New Synthesis of *sn*-1,2- and *sn*-2,3-*O*-Diacylglycerols – Application to the Synthesis of Enantiopure Phosphonates Analogous to Triglycerides: A New Class of Inhibitors of Lipases

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Phosphonate compounds mimic the first transition state occurring during enzymatic carboxyester hydrolysis of natural substrates by forming a covalent bond with the catalytic serine. However, until now the organophosphorus compounds used in the inhibition studies more or less resembled a natural triglyceride substrate. In order to elucidate the interfacial activation and the mechanism of action of lipases, specific inhibitors need to be prepared. To achieve this goal, enantiomerically pure *sn*-1,2- and *sn*-2,3-*O*-didecanoylglycerol compounds were prepared – starting from a C-4 chiral synthon, 3-buten-1,2-diol – and treated with *n*-pentylphosphonic dichloride and *p*-nitrophenol to afford the corresponding diastereomeric phosphonates, which were acylglycerol analogs. Subsequent separation of each of the phosphonate diastereomers **A**/**B** or *ent*-**A**/*ent*-**B**, performed by HPLC, led to four enantiopure stereoisomers that will be investigated as inhibitors of Human Pancreatic Lipase (HPL) and Human Gastric Lipase (HGL) using the monomolecular film technique.

Introduction

The design and synthesis of specific inhibitors of various animal and microbial lipases is of fundamental value for understanding the mechanisms involved in the catalytic activity of lipases. Furthermore, lipase inhibitors should have potential in terms of useful applications in the fields of chemistry, biochemistry, and medicine. Among the range of lipase inhibitors known, phosphonate compounds are of fundamental interest to better characterize the mechanism of catalysis. As revealed by X-ray crystallography,[1] these compounds mimic, in both their charge distribution and geometry, the first transition state occurring during enzymatic carboxyester hydrolysis of natural substrates. They are thus efficient inhibitors of lipases by formation of a covalent bond between the nucleophilic Oγ oxygen of the active serine of the enzyme and the phosphorus atom.[1][2] However, the organophosphorus compounds used in these crystallographic studies to a large extent resemble a natural triacylglycerol substrate. Despite the well recognized importance of these enzymes, relatively little progress has been made towards elucidation of their interfacial activation and mechanism of action. In order to achieve this goal, specific inhibitors need to be prepared.

In an attempt to further characterize the catalytic mechanism of digestive lipases by using phosphorus inhibitors,[2] we have focused our research on a new class of inhibitors of lipases that involve a phosphonate reactive group in a triacylglycerol structure **1** (Figure 1).

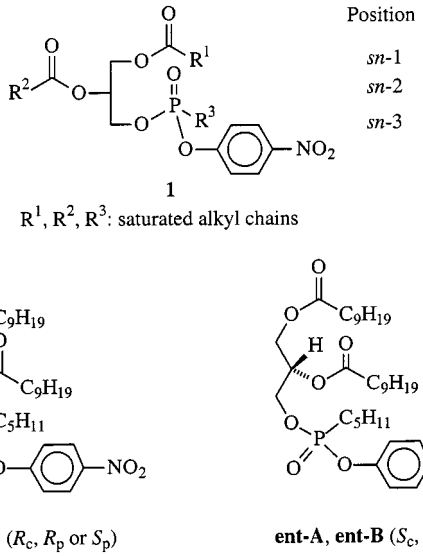

Figure 1. Structures of 1,2- and 2,3-diacyl-*sn*-glycerophosphonates analogous to triglycerides

The phosphonate moiety is located at an external position, *sn*-1 or *sn*-3, of the glycerol backbone, with *p*-nitrophenoxy as a leaving group. We have intentionally retained the two carbonyl ester linkages in order to mimic as closely as possible the structure of acylglycerols (natural substrates of lipases). These molecules have two chiral centers and, as such, exist as four stereomers. Because of the presence of the *p*-nitrophenoxy, which is a good leaving group, on the phosphorus atom, the strategy for the synthesis of these diacylglycerophosphonates **1** requires the formation of the

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phosphonate unit during the last step. The elaboration of the diglyceride unit will then be achieved before the coupling with the phosphorus moiety.

In this article, we describe a new synthesis of racemic and chiral 1,2- and 2,3-diglycerides − starting from a C-4 synthon, 3-buten-1,2-diol − and the isolation of each of the four organophosphorus stereoisomers **1**, which are analogous to triglycerides.

Results and Discussion

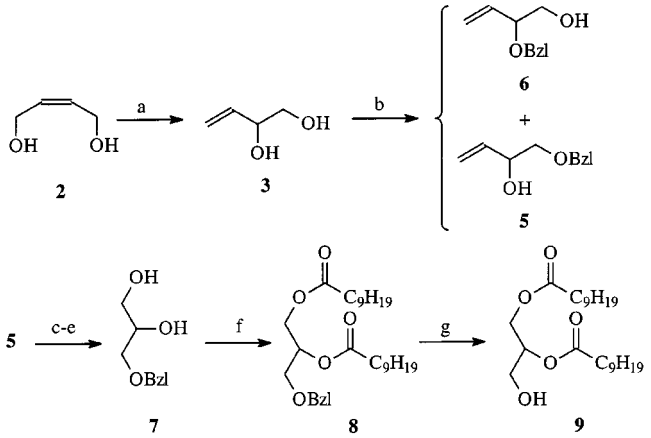
We decided to synthesize a triglyceride analog, in which the two acyl ester chains possess ten carbon atoms, in order to perform specific inhibition tests using the monomolecular film technique.[3] Moreover, because inhibition of digestive lipases is improved with short alkyl chains,[2] the length of the saturated (R^3) alkyl chain on the phosphorus atom was fixed to five carbon atoms.

We began the synthesis with a C-4 synthon: 3-buten-1,2-diol (**3**). This compound was obtained by isomerization of (*Z*)-2-buten-1,4-diol (**2**) in an acidic medium catalysed by mercuric ions.[4] Synthon **3** is important for the elaboration of many building blocks.[5] It has been used as an intermediate in the synthesis of pharmaceutical compounds,[6a,6b] natural products[6c] as well as for the synthesis of (2*R*,3*R*,5*E*)-2-hydroxy-3-methyl-5-heptenal (**4**).[7] This latter compound is an important intermediate in the synthesis of (MeBmt),[4][7] which is a nonnatural amino acid constituting cyclosporine. (*R*)-(+)- and (*S*)-(−)-1-tosyloxy-3-buten-2-ol (**12**), both prepared from **3**, are attractive chiral precursors for the synthesis of chiral phosphonates analogous to triglycerides. Chiron **3** can be efficiently obtained from compound **12** by various pathways: for instance, by a kinetic resolution through a Sharpless asymmetric epoxidation,[8a] or by an enzymatic esterification.[8b,8c] Moreover, compound **3** can also be prepared from butadiene monoepoxide using the method recently described by Jacobsen et al.[8d,8e]

Racemic Synthesis

A synthetic method for the preparation of racemic 1,2-diglycerides **9** is outlined in Scheme 1.

