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A

**SYNTHESES OF
PHOTOACTIVATABLE AND FLUORESCENT LABELED LIPIDS**

by

GUOQING LI

A dissertation submitted to the Graduate Faculty in Chemistry in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

2002

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This manuscript has been read and accepted by the Graduate Faculty in Chemistry in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

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Abstract

Syntheses of Photoactivatable and Fluorescent Labeled Lipids

by

Guoqing Li

Research Advisor: Professor Robert Bittman

This thesis describes synthetic sequences that were used to prepare new photoactivatable and fluorescent lipid probes.

Chapter 1 describes the syntheses of photoactivatable lipid probes of phosphatidylcholine (PC) and ceramide. In these probes, a trifluoromethyldiazirine (TID) group, which is a carbene precursor for covalent labeling, was made from a trifluoroacetyl group via several steps. Stille coupling reaction was employed to convert an iodo group to a tin-substituent and then, and iododestannylation reaction was used to convert tin-substituted probe to an iodine-labeled probe.

Chapter 2 presents the syntheses of novel photoactivatable naphthalene-containing PC and ceramide probes. A new PC analog bearing a fluorophore as well as an aryl azide moiety was also synthesized.

Chapter 3 presents the syntheses of two photoreactive analogs of the antitumor ether lipid 1-*O*-octadecyl-2-*O*-methyl-*rac*-glycero-3-phosphocholine (ET-18-OCH₃) for the first time. One of the photoactivatable ET-18-OCH₃ analogs contained a TID moiety, whereas the other analog did not contain the iodo group.

Chapter 4 describes the syntheses of fluorescent analogs of cholesterol and cholesteryl esters in which the fluorophore is located in the sterol side chain.

Sphingosine is an important bioactive lipid and is also the backbone of sphingolipids such as sphingomyelin, cerebroside, and gangliosides. In Chapter 5, a highly concise synthetic route to *D-erythro*-sphingosine and a short-chain analog of sphingosine was developed employing a sulfoxide intermediate, followed by dehydrosulfenylation, affording a synthon that is readily converted to naturally occurring (2*S*,3*R*,4(*E*))-sphingosine.

Dedicated to my family:

My parents, my wife, my daughters, and my brother

Acknowledgments

I am sincerely grateful to my advisor, Professor Robert Bittman, for his high-standard guidance and encouragement in my whole study and research at CUNY, as well as in the thesis writing. I also express my thanks to his understanding and support in my personal life.

I would like to thank Professor William F. Berkowitz for his valuable advice to my research and his organic synthesis lectures, which dramatically improved my understanding of organic chemistry. I would like to thank Professor Richard W. Franck for his very valuable suggestions and encouragement for my thesis research.

Special thanks to my parents, my wife Xiuhua, and my brother Guolin for their strong support of my graduate studies. A very special thank you to my daughter Joy; she always takes care of her sisters, Michelle and Annie, giving me more time to work on my thesis research.

Thanks to everybody who supported or gave me help throughout my graduate studies.

Portions of this dissertation were published in the following journals.

“Reprinted with permission from *Tetrahedron* and *Tetrahedron Letters*. Copyright 2000, 2001, and 2002 Elsevier Science Ltd.”

Li, G.; Bittman, R. *Tetrahedron Lett.* **2000**, *41*, 6737-6741.

Li, G.; Samadder, P.; Arthur, G.; Bittman, R. *Tetrahedron* **2001**, *57*, 8925-8932.

Chun, J.; Li, G.; Byun, H.-S.; Bittman, R. *Tetrahedron Lett.* **2002**, *43*, 375-377.

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Abbreviations

9-BBN	9-borabicyclo[3.3.1]nonane
CAN	ceric ammonium nitrate
conc.	concentrated
DCC	<i>N,N'</i>-dicyclohexylcarbodiimide
DHP	3,4-dihydro-2<i>H</i>-pyran
DIAD	diisopropyl azodicarboxylate
DIC	<i>N,N'</i>-diisopropylcarbodiimide
DMAP	4-(dimethylamino)pyridine
DMF	<i>N,N</i>-dimethylformamide
DMSO	dimethyl sulfoxide
equiv	equivalent
HMPA	hexamethylphosphoramide
HOBT	1-hydroxybenzotriazole
HRMS	high-resolution mass spectrum
LDA	lithium diisopropylamide
liq.	liquid
lyso-PC	lysophosphatidylcholine
MCPBA	<i>m</i>-chloroperoxybenzoic acid
PC	phosphatidylcholine
Pd(dppf)Cl₂	[1,1'-bis(diphenylphosphino)ferrocene]- dichloropalladium(II)

PMP	<i>p</i> -methoxyphenyl
<i>p</i>-Ts	<i>p</i> -toluenesulfonyl
py	pyridine
rt	room temperature
THF	tetrahydrofuran
THP	tetrahydropyranyl
TLC	thin-layer chromatography

Chapter 1

Synthesis of a Photoactivatable Labeled Phosphatidylcholine and Ceramide

Abstract

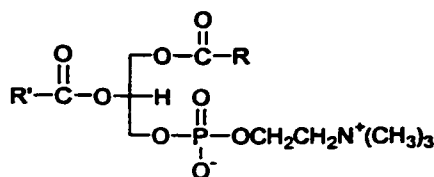
Two novel photoactivatable lipid probes, phosphatidylcholine (PC) analog **1a** and ceramide analog **2a**, were synthesized. In these probes, the trifluoromethyldiazirine group, which is a carbene precursor for covalent labeling of any residues in the vicinity of the diazirine group, was prepared from a trifluoroacetyl group. Use of a phenoxy group instead of a phenyl group in the linker in PC increases the λ_{max} from ~350 nm to ~375 nm (ϵ 284 M⁻¹ cm⁻¹). Formation of PC by acylation of lyso-PC with carboxylic acid **21** (activated as the anhydride in situ) is successful only when sterically unhindered acids **28** and **29** were used. The Stille coupling reaction was employed to introduce a trialkyltin substituent. Bromoaryldiazirine **12** failed to undergo Stille coupling reaction with hexaalkylditin. However, iodoaryldiazirines **19** and **26** were more reactive than **12**, and the desired products (stannylaryldiazirines **20** and **27**) were formed with catalytic Pd(MeCN)₂Cl₂ in HMPA in 50% and 45% yield, respectively. A final iododestannylation reaction (chloramine-T, NaI) converted the tin compound to the desired corresponding iodine-labeled targets **1a** and **2a** in high yield.

Introduction

Photoaffinity labeling is a well-established method for identifying ligand-binding domains in macromolecules.¹ Many investigators consider carbene precursors as the

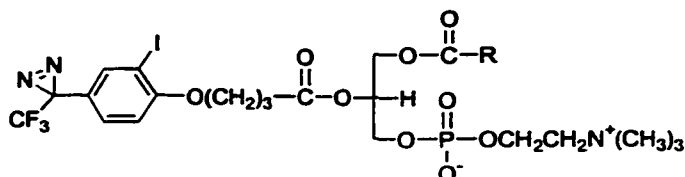
reagents of choice.² Diazirines³ and trifluoromethylphenyl diazirines (which were introduced by Brunner in 1980)⁴ are convenient carbene-generating probes.^{1d,5-7} Since peptide mapping generally employs radioligands (e.g., ¹²⁵I and ¹³¹I)^{1c} to trace the photoadducts, the commercially available hydrophobic photolabel, 3-trifluoromethyl-3-*m*-¹²⁵iodophenyl-3*H*-diazirine (¹²⁵I-TID), is a widely used tool.⁸ Incorporation of the ¹²⁵I-TID label into specific ligands permits characterization of putative membrane receptors and intracellular target proteins.^{1c,d} Although radioiodination has been achieved by aryldiazonium⁸ and arylthallium chemistry,⁹ the chemical yields are very low. As part of the studies to identify the putative glycerophospholipid binding site of phospholipid scramblase,¹⁰ photoactivatable probes of phosphatidylcholine (PC) (and other phospholipids available by transphosphatidylolation)¹¹ were required. In order to photolabel the viral E1 spike glycoprotein of the Semliki Forest virus,¹² a photoactivatable analog of ceramide¹³ was also synthesized.

Chart 1. Phosphatidylcholine and Ceramide

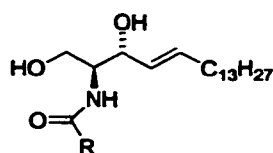


1, Phosphatidylcholine

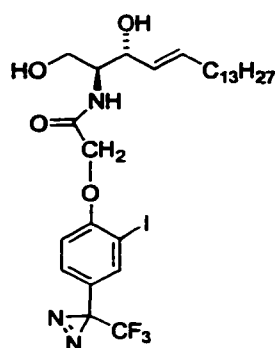
(R, R'= long hydrocarbon chains)



1a, Phosphatidylcholine probe



2, Ceramide



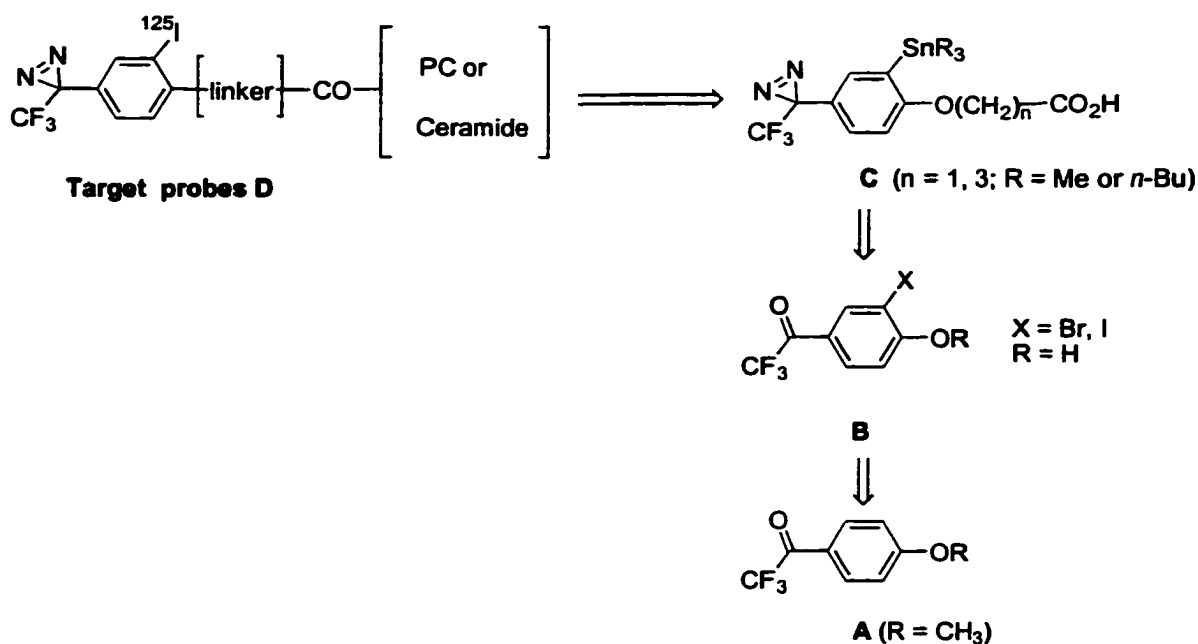
2a, Ceramide probe

Retrosynthetic Plan of Photoactivatable Probes 1a and 2a

This chapter describes the synthesis of photoactivatable PC (1) and ceramide (2). Scheme 1 outlines the preparation of the target probes (**D**) bearing a 3-(trifluoromethyl)-3-(*m*-iodophenyl)-diazirine (TID) moiety as the photolabel. In this synthesis, tin-containing TID-carboxylic acids **C** are needed to prepare PC and ceramide probes. The starting compound **A** was made by a known procedure.⁹ Compounds **B** were made by halogenation of **A** in the presence of HgO. Then, a side chain bearing a terminal carboxyl group was introduced by coupling of the phenol group of **B** with a bromo ester, Br(CH₂)_nCO₂Et. The trifluoromethyldiazirine moiety was prepared from an aryl trifluoromethylketone by using a well-established procedure.¹⁴ After the trifluoromethyldiazirine moiety was installed, the Stille coupling reaction¹⁵ was used to

replace the iodo group with a trialkyltin substituent. Finally, chloramine-T mediated *ipso* iododestannylation afforded the desired iodine-labeled targets.

**Scheme 1. Retrosynthetic Plan for the Preparation of Photoactivatable
Probes of Phosphatidylcholine and Ceramide**



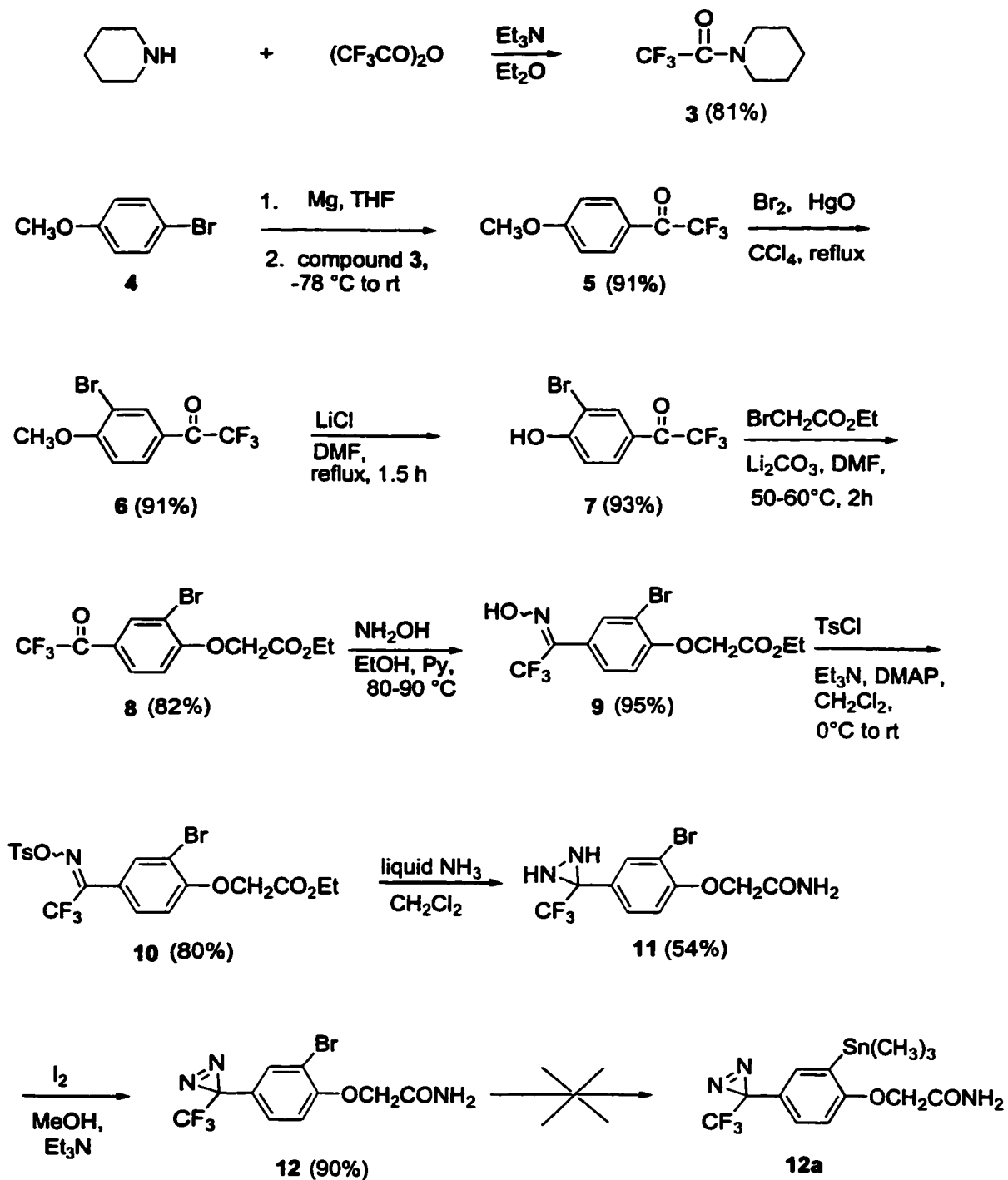
Results and Discussion

A. Synthesis of Ceramide Probe 2a

(1) Attempted synthesis via aryl bromide intermediate 12

As shown in Scheme 2, 4-methoxytrifluoroacetophenone (**5**) was prepared from 4-bromoanisole (**4**) and *N*-trifluoroacetylpiperidine (**3**) as previously reported.⁹ Bromination of aryl trifluoroacetophenone **5** (Br_2 , 2 equiv. of HgO , conc. H_2SO_4 , CCl_4 ,

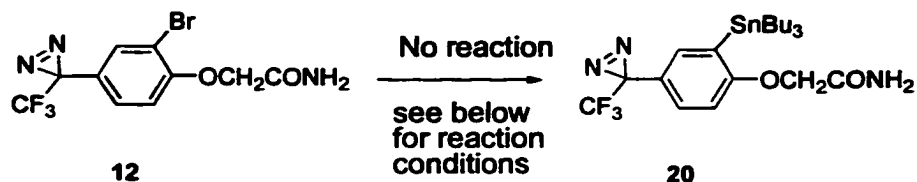
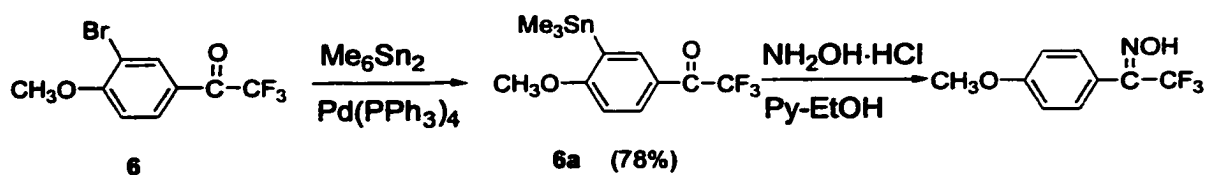
Scheme 2. Synthesis of Bromotrifluoromethyldiaziriny Compound 12



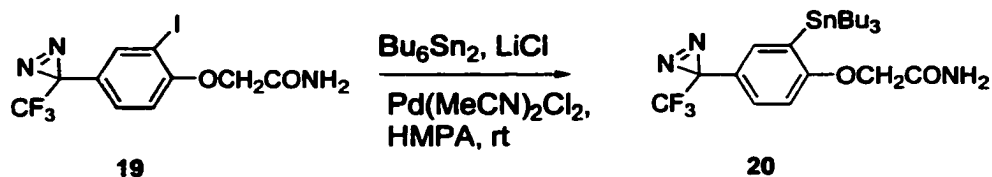
reflux) provided bromide **6** in 91% yield. The only isomer observed was the 3-bromo isomer, since both the methoxy and trifluoroacetyl are favorable to 3-substitution. *O*-Demethylation was carried out with 3 equiv. of LiCl in DMF¹⁶ at reflux for 1.5 h; the electron-withdrawing nature of the 4-trifluoroacetyl group facilitates the demethylation reaction, and phenol **7** was obtained in 93% yield. Coupling of phenol **7** with ethyl bromoacetate (Li₂CO₃/DMF, overnight, at 50-60°C) afforded ester **8** in 82% yield. Diaziridine **12** was prepared by the following reaction sequence:¹⁴ (a) formation of a mixture of *E/Z* oximes (NH₂OH · HCl in dry EtOH/py); (b) *O*-tosylation of the oximes (*p*-TsCl, Et₃N, CH₂Cl₂, 0 °C); (c) reaction of *O*-tosyl oxime **10** with liq. NH₃ in a pressure tube (-78 °C to rt). At the same time, the ethyl esters were converted into amide **11**; (d) oxidation of diaziridine **11** using iodine and 2.5 equiv. of triethylamine afforded the corresponding diazirine **12**. The synthesis of compound **12a** from aryl halides is the key step in this synthesis. However, aryl bromide **12** was subjected to Stille coupling with hexaalkylditin and various Pd catalysts without success (see Scheme 3). Therefore, aryl iodide **14** (see Scheme 4) was used instead.

