Efficient Synthesis of Phospholipids from Glycidyl Phosphates

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New efficient routes to enantiopure phospholipids, starting from (*S*)-glycidol, are described. Lysophosphatidic acids and phosphatidic acids were obtained in good overall yields from (*S*)-glycidol, in only three and four steps, respectively. Moreover, the strategy can also be used to produce phosphatidylcholines in three steps. Using dialkylphosphoramidites, (*S*)-glycidol was phosphorylated to give (*R*)-1-*O*-glycidyl dialkyl phosphates. Regiospecific epoxide opening, using hexadecanol or cesium palmitate, followed by phosphate deprotection, provided lysophosphatidic acids. 2-*O*-Esterification prior to phosphate deprotection provided 1,2-*O*-diacyl and 1-*O*-alkyl-2-*O*-acyl phosphatidic acids. Phosphorylation of (*S*)-glycidol using phosphorus oxychloride followed by in situ treatment with choline tosylate produced (*R*)-glycidyl phosphocholine. Subsequent nucleophilic opening of the epoxide using cesium palmitate produced 1-*O*-palmitoyl-*sn*-glycero-3-phosphocholine, which has been used in syntheses of phosphatidylcholines.

Introduction

Phospholipids, especially phosphatidylcholine and phosphatidylethanolamine, are major constituents of cell membranes. Many phospholipids possess biological activity,[1−4] examples are the hormone platelet activating factor (PAF) and lysophosphatidic acid (LPA)[3−5] (Figure 1). For these lipids, the mechanisms of action are not fully understood. By modification of different parts of the lipid, activity varies from one biological process to the other.[5−7] In addition to structure−activity studies, recent advances in the construction of artificial cell membranes with specific biological functions demand tailor-made glycerol-derived lipids.[8]

In this paper we present efficient methods for synthesis of various phospholipids and analogues thereof, starting from enantiomerically pure (*S*)-glycidol. Up to date several routes to glycerophospholipids have been presented,[9−12] most of them involving derivatization and protecting group manipulations of various selectively protected glycerol derivatives. Methods, for preparing optically active phosphatidylcholine, PAF, and analogues thereof, starting from racemic glycidol and epichlorohydrin,[13] the commercially available (*S*)-glycidol, or (*R*)-glycidyl tosylate have been published.[14−17] Glycidol derivatives, in the form of glycidyl glycosides, have also been used in syntheses of glycosyl glycerolipids.[18−20] The methods involving epoxide opening of enantiopure glycidols have been shown to proceed regiospecifically, and without significant loss of optical purity. Up to date, however, these methods involve protecting group manipulations after epoxide opening.

Surprisingly, direct phosphorylation of glycidol and subsequent opening of the epoxide, has not been published. This approach provides an efficient route, without tedious protecting group manipulations, to various biologically important phospholipids, and analogues thereof.

Results and Discussion

In the routes leading to phosphatidic acids, phosphorylations were performed with two different dialkyl phosphoramidites, dibenzyl-*N*,*N*-diisopropyl, and di-*tert*-butyl-*N*,*N*-diisopropyl phosphoramidite. The amidites were synthesized using known methods.[21−24] The phosphitylation of (*S*)-glycidol using dibenzyl-*N*,*N*-diisopropyl phosphoramidite and subsequent oxidation using *m*-

(1) Snyder, F.; Lee, T.-C.; Blank, M. L. *Ann. N.Y. Acad. Sci.* **1989**, *568*, 35−43.
(2) Racké, K.; Hammermann, R.; Juergens, U. R. *Pulm. Pharmacol. Ther.* **2000**, *13*, 99−114.
(3) Lynch, K. R.; Hopper, D. W.; Carlisle, S. J.; Catalano, J. G.; Zhang, M.; MacDonald, T. L. *Mol. Pharmacol.* **1997**, *52*, 75−81.
(4) Hopper, D. W.; Ragan, S. P.; Hooks, S. B.; Lynch, K. R.; Macdonald, T. L. *J. Med. Chem.* **1999**, *42*, 963−970.
(5) Bandoh, K.; Aoki, J.; Hosono, H.; Kobayashi, S.; Kobayashi, T.; Murakami-Murofushi, K.; Tsujimoto, M.; Arai, H.; Inoue, K. *J. Biol. Chem.* **1999**, *274*, 27776−27785.
(6) Gueguen, G.; Gaige, B.; Grevy, J. M.; Rogalle, P.; Bellan, J.; Wilson, M.; Klaebe, A.; Pont, F.; Simon, M. F.; Chap, H. *Biochemistry* **1999**, *38*, 8440−8450.
(7) Sugiura, T.; Nakane, S.; Kishimoto, S.; Waku, K.; Yoshioka, Y.; Tokumura, A.; Hanahan, D. J. *Biochim. Biophys. Acta* **1999**, *1440*, 194−204.
(8) Stora, T.; Dienes, Z.; Vogel, H.; Duschl, C. *Langmuir* **2000**, *16*, 5471−5478.
(9) Eibl, H. *Chem. Phys. Lipids* **1980**, *26*, 405−429.
(10) Eibl, H. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 257−271.
(11) Ramirez, F.; Marecek, J. F. *Synthesis* **1985**, 449−488.
(12) Martin, S. F.; Josey, J. A.; Wong, Y.-L.; Dean, D. W. *J. Org. Chem.* **1994**, *59*, 4805−4820.
(13) Cimetiere, B.; Jacob, L.; Julia, M. *Bull. Soc. Chim. Fr.* **1991**, *6*, 926−938.
(14) Ali, S.; Bittman, R. *J. Org. Chem.* **1988**, *53*, 5547−5549.
(15) Guivisdalsky, P. N.; Bittman, R. *J. Org. Chem.* **1989**, *54*, 4643−4648.
(16) Guivisdalsky, P. N.; Bittman, R. *J. Org. Chem.* **1989**, *54*, 4637−4642.
(17) Burgos, C. E.; Ayer, D. E.; Johnson, R. A. *J. Org. Chem.* **1987**, *52*, 4973−4977.
(18) Bellucci, G.; Catelani, G.; Chiappe, C.; D'Andrea, F.; Grigò, G. *Tetrahedron Asym.* **1997**, *8*, 765−773.
(19) Suhr, R.; Scheel, O.; Thiem, J. *J. Carbohydr. Chem.* **1998**, *17*, 937−968.
(20) Nishida, Y.; Ohrui, H.; Meguro, H.; Ishizawa, M.; Matsuda, K.; Taki, T.; Handa, S.; Yamamoto, N. *Tetrahedron Lett.* **1994**, *35*, 5465−5468.
(21) Nurminen, E. J.; Mattinen, J. K.; Lönnberg, H. *J. Chem. Soc., Perkin Trans. 2* **1998**, 1621−1628.