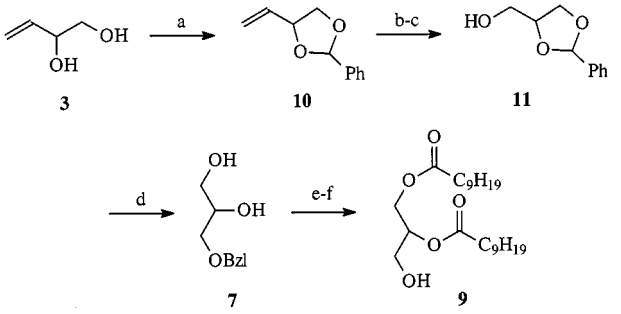
The first step requires the mono-protection of the primary alcohol function of **3** in order to elaborate the 1,2-diacylglycerol unit. We employed benzyl bromide to avoid the isomerization of 1,2-diglyceride into the 1,3-diglyceride during cleavage of the protecting group. The benzylation of diol **3** was not selective and led to a mixture of two isomers in 52% yield: the secondary alcohol **5** and the primary alcohol **6** in a 70:30 ratio. Optimization of the reaction medium (decrease of the temperature of the reaction, slower addition of benzyl bromide) did not significantly improve the selectivity of the reaction (80:20 ratio). Furthermore, under these modified conditions, a marked decrease in the degree of conversion of the reaction was observed with time in comparison with the original compounds. Since alcohols



Scheme 1. Racemic synthesis of 1,2-diglyceride **9** by the benzylation pathway. Reagents and conditions: (a) H_2SO_4, $HgSO_4$, H_2O, microwave oven. − (b) BzlBr, NaH, Bu_4NI, THV. − (c) Pivaloyl chloride, CH_2Cl_2, Pyr, 0°C to room temp. − (d) O_3, Me_2S, CH_2Cl_2, −78°C. − (e) $NaBH_4$, CH_2Cl_2, EtOH, 0°C. − (f) Decanoic acid, DCC, DMAP, Et_2O. − (g) 5% Pd/C, H_2, EtOH.

5 and **6** could not be separated either by distillation nor by silica gel chromatography, we employed selective chemical reagents with respect to primary alcohol functions: TsCl,[9a] PivCl,[9b] TrCl.[9c] Although the PivCl reacted much faster than the other two (4 h for a complete conversion against at least 12 h for TsCl or TrCl), the yields of alcohol **5** were similar with all three reagents (75%). Subsequent ozonolysis of recovered 1-benzyloxy-3-buten-2-ol (**5**) in the presence of dimethyl sulfide, followed by sodium borohydride reduction, led to the 1,2-diol **7** in 84% yield. This compound was then esterified to give 3-*O*-benzyl-1,2-di-*O*-decanoyl-*rac*-glycerol (**8**) in 93% yield. The subsequent hydrogenolysis of **8** with 5% Pd/C in ethanol gave the 1,2-diglyceride compound **9** in 90% yield, without the presence of the 1,3-isomer.

The synthesis has been optimized in order to include a regioselective benzylation step. Several methods were explored and the most interesting one is represented in Scheme 2.



Scheme 2. Racemic synthesis of 1,2-diglyceride **9** by the benzylidene acetal pathway. Reagents and conditions: (a) PhCHO, *p*-TsCOH, PhMe, reflux. − (b) O_3, Me_2S, CH_2Cl_2, −78°C. − (c) $NaBH_4$, CH_2Cl_2, EtOH, 0°C. − (d) Decanoic acid, DCC, DMAP, Et_2O. − (f) 5% Pd/C, H_2, EtOH.

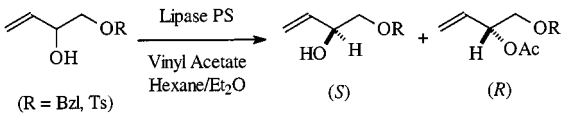
The reaction of diol **3** with benzaldehyde, catalysed by *p*-TsOH, led to two epimers of the benzylidene acetal **10** in 90% yield. Ozonolysis of **10**, followed by sodium borohydride reduction, gave the alcohol **11** in 74% yield. Next, opening of the acetal ring with DIBALH in ether at 0°C led exclusively to compound **7** in 80% yield. The selective formation of **7** can be rationalized in terms of initial formation of aluminium alkoxide.[10] The aluminium atom plays the role of a Lewis acid for the coordination to the neighboring oxygen atom, which allows selective cleavage of the cyclic acetal site to give the vicinal diol **7**. Finally, as previously depicted in Scheme 1, acylation of **7** and subsequent hydrogenolysis led to 1,2-diglyceride **9**.

Enantioselective Synthesis

Enzymatic resolution employing a lipase as a catalyst was used in order to obtain optically pure compounds. Many different alcohols can be resolved easily by this method.[11] Transesterification of 3-buten-1,2-diol (**3**) as well as alcoholysis in an organic medium of the corresponding diacetates catalysed with different lipases are highly regioselective.[12] However, if lipases show only low enantioselectivities during the monoacylation step,[13] the acylation of the second alcohol group is more enantioselective than that of the first alcohol group.[14] Moreover, transesterification or hydrolysis catalysed by lipases of mono-protected diols or corresponding acylated compounds, are performed with high enantioselectivities.[15]

Given the above results, we decided to resolve with lipases the building blocks "benzylic alcohol" **5** or "tosylated alcohol" **12** rather than the diol **3**.[8c]

Resolution of Synthon C-4. We employed a lipase from *Pseudomonas sp*, which shows a high selectivity for the hydrolysis of esters of secondary alcohols, as well as for the corresponding esterification reactions.[16] All crude lipases available from *Pseudomonas sp* have a stereochemical preference for the (*R*) configuration on the reactive center of the secondary alcohol. This stereochemical preference has led to a model for the active site.[17] With lipases, esterifications catalysed in organic solvents are often more enantioselective than the corresponding hydrolysis reactions in aqueous media.[18] Thus, we used irreversible esterification reactions in organic media instead of hydrolysis reactions (Equation 1).



With "benzylic alcohol" **5** (R = Bzl), the two enantiomers were poorly discriminated by lipase PS at room temperature (Table 1, entries 1−2). The enantiomeric excess of remaining substrate as well as the product formed were moderate ($ee_S = 0.70$, $ee_P = 0.79$) for a conversion of 47%, which corresponds to an enantiomer ratio or *E* value[18c] of 18. The addition of molecular sieves into the reaction medium (entry 2) did not have any effect on the enantioselectivity of the enzymatic reaction. Nevertheless, when the reaction was performed at a lower temperature (5°C), chiral discrimination increased to give an *E* value of 59 (entry 3).