The tin substituent was successfully introduced in an earlier step. As shown in Scheme 3, bromotrifluoroacetophenone **6** reacted with hexamethylditin in the presence of Pd(PPh₃)₄ in dioxane to afford **6a** in 78% yield. However, the trimethyltin substituent was lost during the oxime formation step (Scheme 3).

Scheme 3. Stille Coupling Reactions



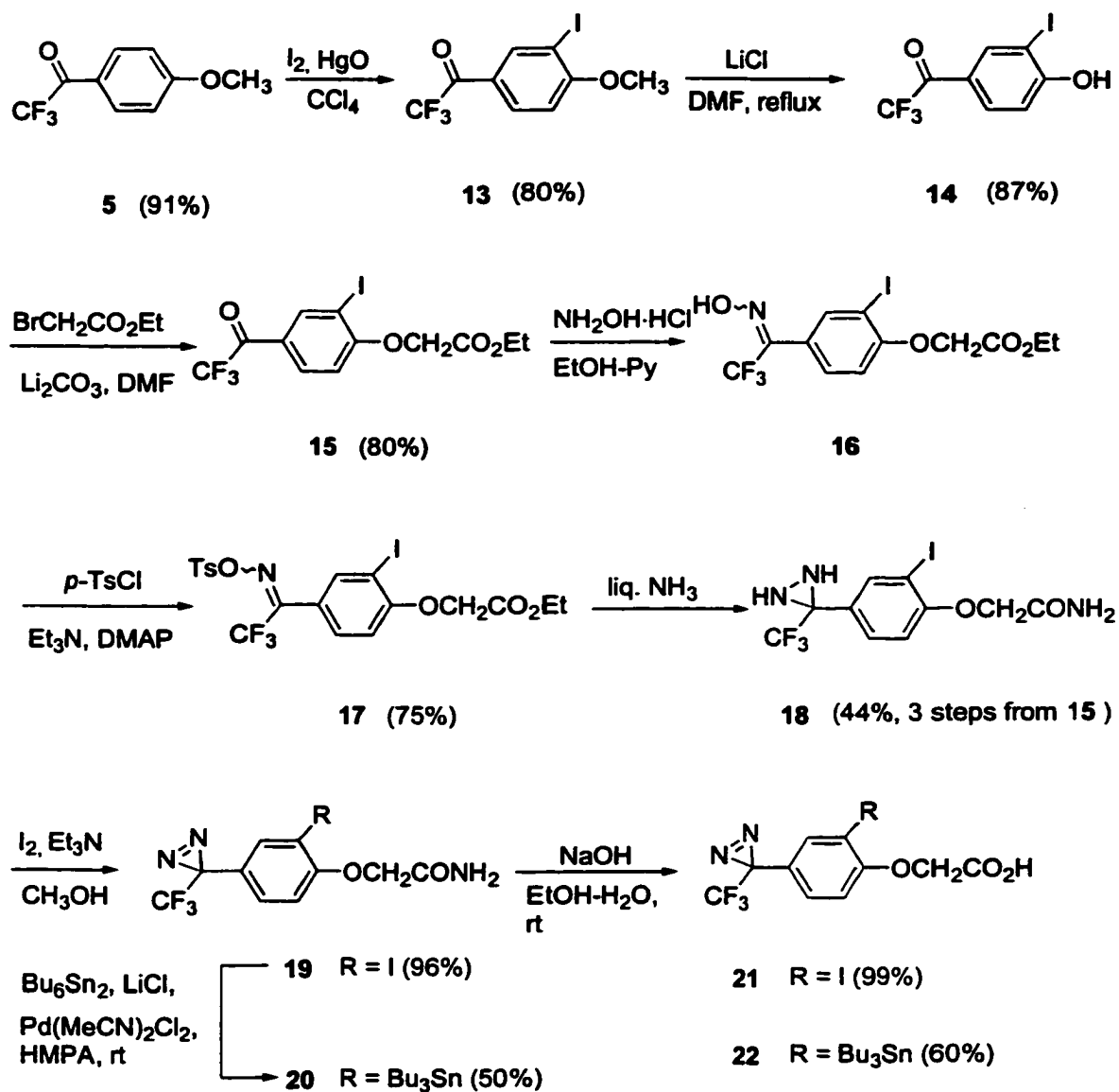
Reaction conditions: i) $\text{Pd}(\text{PPh}_3)_4$, LiCl , Sn_2Me_6 , DMF; ii) $\text{Pd}(\text{PhCN})_2\text{Cl}_2$, LiCl , Sn_2Me_6 , DMF; iii) $\text{Pd}(\text{MeCN})_2\text{Cl}_2$, LiCl , Sn_2Me_6 , DMF; iv) $\text{Pd}(\text{MeCN})_2\text{Cl}_2$, LiCl , Sn_2Bu_6 , HMPA;



(2) Synthetic approach via an aryl iodide intermediate

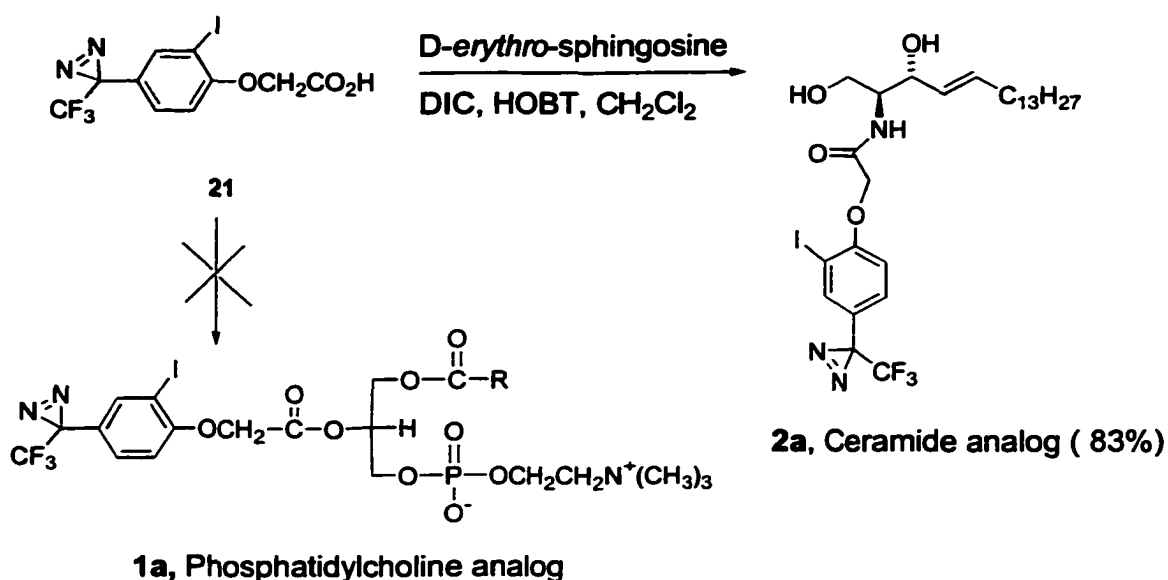
Aryl iodides are normally more reactive than aryl bromides in the Stille coupling reaction.¹⁷ Therefore, an iodo group was used instead of a bromo group, as shown in Scheme 4. Compared to bromination, iodination of aryl trifluoroketone **5** with I_2 in the presence of excess HgO in CCl_4 was slower and the yield of aryl iodide **13** was also lower (80%). *O*-Demethylation (87%) was carried out with 3 equiv. of LiCl in DMF¹⁶ at reflux for 2 h. Coupling of phenol **14** with ethyl bromoacetate ($\text{Li}_2\text{CO}_3/\text{DMF}$, overnight, 50-60 °C) afforded ester **15** in ~80% yield. Diazirine **19** was prepared by the same procedure used in the bromo series.¹⁴

Scheme 4. Synthesis of Iodo-acid 21 and Tin-containing Acid 22



The formation of tin compound **20** from aryl iodide **19** is the key step in the synthesis. The desired product **20** was formed in 50% yield with catalytic $\text{Pd}(\text{MeCN})_2\text{Cl}_2$ in HMPA at room temperature. Then, both iodo- and stannyl acids **21** and **22** were obtained in high yield by hydrolysis of the corresponding amides **19** and **20**. Finally, ceramide probe **2a** was formed successfully with acid **21**, *N,N'*-diisopropylcarbodiimide (DIC), and 1-hydroxybenzotriazole (HOBT) in CH_2Cl_2 (83%).

Scheme 5. Synthesis of Ceramide Probe 2a

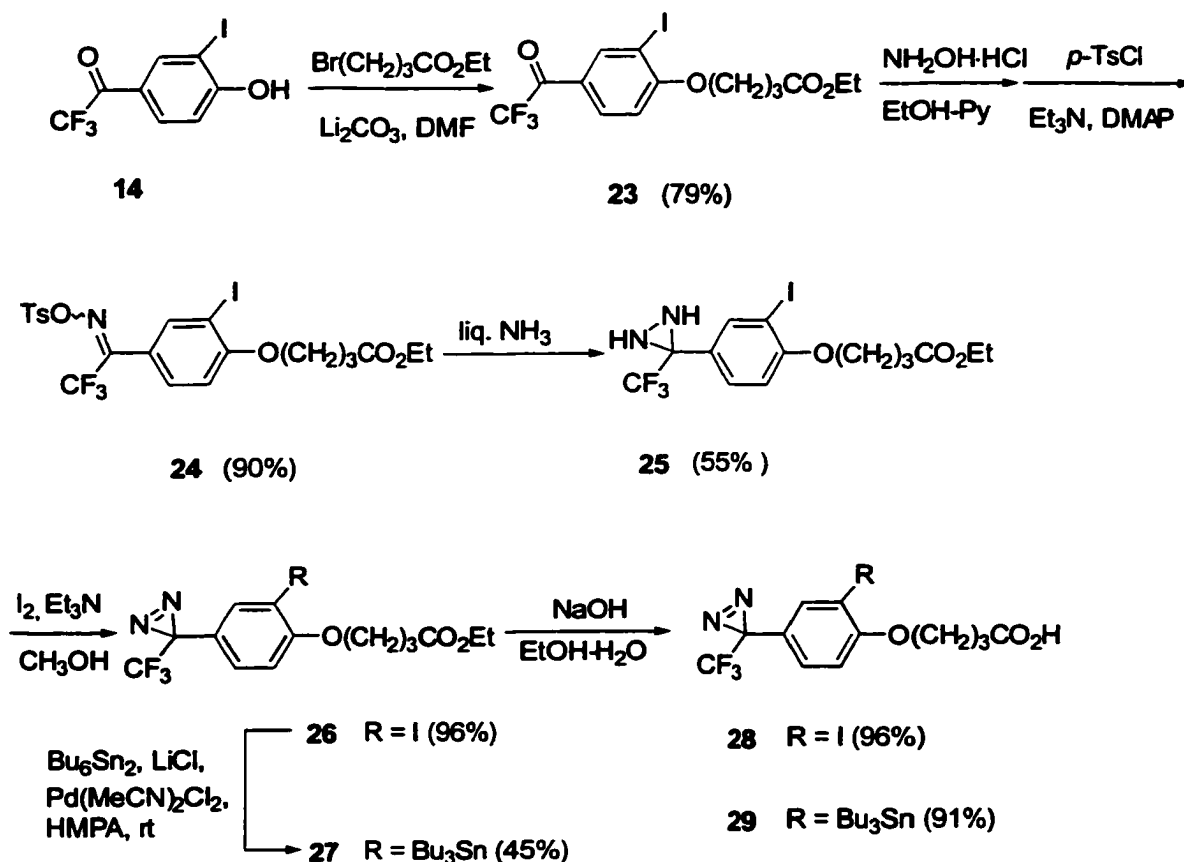


B. Synthesis of Phosphatidylcholine Probe 1a

Acylation of 1-palmitoyl-2-lyso-*sn*-glycero-3-phosphocholine (lyso-PC) with the acyl chloride or anhydride derived from acid **21** was unsuccessful, probably because of steric hindrance. To address this problem, the number of methylene groups between the carboxyl group and the phenoxy group was increased from 1 to 3. As showed in Scheme

6, coupling of phenol **14** with ethyl 4-bromobutyrate ($\text{Li}_2\text{CO}_3/\text{DMF}$, 19 h, 80-90 °C) afforded ester **23** in 79% yield. Diaziridine **25** was obtained as previously described.¹⁴ It should be noted that in the formation of diaziridine in liquid ammonia, no amide formation was observed. Then, iodoester **26** was subjected to Stille coupling with catalytic $\text{Pd}(\text{MeCN})_2\text{Cl}_2$ in HMPA at room temperature, affording tin compound **27** in 45% yield. Both esters **26** and **27** were hydrolyzed to the corresponding acids **28** and **29** in high yield with NaOH in aqueous ethanol.

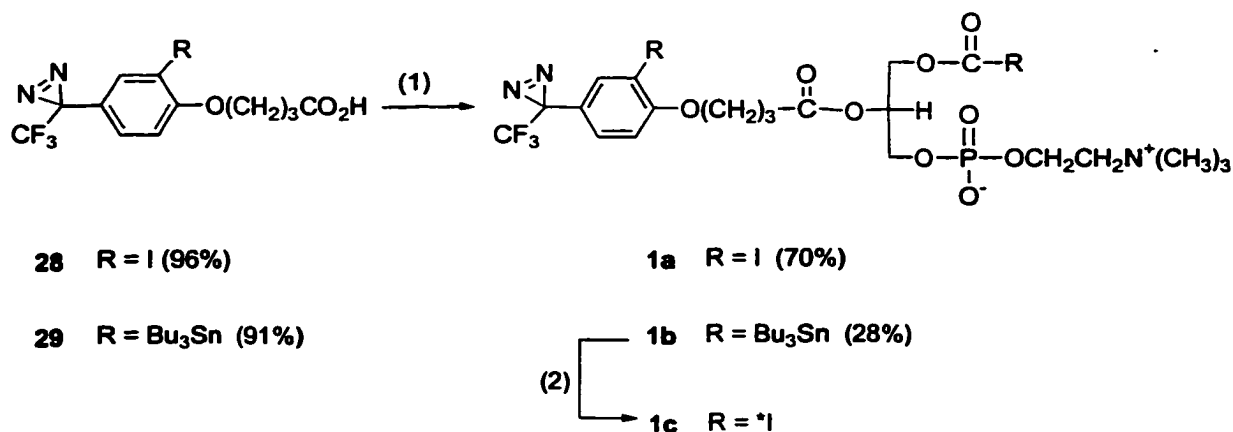
Scheme 6. Synthesis of Iodo-acid **28 and Tin-containing Acid **29****



Reaction of the less hindered iodoaryl acid **28** and arylstannyl acid **29**, via their anhydrides generated in situ, with lyso-PC at room temperature in a water bath afforded PC analogs **1a** (70%) and **1b** (28%), respectively, after chromatography (elution with CHCl_3 ; then with $\text{CHCl}_3/\text{MeOH}$, 10:1; finally with $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$ 65:25:4). The lower yield and longer reaction time (4 days) for **29** may arise from the steric effect of the tri-*n*-butyltin group. It is important to note that the stannyl derivative **1b** is stable for at least 2 months at $-20\text{ }^\circ\text{C}$. The trialkylstannyl group is efficiently replaced by an iodo group as discussed below.

