O exchangeable), 3.48 (m, 1H), 3.63 (dt, *J*=7.0, 1.5 Hz, 1H), 3.94 (m, 1H), 4.04 (m, 1H), 7.27 (d, *J*=9.0 Hz, 2H), 8.27 (d, *J*=9.0 Hz, 2H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 14.5, 23.1, 25.2, 26.1, 26.4, 26.6, 29.2, 29.3, 29.4, 29.5, 29.6, 29.8 30.1, 32.2, 32.5, 34.8, 39.1, 72.0, 74.3, 74.4, 79.5, 79.6, 84.7, 84.8, 122.9, 125.6, 172.0; HRMS (EI) calcd for $C_{24}H_{37}NO_7$ 451.2571, found 451.2659]. Anal. Calcd for $C_{24}H_{37}NO_7$: C, 63.84; H, 8.26; N, 3.10. Found: C, 63.76; H, 8.32; N, 3.24.

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