In contrast, enzymatic resolution of "tosylated alcohol" (R = Ts) **12**, performed under similar conditions as in the case of benzylic alcohol **5**, was more enantioselective (Table 1, entries 4−5). At room temperature (entry 4), the enantiomer ratio was 104 as compared to 18 in the previous case. A decrease in the temperature of the reaction medium (10°C) gave rise to an increase in *E* to 149, coinciding with an excellent chiral discrimination, for a conversion of only 21% (entry 5). Furthermore, in hexane/ether solution we did not observe deactivation of the enzyme by acidic contaminants, which is in contrast to the work described by Boaz et al.[8b]

Elaboration of Chiral 1,2- and 2,3-Diacylglycerols. 1,2-diacyl-*sn*-glycerols are easily available from the chiral pool, starting from D-mannitol,[19] but 2,3-diacyl-*sn*-glycerols are not so easy to synthesize. With the method we have developed, both of these enantiomers are easy to prepare. As the chiral recognition by the lipase PS was more marked with 1-tosyloxy-3-buten-2-ol (**12**) (Table 1), we decided to use (*S*)-**12** for the synthesis of 2,3-diacyl-*sn*-glycerols (*R*)-**9**, as depicted in Scheme 3. In order to avoid formation of epoxybutene during the next step (Scheme 3-b), the alcohol function of (*S*)-**12** was first acetylated to give compound (*S*)-**13**. The tosylate group was then substituted with cesium acetate in DMSO/DMF to form the diacetate (*S*)-**14** in quantitative yield. Saponification with methanolic K_2CO_3 produced the chiral diol (*S*)-**3** in 65% yield. Next, the chemical pathway developed in Scheme 2 for racemic compounds led to 2,3-*O*-didecanoyl-*sn*-glycerol [(*R*)-**9**].

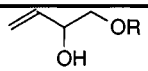
In a same way, compound (*R*)-**13**, obtained by enzymatic resolution of alcohol **12** by lipase PS (Equation 1), led to the 1,2-*O*-didecanoyl-*sn*-glycerol [(*S*)-**9**] enantiomer.

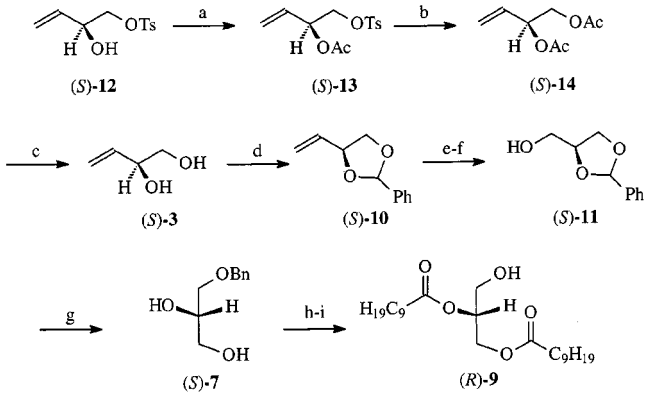
The absolute configurations of the two enantiomers of compound **9** were determined by chemical correlation (see Experimental Section for details). The enantiomeric purity of the tosylated alcohol (*S*)-**12** after enzymatic resolution was determined to be greater than 99% (measured by chiral HPLC), since the 2,3-diglyceride (*R*)-**9** was obtained with 92% ee (measured by ^{19}F-NMR spectroscopy), which corresponded to a 3% racemization. We can assume that this racemization occurred by substitution of the tosylate group during the reaction with cesium acetate. This racemization could have occurred by an SNi reaction catalysed by the cesium ion. The acetate group could then attack either at the primary carbon, with retention of configuration [compound (1), Equation 2], or at the secondary carbon, with inversion of configuration [compound (2), Equation 2].

Synthesis of Compound 1

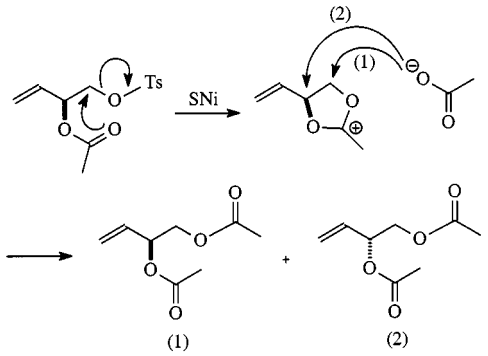
For the elaboration of phosphonates **1**, which are analogous to triglycerides, the synthesis was developed as for more simple alcohols but using alkylphosphonic dichlo-

Table 1. Enzymatic Resolution of alcohols **5** and **12** with Lipase PS

Entry		R	Experimental conditions	Conversion	ee_P	ee_S	*E*
1	**5**	Bzl	Room temp.	0.47	0.79	0.70	18
2	**5**	Bzl	Room temp. + molec. sieves	0.47	0.79	0.70	18
3	**5**	Bzl	5 °C	0.47	0.92	0.80	59
4	**12**	Ts	Room temp.	0.47	0.95	0.84	104
5	**12**	Ts	10 °C	0.21	0.99	0.26	149

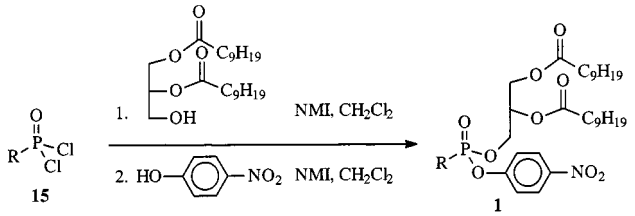


Scheme 3. Enantioselective synthesis of chieral 2,3-diglyderide (*R*)-**9**. Reagents and conditions: (a) Acetyl chloride, CH_2Cl_2, Pyr, 0°C. – (b) Caesium acetata, DMSO, DMF. – (c) K_2CO_3, MeOH, H_2O. – (d) PhCHO, *p*-TsCOH, PhMe, reflux. – (e) O_3, Me_2S, CH_2Cl_2, –78°C. – (f) $NaBH_4$, CH_2Cl_2, EtOH, 0°C. – (g) DIBALF, Et_2O, 0°C to room tem. – (h) Decanoic acid, DCC, DMAP, Et_2O. – (i) 5% Pd/C, H_2, EtOH.



ride.[2a] The alcohol group from 1,2- and 2,3-*O*-didecanoyl-*sn*-glycerol was first allowed to react with 1 equivalent of pentylphosphonic dichloride **15** in the presence of 1.1 equivalents of *N*-methylimidazole (NMI) in dichloromethane. In this case, only monosubstitution was observed. Subsequent addition of 1 equivalent of *p*-nitrophenol in the presence of 1.1 equivalents of NMI led to a mixture of two diacylglycerophosphonate diastereomers (Equation 3), as can be seen by ^{31}P-NMR spectroscopy (two peaks separated by 0.2 ppm). These diastereomers were separated by HPLC (silica column): retention times (24.6 min and 27.3 min) were sufficient for a total separation of the two stereomers.