Finally, *ipso* iododestannylation of **1b** was carried out on a small scale with unlabeled sodium iodide in the presence of an oxidizing agent. The goal was to establish the conditions needed for conversion of **1b** to product **1c** with a minimum of handling and chromatographic procedures so that the reaction could be subsequently carried out using $\text{Na } ^{125}\text{I}$ just prior to use with the biological targets.

Scheme 7. Synthesis of Phosphatidylcholine Probe 1a



Accordingly, two new lipophilic probes (**1a**, **2a**) were synthesized in which a trifluorophenyldiazirine functionality is located near the glycerol backbone (in **1**) or sphingoid base (in **2**). Use of a phenoxy group instead of a phenyl group as the linker in **PC**¹⁸ increases the λ_{max} to ~375 nm (ϵ 284 M⁻¹cm⁻¹). Stille coupling¹⁵ of hexabutyldistannane with aryl iodide **26** afforded arylstannane intermediate **27**. The short half-times of ¹²⁵I (60 days) and ¹³¹I (8.1 days)¹⁹ preclude installation of the radioiodide at the outset of the synthetic route and storage of radioiodinated TID derivatives for significant periods. Stannyl derivative **1b** can be stored under an inert atmosphere for months in the dark and cold without significant decomposition. Iododestannylation²⁰ is a convenient means for introducing a radioiodine atom into photoreactive ligands **1a** and **2a**.

Experimental Section

General procedures: ¹H NMR and ¹³C NMR spectra were recorded at 400 and 100 MHz on a Bruker spectrometer, respectively. The reference chemical shifts for CDCl₃ were at 7.24 (¹H) and 77.0 ppm (¹³C). Deuterated chloroform (CDCl₃) was the solvent used for NMR analyses unless otherwise indicated. The melting points are uncorrected. Chromatography was carried out with Merck 60 (230-400 mesh) silica gel. TLC was carried out on EM 0.25-mm thick silica gel 60 F₂₅₄ aluminum sheets. Solid-phase extraction cartridges (aminopropyl, 500 mg and C-18, 2 g) were purchased from Alltech Associates (Deerfield, IL). The solvents were treated as follows before use: THF (with sodium); chloroform (with P₂O₅); CH₂Cl₂ (with CaH₂); HMPA (redistilled). High-

resolution MS data were recorded at the University of California, Riverside, Mass Spectrometry Facility.

***N*-Trifluoroacetylpiperidine (3).**⁹ To a 1-L three-neck flask, piperidine (118 mL, 1.19 mol), triethylamine (140 mL, 1.0 mol), and Et₂O (200 mL) were added. The mixture was cooled in an ice bath and a solution of trifluoroacetyl anhydride (140 mL) in Et₂O (180 mL) was added dropwise under stirring. After the reaction mixture was stirred at room temperature overnight, the reaction mixture was diluted with Et₂O (500 mL) and washed with HCl (0.2 N) until neutral, then with brine (700 mL × 2), and dried (Na₂SO₄). After the solvent was removed, the residue was distilled under reduced pressure (95-100 °C/30 torr), yielding 145 g (81%) of *N*-trifluoroacetylpiperidine (3) as a colorless liquid. ¹H NMR (CDCl₃): δ 3.63-3.53 (m, 4H), 1.70-1.64 (m, 6H).

4-Methoxytrifluoroacetophenone (5).⁹ The Grignard reagent was made from 4-bromoanisole (4) (37.4 g, 200 mmol) and Mg (5.3 g, 220 mmol) in anhydrous THF (200 mL). To the Grignard reagent was added a solution of 3 (30 g, 166 mmol) in THF (30 mL) dropwise with stirring in an acetone-dry ice bath. After addition, the cooling bath was removed and the mixture was stirred at room temperature overnight. Saturated aqueous NH₄Cl solution (20 mL) was added. The solid was removed by filtration, and the filtrate was concentrated under reduced pressure. The residue including some solid was filtered, through a pad of silica gel (elution with hexane/EtOAc 1:1). The filtrate was concentrated and distilled: bp 70-73 °C/1-2 torr; 31.0 g (yield, 91%). ¹H NMR (CDCl₃): δ 8.07 (d, *J* = 8.6 Hz, 2H), 7.01 (d, *J* = 8.8 Hz, 2H), 3.93 (s, 3H). ¹³C NMR (CDCl₃): δ 178.9 (q, *J*_{C-F} = 35 Hz), 165.3, 132.7, 122.7, 117.0 (q, *J*_{C-F} = 290 Hz), 114.4, 55.6.

3-Bromo-4-methoxytrifluoroacetophenone (6). A mixture of **5** (10.3 g, 50 mmol), HgO (21.6 g, 100 mmol), concentrated H₂SO₄ (5 mL), and bromine (9.0 g, 50 mmol) in CCl₄ (220 mL) was heated to reflux under vigorous stirring for 1 h. After cooling to room temperature, the reaction mixture was poured into saturated aqueous sodium bicarbonate solution (200 mL). The organic layer was washed with sodium bicarbonate solution and brine, dried (MgSO₄), and concentrated. Distillation under reduced pressure gave a pale yellow liquid: bp 94–96°C/1–2 torr; 13.0 g (91%). ¹H NMR (CDCl₃): δ 8.29 (s, 1H), 8.05 (d, *J* = 8.0 Hz, 1H), 7.02 (d, *J* = 8.0 Hz, 1H), 4.04 (s, 3H). ¹³C NMR (CDCl₃): δ 161.4, 135.3, 131.6, 123.6, 115.1, 111.4, 56.8.

3-Bromo-4-hydroxytrifluoroacetophenone (7). A solution of **6** (1.12 g, 3.9 mmol) and lithium chloride (0.50 g, 11.8 mmol) in DMF (10 mL) was heated at reflux for 1.5 h under nitrogen. After cooling to room temperature, the reaction mixture was poured into 10 mL of 0.2 N HCl. The product was extracted with Et₂O (20 mL × 3) and the ether layer was washed three times with brine. The organic layer was dried (Na₂SO₄) and filtered through a pad of silica gel, which was eluted with CH₂Cl₂. The filtrate was concentrated to afford 93% of product. ¹H NMR (CDCl₃): δ 8.24 (m, 1H), 7.98 (m, 1H), 7.15 (d, *J* = 8.8 Hz, 1H), 6.24 (br s, 1H). ¹³C NMR (CDCl₃): δ 158.1, 134.7, 131.8, 124.0, 116.48, (q, *J*_{C-F} = 289 Hz), 116.41, 111.1.

Ethyl 2-[2-Bromo-4-(2, 2, 2-trifluoroacetyl)phenoxy]acetate (8). A mixture of **7** (5.9 g, 21.8 mmol), ethyl bromoacetate (4.36 g, 26.2 mmol), and anhydrous lithium carbonate (2.42 g, 32.7 mmol) in 50 mL of DMF was heated at 50–60 °C for 2 h. The reaction mixture was poured into water and extracted with EtOAc. The organic layer was washed with water and brine, dried (Na₂SO₄), and concentrated. The residue was

purified by silica gel chromatography (elution with CH_2Cl_2), yielding 6.0 g (82%) of product. ^1H NMR (CDCl_3): δ 8.28 (d, $J = 1.4$ Hz, 1H), 7.97 (d, $J = 8.0$ Hz, 1H), 6.83 (dd, $J = 8.0$ Hz, 4.0 Hz, 1H), 4.80 (s, 3H), 4.27 (q, $J = 7.2$ Hz, 2H), 1.28 (t, $J = 7.2$ Hz, 3H). ^{13}C NMR (CDCl_3): δ 178.1 (q, $J_{\text{C-F}} = 35$ Hz), 167.1, 159.9, 135.8, 131.3 (d, $J = 8.4$ Hz), 124.5, 116.5 (q, $J_{\text{C-F}} = 289$ Hz), 113.1, 112.3, 112.2, 65.9, 61.9, 14.1. MS calcd for $(\text{C}_{12}\text{H}_{10}\text{BrF}_3\text{O}_4 + \text{H})^+$ 354.97, found 355.1.

Ethyl 2-[2-Bromo-4-(2,2,2-trifluoroacetyl)phenoxy]acetate Oxime (9). A solution of **8** (6.0 g, 16.9 mmol) and hydroxylamine hydrochloride (1.41 g, 20.3 mmol) in pyridine (22 mL) and anhydrous EtOH (25 mL) was heated at 80-90 °C overnight. The solvents were removed under reduced pressure. The residue was partitioned between water and Et_2O . The aqueous layer was extracted with Et_2O . The combined ethereal layer was washed with HCl (0.1 N) and brine, then dried (MgSO_4). There was obtained 5.9 g (95%) of product, which was used in the next step without further purification. ^1H NMR (CDCl_3): (two *E/Z* isomers) δ 8.51 and 8.33 (2s, 1H), 7.78 and 7.72 (2d, 1H), 7.44-7.41 (m, 1H), 6.85-6.77 (m, 1H), 4.73 and 4.72 (2s, 2H), 4.30-4.24 (m, 2H), 1.32-1.26 (m, 3H). MS calcd for $(\text{C}_{12}\text{H}_{11}\text{BrF}_3\text{NO}_4 + \text{H})^+$ 369.98, found 370.1.

Ethyl 2-[2-Bromo-4-(2,2,2-trifluoroacetyl)phenoxy]acetate *O*-Tosyl Oxime (10). A solution of **9** (1.85 g, 5.0 mmol), triethylamine (1.26 g, 12.5 mmol), and DMAP (31 mg, 0.25 mmol) in CH_2Cl_2 (20 mL) was cooled in an ice bath. *p*-Tosyl chloride (1.0 g, 5.25 mmol) was added slowly. After the mixture was stirred at room temperature for 2 h, it was washed with water, aqueous sodium bicarbonate solution and brine, and dried (MgSO_4). After concentration a pure product was obtained. ^1H NMR (CDCl_3): δ 7.88 (m, 2H), 7.62 (d, $J = 2.4$ Hz, 1H), 7.41-7.37 (m, 3H), 6.84 (d, $J = 8.8$ Hz, 1H), 4.75 (s,

2H), 4.28 (q, $J = 7.2$ Hz, 2H), 2.47 (s, 3H), 1.30 (t, $J = 7.2$ Hz, 3H). ^{13}C NMR (CDCl_3): δ 167.3, 156.7, 146.1, 133.6, 130.9, 129.8, 129.5, 129.2, 129.0, 118.6, 112.7, 66.0, 61.9, 21.9, 14.3. MS calcd for $(\text{C}_{19}\text{H}_{17}\text{BrF}_3\text{NO}_6\text{S}+\text{H})^+$ 523.99, found 524.1.

2-[[2'-Bromo-4'-[(3''-trifluoromethyl)diaziridinyl]]phenoxy]acetamide (11).

To a solution of **10** (2.0 g, 3.8 mmol) in 10 mL of CH_2Cl_2 was added 20 mL of ammonia at -78 °C in a sealed tube. The mixture was stirred at room temperature overnight. The excess ammonia was allowed to evaporate at room temperature. After addition of water, the product precipitated. The precipitate was washed with water and CH_2Cl_2 , providing a solid product (0.7 g, 54%). ^1H NMR ($\text{DMSO}-d_6$): δ 7.67 (d, $J = 2.4$ Hz, 1H), 8.05 (d, $J = 8.0$ Hz, 1H), 7.02 (d, $J = 8.0$ Hz, 1H), 4.60 (s, 2H), 4.13 (d, $J = 8.0$ Hz, 1H), 4.01 (d, $J = 8.0$ Hz, 1H). ^{13}C NMR ($\text{DMSO}-d_6$): δ 169.1, 155.3, 133.0, 129.5, 126.3, 124.2 (q, $J_{\text{C-F}} = 276$ Hz), 113.7, 110.7, 67.5, 56.7 (q, $J_{\text{C-F}} = 35$ Hz). MS calcd for $(\text{C}_{10}\text{H}_9\text{BrF}_3\text{N}_3\text{O}_2+\text{H})^+$ 339.98, found 340.1.

2-[[2'-Bromo-4'-(3''-trifluoromethyl)diaziriny]]phenoxy]acetamide (12).

To a solution of **11** (0.51 g, 1.5 mmol) in MeOH (5 mL), triethylamine (380 mg, 3.7 mmol) was added. Iodine (0.40 g, 1.6 mmol) was added with stirring. The reaction mixture was stirred at room temperature for 1 h. The resulting pale yellow solution was added to water. After concentration, the product (0.45 g, 90%) was obtained by filtration, washing with water, and drying under vacuum. ^1H NMR ($\text{DMSO}-d_6$): δ 7.45 (d, $J = 2.0$ Hz, 1H), 7.42 (br s, 1H), 7.31-7.29 (m, 2H), 7.02 (d, $J = 8.4$ Hz, 1H), 4.59 (s, 2H). ^{13}C NMR ($\text{DMSO}-d_6$): δ 168.9, 156.1, 131.4, 128.1, 122.0 (q, $J_{\text{C-F}} = 273$ Hz), 121.3, 114.6, 112.0, 67.5, 27.6 (q, $J_{\text{C-F}} = 40$ Hz). MS calcd for $(\text{C}_{10}\text{H}_7\text{BrF}_3\text{N}_3\text{O}_2+\text{Na})^+$ 359.96, found 360.1; calcd for $(\text{C}_{10}\text{H}_7\text{BrF}_3\text{N}_3\text{O}_2-\text{N}_2+\text{H})^+$ 309.97, found 310.1.

3-Iodo-4-methoxytrifluoroacetophenone (13). A mixture of **5** (1.03 g, 50 mmol), HgO (2.16 g, 100 mmol), concentrated H₂SO₄ (0.5 mL), and iodine (1.27 g, 50 mmol) in CCl₄ (25 mL) was heated to reflux with vigorous stirring for 5 h. The reaction mixture was cooled to room temperature, and then filtered through a pad of Celite. The filtrate was washed with 2 N sodium thiosulfate solution and brine, dried (Na₂SO₄), and concentrated. The resulting residue was purified on silica gel (elution with hexane/EtOAc 9:1), affording 1.32 g (80%) of **13** as a white solid; mp 52-54 °C; R_f 0.22 (hexane/EtOAc 9:1). ¹H NMR (CDCl₃): δ 8.47 (s, 1H), 8.04 (d, *J* = 8.8 Hz, 1H), 6.89 (d, *J* = 8.8 Hz, 1H), 3.98 (s, 3H). ¹³C NMR (CDCl₃): δ 177.9 (q, *J*_{C-F} = 35 Hz), 163.6, 141.7, 132.7 (d, *J* = 8.4 Hz), 124.3, 116.6 (q, *J*_{C-F} = 145 Hz), 110.3, 86.5, 56.9.

4-Hydroxy-3-iodo-trifluoroacetophenone (14). To a solution of **13** (3.35 g, 10.0 mmol) in 25 mL of DMF, LiCl (1.3 g, 31 mmol) was added. The mixture was refluxed under argon for 2 h, then cooled to room temperature, poured into water (100 mL), and acidified with 10% HCl. The mixture was extracted with Et₂O (2 x 75 mL). The ethereal layer was washed with brine, dried (MgSO₄), and concentrated. The residue was dried under high vacuum to afford 2.8 g (87%) of **14**; R_f 0.45 (CH₂Cl₂). A small amount of **14** was purified on silica gel (elution with CH₂Cl₂) to give a white solid; mp 87-88 °C. ¹H NMR (CDCl₃): δ 8.40 (d, *J* = 0.4 Hz, 1H), 7.98 (d, *J* = 8.6 Hz, 1H), 7.09 (d, *J* = 8.8 Hz, 1H), 6.13 (s, 1H). ¹³C NMR (CDCl₃): δ 178.4 (q, *J*_{C-F} = 35 Hz), 160.8, 141.2 (d, *J* = 1.6 Hz), 132.9 (d, *J* = 1.0 Hz), 125.9, 124.6, 118.0 (q, *J*_{C-F} = 290 Hz), 115.3, 86.3. DEI-MS 316 (M)⁺; HRMS calcd for (C₈H₄F₃IO₂)⁺ 315.9208, found 315.9208.

Ethyl 2-[[2-Iodo-4-(trifluoroacetyl)]phenoxy]acetate (15). A mixture of **14** (0.95 g, 3.0 mmol), ethyl bromoacetate (0.60 g, 3.6 mmol), and anhydrous lithium

carbonate (0.33 g, 4.5 mmol) in DMF (6 mL) was heated overnight at 50–60 °C. The reaction mixture was poured into water and extracted with EtOAc. The organic layer was washed with water and brine, dried (Na₂SO₄), and concentrated. The residue obtained after concentration was purified by silica gel chromatography (elution with CH₂Cl₂/hexane 1:1), providing 0.96 g (80%) of product. ¹H NMR (CDCl₃): δ 8.50 (d, *J* = 1.5 Hz, 1H), 7.01 (dd, *J* = 8.8 Hz, 0.8 Hz, 1H), 6.75 (d, *J* = 8.0 Hz, 1H), 4.79 (s, 3H), 4.26 (q, *J* = 7.2 Hz, 2H), 1.28 (t, *J* = 7.2 Hz, 3H). ¹³C NMR (CDCl₃): δ 177.0 (q, *J*_{C-F} = 35 Hz), 167.1, 162.0, 142.1, 132.3, 125.1, 116.5 (q, *J*_{C-F} = 289 Hz), 111.2, 86.7, 66.1, 61.9, 14.1. MS calcd for (C₁₂H₁₀F₃IO₄+H)⁺ 402.96, found 403.1.

2-[[2'-Iodo-4'-[(3"-trifluoromethyl)diaziridinyl]]phenoxy]acetamide (18).