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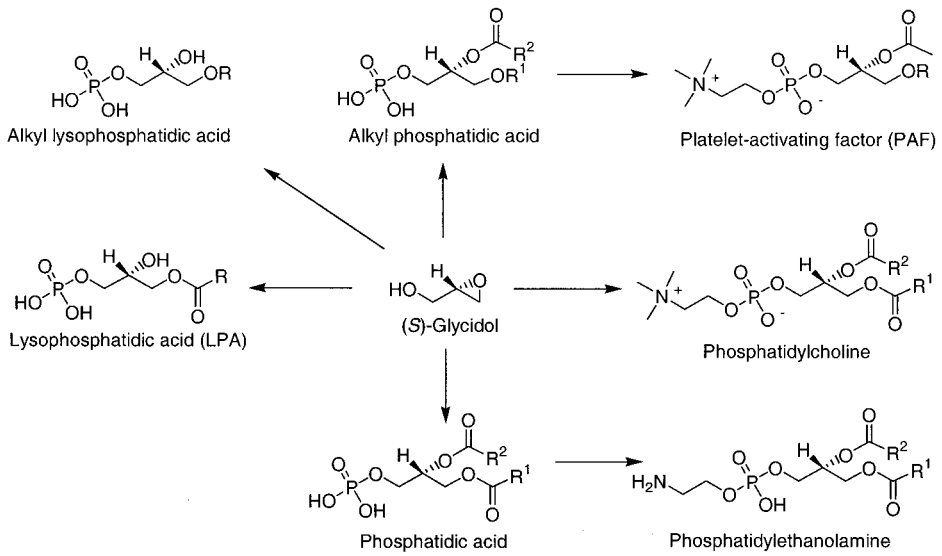
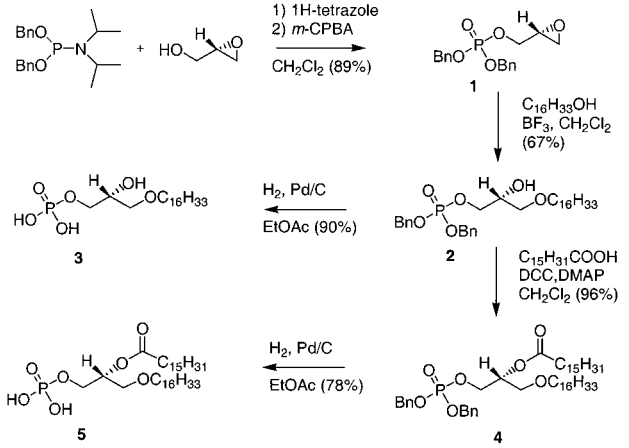
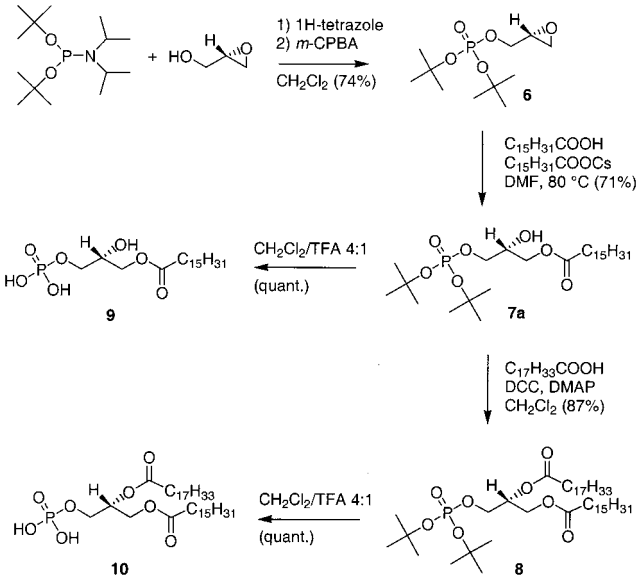


Figure 1. Efficient and short synthetic routes from commercially available (*S*)-glycidol to many bioactive and/or naturally occurring glycerophospholipids, developed in this work.

Scheme 1



Scheme 2



CPBA, produced the phosphorylated glycidol derivative **1** in 89% yield (Scheme 1). Lewis acid-catalyzed epoxide opening of the corresponding tosylate, using hexadecanol and BF_3, has been shown to proceed regio- and stereospecifically.[15] Opening of the epoxide in **1** using similar conditions proceeded with complete regiospecificity to produce the dibenzyl-protected lysoalkyl phosphatidic acid **2** in 67% yield. Deprotection of the phosphate gave compound **3**, in 53% overall yield from (*S*)-glycidol. DCC-promoted esterification of **2** with palmitic acid in almost quantitative yield, and subsequent debenzylation of the phosphate produced the 1-*O*-alkyl-2-*O*-acyl-phosphatidic acid **5**, in 40% overall yield from (*S*)-glycidol.