Cyclodextrin inclusion complexes prepared to obtain suitable crystals for elucidation of the stereochemistry by



X-ray diffraction, or NOE experiments did not allow us to determine the absolute configuration at the phosphorus atom of these triglyceride analogs. However, further experiments are currently in progress in order to make this assignment.

By convention, and until the absolute configuration at phosphorus can be made, we have designated as isomer **A** the one that resonates at lower fields in ^{31}P NMR, and isomer **B** the one at higher fields. Thus, 1,2-didecanoyl-*sn*-glycerol led to two diastereomers named **A** and **B**, and 2,3-didecanoyl-*sn*-glycerol to two other diastereomers named ***ent*-A** and ***ent*-B**, enantiomers of **A** and **B**, respectively (Figure 1).

Conclusion

We have described the enantioselective synthesis of optically pure 1,2- and 2,3-*O*-didecanoyl-*sn*-glycerophosphonates, which exist as two pairs of enantiomers, starting from a chiral diol obtained by an irreversible enzymatic acylation of (*R*,*S*)-**12**: (*R*)- and (*S*)-**3**, respectively. The strategy depicted in Scheme 3, which leads to enantiopure diglycerides **9**, could also be applied to the synthesis of various mixed diglyceride compounds.

Inhibition studies with each enantiomer **A**, ***ent*-A**, **B**, ***ent*-B** on Human Pancreatic Lipase (HPL) and Human Gastric Lipase (HGL) are under currently under way.

Experimental Section

General Remarks: Unless stated otherwise, all starting materials were obtained from commercial suppliers and were used without further purification. Tetrahydrofuran (THF) was dried by distillation from potassium/benzophenone under nitrogen. Diethyl ether (ether) was distilled from sodium. Ethanol was removed from dichloromethane (CH_2Cl_2) by passing it through a silica column immediately prior to use. *N*,*N*-Dimethylformamide (DMF) was distilled under reduced pressure from barium oxide immediately prior

to use. Dimethylsulfoxide (DMSO) was dried with CaH_2, then distilled under reduced pressure immediately prior to use. Reactions involving phosphorus compounds were performed under an inert atmosphere of dry nitrogen. − Flash-chromatography was performed as described by Still et al.[21] using silica gel 60 (70−230 mesh, Merck). Thin-layer chromatography (TLC) was carried out on silica gel plates (0.25 mm, Merck), and the following detection methods were used: UV lamp (254 nm); PMA: TLC plate dipped into a solution containing 5% phosphomolybdic acid in absolute ethanol, and heated on a hot plate; PAA: TLC plate dipped into a solution containing 3% *p*-anisaldehyde, 4% concentrated H_2SO_4, 1% acetic acid in 95% ethanol, and heated on a hot plate. − ^{1}H-NMR spectra were obtained on a Bruker AC 200 using $CDCl_3$ as solvent and tetramethylsilane (TMS) as an internal standard. ^{13}C-NMR spectra were obtained at 50.36 MHz. ^{31}P-NMR spectra were obtained on a Bruker AC 100 at 40.54 MHz (85% H_3PO_4 aqueous solution was used as an external standard) and ^{19}F-NMR spectra at 94.22 MHz ($CFCl_3$ was used as an external standard). Spectral splitting patterns are designated as s, singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet. − Elemental analyses were performed at the *Service Commun de Microanalyse*, University of Aix-Marseille III, France. All compounds were characterized by a combination of $^{1\,H,\ 13\,C,\ 31}$P NMR, elemental analyses and $[\alpha]_D^{20}$.

Absolute Configuration: The derivatization of 1,2-diacylglycerol **9** with a chiral derivative agent (*o*-fluorophenoxy lactic acid)[22] allows us to differentiate, by ^{19}F-NMR spectroscopy, the two diastereomers. As D-mannitol leads specifically to 1,2-*O*-diacyl-*sn*-glycerol, it is possible to assign to each NMR signal the absolute configuration of the *sn*-2 carbon of the glycerol backbone, and in this way to determine the absolute configuration of the asymmetric center formed during the enantioselective step.

The lipase PS has a stereopreference for the (*R*) enantiomer with each synthon **5** and **12**. This is in agreement with the studies undertaken with crude lipases from *Pseudomonas sp.*, which all have a stereochemical preference for the (*R*) configuration on the reactive center of the secondary alcohol.

Preparation of the Diastereomeric Esters for ee Measurements of Alcohols 5 and 9: The appropriate alcohol (0.175 mmol) and *o*-fluorophenoxy lactic acid (48.2 mg, 0.262 mmol) were dissolved in anhydrous ether (2 mL) and the mixture cooled to 0°C. DCC (54.2 mg, 0.262 mmol) and DMAP (3.2 mg, 0.026 mmol) in ether (3 mL) were added. The cooling bath was removed and the mixture was stirred for 12 h. The solution was then carefully filtered through a pressed cotton plug and diluted with ether (5 mL). The solution was washed successively with saturated NH_4Cl (5 mL), 10% $NaHCO_3$ (2× 5 mL), water (5 mL), and brine (5 mL). The organic layer was dried with $MgSO_4$, filtered, and concentrated. The crude product (yield 80−90%) was analysed by ^{19}F-NMR spectroscopy.

Enantiomeric Purity: Analysis of enantiomeric purity was determined by NMR in the presence of a chiral derivative agent or by HPLC with chiral columns.

(a) NMR Analysis was performed on compounds **5** and **9**, onto which was added *o*-fluorophenoxy lactic acid.[22] The two diastereomers formed were analysed and quantified by NMR ($^{1\,H,\ 13\,C,\ 19}$F). The fluorine resonance of each enantiomer is separated by 9.8 Hz for **5** and 15.3 Hz for **9**.

(b) HPLC. Enantiomers of **12** were separated on a Chiracel OD-H column (J. T. Baker) with hexane/2-propanol (98:2) as eluent, at 25°C. The (*R*) enantiomer eluted first. Retention times: t_1 = 34.0 min; t_2 = 38.6 min.

Enantiomers of **13** were separated on a Chiralpak AS column (Daicel Chemical Industries) eluted with hexane/2-propanol (90:10) at 25°C. The (*R*) enantiomer eluted first. Retention times: t_1 = 21.4 min; t_2 = 26.1 min.