A solution of **15** (3.0 g, 7.5 mmol) and hydroxylamine hydrochloride (0.62 g, 9.0 mmol) in pyridine (10 mL) and anhydrous ethanol (12 mL) was heated at reflux for 1.5 h. The solvents were removed under reduced pressure. The residue was partitioned between water and Et₂O. The aqueous layer was extracted with Et₂O. The combined ethereal layer was washed with 0.1 N HCl, brine, and dried (MgSO₄). The crude product oxime **16** (3.09 g, 99%) was used directly in the next step. A solution of **16** (from the previous step), triethylamine (1.9 g, 18.6 mmol), and DMAP (46 mg, 3.8 mmol) in 20 mL of CH₂Cl₂ was cooled in an ice bath. To the solution *p*-tosyl chloride (1.57 g, 8.3 mmol) was added slowly. The mixture was stirred at room temperature for 2 h. The resulting solution was washed with water, aqueous sodium bicarbonate solution and brine, dried (MgSO₄), and concentrated, providing 3.2 g (75%) of crude *O*-tosyl oxime **17**. MS calcd for (C₁₉H₁₇F₃INO₆S+H)⁺ 571.98, found 572.1.

To a solution of **17** (from the previous step) in 10 mL of CH₂Cl₂, ammonia (20 mL) was added at -78 °C in a pressure tube. The mixture was stirred overnight at room temperature. The excess ammonia was allowed to evaporate at room temperature. On addition of water (30 mL), a solid was obtained, which was filtered and washed with water and CH₂Cl₂, providing 1.27 g of **18** (overall yield of 44% for the 3 steps from compound **15**). ¹H NMR (DMSO-d₆): δ 7.88 (d, *J* = 1.8 Hz, 1H), 7.53 (m, 2H), 7.26 (s, 1H), 6.95 (d, *J* = 8.6 Hz, 1H), 4.60 (s, 2H), 4.11 (d, *J* = 8.0 Hz, 1H), 4.00 (d, *J* = 8.0 Hz, 1H). ¹³C NMR (DMSO-d₆): δ 170.4, 158.8, 140.2, 131.5, 128.0, 125.4 (q, *J*_{C-F} = 276 Hz), 113.7, 87.5, 68.9, 57.7 (q, *J*_{C-F} = 35 Hz). MS calcd for (C₁₀H₉F₃IN₃O₂+H)⁺ 387.97, found 388.1.

2-[[2'-Iodo-4'-[(3"-trifluoromethyl)diazirinyl]]phenoxy]acetamide (19). To solution of **18** (774 mg, 2.0 mmol) in 6 mL of MeOH, triethylamine (500 mg, 5.0 mmol) was added. Iodine (534 mg, 2.1 mmol) was added to the mixture with stirring. After the reaction mixture was stirred for 1 h at room temperature, water was added and the solution was concentrated. The residue was washed with water, and then dried under vacuum, affording 714 mg (96%) of the product. ¹H NMR (DMSO-d₆): δ 7.64 (s, 1H), 7.52 (br s, 1H), 7.35 (d, *J* = 8.4 Hz, 1H), 7.27 (br s, 1H), 6.96 (d, *J* = 8.6 Hz, 1H), 4.60 (s, 2H). ¹³C NMR (DMSO-d₆): δ 168.9, 158.4, 137.1, 128.8, 122.0 (q, *J*_{C-F} = 273 Hz), 121.7, 113.3, 87.7, 67.7, 27.3 (q, *J*_{C-F} = 40 Hz). MS calcd for (C₁₀H₇F₃IN₃O₂+H)⁺ 385.95, found 386.1.

2-[[2'-Tri-*n*-butylstannyl-4'-[(3"-trifluoromethyl)diazirinyl]]phenoxy]acetamide (20). A mixture of **19** (50 mg, 0.13 mmol), hexabutyltin (87 mg, 0.15 mmol), lithium chloride (15 mg, 0.36 mmol), and Pd(CH₃CN)₂Cl₂ (5 mg, 0.019 mmol) in 1 mL

of HMPA was stirred for 18 h under argon at room temperature. The reaction mixture was diluted with 50 mL of CH₂Cl₂ and 10 mL of water. The organic layer was washed with water twice and dried over sodium sulfate. The product was purified by chromatography (elution with CH₂Cl₂), providing 36 mg (50%) of product **20**. ¹H NMR (CDCl₃): δ 7.22 (d, *J* = 1.6 Hz, 1H), 7.15 (dd, *J* = 8.4 Hz, 1.6 Hz, 1H), 6.79 (d, *J* = 8.8 Hz, 1H), 6.23 (br s, 1H), 5.62 (br s, 1H), 4.50 (s, 2H), 1.53-1.47 (m, 6H), 1.38-1.29 (m, 6H), 1.08 (t, *J* = 8.0 Hz, 6H), 0.88 (t, *J* = 7.3 Hz, 9H). ¹³C NMR (CDCl₃): δ 170.8, 162.8, 135.9, 132.1, 129.3, 123.3, 110.8, 68.0, 29.5, 27.7, 14.0, 10.4.

2-[[2'-Tri-*n*-butylstannyl-4'-[(3"-trifluoromethyl)diaziriny]]phenoxy]acetic Acid (22). To a solution of **20** (100 mg, 0.26 mmol in 10 mL of EtOH) was added 5.6 mL of 1 N NaOH. After the reaction mixture was stirred at room temperature for 2 days, the mixture was acidified with 1.0 M citric acid and extracted with CH₂Cl₂ (50 mL). The organic layer was washed with water and brine, dried (MgSO₄), filtered, and concentrated. The residue was purified by chromatography (elution with hexane/EtOAc 3:1), providing 60 mg (60%) of product **22**. ¹H NMR (CDCl₃): δ 7.14 (s, 1H), 7.05 (d, *J* = 8.4 Hz, 1H), 6.61 (d, *J* = 8.4 Hz, 1H), 4.54 (s, 2H), 1.53-1.47 (m, 6H), 1.31-1.25 (m, 9H), 1.07-1.03 (t, *J* = 8.0 Hz, 6H), 0.89-0.84 (m, 9H). ¹³C NMR (CDCl₃): δ 135.6, 132.5, 128.6, 126.5, 124.0, 121.4, 110.0, 65.9, 29.4, 27.7, 14.1, 10.2.

2-[[2'-Iodo-4'-[(3"-trifluoromethyl)diaziriny]]phenoxy]acetic Acid (21). This compound was prepared (99%) from **19** by using a similar procedure to that used to prepare **22**. ¹H NMR δ 7.60 (d, *J* = 2.1 Hz, 1H), 7.21 (dd, *J* = 8.6 Hz, 2.0 Hz, 1H), 6.72 (d, *J* = 8.6 Hz, 1H), 4.75 (s, 2H). ¹³C NMR δ 171.8, 157.3, 138.1, 128.3, 124.4 (q, *J*_{C-F} = 290 Hz), 124.3, 111.8, 86.7, 65.5.

***N*-[2-[[2'-Iodo-4'-[(3"-trifluoromethyl)diaziriny]]phenoxy]acetyl]-*D*-erythro-sphingosine (2a).** To a mixture of *D*-erythro-sphingosine (14 mg, 0.047 mmol), **21** (20 mg, 0.052 mmol), and HOBt (7 mg, 0.052 mmol) in 2 mL of CH₂Cl₂ was added DIC (8.5 μL, 0.054 mmol). After the reaction mixture was stirred overnight, it was diluted with 10 mL of CH₂Cl₂, washed with H₂O, and then dried (MgSO₄). The residue after concentration was purified by chromatography (elution with CH₂Cl₂/MeOH 100:1, then 50:1) to afford product **2a** (26 mg, 83%); *R*_f 0.70 (CH₂Cl₂/MeOH 10:1). ¹H NMR (CDCl₃): δ 7.64 (d, *J* = 8.0 Hz, 1H), 7.56 (d, *J* = 2.0 Hz, 1H), 7.21 (dd, *J* = 8.0 Hz, 2.0 Hz, 1H), 6.75 (d, *J* = 8.0 Hz, 1H), 5.78 (dt, *J* = 15.0 Hz, 7.0 Hz, 1H), 5.53 (dd, *J* = 15.0 Hz, *J* = 7.0 Hz, 1H), 4.52 (d, *J* = 2.0 Hz, 2H), 4.37 (t, *J* = 5.0 Hz, 1H), 4.02-3.98 (m, 2H), 3.80-3.73 (m, 1H), 2.04-2.00 (m, 2H), 1.33-1.10 (m, 22 H), 0.85 (t, *J* = 7.0 Hz, 3H). ¹³C NMR (CDCl₃): δ 167.1, 157.0, 156.7, 137.6, 134.7, 128.6, 128.5, 124.3, 121.9 (q, *J*_{C-F} = 270 Hz), 111.9, 86.8, 74.3, 67.8, 62.2, 54.3, 42.3, 32.3, 31.9, 29.7, 29.6, 29.5, 29.3, 29.2, 29.1, 27.4 (q, *J*_{C-F} = 40 Hz), 23.4, 22.7, 14.1.

Ethyl 4-(2-Iodo-4-trifluoroacetylphenoxy)butyrate (23). To a solution of **14** (2.68 g, 8.4 mmol) and ethyl 4-bromobutyrate (2.34 g, 12.0 mmol) in 25 mL of DMF was added Li₂CO₃ (1.11 g, 15.0 mmol). The mixture was stirred at 80-90 °C for 19 h. After cooling to room temperature, the mixture was poured into water (100 mL) and extracted with Et₂O (2 x 75 mL). The ether layer was washed with brine, dried (MgSO₄), and concentrated. The product was purified by chromatography (elution with hexane/EtOAc 6:1) to afford 2.85 g (79%) of **23** as a liquid; *R*_f 0.15 (hexane/EtOAc 6:1). ¹H NMR (CDCl₃): δ 8.45 (d, *J* = 4.8 Hz, 1H), 8.00 (d, *J* = 7.0 Hz, 1H), 6.85 (dd, *J* = 2.4 Hz, 8.8 Hz, 1H), 4.15 (m, 4H), 2.58 (m, 2H), 2.18 (m, 2H), 1.22 (m, 3H). ¹³C NMR (CDCl₃): δ

177.8 (q, $J_{\text{C-F}} = 34.8$ Hz), 172.9, 162.8, 141.6, 132.6, 119.5 (q, $J_{\text{C-F}} = 290$ Hz), 111.0, 86.9, 60.6, 30.2, 24.1, 14.2.

4-[(3-Ethoxycarbonyl)propyloxy]-3-iodo-trifluoroacetophenone *O*-Tosyl

Oxime (24). A solution of **4** (2.8 g, 6.5 mmol) and $\text{NH}_2\text{OH}\cdot\text{HCl}$ (0.54 g, 7.8 mmol) in 10 mL of absolute ethanol and 10 mL of pyridine was heated at 80-90 °C for 4 h. The solvents were evaporated under reduced pressure. The residue was partitioned between water (30 mL) and Et_2O (150 mL). The ether layer was washed with brine, dried (MgSO_4), and concentrated. After drying under high vacuum, the residue was dissolved in CH_2Cl_2 (30 mL). To the solution, *p*-TsCl (1.30 g, 6.8 mmol) was added portionwise with stirring at 0 °C. After the reaction mixture was stirred for 1 h at room temperature, it was washed with water, 10% HCl, saturated aqueous NaHCO_3 solution and brine, then dried (MgSO_4). There was obtained 3.5 g (90% yield) of crude product. A portion of product was further purified by chromatography (elution with CH_2Cl_2 /hexane 1:1); R_f 0.40. ^1H NMR (CDCl_3): δ 7.90-7.88 (m, 2H), 7.69 (d, $J = 2.0$ Hz, 1H), 7.45-7.36 (m, 3H), 6.86-6.79 (2d, 1H), 4.14 (m, 4H), 2.63-2.58 (m, 2H), 2.47-2.48 (2s, 3H), 1.26 (m, 3H). ^{13}C NMR (CDCl_3): δ 173.0, 160.1, 159.7, 151.9, 146.2, 146.1, 139.8, 139.2, 131.3, 131.1, 130.8, 130.6, 129.9, 129.3, 129.2, 121.6, 118.2, 111.3, 111.2, 86.5, 68.20, 68.17, 60.6, 30.50, 30.48, 24.2, 21.8, 14.2. MS calcd for $(\text{C}_{21}\text{H}_{21}\text{F}_3\text{INO}_6\text{S}+\text{H})^+$ 600.01, found 600.1.

Ethyl 4-[2'-Iodo-4'-[(3"-trifluoromethyl)diaziridinyl]]phenoxybutyrate (25).

To a solution of **24** (3.3 g, 5.5 mmol) in 40 mL of CH_2Cl_2 was added 15 mL of liquid ammonia at -78 °C in a pressure tube. The mixture was stirred at room temperature overnight. After the excess ammonia was released at room temperature, 50 mL of water

and 100 mL of CH₂Cl₂ were added. The organic layer was washed with water and brine, dried (MgSO₄), and concentrated. The product (1.35 g, 55%) was purified by chromatography (elution with CH₂Cl₂); *R_f* 0.18. ¹H NMR (CDCl₃): δ 7.98 (s, 1H), 7.52 (d, *J* = 8.4 Hz, 1H), 6.78 (d, *J* = 8.5 Hz, 1H), 4.14–4.07 (m, 4H), 2.60–2.56 (m, 3H), 2.15–2.14 (m, 3H), 1.23 (t, *J* = 7.0 Hz, 3H). ¹³C NMR (CDCl₃): δ 173.1, 158.6, 139.0, 129.5, 125.6, 123.4 (q, *J*_{C-F} = 475 Hz), 111.4, 86.5, 68.0, 60.5, 56.9 (q, *J*_{C-F} = 36 Hz), 30.5, 24.3, 14.2. MS calcd for (C₁₄H₁₆F₃IN₂O₃+H)⁺ 445.02, found 445.1.

Ethyl 4-[2'-Iodo-4'-[(3"-trifluoromethyl)diaziriny]]phenoxybutyrate (26).

To a solution of **25** (1.32 g, 2.97 mmol) in 10 mL of MeOH, triethylamine (758 mg, 9.0 mmol) was added, followed by iodine (830 mg, 3.3 mmol). The mixture was stirred at room temperature for 1 h. The mixture was concentrated and the resulting residue was dissolved in 150 mL of CH₂Cl₂. The solution was washed with 50 mL of 1 N Na₂S₂O₃, 1 N HCl, saturated aqueous NaHCO₃ solution, and brine, and then dried (MgSO₄). Purification of the residue on silica gel (elution with CH₂Cl₂: hexane 1:1) gave 1.28 g (96%) of **7**; *R_f* 0.8 (CH₂Cl₂). ¹H NMR (CDCl₃): δ 7.52 (d, *J* = 1.9 Hz, 1H), 7.17 (d, *J* = 8.6 Hz, 1H), 6.75 (d, *J* = 8.7 Hz, 1H), 4.12 (q, *J* = 7.1 Hz, 2H), 4.05 (t, *J* = 6.0 Hz, 2H), 2.57 (t, *J* = 7.2 Hz, 2H), 2.16–2.10 (m, 2H), 1.25–1.20 (t, *J* = 7.2 Hz, 3H). ¹³C NMR (CDCl₃): δ 173.0, 158.5, 137.6, 128.3, 122.8, 121.9 (q, *J*_{C-F} = 273 Hz), 111.5, 86.8, 68.1, 65.8, 60.5, 30.5, 27.5 (q, *J*_{C-F} = 40.5 Hz), 24.2, 15.2, 14.2. MS calcd for (C₁₄H₁₄F₃IN₂O₃+H)⁺ 443.00, found 443.1.

Ethyl 4-[2'-Tri-*n*-butylstannyl-4'-[(3"-trifluoromethyl)diaziriny]]phenoxybutyrate (27). To a solution of **26** (442 mg, 1.0 mmol) and hexabutylditin (696 mg, 1.2 mmol) in 2 mL of HMPA, Pd(CH₃CN)₂Cl₂ (50 mg, 0.19 mmol) was added under argon.

The mixture was stirred overnight at room temperature, then diluted with Et₂O (100 mL) and washed with 1 M KF solution. The organic layer was washed with water and dried (MgSO₄). The residue after concentration was purified by chromatography (elution with hexane/EtOAc 100:1) to afford 270 mg (45%) of **27** as an oil; *R*_f 0.9 (CH₂Cl₂). ¹H NMR (CDCl₃): δ 7.15 (d, *J* = 1.9 Hz, 1H), 7.09 (dd, *J* = 1.9 Hz, 8.7 Hz, 1H), 6.75 (d, *J* = 8.7 Hz, 1H), 4.13 (q, *J* = 7.1 Hz, 2H), 3.95 (t, *J* = 6.3 Hz, 2H), 2.48 (t, *J* = 7.3 Hz, 2H), 2.09 (q, *J* = 6.7 Hz, 2H), 1.50-1.45 (m, 6H), 1.32-1.22 (m, 12H), 1.03 (t, *J* = 8.2 Hz, 3H), 0.86 (t, *J* = 7.3 Hz, 9H). ¹³C NMR (CDCl₃): δ 172.9, 163.9, 135.1, 131.5, 128.5, 122.4 (q, *J*_{C-F} = 273 Hz), 121.1, 109.4, 66.7, 60.5, 30.6, 28.5, 24.6, 14.2, 13.6, 9.9.