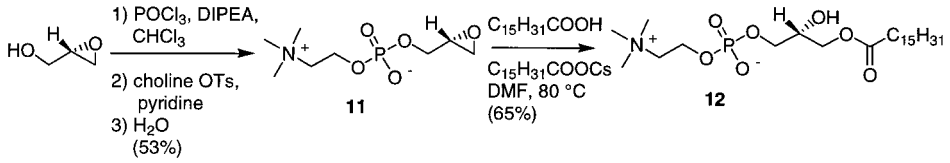
Attempts to produce 1-*O*-acyl-3-*O*-dibenzylphosphoryl-*sn*-glycerols by nucleophilic opening of the epoxide **1** using cesium palmitate produced only decomposed material and benzyl palmitate, due to carboxylate attack on the benzyl groups. At this stage our attention was turned to other phosphate protecting groups, not so easily attacked by nucleophiles. The *tert*-butyl group seemed like a reasonable choice. Di-*tert*-butyl-*N*,*N*-diisopropyl phosphoramidite was used for the oxidative phosphorylation of (*S*)-glycidol to produce (*R*)-di-*tert*-butyl-phosphorylglycidol **6** in 74% yield (Scheme 2). Regioselective opening of the epoxide with cesium palmitate produced **7a** in 71% yield along with minor amounts of the regioisomeric 3-*O*-di-*tert*-butylphosphoryl-2-*O*-palmitoyl-*sn*-glycerol.

Esterification of **7a** with oleic acid produced the di-*tert*-butyl protected mixed 1,2-diacyl phosphatidic acid **8** in 87% yield. Treatment of **7a** and **8** with TFA in CH_2Cl_2 cleanly produced the deprotected lysophosphatidic acid **9** and the mixed 1,2-diacyl phosphatidic acid **10** in quantitative yields. The overall yields were 53% and 46%, respectively. Besides from being present in biological membranes, these types of compounds have been used in syntheses of phosphatidylcholines and phosphatidyl ethanolamines, by coupling with various choline- and ethanolamine derivatives.[14,25−28]

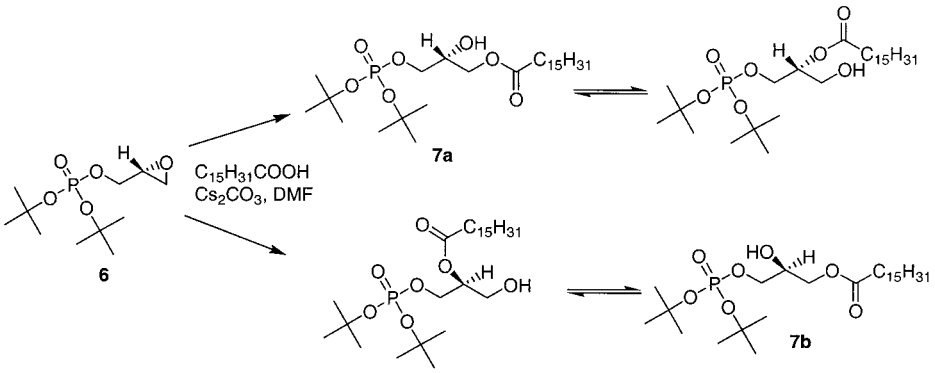
To find a shorter route to choline derivatives, (*S*)-glycidol was phosphorylated using phosphorus oxychlo-

(22) Tanaka, T.; Tamatsukuri, S.; Ikehara, T. *Tetrahedron Lett.* **1986**, *27*, 199.
(23) Ullman, E.; Engels, J. *Tetrahedron Lett.* **1986**, *27*, 1023.
(24) Kuo-Long, Y.; Fraser-Reid, B. *Tetrahedron Lett.* **1988**, *29*, 979–982.

Scheme 3



Scheme 4



ride. Subsequent in situ treatment with choline tosylate produced the choline derivative **11** in 53% yield. Nucleophilic opening of the epoxide using cesium palmitate produced lysophosphatidylcholine **12** (Scheme 3), which has been used in syntheses of phosphatidylcholine.[13,29,30]

The minor amounts of the regioisomeric 2-*O*-palmitoyl derivative, produced in the nucleophilic opening of the epoxide in Scheme 2, was thought to be the result of acyl migration from the primary position. There is, of course, a possibility that part of the product is a result of nucleophilic attack on the secondary carbon, followed by equilibration of the regioisomers as depicted in Scheme 4. This would give a partially racemized product. Therefore the optical purity of **7a** had to be evaluated.

7a was converted to Mosher's acid derivative **13a** (Figure 2) by treatment with (*R*)-α-methoxy-α-(trifluoromethyl)phenylacetic acid chloride ((*R*)-Mosher's acid chloride) in pyridine. The enantiomeric **7b** was synthesized analogously from (*R*)-glycidol, and derivatized with (*R*)-Mosher's acid to produce **13b** (Figure 2).

The optical purity was determined by ^{1}H and ^{19}F NMR. The double doublet at 4.38 ppm in **13a** (Figure 2B) shifted to 4.48 in **13b** (Figure 2D) and the trifluoromethyl peak from 104.44 to 104.36 (Figures 2A and 2C). Only traces of the double doublet at 4.48 ppm in Figure 2B and the double doublet at 3.38 in Figure 2D could be detected, indicating that no loss of optical purity had occurred. The ^{19}F spectra (Figures 2A and 2C) indicated >96% ee. Considering the optical purity of the starting compouds, (*S*)-glycidol (98% ee) and (*R*)-Mosher's acid chloride (99% ee), no significant loss of optical purity is observed.