3-Buten-1,2-diol (3): A mixture of 20.0 g (227 mmol) of (*Z*)-2-buten-1,4-diol (**2**), mercuric sulfate (80 mg) and concentrated sulfuric acid (0.12 mL) in water (10 mL) was kept in a microwave oven for 3−5 min (temperature of the reaction mixture was ca. 50°C). After the usual workup procedure,[5a] 13.3 g (66%) of 3-buten-1,2-diol (**3**) was isolated as a colorless liquid by fractional distillation (bp 78−84°C/10 Torr). − ^{1}H NMR ($CDCl_3$) δ = 5.9/5.7 (m, 1 H), 5.4/5.1 (m, 2 H), 4.2/4.0 (m, 3 H), 3.6/3.3 (m, 2 H). − ^{13}C NMR ($CDCl_3$) δ = 136.7, 116.6, 73.4, 66.2. − $C_4H_8O_2$ (88.11): calcd. C 54.5, H 9.2; found, C 53.2, H 8.9.

1-Benzyloxy-3-buten-2-ol (5): A solution of 3-buten-1,2-diol (**3**) (3.60 g, 41 mmol) in anhydrous THF (50 mL) at 0°C was added to a stirred suspension of sodium hydride (1.0 g, 43 mmol) in anhydrous THF (200 mL) under nitrogen. The reaction mixture was cooled to −30°C. Tetrabutylammonium iodide (320 mg, 0.9 mmol) was added followed by a solution of benzyl bromide (7.50 g, 44 mmol) in anhydrous THF (100 mL). After stirring for 20 h, the reaction mixture was neutralized with water (40 mL) and then the pH adjusted with concentrated HCl to give pH 7. The aqueous layer was saturated with NaCl and extracted with CH_2Cl_2 (3× 40 mL). The organic layers were combined, dried with Na_2SO_4, filtered, and concentrated. The residue was purified by flash chromatography eluting with pentane/EtOAc (7:3) to give 3.8 g (52%) of a mixture of 1-benzyloxy-3-buten-2-ol (**5**) (R_f = 0.44, 30% EtOAc in pentane, PAA) and 2-benzyloxy-3-buten-1-ol (**6**) (R_f = 0.40, 30% EtOAc in pentane, PAA) in an 80:20 ratio.

Separation of the Two Isomers: to a solution of the two isomers **5** and **6** (240 mg, 1.35 mmol) in CH_2Cl_2 (4.0 mL) and pyridine (1.0 mL) was added pivaloyl chloride (82 mg, 0.7 mmol) at 0°C. After stirring the mixture at room temperature for 15 h, CH_2Cl_2 (10 mL) was added and the solution was washed with 10% K_2CO_3 (2× 5 mL), saturated $CuSO_4$ (2× 5 mL), saturated NH_4Cl (5 mL), and brine (5 mL). The organic layer was dried with Na_2SO_4, filtered, and concentrated. The residue was purified by flash chromatography eluting with pentane/EtOAc (7:3) to give 150 mg (63%) of 1-benzyloxy-3-buten-2-ol (**5**) (R_f = 0.44, 30% EtOAc in pentane, PAA). − ^{1}H NMR ($CDCl_3$) δ = 7.5 (m, 5 H), 6.1/7.8 (m, 1 H), 5.5/5.3 (m, 2 H), 4.7 (s, 2 H), 4.5 (m, 1 H), 3.7/3.4 (m, 2 H), 3.2 (d, 1 H). − ^{13}C NMR ($CDCl_3$) δ = 137.7, 136.7, 128.3, 127.7, 116.1, 74.0, 73.2, 71.3. − $C_{11}H_{14}O_2$ (178.23): calcd. C 74.1, H 7.9; found, C 74.6, H 8.2.

1-Benzyloxypropan-2,3-diol (7): A solution of 1-benzyloxy-3-buten-2-ol (**5**) (640 mg, 3.6 mmol) in CH_2Cl_2 (80 mL) was introduced into an ozonolysis tube. The solution was cooled to −78°C and ozone was bubbled into the solution. After 15 min, the reaction was a deep blue color, which was an indication of saturation with ozone. The solution was then purged at −78°C with nitrogen and Me_2S (660 μL) was added. The cooling bath was removed and the solution was stirred for 1 h. The mixture was then concentrated to an approximate volume of 50 mL, and ethanol (20 mL) was added. The solution was cooled to 0°C and sodium borohydride (120 mg, 3.1 mmol) was added. After 20 min, solvents were removed and the residue was dissolved in methanol (10 mL) and CH_2Cl_2 (20 mL). After stirring for 1 h, CH_2Cl_2 (40 mL) was added and the organic layer was washed with brine (3× 10 mL), dried with Na_2SO_4, filtered, and concentrated. The residue was purified by flash chromatography eluting with EtOAc to give 550 mg (84%) of 1-benzyloxypropan-2,3-diol (**7**) (R_f = 0.43, EtOAc, PMA). − ^{1}H NMR

($CDCl_3$) δ = 7.3 (s, 5 H), 4.5 (s, 2 H), 3.8 (m, 1 H), 3.6/3.4 (m, 4 H), 2.9 (s, 2 H). − ^{13}C NMR ($CDCl_3$) δ = 137.7, 128.5, 127.8, 73.5, 71.6, 70.8, 63.9. − $C_{10}H_{14}O_3$ (182.22): calcd. C 65.9, H 7.7; found, C 66.1, H 7.5.

1,2-*O*-Didecanoyl-3-*O*-benzyl-*rac*-glycerol (8): To a solution of l-benzyloxypropan-2,3-diol (**7**) (1.35 g, 7.4 mmol) in pentane (60 mL) was added successively capric acid (2.48 g, 14.4 mmol), DMAP (180 mg, 22 mmol), DCC (3.06 g, 14.8 mmol) and the resulting suspension was stirred for 12 h. The solid was removed by filtration through celite and the filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography eluting with pentane/ether (9:1) to give 3.40 g (93%) of 1,2-*O*-didecanoyl-3-*O*-benzyl-*rac*-glycerol (**8**) (R_f = 0.30, 10% ether in pentane, PMA). − ^1H NMR ($CDCl_3$) δ = 7.31 (s, 5 H, C_6H_5), 5.24 (app. quintet, 1 H, *J* = 3.9 Hz, *sn*-2 C*H*), 4.54 (s, 2 H, $CH_2-C_6H_5$), 4.35 and 4.20 (dd, 1 H, *J* = 3.9 Hz and *J* = 11.9 Hz, and, dd, 1 H, *J* = 6.3 Hz and *J* = 11.9 Hz, CH_2-O-CO), 3.58 (d, 2 H, *J* = 5.2 Hz, $CH_2-O-Bzl$), 2.32 (t, 2 H, *J* = 7.6 Hz, CH_2-CO) and 2.28 (t, 2 H, *J* = 7.6 Hz, CH_2-CO), 1.60 (m, 4 H, CH_2-CH_2-CO), 1.40/1.10 [m, 24 H, $(CH_2)_6$], 0.88 (t, 6 H, *J* = 6.0 Hz, CH_3). − ^{13}C NMR ($CDCl_3$) δ = 173.4, 173.1, 137.8, 128.5, 127.8, 127.7, 73.3, 70.1, 68.3, 62.7, 34.4, 34.1, 31.9, 29.5, 29.3, 29.2, 25.0, 24.9, 22.7, 14.1. − $C_{30}H_{50}O_5$ (490.72): calcd. C 73.4, H 10.3; found, C 72.9, H 9.9.