4-[2'-Tri-*n*-butylstannyl-4'-[(3"-trifluoromethyl)diaziriny]]phenoxybutyric Acid (29). To a solution of NaOH (72 mg, 1.8 mmol) in 3 mL of EtOH and 1 mL of water was added **27** (218 mg, 0.36 mmol). After the mixture was stirred for 3 h at room temperature, it was acidified with 1 N citric acid and extracted with Et₂O (2 x 50 mL). The ethereal layer was washed with brine and dried (MgSO₄). The solution was concentrated under reduced pressure and lyophilized with 3 mL of benzene to afford 200 mg (96%) of **29**. ¹H NMR (CDCl₃): δ 7.16 (d, *J* = 2.0 Hz, 1H), 7.09 (dd, *J* = 8.6 Hz, 2.0 Hz, 1H), 6.75 (d, *J* = 8.6 Hz, 1H), 3.97 (t, *J* = 6.2 Hz, 2H), 2.56 (t, *J* = 7.3 Hz, 2H), 2.10 (m, 2H), 1.51-1.44 (m, 6H), 1.34-1.24 (m, 9H), 1.02 (t, *J* = 8.2 Hz, 6H), 0.86 (t, *J* = 7.3 Hz, 9H). ¹³C NMR (CDCl₃): δ 178.3, 163.8, 135.1, 131.4, 128.3, 122.3 (q, *J*_{C-F} = 273.5 Hz), 121.2, 109.0, 66.5, 30.3, 29.1, 27.3, 24.3, 13.6, 9.9.

4-[2'-Iodo-4'-[(3"-trifluoromethyl)diaziriny]]phenoxybutyric Acid (28). This compound was prepared in 91% yield. ¹H NMR (CDCl₃): δ 7.55 (d, *J* = 2.2 Hz, 1H), 7.17 (dd, *J* = 2.1 Hz, 8.6 Hz, 1H), 6.75 (d, *J* = 8.6 Hz, 1H), 4.07 (t, *J* = 5.9 Hz, 2H), 2.66

(t, $J = 7.2$ Hz, 2H), 2.16 (m, 2H). ^{13}C NMR (CDCl_3): δ 178.9, 158.4, 137.7, 128.3, 122.9, 122.0 (q, $J_{\text{C-F}} = 273$ Hz), 111.5, 86.8, 67.8, 30.3, 27.5 (q, $J_{\text{C-F}} = 40.5$ Hz), 24.0.

1-*O*-Palmitoyl-2-*O*-[4-[2'-iodo-4'-[(3"-trifluoromethyl)diaziriny]]phenoxybutyryl]-*sn*-glycero-3-phosphocholine (1a). A solution of acid **28** (83 mg, 0.20 mmol) and DCC (25.0 mg, 0.60 mmol) in 5 mL of CHCl_3 was stirred at room temperature for 7 h. Then, a solution of lyso-PC (35 mg, 0.067 mmol) and DMAP (36 mg, 0.30 mmol) in 4 mL of alcohol-free, dry CHCl_3 was added. The mixture was stirred for 16 h in a water bath at room temperature. After filtration, the filtrate was concentrated and passed through a TMD-8 column (a mixed-bed ion-exchange resin, purchased from Aldrich, elution with $\text{THF}/\text{H}_2\text{O}$ 9:1). The residue obtained after concentration was purified by chromatography (elution with CHCl_3 ; then with $\text{CHCl}_3/\text{MeOH}$ 10:1; finally with $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$ 65:25:4), affording 42 mg (70%) of **1a**; R_f 0.28 ($\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$ 65:25:4). ^1H NMR (CDCl_3): δ 7.52 (d, $J = 2.0$ Hz, 1H), 7.15 (dd, $J = 1.7$ Hz, 8.5 Hz, 1H), 6.76 (d, $J = 8.7$ Hz, 1H), 5.18 (m, 1H), 4.38-4.37 (m, 1H), 4.26 (m, 2H), 4.11-4.01 (m, 3H), 3.91 (br s, 2H), 3.76 (br s, 2H), 3.32 (s, 9H), 2.57 (t, $J = 7.3$ Hz, 2H), 2.19 (t, $J = 7.6$ Hz, 2H), 2.09 (m, 2H), 1.50 (m, 2H), 1.34-1.20 (m, 24H), 0.84 (t, $J = 6.8$ Hz, 3H). ^{13}C NMR (CDCl_3): δ 173.5, 172.4, 158.3, 137.5, 128.3, 122.8, 121.9 (q, $J_{\text{C-F}} = 273$ Hz), 111.7, 86.9, 70.9, 67.9, 66.3, 63.4, 62.7, 59.3, 54.3, 34.0, 31.9, 30.6, 29.7-29.1 (m), 27.4 (q, $J_{\text{C-F}} = 40.5$ Hz), 24.8, 24.3, 22.6, 14.1. UV λ_{max} 375 nm (ϵ 284 $\text{M}^{-1} \text{cm}^{-1}$) in absolute ethanol.

1-*O*-Palmitoyl-2-*O*-[4-[2'-tri-*n*-butylstannyl-4'-[(3"-trifluoromethyl)diaziriny]]phenoxybutyryl]-*sn*-glycero-3-phosphocholine (1b). Similarly, **1b** was obtained by reaction of **29** with lyso-PC for 4 days (yield, 28%). ^1H NMR (CDCl_3): δ

7.15 (s, 1H), 7.06 (d, $J = 8.5$ Hz, 1H), 6.76 (d, $J = 8.6$ Hz, 1H), 5.19 (br s, 1H), 4.41-4.29 (m, 3H), 4.09-3.92 (m, 5H), 3.74-3.71 (m, 2H), 3.31 (br s, 9H), 2.48 (t, $J = 7.3$ Hz, 2 H), 2.19 (t, $J = 7.5$ Hz, 2H), 2.05 (m, 2H), 1.51-1.43 (m, 7H), 1.32-1.21 (m, 32H), 1.03-0.99 (m, 5H), 0.90-0.83 (m, 12H). ^{13}C NMR (CDCl_3): δ 173.6, 172.3, 163.9, 135.1, 131.4, 128.5, 125.9, 121.2, 109.5, 68.0, 66.7, 66.4, 63.3, 62.9, 59.3, 54.4, 34.0, 31.9, 30.6, 29.7-28.9 (m), 27.5, 27.3, 27.0, 25.6, 24.8, 24.5, 22.7, 14.1, 13.7, 9.9.

Iododestannylation of 1b. The following procedure was used to incorporate radioiodine into the derivatized 1c. The method described here was carried out with nonradioactive iodine. In a 1-mL cone-shaped vial, 100 μL (0.093 μmol) of a solution of 1b (1.7 mg of 1b in 2 mL of absolute EtOH), 50 μL (10 μmol) of a solution of NaH_2PO_4 (276 mg of $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ in 10 mL of H_2O), and 50 μL (0.23 μmol) of NaI (13.8 mg of NaI in 20 mL of H_2O) were added. Then 50 μL (0.23 μmol) of chloramine-T (from a freshly prepared stock solution of 13 mg of chloramine-T trihydrate in 10 mL of absolute EtOH) was added with stirring. The solution, which became pale yellow, was stirred for 30 min. The reaction was quenched with 50 μL of NaHSO_3 solution (prepared by dissolving 600 mg of NaHSO_3 in 2 mL of H_2O). After 200 μL of chloroform was added, the mixture was shaken. The bottom layer was collected and concentrated to dryness with a stream of nitrogen. The residue was dissolved in 100 μL of chloroform and loaded onto a “Bond-Elut” solid phase cartridge (aminopropyl, 500 mg). The vial was washed with an additional 100 μL of chloroform. The latter solution was also loaded onto the cartridge. Elution with 4 mL of chloroform removed the byproduct. Then elution with

1:1 CHCl₃/MeOH (4 mL) afforded product **1c**. To obtain the ¹H NMR spectrum, the reaction to prepare **1a** from **1b** was carried out on a 10-mg scale.

References and Notes

- (1). (a) Bayley, H. *Photogenerated Reagents in Biochemistry and Molecular Biology*, North-Holland/Elsevier: Amsterdam, 1983. (b) Fedan, J. S.; Hogaboom, G. K.; O'Donnell, J. P. *Biochem. Pharmacol.* **1984**, *33*, 1167-1180. (c) Brunner, J. *Methods Enzymol.* **1989**, *172*, 628-687. (d) Brunner, J. *Annu. Rev. Biochem.* **1993**, *62*, 483-514. (e) Kotzyba-Hilbert, F.; Kapfer, I.; Goeldner, M. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1296-1312. (f) Fleming, S. A. *Tetrahedron* **1995**, *51*, 12479-12520. (g) Dormann, G.; Prestwich, G. D. *Trends Biotechnol.* **2000**, *18*, 64-77.
- (2). Diffusion of carbenes from the site of formation is limited because of their short lifetimes. Carbenes insert randomly into the functional groups found in biomolecules, including normally unreactive saturated C-H bonds of apolar amino acid side chains at or near the ligand-binding site.
- (3). (a) Schmitz, E. in Houben-Weyl, *Methoden der Organischen Chemie*, Klamann, D., Ed.; G. Thieme Verlag: Stuttgart, 1982; Vol. E 16c, pp 678-728. (b) Liu, M. T. H., Ed., *Chemistry of Diazirines*; CRC Press: Boca Raton, FL, 1987.
- (4). Brunner, J.; Senn, H.; Richards, F. M. *J. Biol. Chem.* **1980**, *255*, 3313-3318.
- (5). Hatanaka, Y.; Nakayama, H.; Kanaoka, Y. *Rev. Heteroatom. Chem.* **1996**, *14*, 213-243.
- (6). The electron-withdrawing trifluoromethyl group suppresses photorearrangement to a linear diazo compound and other intramolecular rearrangements that can occur in carbenes after photolysis.^{1c}
- (7). Advantages of trifluoromethylphenyl diazirines: photoactivation at $\lambda > 350$ nm, avoiding protein-damaging wavelengths; high chemical stability in subdued ambient lighting toward mild reducing agents, oxidizing agents, acidic and basic conditions, and elevated temperatures.¹
- (8). Brunner, J.; Semenza, G. *Biochemistry* **1981**, *20*, 7174-7182.
- (9). Hatanaka, Y.; Hashimoto, M.; Kurihara, H.; Nakayama, H.; Kanaoka, Y. *J. Org. Chem.* **1994**, *59*, 383-387.
- (10). Bevers, E. M.; Comfurius, P.; Dekkers, D. W.; Zwaal, R. F. *Biochim. Biophys. Acta* **1999**, *1439*, 317-330.

- (11). For a review of transphosphatidylation, see: Bittman, R. In *Lipid Synthesis and Manufacture*, Gunstone, F. D., Ed.; Marcel Dekker: New York, 1999, pp. 125-144.
- (12). Sphingolipid specificity of Semliki Forest virus fusion: e.g., Moesby, L.; Corver, J.; Erukulla, R. K.; Bittman, R.; Wilschut, J. *Biochemistry* **1995**, *34*, 10319-10324.
- (13). For a previous synthesis of a radioiodinated TID photoaffinity analog of ceramide, see: Huwiler, A.; Brunner, J.; Hummel, R.; Vervoordeldonk, M.; Stabel, S.; Van den Bosch, H.; Pfeilschifter, J. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 6959-6963. For a radioiodinated aryl azido analog of ceramide, see: Zegers, M. M. P.; Kok, J. W.; Hoekstra, D. *Biochem. J.* **1997**, *328*, 489-498.
- (14). Weber, T.; Brunner, J. *J. Am. Chem. Soc.* **1995**, *117*, 3084-3095.
- (15). Farina, V.; Krishnamarthy, V.; Scott, W. J. *Org. React.* **1997**, *50*, 1-652.
- (16). Bernard, A. M.; Ghiani, M. R.; Piras, P. P.; Rivoldini, A. *Synthesis* **1989**, 287-289.
- (17). Angara, G. J.; Bovonsombat, P.; McNelis, E. *Tetrahedron Lett.* **1992**, *33*, 2285-2287.
- (18). [¹²⁵I-TID]-PC: (a) Ref. 1d. (b) Durrer, P.; Gaudin, Y.; Ruigrok, R. W.; Graf, R.; Brunner, J. *J. Biol. Chem.* **1995**, *270*, 17575-17581. (c) Goldmann, W. H.; Niles, J. L.; Arnaout, M. A. *Eur. J. Biochem.* **1999**, *261*, 155-162.
- (19). Boyer, R. F. *Modern Experimental Biochemistry*, 2nd Ed., Benjamin: Redwood City, p. 176.
- (20). Gallant, M.; Sawyer, N.; Metters, K.; Zamboni, R. *Bioorg. Med. Chem.* **1998**, *6*, 63-72.

Chapter 2

Part 1. Synthesis of Photoactivatable Naphthalene-containing Fluorescent Phosphatidylcholine and Ceramide

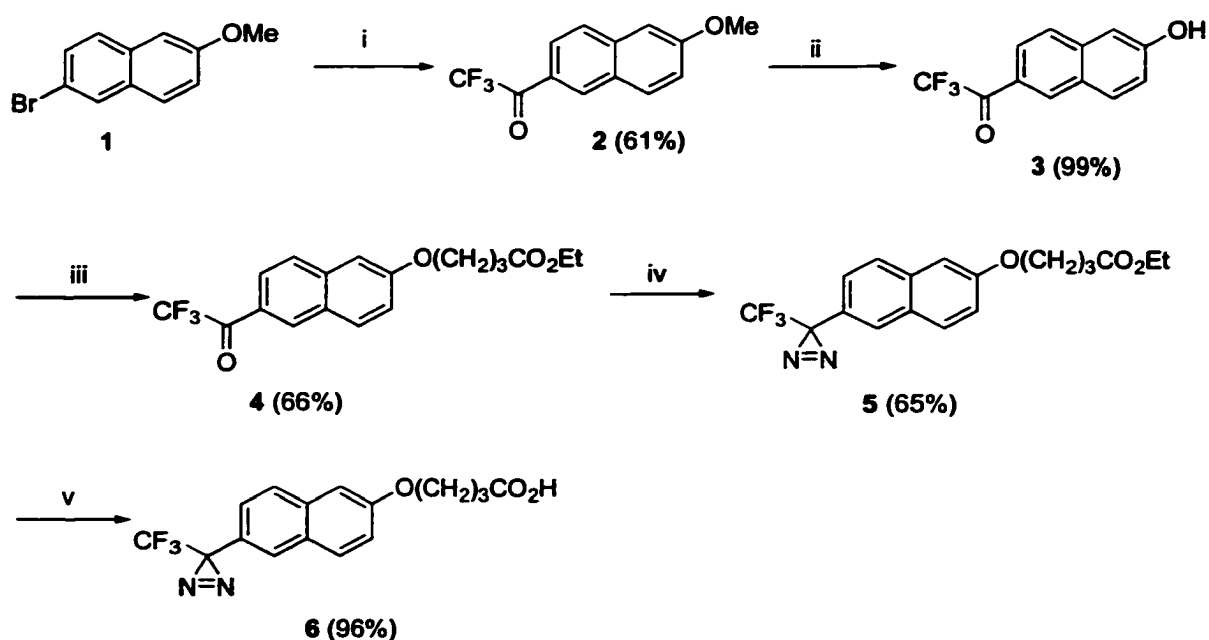
Abstract

Chapter 1 describes the synthesis of iodine-containing photoactivatable lipids. Part 1 of this chapter presents the syntheses of fluorescent-labeled probes of phosphatidylcholine and ceramide. Two novel photoactivatable naphthalene-containing probes, **7** and **8**, were synthesized. These compounds may be used for fluorescent detection of proteins that interact with phosphatidylcholine or ceramide. Thus, they represent potential alternatives to the use of radiolabeled photoactivatable probes for labeling of membrane-bound proteins. The UV spectrum of diazirine-ester **5** indicated that this compound has about 3 times the absorbance (ϵ 950 M⁻¹ cm⁻¹) at ~378 nm compared to the corresponding phenoxy compound (λ ~375 nm, ϵ 284 M⁻¹ cm⁻¹); however, the UV absorption is too low to permit detection of protein bands on polyacrylamide gels by UV detection. Probe **7** has an excitation maximum at 236 nm and an emission maximum at 350 nm. Since fluorescence detection is more sensitive than UV detection, aryl azide fluorophores were synthesized (Part 2 of this Chapter).