Conclusions

Both natural and unnatural enantiomers of a broad range of biologically important phospholipids, and analogues thereof, can be obtained in high optical purity from commercially available (*S*)- or (*R*)-glycidol. The routes are short and efficient and proceed in good overall yields.

Phosphorylation of (*S*)-glycidol using dialkyl phosphoramidites, followed by opening of the epoxide, using alcohol or cesium carboxylate, and subsequent phosphate deprotection is an effective route to enantiopure lysophosphatidic acids. Moreover, 2-*O*-esterification of the lysophosphatidic acids prior to phosphate deprotection, provides phosphatidic acids in only four steps.

Variation of the phosphorylation procedure enables synthesis of other classes of phospholipids. Phosphorylation of (*S*)-glycidol using phosphorus oxychloride followed by in situ treatment with choline tosylate, and subsequent nucleophilic opening of the epoxide using cesium palmitate, produced the lysophosphatidylcholine 1-*O*-Palmitoyl-*sn*-glycero-3-phosphocholine, which has been used in syntheses of phosphatidylcholines.[13,29,30]

Experimental Section

General Methods. Organic phases were dried over $MgSO_4$, filtered, and concentrated in vacuo below 40 °C. TLC: 0.25 mm precoated silica gel plates (MERCK silica gel $60F_{254}$); Detection by spraying the plates with AMC solution [$(NH_4)_2$-MoO_4 100 g and Ce(IV)SO_4 2 g dissolved in 10% H_2SO_4, 2 L] followed by heating at ~250 °C. Optical rotations were recorded at room temperature with a Perkin-Elmer 241 polarimeter. Melting points were recorded with a Gallenkamp melting point apparatus. Flash chromatography (FC): Silica gel MERCK 60 (0.040−0.063 mm). ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury 300 at 300 MHz (^{1}H), 75 MHz (^{13}C), 121 MHz (^{31}P) and 282 MHz (^{19}F), temp 25 °C. Chemical shifts are given in ppm with TMS as internal standard ($\delta = 0.00$); ^{31}P, 85% H_3PO_4 ($\delta = 0.00$); ^{19}F, $CFCl_3$ ($\delta = 0.00$). MALDI-TOF mass-spectra were recorded with a Voyager-DE STR Biospectrometry Workstation, in positive mode, using an α-cyano-4-hydroxycinnamic acid matrix. (*S*)-glycidol (98% ee) and (*R*)-glycidol (98% ee) were purchased from Aldrich. (*R*)-α-methoxy-α-(trifluoromethyl)phenylacetic acid chloride (99% ee) was purchased from Fluka.

(25) Aneja, R.; Chadha, J. S.; Davies, A. P. *Tetrahedron Lett.* **1969**, *48*, 4183−4186.
(26) Rakhit, S.; Bagli, J. F.; Deghenghi, R. *Can. J. Chem.* **1969**, *47*, 2906−2910.
(27) Bersch, B.; Starck, J. P.; Milon, A.; Nakatani, Y.; Ourisson, G. *Bull. Soc. Chim. Fr.* **1993**, *130*, 575−583.
(28) Akita, H.; Umezawa, I.; Matsukura, H.; Oishi, T. *Chem. Pharm. Bull.* **1992**, *40*, 318−324.
(29) Xia, J.; Hui, Y.-Z. *Tetrahedron Asym.* **1997**, *8*, 451−458.
(30) Vodovozova, E. L.; Molotkovsky, J. G. *Tetrahedron Lett.* **1994**, *35*, 1933−1936.