1,2-*O*-Didecanoyl-*rac*-glycerol (9): 1,2-*O*-didecanoyl-3-*O*-benzyl-*rac*-glycerol (**8**) (265 mg, 0.54 mmol) was dissolved in 95% ethanol (12 mL) containing 5% Pd/C (210 mg), and the mixture was stirred under an atmosphere of H_2 at room temperature. After 1 h, the catalyst was removed by filtration through celite and the filtrate was concentrated under reduced pressure with a water bath temperature below 20°C. The residue was purified by flash chromatography eluting with pentane/ether (6:4) to give 195 mg (90%) of 1,2-*O*-didecanoyl-*rac*-glycerol (**9**) (R_f = 0.25, 40% ether in pentane, PMA). − ^1H NMR ($CDCl_3$) δ = 5.08 (m, 1 H, *J* = 4.8 Hz, *sn*-2 C*H*), 4.32 and 4.20 (dd, 1 H, *J* = 4.4 Hz and *J* = 11.9 Hz, and dd, 1 H, *J* = 5.7 Hz and *J* = 11.9 Hz, CH_2-O-CO), 3.73 (t, 2 H, *J* = 5.5 Hz, CH_2-OH), 2.35 (t, 2 H, *J* = 7.3 Hz, CH_2-CO) and 2.32 (t, 2 H, *J* = 7.3 Hz, CH_2-CO), 2.27 (s, 1 H, OH), 1.62 (m, 4 H, CH_2-CH_2-CO), 1.40/1.10 [m, 24 H, $(CH_2)_6$], 0.88 (t, 6 H, *J* = 6.1 Hz, CH_3). − ^{13}C NMR ($CDCl_3$) δ = 173.4, 173.7, 72.1, 62.2, 61.2, 34.0, 31.8, 29.4, 29.2, 29.1, 24.8, 22.6, 14.0. − $C_{23}H_{44}O_5$ (400.60): calcd. C 68.9, H 11.1; found, C 69.1, H 10.8.

1,2-Benzylidene-3-buten-1,2-diol (10): 3-buten-1,2-diol (**3**) (500 mg, 5.7 mmol) was dissolved in toluene (10 mL) containing benzaldehyde (600 mg, 6.3 mmol) and a catalytic amount of *p*-TsOH (20 mg). The mixture was refluxed for 3 h. The solution was then concentrated and the residue was distilled with a bulb-to-bulb oven to give 900 mg (90%) of an epimeric mixture of 1,2-benzylidene-3-buten-1,2-diol (**10**) (bp 110−112°C/7.6·10^{-3} Torr). − R_f = 0.56 and 0.62, 10% EtOAc in pentane, PAA. − ^1H NMR ($CDCl_3$) δ = 7.5/7.1 (m, 5 H), 6.0/5.7 (m, 2 H), 5.4/5.1 (m, 2 H), 4.6/4.5 (m, 1 H), 4.3/4.1 (m, 1 H), 3.8/3.5 (m, 1 H). − ^{13}C NMR ($CDCl_3$) δ = 137.8−137.1, 135.5−135.3, 129.3−129.1, 128.4, 126.6−126.4, 118.5−118.1, 104.4−103.8, 78.4−77.4, 70.4−70.0. − $C_{11}H_{12}O_2$ (176.22): calcd. C 75.0, H 6.9; found, C 75.2, H 6.8.

1,2-Benzylideneglycerol (11): In an ozonolysis tube was introduced 1,2-benzylidene-3-buten-1,2-diol (**10**) (900 mg, 5.1 mmol) in CH_2Cl_2 (80 mL). The solution was cooled to −78°C and ozone was bubbled into the solution. After 15 min, the reaction was a deep blue color, which was an indication of saturation with ozone. The solution was then purged at −78°C with nitrogen and Me_2S (750 μL) was added. The cooling bath was removed and the solution was stirred for 1 h. The mixture was then evaporated to an approximate volume of 50 mL and ethanol (50 mL) was added. The solution was cooled to 0°C and sodium borohydride (108 mg, 2.6 mmol) was added. After 20 min, the solvents were removed and the residue was dissolved in methanol (10 mL) and CH_2Cl_2 (20 mL). After stirring for 1 h, CH_2Cl_2 (50 mL) was added and the organic layer was washed with brine (10 mL), dried with Na_2SO_4, filtered, and concentrated. The residue was purified by flash chromatography eluting with pentane/ether (5:5) to give 680 mg (74%) of 1,2-benzylidene glycerol (**11**) (R_f = 0.15, 50% ether in pentane, PMA). − ^1H NMR ($CDCl_3$) δ = 7.5/7.3 (m, 5 H), 5.9 (s, 0.5 H), 5.8 (s, 0.5 H), 4.4/3.6 (m, 5 H), 2.7 (t, 0.5 H), 2.6 (t, 0.5 H). − ^{13}C NMR ($CDCl_3$) δ = 137.7−137.0, 129.5−129.2, 128.3, 125.6−126.3, 104.2−103.7, 76.9−76.5, 66.9−66.7, 63.1−62.5. − $C_{10}H_{12}O_3$ (180.20): calcd. C 66.6, H 6.7; found, C 66.1, H 6.9.

1-Benzyloxypropan-2,3-diol (7): To a solution of DIBALH (1 M/CH_2Cl_2, 5 mmol) in anhydrous ether (10 mL) was added dropwise at 0°C a solution of 1,2-benzylidene glycerol (**11**) (300 mg, 1.67 mmol) in anhydrous ether (3 mL). The mixture was stirred for 4 h at room temperature and then methanol (2 mL) and saturated NH_4Cl (10 mL) were added. After 15 min, the solution was centrifuged and the solvents removed. Ether (10 mL) was added and the aqueous layer was extracted with CH_2Cl_2 (2× 10 mL). The organic layers were combined, dried with Na_2SO_4, filtered, and concentrated to give 240 mg (80%) of 1-benzyloxypropan-2,3-diol (**7**) (R_f = 0.43, EtOAc, PMA). − ^1H NMR ($CDCl_3$) δ = 7.3 (s, 5 H), 4.5 (s, 2 H), 3.8 (m, 1 H), 3.6/3.4 (m, 4 H), 2.9 (s, 2 H). − ^{13}C NMR ($CDCl_3$) δ = 137.7, 128.5, 127.8, 73.5, 71.6, 70.8, 63.9. − $C_{10}H_{14}O_3$ (182.22): calcd. C 65.9, H 7.7; found, C 66.1, H 7.5.