Results and Discussion

Novel photoactivatable naphthalene-containing probes of PC and ceramide were synthesized. Trifluoroacetylation of the Grignard reagent derived from commercially available 2-bromo-6-methoxynaphthalene (**1**) with *N*-(trifluoroacetyl)-

Scheme 1. Synthesis of Photoactivatable Acid **6**

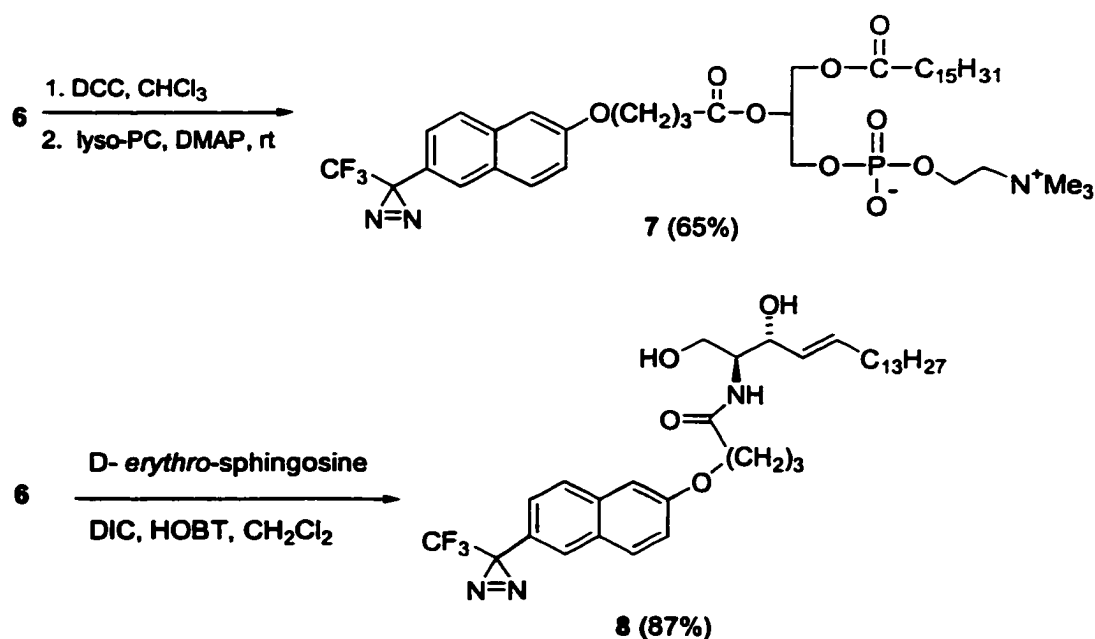


(i) (a) Mg, THF; (b) piperidine-COCF₃, -78 °C to r.t.; (ii) 48% HBr, HOAc, reflux, 36 h; (iii) Br(CH₂)₃CO₂Et, K₂CO₃, 18-crown-6, 60-70 °C, DMSO, 2 h; (iv) (a) NH₂OH·HCl, py-EtOH, 80-90 °C, 6 h; (b) *p*-TsCl, Et₃N, DMAP, 0 °C, 1 h; (c) liq. NH₃, CH₂Cl₂, -78 °C to rt; (d) I₂, Et₃N, MeOH; (v) NaOH, EtOH-H₂O, 2.5 h, rt

piperidine afforded ketone **2** in 61% yield. No *O*-demethylation of **2** was observed using conditions that were successful with anisole derivatives (Chapter 1, Schemes 2 and 4, LiCl/DMF, 100-110 °C or reflux). An attempt to carry out the demethylation reaction

with BBr_3 at 0 °C in CH_2Cl_2 was also unsuccessful. As shown in Scheme 1, *O*-demethylation of **2** was carried out in very high yield with 48% HBr in acetic acid at reflux.¹ *O*-Alkylation of **3** with ethyl 4-bromobutyrate in the presence of K_2CO_3 /18-crown-6 in DMSO at 60-70 °C gave product **4** in 66% yield. Trifluoromethylnaphthyldiazirine **5** was formed from ketone **4** by a standard procedure^{2,3} in good overall yield (65% for four steps). The UV spectrum of **5** indicates that naphthalene-diazirine has a 3-fold higher absorbance at ~375 nm than an analogous phenoxydiazirine compound (see Chapter 1). Hydrolysis of ester **5** with NaOH in aqueous ethanol afforded the important intermediate diazirine acid **6**, which was coupled to lipids via ester or amide bonds (see Scheme 2). Fluorescent-labeled PC **7** and ceramide **8** were synthesized in good yields.

Scheme 2. Synthesis of Photoactivatable Phosphatidylcholine and Ceramide



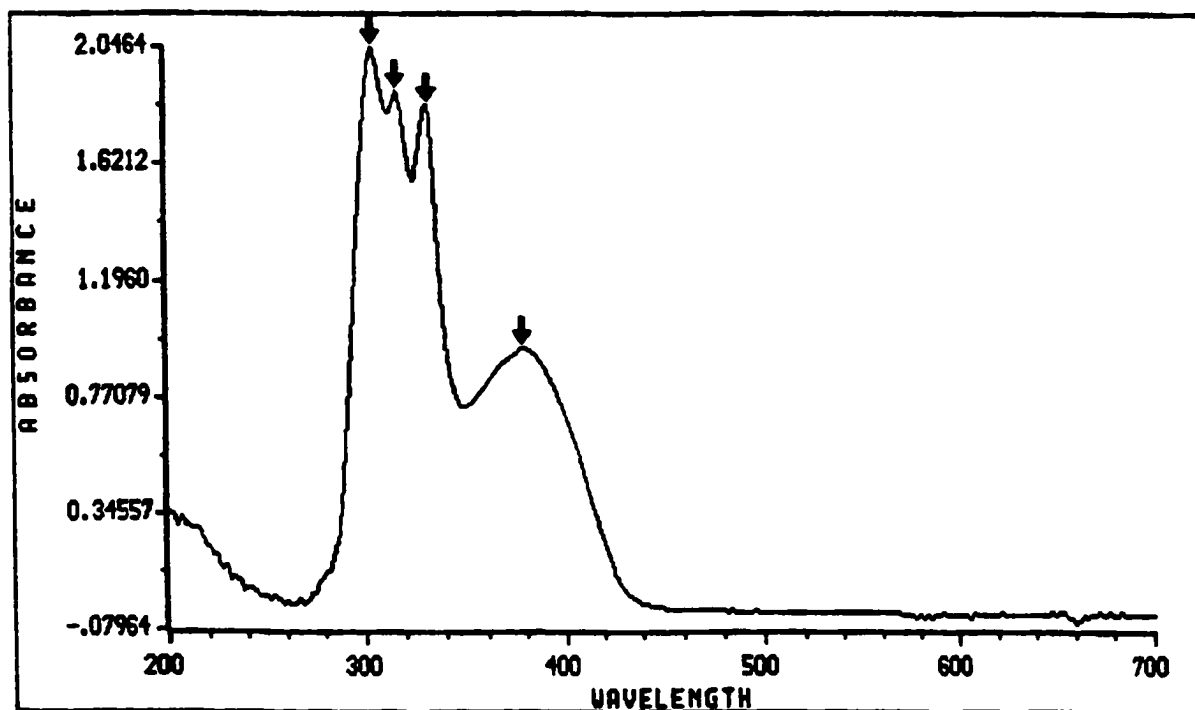


Figure 1. UV Spectrum of Naphthyldiazirine 5

Experimental Section

General procedures: See the previous chapter for detailed information. Anhydrous DMSO and DMF were purchased from Acros. Lyso-PC was purchased from Sigma. *D-erythro*-Sphingosine was purchased from Matreya.

2-Methoxy-6-trifluoroacetylnaphthalene (2). The Grignard reagent of 2-bromo-6-methoxynaphthalene (**1**) was prepared from 4.74 g (20.0 mmol) of bromide **1** and Mg (535 mg, 22.0 mmol) in 30 mL of anhydrous THF at -78°C . When most of the Mg turnings had dissolved, a solution of *N*-trifluoroacetylpyrrolidine⁴ (3.60 g, 20.0 mmol) in 10 mL of THF was added dropwise. The reaction mixture was stirred at room temperature overnight. The reaction was quenched with 50 mL of saturated aqueous

NH_4Cl solution, and the product was extracted with EtOAc (3 x 100 mL). The organic layer was washed (brine) and dried (MgSO_4). After concentration, the residue was purified by chromatography (elution with hexane, then with hexane/EtOAc 100:1) to give 3.1 g (61%) of **2**; mp 66-67 °C; R_f 0.3 (hexane/EtOAc 8:1); ^1H NMR (CDCl_3): δ 8.53 (s, 1H), 8.38 (dd, $J = 8.7$ Hz, 0.8 Hz, 1H), 7.89 (d, $J = 8.8$ Hz, 1H), 7.81 (d, $J = 8.7$ Hz, 1H), 7.25 (dd, $J = 8.8$ Hz, 2.0 Hz, 1H), 7.17 (d, $J = 2.0$ Hz, 1H), 3.93 (s, 1H). ^{13}C NMR (CDCl_3): δ 180.1 (q, $J_{\text{C-F}} = 34.4$ Hz), 161.1, 138.5, 133.01, 132.98, 131.9, 127.7, 127.5, 125.1, 120.4, 117.0 (q, $J_{\text{C-F}} = 289.7$ Hz), 105.9, 55.6. EI-MS 254 (M^+); HRMS calcd for $(\text{C}_{13}\text{H}_9\text{F}_3\text{O}_2)^+$ 254.0555, found 254.0553.

2-Hydroxy-6-trifluoroacetylnaphthalene (3). A mixture of **2** (1.50 g, 5.9 mmol), HBr (48%, 6 mL, 53 mmol), and HOAc (60 mL) was heated to reflux for 36 h. After concentration, 50 mL of H_2O was added. The mixture was extracted with EtOAc (3 x 100 mL). The organic layer was washed with saturated aqueous NaHCO_3 solution and brine, and then dried (MgSO_4). After evaporation of the solvent, the residue was purified by chromatography (elution with hexane/EtOAc 6:1) to give 1.4 g (99%) of **3** as a yellow solid; mp 146-147 °C; R_f 0.25 (hexane/EtOAc 4:1). ^1H NMR (CDCl_3): δ 8.04 (s, 1H), 7.75 (d, $J = 8.8$ Hz, 1H), 7.68 (d, $J = 8.8$ Hz, 1H), 7.58 (d, $J = 8.8$ Hz, 1H), 7.17-7.12 (m, 2H). ^{13}C NMR (CDCl_3): δ 157.4, 136.8, 131.2, 130.2, 129.2, 129.1, 127.1, 126.4, 124.7 (q, $J_{\text{C-F}} = 285$ Hz), 119.8, 109.7. EI-MS 240 (M^+); HRMS calcd for $(\text{C}_{12}\text{H}_7\text{F}_3\text{O}_2)^+$ 240.0398, found 240.0402.

Ethyl 4-[(6-Trifluoroacetylnaphthalen-2-yloxy)]butyrate (4). A mixture of **3** (120 mg, 0.50 mmol), ethyl 4-bromobutyrate (146 mg, 1.5 mmol), anhydrous K_2CO_3 (138 mg, 1.0 mmol), and 18-crown-6 (4.0 mg, 0.015 mmol) in 3 mL of DMSO was

heated to 60-70 °C for 2 h. After addition of 20 mL of H₂O, the mixture was extracted with EtOAc (3 x 30 mL). The organic layer was washed with brine and dried (MgSO₄). Concentration, followed by chromatography (elution with hexane/EtOAc 10:1), gave 117 mg (66%) of **4**; mp 64-65 °C; *R*_f 0.40 (hexane/EtOAc 4:1). ¹H NMR (CDCl₃): δ 8.53 (s, 1H), 8.03 (d, *J* = 8.7 Hz, 1H), 7.89 (d, *J* = 8.8 Hz, 1H), 7.79 (d, *J* = 8.8 Hz, 1H), 7.23 (dd, *J* = 2.4 Hz, 8.8 Hz, 1H), 7.16 (d, *J* = 2.4 Hz, 1H), 4.23-4.14 (m, 4H), 2.57 (t, *J* = 7.2 Hz, 2H), 2.21 (m, 2H), 1.27 (t, *J* = 7.2 Hz, 3H). ¹³C NMR (CDCl₃): δ 180.1 (q, *J*_{C-F} = 34 Hz), 173.1, 160.3, 138.5, 133.00, 132.97, 131.9, 127.7, 127.5, 125.14, 125.10, 120.5, 117.0 (q, *J*_{C-F} = 290 Hz), 67.1, 60.6, 30.7, 24.5, 14.2. DEI-MS 354 (M⁺); HRMS calcd for (C₁₈H₁₇F₃O₄)⁺ 354.1079, found 354.1070.

Ethyl 4-[6-[(3'-Trifluoromethyl)diazirinylnaphthalen-2-yloxy]butyrate (5**).**

A solution of **4** (1.07 g, 3.0 mmol) and NH₂OH·HCl (252 mg, 3.6 mmol) in 5 mL of absolute ethanol and 5 mL of pyridine was heated at 80-90 °C for 6 h. The solvents were evaporated under reduced pressure. The residue was partitioned between water (30 mL) and Et₂O (100 mL). The ether layer was washed with brine, dried (MgSO₄), and concentrated. After drying under high vacuum, the residue was dissolved in anhydrous CH₂Cl₂ (20 mL), and Et₃N (900 mg, 8.9 mmol) and DMAP (10 mg, 0.082 mmol) were added. To the solution, *p*-TsCl (630 mg, 3.3 mmol) was added portionwise with stirring at 0 °C. The solution was stirred for 30 min at room temperature. After addition of 80 mL of CH₂Cl₂, the reaction mixture was washed with water, 10% HCl, saturated aqueous NaHCO₃ solution, and brine, and then dried (MgSO₄). A crude product (two stereoisomers of the *O*-tosyloxime) (1.19 g) was obtained. To a solution of the crude product (1.0 g, 1.91 mmol) in 40 mL of CH₂Cl₂ was added 15 mL of liquid ammonia at

-78 °C in a pressure tube. The mixture was stirred at room temperature for 3 h. After the excess ammonia was allowed to evaporate at room temperature, 40 mL of water and 50 mL of CH₂Cl₂ were added. The organic layer was washed with water and brine, dried (MgSO₄), and concentrated. The residue was dissolved in 10 mL of MeOH containing Et₃N (450 mg, 4.5 mmol). After iodine (480 mg, 1.9 mmol) was added, the mixture was stirred for 30 min at room temperature and concentrated. To the residue, 100 mL of Et₂O and 20 mL of H₂O were added. The organic layer was washed with 1 N Na₂S₂O₃ (20 mL) and brine, then concentrated. Purification of the residue by chromatography (elution with hexane/CH₂Cl₂ 1:1) afforded 0.60 g (65%) of **5**; *R_f* 0.50 (CH₂Cl₂). ¹H NMR (CDCl₃): δ 7.72 (d, *J* = 5.8 Hz, 1H), 7.71 (d, *J* = 6.2 Hz, 1H), 7.52 (s, 1H), 7.28 (dd, *J* = 5.8 Hz, 8.8 Hz, 1H), 7.17 (dd, *J* = 2.4 Hz, 8.8 Hz, 1H), 7.10 (d, *J* = 2.4 Hz, 1H), 4.18-4.11 (m, 4H), 2.56 (t, *J* = 7.2 Hz, 2H), 2.18 (m, 2H), 1.26 (t, *J* = 7.2 Hz, 3H). ¹³C NMR (CDCl₃): δ 173.1, 158.0, 134.7, 129.7, 128.1, 127.6, 126.5, 124.1, 123.4, 122.2 (q, *J*_{C-F} = 290 Hz), 120.2, 106.3, 66.8, 60.5, 30.8, 24.5, 14.2. UV (MeOH) λ_{max} (ε, M⁻¹cm⁻¹) 304 nm (2.0 × 10³), 316 (1.9 × 10³), 332 (1.8 × 10³), 378 (950). DEI-MS 367 (MH⁺); HRMS calcd for (C₁₈H₁₈F₃N₂O₃)⁺ 367.1270, found 367.1269.

4-[6-[(3'-Trifluoromethyl)diazirinylnaphthalen-2-yloxy]butyric acid (6). To a solution of NaOH (100 mg, 2.5 mmol) in 5.0 mL of MeOH and 2.5 mL of H₂O, 193 mg (0.50 mmol) of **5** was added. The mixture was stirred for 3 h at room temperature, then acidified with 1 N citric acid and extracted with Et₂O (2 x 50 mL). The ethereal layer was washed with brine and dried (MgSO₄). The solution was concentrated under reduced pressure and lyophilized from 3 mL of benzene to afford 170 mg (96%) of diazirine **6**; *R_f* 0.55 (CH₂Cl₂/MeOH 5:1). ¹H NMR (CDCl₃): δ 7.70 (d, *J* = 8.8 Hz, 1H), 7.69 (d, *J* = 8.8

Hz, 1H), 7.50 (s, 1H), 7.26 (m, 1H), 7.15 (dd, $J = 2.4$ Hz, 8.8 Hz, 1H), 7.08 (d, $J = 2.4$ Hz, 1H), 4.12 (t, $J = 6.0$ Hz, 2H), 2.62 (t, $J = 7.2$ Hz, 2H), 2.17 (m, 2H). ^{13}C NMR (CDCl_3): δ 178.5, 157.9, 134.7, 129.7, 128.2, 127.6, 126.6, 124.1, 123.5, 122.4 (q, $J_{\text{C-F}} = 290$ Hz), 120.1, 106.3, 66.6, 30.4, 24.2. EI-MS 338 (M^+); HRMS calcd for $(\text{C}_{16}\text{H}_{13}\text{F}_3\text{N}_2\text{O}_3)^+$ 338.0878, found 338.0889.

1-*O*-Palmitoyl-2-*O*-[4-[6-[(3'-trifluoromethyl)diaziriny]naphthalen-2-yloxy]butyryl]-*sn*-glycero-3-phosphocholine (7). A solution of acid 6 (84.5 mg, 0.25 mmol) and DIC (17.7 mg, 0.14 mmol) in 3 mL of CHCl_3 was stirred at room temperature for 4 h. A solution of lyso-PC (52 mg, 0.10 mmol) and DMAP (46 mg, 0.38 mmol) in 5 mL of alcohol-free CHCl_3 was added. The mixture was stirred for 24 h in a water bath at room temperature. The reaction mixture was concentrated and passed through a TMD-8 column (elution with THF/ H_2O 9:1). The residue obtained after concentration was purified by chromatography (elution with CHCl_3 , then with $\text{CHCl}_3/\text{MeOH}$ 10:1, finally with $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$ 65:25:4), affording 55 mg (65%) of PC 7 after removal of suspended silica gel in CHCl_3 by filtration through a Gelman 0.25- μm filter; R_f 0.30 ($\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$ 65:25:4). ^1H NMR (CDCl_3): δ 7.70 (m, 2H), 7.51 (s, 1H), 7.21-7.10 (m, 3H), 5.23 (br s, 1H), 4.41-4.38 (m, 1H), 4.31 (br s, 2H), 4.21-4.08 (m, 3H), 3.98 (br s, 2H), 3.79 (br s, 2H), 3.32 (br s, 9H), 2.57 (t, $J = 7.2$ Hz, 2H), 2.26-2.12 (m, 4H), 1.51 (m, 2H), 1.24-1.15 (m, 24H), 0.87 (t, $J = 6.8$ Hz, 3H). ^{13}C NMR (CDCl_3): δ 173.6, 172.5, 157.9, 134.7, 129.7, 128.1, 127.6, 126.5, 124.2, 123.5, 120.1, 106.3, 70.8, 66.7, 66.3, 63.5, 62.8, 58.5, 54.4, 34.0, 31.9, 30.8, 29.69, 29.64, 29.5, 29.34, 29.28, 29.1, 24.8, 24.5, 22.7, 14.1. FAB-MS 816 (MH^+); HRMS calcd for $(\text{C}_{40}\text{H}_{62}\text{F}_3\text{N}_3\text{O}_9\text{P})^+$ 816.4176, found 816.4180.