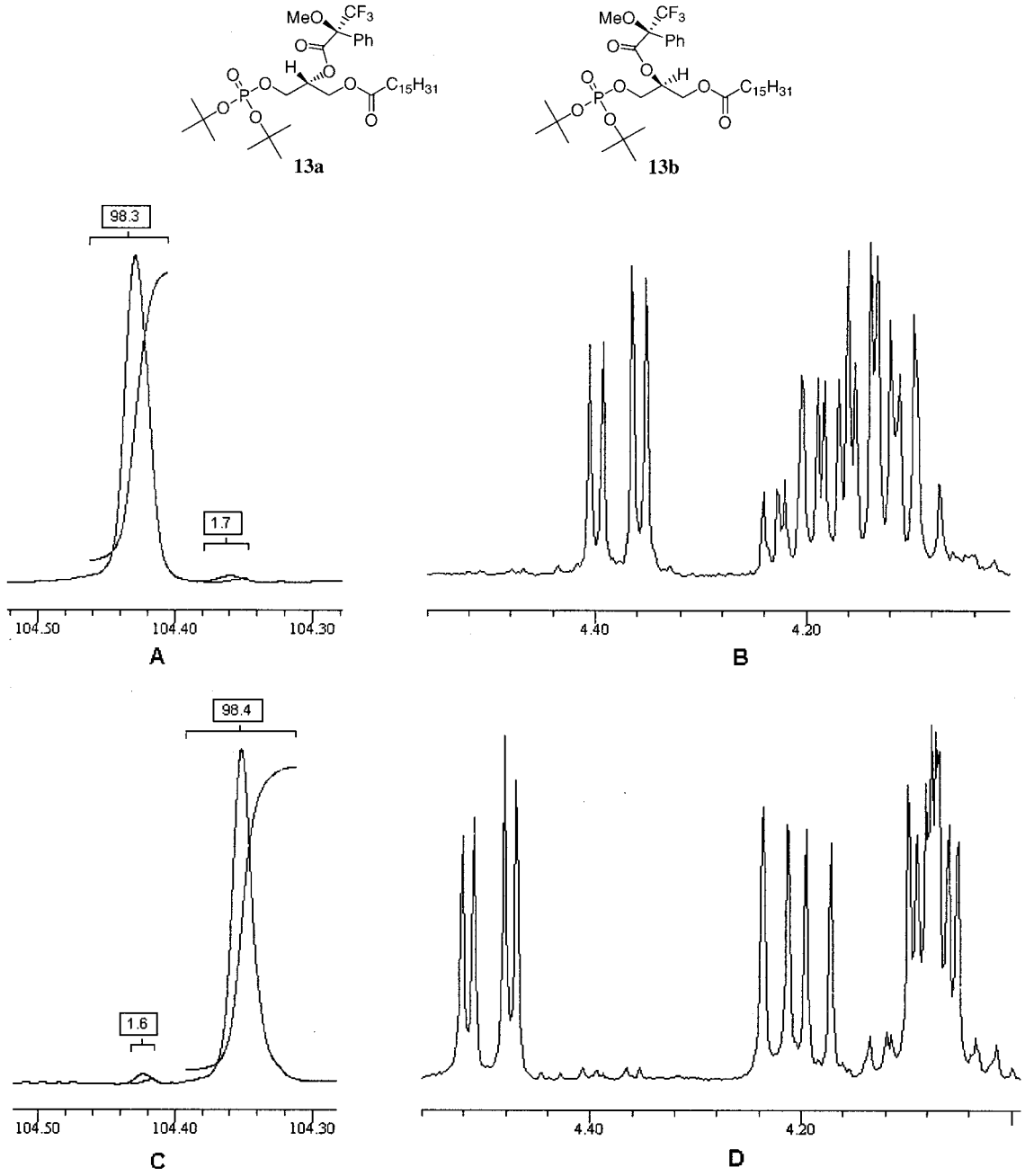


Figure 2. ^{1}H and ^{19}F NMR spectra of the (*S*)-Mosher's acid derivatives **13a** (A+B) and **13b** (C+D).

(*R*)-Dibenzylphosphorylglycidol (1). To a stirred solution of (*S*)-glycidol (253 mg, 3.42 mmol) and dibenzyl-*N*,*N*-diisopropyl phosphoramidite (2.50 g, 6.92 mmol) in CH_2Cl_2 (100 mL) was added 1*H*-tetrazole (800 mg, 11.4 mmol). After 30 min, the mixture was cooled to 0 °C and *m*-CPBA (1.75 g, 10 mmol) was added. The mixture was stirred for 40 min, washed with 10% aqueous $Na_2S_2O_3$ and $NaHCO_3$ (sat.), dried, filtered, and concentrated. FC (toluene/EtOAc 3:2, 1% NEt_3) gave **1** as a colorless oil (1.02 g, 3.05 mmol, 89%). R_f 0.38 (toluene/EtOAc 1:1); $[\alpha]_D$ −8.9 (*c* 0.88, $CHCl_3$); ^{1}H and ^{13}C NMR spectra were identical to those published for the racemic compound.[31]

3-*O*-Dibenzylphosphoryl-1-*O*-hexadecyl-*sn*-glycerol (2). To a solution of **1** (80 mg, 0.24 mmol) and hexadecanol 116 mg, 0.48 mmol) in CH_2Cl_2 (3 mL) was added a 10% v/v solution of BF_3−diethyl ether complex in CH_2Cl_2 (40 μL). After 10 h, the mixture was concentrated. FC (toluene/EtOAc 2:1) of the residue gave crystalline **2** (93 mg, 0.16 mmol, 67%). R_f 0.43 (toluene/EtOAc 1:1): mp 44−45 °C (from hexane); $[\alpha]_D$ −2.7 (*c* 1.1, $CHCl_3$); NMR ($CDCl_3$): ^{1}H, δ 0.88 (t, 3H, J = 6.4 Hz), 1.26 (br, 26H), 1.53 (m, 2H), 3.37−3.42 (m, 4H), 3.87−4.13 (m, 3H), 5.05 (d, 2H, J = 8.4 Hz), 5.05 (d, 2H, J = 8.4 Hz), 7.34 (br, 10H); ^{13}C, δ 14.1, 22.7, 26.1, 29.3, 29.5, 29.6, 29.7, 31.9, 69.1 (d, J = 5.5 Hz), 69.3 (d, J = 7.4 Hz), 70.7, 71.7, 128.0, 128.6, 135.7 (d, J = 5.5 Hz); ^{31}P, δ 0.58; Anal. Calcd for $C_{33}H_{53}O_6P$: C 68.7; H 9.3 Found C 68.8; H 8.9.

1-*O*-Hexadecyl-3-*O*-phosphoryl-*sn*-glycerol (3). A mixture of **2** (26 mg, 0.045 mmol) and 10% Pd on carbon (18 mg) in EtOAc (2 mL) was stirred under H_2 (1 atm) for 2 h, filtered through Celite, and concentrated to yield crystalline **3** (16 mg, 0.040 mmol, 90%). R_f 0.12 ($CHCl_3$/MeOH/H_2O 65:25:4); mp. 66−67 °C ($CHCl_3$); $[\alpha]_D$ −2.0 (*c* 1.1, DMSO); ^{1}H and ^{13}C NMR spectra were identical to those published for the racemic compound.[32]

(31) De Viguerie, N. L.; Willson, M.; Périé, J. *New J. Chem.* **1994**, *18*, 1183−1195.

(32) Malekin, S. I.; Khromova, N. Y.; Kisin, A. V.; Kruglyak, Y. L.; Kurochkin, V. K. *Russ. J. Bioorg. Chem.* **1997**, *23*, 677−682.