1-Tosyloxy-3-buten-2-ol (12): To a solution of 3-buten-1,2-diol (**3**) (2.0 g, 23 mmol) in CH_2Cl_2 (30 mL) and pyridine (20 mL) was added *p*-TsOH (4.7 g, 25 mmol). The mixture was stirred for 2 d at room temperature. CH_2Cl_2 (100 mL) was then added and the solution was washed with 2% HCl (3× 30 mL), saturated $CuSO_4$ (5× 30 mL), 10% K_2CO_3 (2× 30 mL), and brine (30 mL). The solution was centrifuged and the organic layer was dried with Na_2SO_4, filtered, and concentrated. The residue was purified by flash chromatography eluting with pentane/ether (7:3) to give 3.80 g (68%) of 1-tosyloxy-3-butene (**12**) (R_f = 0.27, 30% ether in pentane, PAA). − ^1H NMR ($CDCl_3$) δ = 7.85 (d, 2 H, *J* = 8 Hz), 7.40 (d, 2 H, *J* = 8 Hz), 5.9/5.7 (m, 1 H), 5.5/5.3 (m, 2 H), 4.4 (m, 1 H), 4.1/3.9 (m, 2 H), 2.7 (s, 1 H), 2.5 (s, 3 H). − ^{13}C NMR ($CDCl_3$) δ = 145.1, 134.7, 132.6, 130.0, 128.0, 118.1, 73.0, 70.4, 21.7. − $C_{11}H_{14}O_4S$ (242.29): calcd. C 54.5, H 5.8, S 13.3; found, C 54.2, H 6.3, S 13.1.

Lipase-Mediated Acylation of Alcohol 5: To a solution of 1-benzyloxy-3-buten-2-ol (**5**) (150 mg, 0.84 mmol) in hexane (10 mL) was added successively vinyl acetate (300 mg, 3.36 mmol) and lipase PS (75 mg). The mixture was vigorously stirred for 4 h at room temperature. The reaction was then stopped by filtration through celite. The solvent was removed and the residue was purified by flash chromatography eluting with pentane/EtOAc (8:2) to give 84 mg (45%) of acetate of **5** (79% ee by ^{19}F NMR) and 82 mg (55%) of alcohol **5** (70% ee by ^{19}F NMR). − R_f = 0.50 for acetate and 0.18 for alcohol, 20% EtOAc in pentane, PAA. − ^1H NMR ($CDCl_3$) δ = 7.3 (s, 5 H), 6.0/5.7 (m, 1 H), 5.6/5.4 (m, 2 H), 5.2 (m, 1 H), 4.6 (s, 2 H), 3.6 (d, 2 H, 5.5 Hz), 2.0 (s, 3 H). − ^{13}C NMR ($CDCl_3$) δ = 170.1, 137.9, 133.3, 128.3, 127.7, 127.6, 117.9, 73.1, 71.2, 21.1. − $C_{13}H_{16}O_3$ (220.26): calcd. C 70.9, H 7.3; found C 69.7, H 6.9.

Lipase-Mediated Acylation of Alcohol 12: To a solution of 1-tosyloxy-3-buten-2-ol (**12**) (1.0 g, 4.13 mmol) in hexane/ether (1:1, 50 mL) was added successively vinyl acetate (1.44 g, 16.5 mmol) and lipase PS (500 mg). The mixture was vigorously stirred for 3 h at

room temperature. The reaction was then stopped by filtration through celite. The solvent was removed and the residue was purified by flash chromatography eluting with pentane/ether (7:3) to give 570 mg (2 mmol) of acetate **13** (95% ee by HPLC using Chiralpak AS, eluent: 10% *i*PrOH in hexane) and 490 mg (2.03 mmol) of alcohol **12** (84% ee by HPLC using Chiracel OD-H, eluent: 2% *i*PrOH in hexane). – R_f = 0.43 for **13** and 0.27 for **12**, 30% ether in pentane, PAA. – ^{1}H NMR ($CDCl_3$) δ = 7.7 (d, 2 H, *J* = 8 Hz), 7.3 (d, 2 H, *J* = 8 Hz), 5.7/5.5 (m, 1 H), 5.4/5.1 (m, 3 H), 4.0 (m, 2 H), 2.4 (s, 3 H), 1.9 (s, 3 H). – ^{13}C NMR ($CDCl_3$) δ = 169.8, 145.1, 132.8, 131.2, 130.0, 128.0, 119.8, 71.5, 69.8, 21.7, 20.9. – $C_{13}H_{16}O_5S$ (284.33): calcd. C 54.9, H 5.7, S 11.3; found, C 55.2, H 5.5, S 11.5.

(*S*)-1-Tosyloxy-2-acetyloxy-3-butene [(*S*)-13]: To a solution of 1-tosyloxy-3-buten-2-ol [(*S*)-**12**] (440 mg, 1.81 mmol) in CH_2Cl_2 (6 mL) at 0°C was added pyridine (180 mg, 2.1 mmol) and acetyl chloride (180 mg, 2.1 mmol). The mixture was stirred for 30 min. The solution was then concentrated and ether (5 mL) was added. After filtration and concentration, the residue was purified by flash chromatography eluting with pentane/ether (7:3) to give 480 mg (93%) of (*S*)-1-tosyloxy-2-acetyloxy-3-butene [(*S*)-**13**] (R_f = 0.43, 30% ether in pentane, PAA). – ^{1}H NMR ($CDCl_3$) δ = 7.7 (d, 2 H, *J* = 8 Hz), 7.3 (d, 2 H, *J* = 8 Hz), 5.7/5.5 (m, 1 H), 5.4/5.1 (m, 3 H), 4.0 (m, 2 H), 2.4 (s, 3 H), 1.9 (s, 3 H). – ^{13}C NMR ($CDCl_3$) δ = 169.8, 145.1, 132.8, 131.2, 130.0, 128.0, 119.8, 71.5, 69.8, 21.7, 20.9. – $C_{13}H_{16}O_5S$ (284.33): calcd. C 54.9, H 5.7, S 11.3; found, C 55.2, H 5.5, S 11.5.