***N*-[4-[6-[(3'-Trifluoromethyl)diaziriny]naphthalen-2-yloxy]butyryl]-D-*erythro*-sphingosine (Ceramide 8).** To a mixture of D-*erythro*-sphingosine (25 mg, 0.084 mmol), acid 6 (31 mg, 0.092 mmol), and HOBT (12.4 mg, 0.092 mmol) in 3 mL of CH₂Cl₂ was added DIC (12.6 mg, 0.10 mmol). The reaction mixture was stirred at room temperature overnight, then diluted with 30 mL of CHCl₃ and washed with H₂O (2 x 20 mL). The aqueous layer was extracted with CHCl₃ (2 x 10 mL). The combined organic layers were dried (MgSO₄) and concentrated. The residue was purified by chromatography (elution with CH₂Cl₂/MeOH 100:1, then 100:3) to afford 45 mg (87%) of ceramide 8 after removal of suspended silica gel by filtration through a Gelman 0.25- μ m filter; R_f 0.50 (CH₂Cl₂/MeOH 10:1). ¹H NMR (CDCl₃): δ 7.71 (d, *J* = 8.4 Hz, 1H), 7.70 (d, *J* = 8.8 Hz, 1H), 7.52 (s, 1H), 7.27 (d, *J* = 8.8 Hz, 1H), 7.18 (dd, *J* = 2.4 Hz, 8.8 Hz, 1H), 7.10 (d, *J* = 2.4 Hz, 1H), 6.41 (d, *J* = 7.3 Hz, 1H), 5.74 (m, 1H), 5.49 (dd, *J* = 6.4 Hz, 15.4 Hz, 1H), 4.29 (m, 1H), 4.13 (t, *J* = 6.0 Hz, 2H), 3.95 (m, 2H), 3.68 (dd, *J* = 4.4 Hz, 12.4 Hz, 1H), 2.50 (t, *J* = 11.0 Hz, 2H), 2.20 (m, 2H), 2.00 (m, 2H), 1.31-1.24 (m, 22H), 0.88 (t, *J* = 6.8 Hz, 3H). ¹³C NMR (CDCl₃): δ 173.6, 172.5, 157.9, 134.7, 129.7, 128.1, 127.6, 126.5, 124.2, 123.5, 120.1, 106.3, 70.8, 66.7, 66.3, 63.5, 62.8, 58.5, 54.4, 34.0, 31.9, 30.8, 29.69, 29.64, 29.50, 29.34, 29.28, 29.10, 24.8, 24.5, 22.7, 14.1. FAB-MS 620 (MH⁺); HRMS calcd for (C₃₄H₄₉F₃N₃O₄)⁺ 620.3675, found 620.3666.

References

- (1). Landini, D.; Montanri, F.; Rolla, F. *Synthesis* **1978**, 771-773.
- (2). Weber, T.; Brunner, J. *J. Am. Chem. Soc.* **1995**, *117*, 3084-3095.
- (3). Li, G.; Bittman, R. *Tetrahedron Lett.* **2000**, *41*, 6737-6741.

- (4). Hatanaka, Y.; Hashimoto, M.; Kurihara, H.; Nakayama, H.; Kanaoka, Y. *J. Org. Chem.* **1994**, *59*, 383-387.

Part 2. Synthesis of a Photoactivatable Fluorescent Phosphatidylcholine Bearing an Azido-naphthalimide Moiety

Abstract

A very concise synthesis of photoactivatable fluorescent phosphatidylcholine probe **5** is presented in this part. An aryl azido group was the photoactive group, whereas the fluorophore was 1,8-naphthalimide. *N*-(10-Carboxydecyl)-4-azido-1,8-naphthalimide (**4**) was synthesized in three steps in 58% overall yield and with an easy work-up procedure. This photoactivatable fluorescent fatty acid is a useful precursor for many bioactive probes, as exemplified here for phosphatidylcholine probe **5**.

Introduction

Aryl azides have been the most frequently used photoactivatable probes; they were discovered in 1969 by Knowles et al.¹ Photolysis with UV converts an aryl azide (with loss of nitrogen) to an aryl nitrene, which is highly reactive and may bind covalently to nearby residues in biological molecules. Many integral membrane proteins have been labeled with radiolabeled aryl azides.²

In this chapter, 1,8-naphthalimide was chosen as the fluorophore for the design of a highly fluorescent aryl nitrene precursor of a phospholipid derivative. The selection of this fluorophore is based on the following considerations: (1) its excellent fluorescence intensity, (2) its ease of synthesis, and (3) its moderate polarity.

It was reported that 1,8-naphthalimides bearing electron-withdrawing groups exhibit low fluorescence intensity, whereas 1,8-naphthalimides and their derivatives with

electron-donating groups show very strong fluorescence intensity.³ 4-Azido-1,8-naphthalimido-PC **5** was synthesized for use in covalent modification of proteins that bind to PC, with detection based on fluorescence.

Results and Discussion

Scheme 1. Synthesis of Photoactivatable Fluorescent Azido Phosphatidylcholine

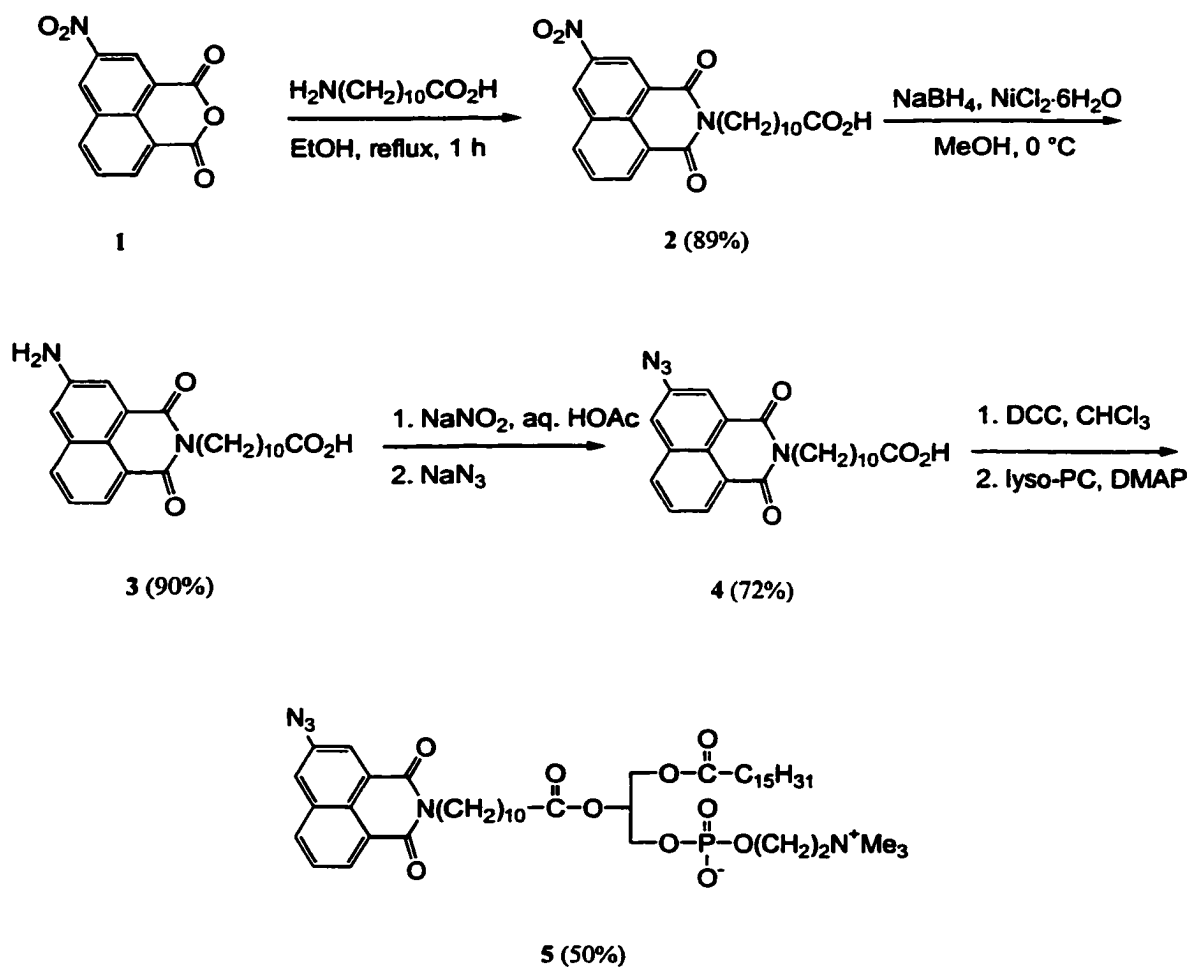


Figure 2. Excitation Spectrum of Azido-PC 5 at $\lambda_{em} = 415$ nm

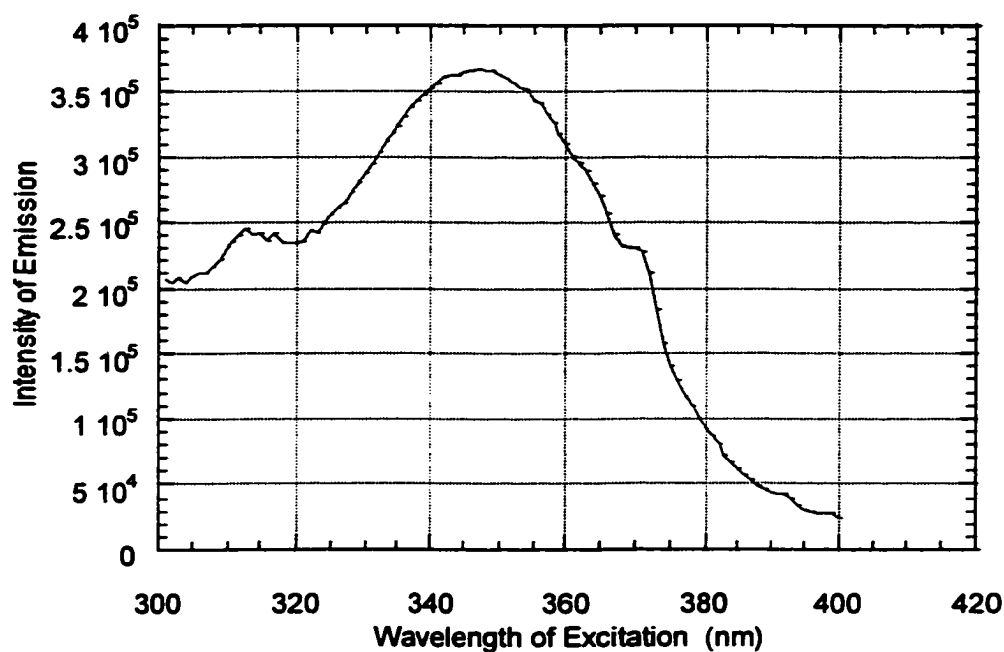
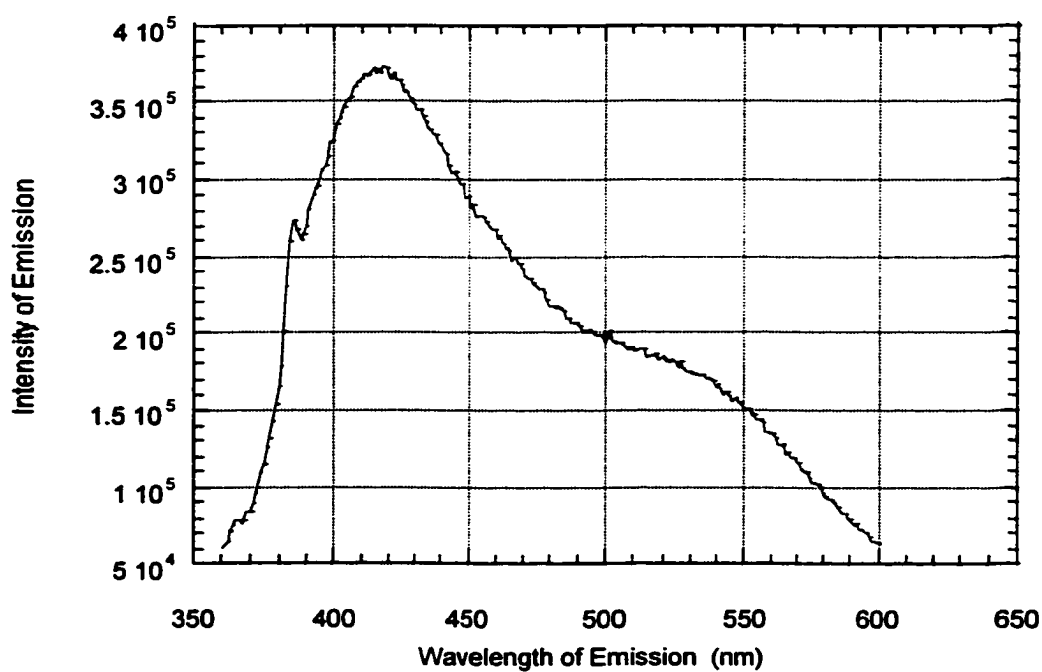


Figure 3. Emission Spectrum of Azido-PC 5 at $\lambda_{ex} = 347$ nm



4-Nitro-1,8-naphthalimide derivative **2** was obtained in 89% yield from commercially available 3-nitro-1,8-naphthalic anhydride (**1**) and 11-aminoundecanoic acid by heating in ethanol at reflux for 1 h. Nitro compound **2** did not fluoresce. However, reduction with sodium borohydride and $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ in MeOH at 0 °C afforded 4-amino-1,8-naphthalimide **3** (90% yield), which displayed a strong yellow fluorescence (excitation at $\lambda \sim 350$ nm). Aryl amine **3** was treated with sodium nitrite in 80% aqueous HOAc,⁴ followed by sodium azide, to form 4-azido compound **4** (72% yield), which displayed a weak fluorescence. The azido group is a weakly electron-withdrawing group. Finally, PC probe **5** was synthesized by acylation of lyso-PC.⁵ Figure 2 and 3 show the fluorescence properties of azido-PC **5**, which has $\lambda_{\text{max}}(\text{excitation})$ at 347 nm and $\lambda_{\text{max}}(\text{emission})$ at about 418 nm in MeOH.

Experimental Section

General procedures: See the previous chapter for detailed information.

***N*-(10-Carboxydecyl)-4-nitro-1,8-naphthalimide (**2**).** A solution of 3-nitro-1,8-naphthalic anhydride (2.43 g, 10.0 mmol) and 11-aminoundecanoic acid (2.11 g, 11.0 mmol) in 30 mL of absolute EtOH was heated to reflux for 1 h. After concentration, 100 mL of 1 N HCl was added to the residue. A yellow-brown solid product **2** (3.8 g, 89%) was collected by filtration; R_f 0.65 ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 9:1). ^1H NMR ($\text{DMSO}-d_6$): δ 11.99 (br s, 1H), 9.38 (d, $J = 2.0$ Hz, 1H), 8.91 (d, $J = 2.0$ Hz, 1H), 8.69 (d, $J = 8.4$ Hz, 1H), 8.59 (d, $J = 7.2$ Hz, 1H), 7.99 (t, $J = 8.0$ Hz, 1H), 3.99 (t, $J = 7.2$ Hz, 2H), 2.16 (t, $J = 7.2$ Hz, 2H), 1.63-1.23(m, 16H). MS calcd for $(\text{C}_{23}\text{H}_{26}\text{N}_2\text{O}_6+\text{H})^+$ 427.18, found 427.1.

***N*-(10-Carboxydecyl)-4-amino-1,8-naphthalimide (3).** To a solution of **2** (820 mg, 1.92 mmol) and $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ (877 mg, 3.84 mmol) in 70 mL of MeOH was added sodium borohydride (291 mg, 7.70 mmol) portionwise at 0 °C. The mixture was stirred for 1 h at this temperature and then filtered. To the residue obtained by concentration was added 100 mL of 1 N HCl. Filtration, washing with H_2O , and drying (high vacuum) afforded 689 mg (90%) of product **3** as a solid; R_f 0.40 ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 9:1). ^1H NMR (CD_3OD): δ 8.18 (d, J = 7.6 Hz, 1H), 8.02 (d, J = 6.4 Hz, 1H), 7.96 (d, J = 8.4 Hz, 1H), 7.59 (t, J = 8.0 Hz, 1H), 7.35 (d, J = 2.4 Hz, 1H), 4.10 (t, J = 7.4 Hz, 2H), 2.26 (t, J = 7.6 Hz, 2H), 1.69-1.58 (m, 4H), 1.38-1.30 (m, 12H). MS calcd for $(\text{C}_{23}\text{H}_{28}\text{N}_2\text{O}_2 + \text{H})^+$ 397.20, found 397.1. The fluorescence spectra showed a λ_{max} (excitation) at 421 nm and λ_{max} (emission) at 549 nm in MeOH.