3-*O*-Dibenzylphosphoryl-1-*O*-hexadecyl-2-*O*-palmitoyl-*sn*-glycerol (4). A solution of **2** (40 mg, 0.069 mmol), palmitic acid (28 mg, 0.11 mmol), dicyclohexylcarbodiimide (23 mg, 0.11 mmol), and 4-(dimethylamino)pyridine (1 mg, 0.008 mmol) in CH_2Cl_2 (2 mL) was stirred for 22 h, filtered through Celite, and concentrated. FC (toluene/EtOAc 8:1) of the residue gave crystalline **4** (54 mg, 0.066 mmol, 96%). R_f 0.27 (toluene/EtOAc 8:1); mp 37−38 °C (from ethanol); $[\alpha]_D$ −3.1 (*c* 1.0, $CHCl_3$); NMR ($CDCl_3$): ^{1}H, δ 0.88 (m, 6H), 1.25 (br, 52H), 1.45−1.62 (m, 4H), 2.27, (t, 2H, J = 7.7 Hz), 3.35−3.41 (m, 2H), 3.49 (d, 2H, J = 5.5 Hz), 4.08−4.23 (m, 2H), 5.03 (d, 2H, J = 8.0 Hz), 5.04 (d, 2H, J = 8.0 Hz), 5.11 (m, 1H), 7.34 (br, 10H); ^{13}C, δ 14.1, 22.7, 24.9, 26.1, 29.1, 29.3, 29.4, 29.7, 31.9, 34.3, 66.1 (d, J = 5.5 Hz), 68.3, 69.3, 69.4. 70.6 (d, J = 7.4 Hz), 71.8, 127.9, 128.5, 128.6, 135.8 (d, J = 7.4 Hz), 173.1; ^{31}P, δ 0.53. Anal. Calcd for $C_{49}H_{83}O_7P$: C 72.2; H 10.3. Found: C 72.2; H 10.1.

1-*O*-Hexadecyl-2-*O*-palmitoyl-3-*O*-phosphoryl-*sn*-glycerol (5). A mixture of **4** (33 mg, 0.040 mmol) and 10% Pd on carbon (14 mg) in EtOAc (2 mL) was stirred under H_2 (1 atm) for 2 h, filtered through Celite, and concentrated to yield crystalline **5** (20 mg, 0.032 mmol, 78%). R_f 0.36 ($CHCl_3$/MeOH/H_2O 65:25:4); mp 78−80 °C (from EtOAc/hexane); $[\alpha]_D$ −1.3 (*c* 1.7, $CHCl_3$); ^{1}H and ^{13}C NMR of the pyridinium salt were in accordance with those published for the racemic compound.[33]

(*R*)-Di-*tert*-butylphosphorylglycidol (6). To a stirred solution of (*S*)-glycidol (238 mg, 3.21 mmol) and di-*tert*-butyl-*N*,*N*-diisopropyl phosphoramidite (1.58 g, 5.70 mmol) in CH_2Cl_2 (100 mL) was added 1*H*-tetrazole (760 mg, 10.9 mmol). After 30 min, the mixture was cooled to 0 °C and *m*-CPBA (1.70 g, 9.85 mmol) was added. The mixture was stirred for 40 min, washed with 10% aqueous $Na_2S_2O_3$ and $NaHCO_3$ (sat.), dried, filtered, and concentrated. FC (gradient petroleum ether 65−75/EtOAc 3:1 → 1:1, 0.1% NEt_3) gave **6** as a colorless oil (635 mg, 2.38 mmol, 74%). R_f (toluene/EtOAc 1:1) 0.24; $[\alpha]_D$ −5.0 (*c* 1.1, $CHCl_3$); ^{1}H NMR (CCl_4) were in accordance with that published for the racemic compound.[34] NMR ($CDCl_3$): ^{1}H, δ 1.52 (s, 18H), 2.68 (dd, 1H, J = 4.7, 2.6 Hz), 2.84 (dd, 1H, J = 4.6, 4.6 Hz), 3.25 (m, 1H), 3.91 (ddd, 1H, J = 11.7, 8.0, 5.8 Hz), 4.19 (ddd, 1H, J = 11.7, 3.3, 6.9); ^{13}C, δ 29.9 (d, J = 3.7 Hz), 44.7, 50.2 (d, J = 9.2 Hz), 67.4 (d, J = 5.6 Hz), 82.8 (d, J = 3.8 Hz), 83.0 (d, J = 3.7); ^{31}P, δ −9.09.

3-*O*-Di-*tert*-butylphosphoryl-1-*O*-palmitoyl-*sn*-glycerol (7a). A mixture of **6** (127 mg, 0.48 mmol), cesium palmitate (557 mg, 1.43 mmol), and palmitic acid (122 mg, 0.48 mmol) in DMF (5 mL) was stirred at 80 °C. After 10 h, the mixture was allowed to attain room temperature, and the precipitate filtered off. Concentration and FC (toluene/EtOAc 2:1 1% NEt_3) gave **7a** as a waxy solid (178 mg, 0.34 mmol, 71%). R_f 0.52 (toluene/EtOAc 1:3); $[\alpha]_D$ −1.7 (*c* 1.5, $CHCl_3$); NMR ($CDCl_3$): ^{1}H, δ 0.88 (t, 3H, J = 6.6 Hz), 1.26 (br, 26H), 1.50 (s, 18H), 1.62 (m, 2H), 2.34 (t, 2H, J = 7.5 Hz), 3.98−4.17 (m, 5H); ^{13}C, δ 14.1, 22.7, 24.9, 29.2, 29.3, 29.4, 29.5, 29.6, 29.7, 29.8, 29.9, 31.9, 34.2, 64.5, 68.2 (d, J = 7.4 Hz), 68.9 (d, J = 5.5 Hz), 83.2 (d, J = 7.4 Hz), 173.8; ^{31}P, δ −7.32. Anal. Calcd for $C_{27}H_{55}O_7P$: C 62.0; H 10.6. Found: C 61.9; H 10.4.