(*S*)-1,2-Diacetyloxy-3-butene [(*S*)-14]: To a solution of 1-tosyloxy-2-acetyloxy-3-butene [(*S*)-**13**] (100 mg, 0.35 mmol) in DMSO/DMF (4:1, 4 mL) under nitrogen was introduced cesium acetate (140 mg, 0.70 mmol). The mixture was stirred for 3 d at room temperature. Water (10 mL) was then added and the mixture was extracted with ether (3× 20 mL). The organic layer was dried with $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography eluting with pentane/ether (7:3) to give 60 mg (99%) of (*S*)-1,2-diacetyloxy-3-butene [(*S*)-**14**] (R_f = 0.30, 30% ether in pentane, PAA). – ^{1}H NMR ($CDCl_3$) δ = 5.9/5.7 (m, 1 H), 5.5 (m, 1 H), 5.4/5.2 (m, 2 H), 4.3 (m, 1 H), 4.1 (m, 1 H), 2.1 (s, 1 H), 2.0 (s, 1 H). – ^{13}C NMR ($CDCl_3$) δ = 170.6, 170.0, 132.3, 118.8, 72.0, 64.7, 21.0, 20.7. – $C_8H_{12}O_4$ (172.18): calcd. C 55.8, H 7.0; found, C 56.1, H 7.3.

(*S*)-3-Buten-1,2-diol [(*S*)-3]: To a stirred solution of 1,2-diacetyloxy-3-butene [(*S*)-**14**] (270 mg, 1.57 mmol) in methanol/water (96:4, 7 mL) was added potassium carbonate (480 mg, 3.5 mmol). The mixture was stirred for 1 h and then filtered through celite. The reaction was neutralized to pH 7. After filtration, the mixture was concentrated and the residue was purified by flash chromatography eluting with EtOAc to give 90 mg (65%) of (*S*)-3-buten-1,2-diol [(*S*)-**3**] (R_f = 0.45, EtOAc, PMA). – ^{1}H NMR ($CDCl_3$) δ = 5.9/5.7 (m, 1 H), 5.4/5.1 (m, 2 H), 4.2/4.0 (m, 3 H), 3.6/3.3 (m, 2 H). – ^{13}C NMR ($CDCl_3$) δ = 135.7, 115.5, 73.4, 55.2. – $C_4H_8O_2$ (88.11): calcd. C 54.5, H 9.2; found, C 53.2, H 8.9.

***O*-[(1,2-Didecanoyl)glyceryl] *O*-(*p*-nitrophenyl) *n*-pentylphosphonate (1):** Under nitrogen, a mixture of (*S*)-1,2-*O*-didecanoyl-*sn*-glycerol [(*S*)-**9**] (500 mg, 1.25 mmol), *N*-methylimidazole (125 mg, 1.5 mmol) and CH_2Cl_2 (10 mL) was slowly added dropwise to a solution of pentylphosphonic dichloride **15** (237 mg, 1.25 mmol) in CH_2Cl_2 (10 mL). The reaction was then stirred for a further 30 min. A solution of *p*-nitrophenol (174 mg, 1.25 mmol), *N*-methylimidazole (125 mg, 1.5 mmol) and CH_2Cl_2 (8 mL) was then slowly added dropwise. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography eluting with pentane/EtOAc (8:2) (R_f = 0.30, 20% EtOAc in pentane, PMA). The product was dissolved in ether (10 mL) and washed with 5% K_2CO_3 (5× 3 mL), saturated NH_4Cl (3 mL), and brine (3 mL). The organic layer was dried with $MgSO_4$, filtered, and concentrated under reduced pressure to give 175 mg (22%) of *O*-[(1,2-didecanoyl)glyceryl] *O*-(*p*-nitrophenyl) *n*-pentylphosphonate (**1**). – ^{31}P NMR ($CDCl_3$) δ = 30.4, 30.2. – ^{1}H NMR ($CDCl_3$) δ = 8.24 (d, 2 H, *J* = 9.1 Hz, *H*-C_{ar}), 7.37 (d, 2 H, *J* = 9.1 Hz, *H*-C_{ar}), 5.23 (app. quintet, 1 H, *J* = 5.0 Hz, *sn*-2 C*H*), 4.35/4.20 (m, 2 H, CH_2–O–CO), 4.20/4.05 (m, 2 H, CH_2–O–PO), 2.29 (t, 4 H, *J* = 7.5 Hz, CH_2–CO), 1.92 (m, 2 H, CH_2–PO), 1.62 (m, 6 H, CH_2–CH_2–CO and CH_2–CH_2–PO), 1.40/1.05 (m, 28 H), 0.88 (m, 9 H, CH_3). – ^{13}C NMR ($CDCl_3$) δ = 173.0, 172.6, 155.3 (d, *J* = 8.5 Hz), 144.6, 125.5, 120.9 (d, *J* = 4.2 Hz), 69.4 (d, *J* = 5.8 Hz), 64.2, 61.6, 34.0, 33.9, 32.4 (d, *J* = 17.2 Hz), 31.8, 29.3, 29.2, 29.1, 25.7 (d, *J* = 139.3 Hz), 24.7, 22.6, 22.0, 21.7 (d, *J* = 7.2 Hz), 14.0, 13.6. – $C_{34}H_{58}NO_9P$ (655.81): calcd. C 62.3, H 8.9, N 2.1, P 4.7; found, C 62.5, H 8.6, N 1.9, P 5.2.

Separation of Diastereomers A/B (or *ent*-A/*ent*-B): Separation of diastereomers **A/B** (or ***ent*-A/*ent*-B**) was performed by HPLC using a silica column (250 × 10 mm, particle size: 5 μm) from S.F.C.C. under semi-preparative conditions:

- room temperature (25°C)
- solvent: heptane/2-propanol (99.05/0.95)
- UV detection at λ = 234 nm
- flow rate: 3 mL/min

15 injections of 10 μL each (125 mg/mL) led to the recovery of both enantiomers: t_1 (**A** or ***ent*-A**) = 24.5 min, ^{31}P NMR: δ = 30.4. – t_2 (**B** or ***ent*-B**) = 27.3 min, ^{31}P NMR: δ = 30.2.

Optical rotations were measured using a Perkin–Elmer 241 MC polarimeter and gave the following values:

$[\alpha(\mathbf{A})]^{20}_D$ = +7.45 (*c* = 0.5, CH_2Cl_2)
$[\alpha(\textit{ent}\text{-}\mathbf{A})]^{20}_D$ = –7.25 (*c* = 0.4, CH_2Cl_2)
$[\alpha(\mathbf{B})]^{20}_D$ = +1.28 (*c* = 0.5, CH_2Cl_2)
$[\alpha(\textit{ent}\text{-}\mathbf{B})]^{20}_D$ = –1.25 (*c* = 0.4, CH_2Cl_2)

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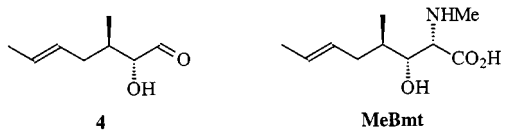
[7] **MeBmt**: (4*R*)-4-[(*E*)-2-butenyl]-4,*N*-dimethyl-L-threonine.



4 **MeBmt**

Figure 2. Structures of synthon **4** and **MeBmt**

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