3-*O*-Di-*tert*-butylphosphoryl-2-*O*-oleoyl-1-*O*-palmitoyl-*sn*-glycerol (8). A solution of **7a** (51 mg, 0.098 mmol), oleic acid (74 mg, 0.26 mmol), dicyclohexylcarbodiimide (54 mg, 0.262 mmol), and 4-(dimethylamino)pyridine (2 mg, 0.016 mmol) in CH_2Cl_2 (3 mL) was stirred for 20 h, filtered through Celite, and concentrated. FC (toluene/EtOAc 8:1, 0.1% NEt_3) gave **8** (67 mg, 0.085 mmol, 87%) as a colorless syrup. R_f 0.32 (toluene/EtOAc 4:1); $[\alpha]_D$ +4.9 (*c* 0.9, $CHCl_3$); NMR ($CDCl_3$): ^{1}H, δ 0.88 (m, 6H), 1.26 (br, 48H), 1.48 (s, 18H), 1.61 (m, 4H), 2.01 (m, 4H), 2.31 (t, 2H, J = 7.5 Hz), 2.32 (t, 2H, J = 7.5 Hz), 4.05−4.10 (m, 2H), 4.17 (dd, 1H, J = 11.9, 6.0 Hz), 4.36 (dd, 1H, J = 11.9, 4.2 Hz), 5.18−5.41 (m, 3H); ^{13}C, δ 14.1, 22.7, 24.9, 27.2, 27.3, 29.1, 29.2, 29.3, 29.5, 29.6, 29.7, 29.8, 29.9, 31.9, 34.1, 34.2, 62.0, 64.4 (d, J = 5.5 Hz), 69.6 (d, J = 9.2 Hz), 82.7 (d, J = 7.4 Hz), 82.8 (d, J = 7.4 Hz), 129.7, 130.0, 172.9, 173.3; ^{31}P, δ −9.02. Anal. Calcd for $C_{45}H_{87}O_8P$: C 68.7; H 11.1. Found: C 68.3; H 11.0.

1-*O*-Palmitoyl-3-*O*-phosphoryl-*sn*-glycerol (9). To **7a** (37 mg, 0.071 mmol) in CH_2Cl_2 (8 mL) was added TFA (2 mL). After 10 min was added MeOH (0.10 mL). After an additional 10 min, the mixture was diluted with toluene (20 mL) and concentrated to yield crystalline **9** (29 mg, 0.071 mmol, quant.). R_f 0.12 ($CHCl_3$/MeOH/H_2O 65:25:4); mp 79−80 °C (from $CHCl_3$); $[\alpha]_D$ +5.0 (*c* 0.54, DMSO), lit.[35] (sodium salt, $CHCl_3$) $[\alpha]_D$ +13.2; NMR (DMSO): ^{1}H, δ 0.84 (t, 3H, J = 5.8 Hz), 1.22 (br, 24H), 1.50 (m, 2H), 2.28 (t, 2H, J = 7.4 Hz), 3.72−3.85 (m, 3H), 3.93 (dd, 1H, J = 11.2, 5.6 Hz), 4.02 (dd, 1H, J = 11.2, 4.1 Hz); ^{13}C, δ 13.8, 22.0, 24.3, 28.4, 28.59, 28.61, 28.8, 28.89, 28.94, 31.2, 33.3, 64.7, 66.1 (d, J = 5.4 Hz), 67.2 (d, J = 8.0 Hz), 172.7; ^{31}P, δ 0.25; MALDI-TOF Calcd for $C_{19}H_{39}O_7P$: $[M + Na]^+$ 433.2. Found: $[M + Na]^+$ 433.2.

2-*O*-Oleoyl-1-*O*-palmitoyl-3-*O*-phosphoryl-*sn*-glycerol (10). To **8** (32 mg, 0.041 mmol) in CH_2Cl_2 (8 mL) was added TFA (2 mL). After 10 min was added MeOH (0.1 mL). After an additional 10 min, the mixture was diluted with toluene (20 mL) and concentrated to yield **10** (28 mg, 0.041 mmol, quant.). R_f 0.64 ($CHCl_3$/MeOH/H_2O 65:25:4); mp diammonium salt 185−187 °C (from $CHCl_3$/Acetone), lit.[36] 192−194 °C; $[\alpha]_D$ +7.2 (*c* 3.0, $CHCl_3$), lit.[36] $[\alpha]_D$ +4.5; NMR ($CDCl_3$/CD_3OD 4:1): ^{1}H, δ 0.81−0.91 (m, 6H), 1.2−1.4 (m, 48H), 1.55−1.68 (m, 4H), 1.97−2.06 (m, 4H), 2.30−2.37 (m, 6H), 4.08−4.12 (m, 2H), 4.19 (dd, 1H, J = 12.1, 6.3 Hz), 4.38 19 (dd, 1H, J = 12.0, 3.7 Hz), 5.20−5.26 (m, 1H), 5.32−5.37 (m, 2H); ^{13}C, δ 14.2, 22.9, 25.1, 27.4, 29.3−30.0, 32.1, 34.3, 34.4, 62.4, 64.5 (d, J = 5.2 Hz), 70.1 (d, J = 7.4 Hz), 129.9, 130.2, 173.7, 174.2; ^{31}P ($CDCl_3$), δ 1.62.

(*R*)-Glycidyl Phosphocholine (11). To a stirred solution of (*S*)-glycidol (670 mg, 9.04 mmol) and ethyl *N*,*N*-diisopropylamine (6.5 mL, 9.5 mmol) in $CHCl_3$ (20 mL) at 0 °C under an argon atmosphere was added phosphorus oxychloride (1.40 g, 9.13 mmol). After 2 h, pyridine (2.0 mL) and choline tosylate (2.89 g, 10.5 mmol) were added, and the mixture was allowed to attain room temperature. After an additional 5 h, water was added (0.50 mL), and the stirring was continued for an additional 1 h. Concentration and FC (70% EtOH) gave **11** (1.14 g, 4.76 mmol, 53%). R_f 0.19 (70% EtOH); $[\alpha]_D$ −15 (*c* 1.8, MeOH); NMR (CD_3OD): ^{1}H, δ 2.68 (dd, 1H, J = 5.0, 2.7 Hz), 2.82 (dd, 1H, J = 5.0, 4.1 Hz), 3.21−3.26 (m, 1H), 3.24 (s, 9H), 3.65−3.70 (m, 3H), 4.19 (ddd, 1H, J = 12.0, 6.9, 2.5 Hz), 4.26−4.33 (m, 2H); ^{13}C, δ 45.0, 52.0 (d, J = 8.3 Hz), 54.6, 54.7, 54.8, 60.4 (d, J = 5.2 Hz), 67.5, 67.7 (d, J = 5.4 Hz); ^{31}P, δ 0.47; MALDI-TOF Calcd for $C_8H_{19}NO_5P$: $[M + H]^+$ 240.1. Found: $[M + H]^+$ 240.1.

1-*O*-Palmitoyl-*sn*-glycero-3-phosphocholine (12). A mixture of **11** (107 mg, 0.45 mmol), cesium palmitate (174 mg, 0.45 mmol) and palmitic acid (115 mg, 0.55 mmol) in DMF (5 mL) was stirred at 80 °C. After 26 h, the mixture was concentrated. FC (MeOH/H_2O 5:1) gave crystalline **12** (143 mg, 0.29 mmol, 65%). R_f 0.29 (MeOH/H_2O 5:1); mp 250−253 °C (from MeOH), lit.[37] 243−245 °C; $[\alpha]_D$ −2.8 (*c* 7.7, $CHCl_3$/MeOH 9:1), lit. for the enantiomer 3-*O*-palmitoyl-*sn*-glycero-1-phosphocholine.[37] $[\alpha]_D$ +2.8; ^{1}H and ^{13}C NMR spectra were identical to those previously published.[38] ^{31}P (CD_3OD), δ 1.22.

3-*O*-Di-*tert*-butylphosphoryl-2-*O*-[(*S*)-α-methoxy-α-(trifluoromethyl)phenylacetyl]-1-*O*-palmitoyl-*sn*-glycerol (13a). A solution of **7a** (17 mg, 0.033 mmol) and (*R*)-α-methoxy-α-(trifluoromethyl)phenylacetic acid chloride (32 mg, 0.13 mmol) in pyridine (2 mL) was stirred for 21 h. The mixture was diluted with CH_2Cl_2, washed with aqueous $NaHCO_3$, dried, filtered, and concentrated. FC (toluene → toluene/EtOAc 2:1 0.1% NEt_3) gave **13a** as a white solid (23 mg, 0.31 mmol,

(33) Malekin, S. I.; Kruglyak, Y. L.; Khromova, N. Y.; Aksenova, M. Y.; Sokolov, V. P. *Russ. J. Bioorg. Chem.* **1997**, *23*, 591−596.
(34) Kluba, M.; Zwierak, A. *Roczniki Chem.* **1974**, *48*, 1603−1608.
(35) Stoffel, W.; Wolf, G. D. *Hoppe-Seyler's Z. Physiol. Chem.* **1966**, *347*, 94−101.
(36) Molotkowsky, J. G.; Nikulina, L. F.; Bergelson, L. D. *Chem. Phys. Lipids* **1976**, *17*, 108−110.
(37) Eibl, H.; Westphal, O. *Liebings Ann. Chem.* **1970**, *738*, 161−169.
(38) Noda, N.; Tanaka, R.; Nishi, M.; Inoue, S.; Miyahara, K. *Chem. Pharm. Bull.* **1993**, *41*, 1366−1368.

96%). R_f 0.44 (toluene/EtOAc 2:1); NMR: ^{1}H ($CDCl_3$), δ 0.88 (t, 3H, J = 6.6 Hz), 1.26 (br, 24H), 1.46 (br, 20H), 2.22 (t, 2H, J = 8.1 Hz), 3.58 (s, 3H), 4.05−4.24 (m, 3H), 4.38 (dd, 1H, J = 4.0, 12.4 Hz), 5.51 (m, 1H), 7.40 (m, 3H), 7.54 (m, 2H); ^{19}F (CCl_4), δ 104.36.

1-*O*-Di-*tert*-butylphosphoryl-2-*O*-[(*S*)-α-methoxy-α-(trifluoromethyl)phenylacetyl]-3-*O*-palmitoyl-*sn*-glycerol (13b). 13b was synthesized by the same method as **13a**, starting from (*R*)-glycidol. R_f 0.44 (toluene/EtOAc 2:1); NMR: ^{1}H ($CDCl_3$), δ 0.88 (t, 3H, J = 7.0 Hz), 1.25 (br, 24H), 1.44 (br, 20H), 2.28 (t, 2H, J = 7.7 Hz), 3.57 (s, 3H), 4.0−4.25 (m, 3H), 4.48 (dd, 1H, J = 3.3, 12.1 Hz), 5.51 (m, 1H), 7.41 (m, 3H), 7.55 (m, 2H); ^{19}F (CCl_4), δ 104.44.

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