***N*-(10-Carboxydecyl)-4-azido-1,8-naphthalimide (4).** To a solution of **3** (218 mg, 0.55 mmol) in 50 mL of 80% aq. HOAc was added sodium nitrite (46 mg, 0.66 mmol) at 0 °C. After the reaction mixture was stirred for 30 min, sodium azide (54 mg, 0.83 mmol) was added. The reaction mixture was stirred for 4 h and concentrated. To the residue was added 10 mL of 1 N HCl. The solid was collected by filtration. The product (168 mg, 72%) was purified by chromatography (elution with CH_2Cl_2 , then with $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 95:5); R_f 0.60 ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 9:1). ^1H NMR (CDCl_3): δ 11.18 (br s, 1H), 8.41 (d, J = 7.2 Hz, 1H), 8.10 (d, J = 2.4 Hz, 1H), 8.01 (d, J = 8.4 Hz, 1H), 7.67 (t, J = 7.2 Hz, 1H), 7.63 (d, J = 2.4 Hz, 1H), 4.08 (t, J = 7.6 Hz, 2H), 2.30 (t, J = 7.6 Hz, 2H), 1.68-1.57 (m, 4H), 1.37-1.25 (m, 12H). ^{13}C NMR (CDCl_3): δ 179.6, 163.3, 162.9, 139.1, 132.3, 132.2, 130.0, 127.7, 125.2, 124.2, 122.8, 122.4, 120.8, 40.6, 34.1, 29.4, 29.34, 29.27, 29.18, 29.0, 28.0, 27.1, 24.7. MS calcd for $(\text{C}_{23}\text{H}_{26}\text{N}_4\text{O}_4 + \text{H})^+$ 423.20, found 423.1.

1-*O*-Palmitoyl-2-*O*-[11-(4-azido-1,8-naphthalimido)undecanoyl]-*sn*-glycero-3-phosphocholine (5). To a solution of **4** (106 mg, 0.25 mmol) in 5 mL of CHCl₃ was added 0.75 mL (0.75 mmol) of a 1.0 M solution of DCC in CH₂Cl₂. The mixture was stirred for 4 h at room temperature. Then, DMAP (50 mg, 0.40 mmol) and lyso-PC (50 mg, 0.10 mmol) in 5 mL of alcohol-free, dry CHCl₃ were added to the reaction mixture. The mixture was stirred in a water bath at room temperature overnight. The reaction mixture was concentrated and passed through a TMD-8 column (elution with THF/H₂O 9:1). The residue obtained after concentration was purified by chromatography (elution with CHCl₃, then with CHCl₃/MeOH 10:1, finally with CHCl₃/MeOH/H₂O 65:25:4), affording 45 mg (50%) of PC **5** after removal of suspended silica gel by filtration through a Gelman 0.25- μ m filter; *R_f* 0.30 (CHCl₃/MeOH/H₂O 65:25:4). ¹H NMR (CDCl₃): δ 8.49 (d, *J* = 7.6 Hz, 1H), 8.22 (d, *J* = 2.0 Hz, 1H), 8.08 (d, *J* = 8.0 Hz, 1H), 7.73 (t, *J* = 7.6 Hz, 2H), 5.18 (br s, 1H), 4.40-4.08 (m, 5H), 3.93 (br s, 2H), 3.82 (br s, 2H), 3.36 (br s, 10H), 2.29-2.23 (m, 4H), 1.71-1.55 (m, 6H), 1.39-1.22 (m, 36H), 0.85 (t, *J* = 6.8 Hz, 3H). MS calcd for (C₄₇H₇₄N₅O₁₀P+H)⁺ 900.52, found 901.2. The fluorescence spectra showed a λ_{max} (excitation) at 347 nm and λ_{max} (emission) at 418 nm in MeOH.

References and Notes

- (1). Fleet, G. W. J.; Porter, R. P.; Knowles, J. R. *Nature* **1969**, *224*, 511-512.
- (2). Kotzyba-Hibert, F.; Kapfer, I. Goeldner, M. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1296-1312.
- (3). Middleton, R.; Parrick, J.; Wardman, P. *J. Heterocyclic Chem.* **1986**, *23*, 849-855.
- (4). Kalai, T.; Rozsnyai, B.; Jerkovich, G.; Hideg, K. *Synthesis* **1994**, 1079-1082.
- (5). See the procedure for compound **7** in Part 1 of this chapter.

Chapter 3

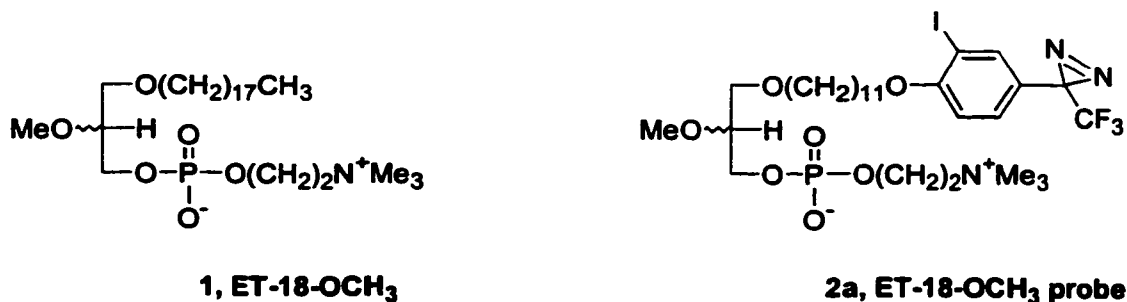
Part 1. Synthesis and Antiproliferative Properties of a Photoactivatable

Analog of ET-18-OCH₃

Abstract

A photoreactive analog of the antitumor ether lipid 1-*O*-octadecyl-2-*O*-methyl-*rac*-glycero-3-phosphocholine (ET-18-OCH₃, **1**) was synthesized for the first time. The 3-(trifluoromethyl)-3-(*m*-iodophenyl)diazirine (TID) moiety was used as the photolabel in ether lipid **2a**. The TID group was tethered through an *O*-undecyloxy linkage to the *sn*-1 position of the ether lipid. Compound **2a** was found to qualitatively mimic the antiproliferative effects of **1** in the dark (i.e., in the absence of carbene generation), suggesting that this photoactivatable probe may be suitable for identification of the proteins that mediate the biological activities of **1**. Analog **2a** was converted by Stille reaction to stannane intermediate **2b**. On *ipso* [¹²⁵I]-iododestannylation of **2b**, [¹²⁵I]-labeled ether lipid **2c** was obtained. Photolysis of **2c** in the presence of cytosolic proteins isolated from MCF-7 cells indicated that **2c** has utility in the characterization of the putative proteins that interact specifically with antitumor ether lipid **1**.

Chart 1. ET-18-OCH₃ and Its Photoactivatable Analog **2a**



Introduction

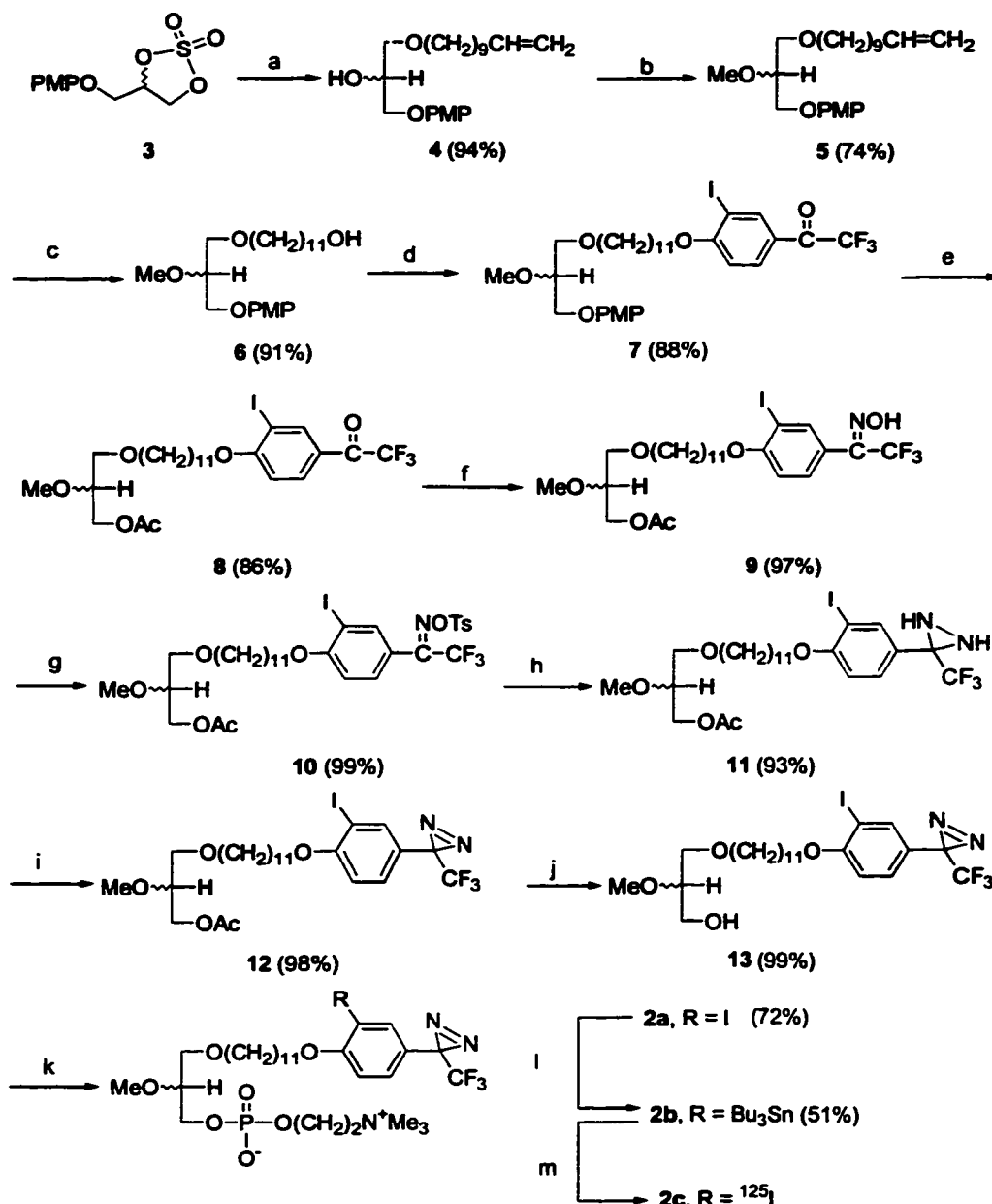
The antitumor ether lipid (AEL) 1-*O*-octadecyl-2-*O*-methyl-glycero-3-phosphocholine (1), also known as ET-18-OCH₃ and edelfosine, inhibits tumor cell growth by a multitude of processes.¹⁻³ Like other alkyl-lysophospholipids and unlike many other anticancer agents, 1 does not directly target cellular DNA. Although the principal mechanism of action of ET-18-OCH₃ has not yet been fully established, it is clear that the drug perturbs intracellular signaling transduction events after it is taken up spontaneously into cells.⁴ It was shown that ET-18-OCH₃ inhibits the translocation of Raf-1 to the plasma membrane of the mammary epithelial cancer cell line MCF-7, thereby directly blocking Ras-dependent activation of Raf-1 and subsequently blocking mitogen-activated protein (MAP) kinase activity and the Raf-1/MEK/ERK cascade.^{5,6} ET-18-OCH₃ is also implicated in the c-Jun kinase (JNK) signaling pathway, since it activated JNK in leukemic cells.⁷ These findings raise the possibility that the antiproliferative effects of ET-18-OCH₃ may arise, at least in part, from its ability to interfere with the activities of key proteins in various mitogenic signaling pathways of cells that are sensitive to ET-18-OCH₃. To examine protein-ET-18-OCH₃ interactions and thereby enhance our understanding of the drug's mechanism(s) of action, we seek to identify the intracellular signaling proteins that are inhibited by ET-18-OCH₃.

Photoactivatable analogs of biologically active compounds are an attractive means for determining the macromolecular targets of the parent compounds.⁸ Photoreactive analogs generate highly reactive intermediates on activation with UV light. Carbene precursors have been used extensively to identify ligand-binding domains. The 3-trifluoromethyl-3-*m*-iodophenyl-3*H*-diazirine (TID) moiety is readily incorporated into

the hydrophobic region of membranes,⁹ and is thus a useful probe for the study of lipid-protein interactions.^{8,10} This chapter presents the first synthesis of a photoactivatable probe of ET-18-OCH₃ (**2a**), which bears a trifluoromethyl¹¹ iodophenoxydiazirine (TID) group at the terminus of the *sn*-1 *O*-alkyl moiety. To test whether the TID photolabel in **2a** perturbs its biological activities as an antitumor agent, the antiproliferative activity of **2a** with that of the parent compound (**1**) were compared. Thus, Professor Gilbert Arthur's group (University of Manitoba, Winnipeg, Canada) incubated a variety of epithelial cancer cell lines with **2a** in the dark, i.e., under conditions in which photoactivation of **2a** to produce the reactive carbene functionality is avoided. Their findings indicate that probe **2a** mimics the antiproliferative properties of **1** in the epithelial cancer cells tested. To illustrate the utility of the photoactivatable probe, **2a** was converted (at the University of Manitoba) in two steps into **2c**, the radioiodinated form of **2a**. Compound **2c** was activated with UV light in the presence of cytosolic proteins derived from MCF-7 cells. Two proteins were prominently photolabeled; moreover, the extent of labeling was reduced markedly in competition experiments carried out with an excess of **2a**, indicative of a specific interaction with cellular proteins.

Results and Discussion

Probes **2a** and **2c** were synthesized as follows. Ring-opening reactions of 1-(4'-methoxyphenyl)-*rac*-glycerol 2,3-cyclic sulfate (**3**)¹² with long-chain nucleophiles were recently shown to provide a convenient entry to protected glycerol derivatives.^{12,13} The reaction of lithium 11-undecenoxide (2 equiv) with racemic cyclic sulfate **3** proceeded smoothly in THF at 0 °C to give the *p*-methoxyphenyl (PMP)-protected glycerol

Scheme 1. Synthesis of Photoactivatable ET-18-OCH₃ Analog 2


a. $\text{CH}_2=\text{CH}(\text{CH}_2)_9\text{OH}$, $n\text{-BuLi}$, THF; b. NaH , CH_3I , Bu_4NBr , THF; c. (i) 9-BBN, (ii) NaOH , H_2O_2 ; d. 17 (Scheme 2), Ph_3P , DIAD, THF, rt, 18 h; e. (i) CAN , $\text{CH}_3\text{CN-H}_2\text{O}$ (2:1), (ii) Ac_2O , DMAP , Et_3N , Et_2O ; f. $\text{NH}_2\text{OH}\cdot\text{HCl}$, Py-EtOH (1:6), 50-60 °C, 30 h; g. $p\text{-TsCl}$, Et_3N , DMAP , CH_2Cl_2 ; h. liq. NH_3 , CH_2Cl_2 ; i. I_2 , Et_3N , MeOH ; j. NaOMe , MeOH ; k. (i) POCl_3 , Et_3N , -10 °C; (ii) choline tosylate, Py , CHCl_3 ; l. Bu_6Sn_2 , $\text{Pd}(\text{MeCN})_2\text{Cl}_2$, HMPA , 18 h, rt; m. Na^{125}I , chloramine-T, NaH_2PO_4

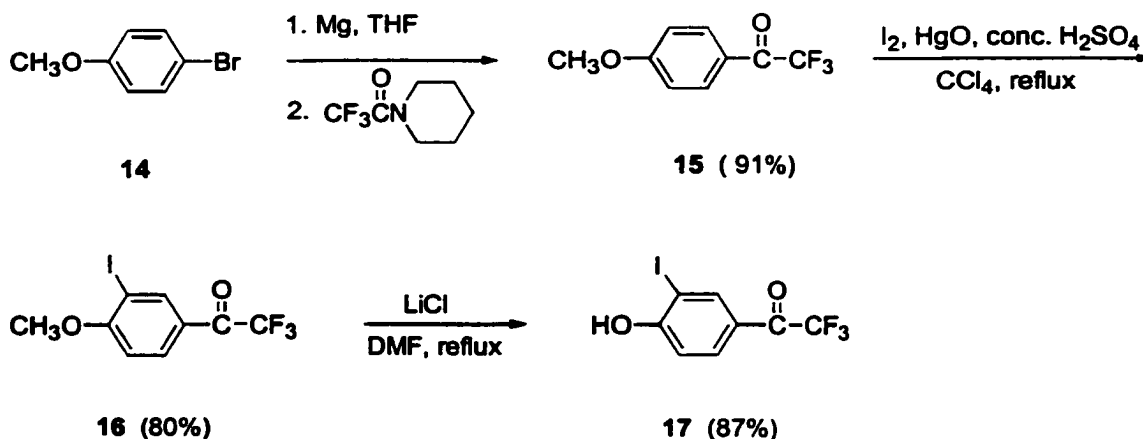
derivative **4** in 94% yield. After the unreacted 11-undecenol was removed by converting it into 11-undecenyl methyl ether, alcohol **4** was *O*-methylated under phase-transfer conditions (excess NaH, MeI, cat. Bu₄NBr). Hydroboration of methoxy derivative **5** using 9-borabicyclo[3.3.1]nonane (9-BBN) afforded ω-alcohol **6**. Mitsunobu reaction¹⁴ (DIAD/Ph₃P) of phenol derivative **15** (prepared as outlined in Scheme 2) with primary alcohol **6** afforded the coupling product **7** in 88% yield. The PMP ether group in **7** was removed with CAN in aqueous acetonitrile to give the free C-3 alcohol, which was acetylated to give **8** in 86% overall yield. The trifluoroacetyl group of acetate **8** was converted into the trifluoromethyldiaziriny group in **12** by using conventional procedures.¹⁵ Base-catalyzed hydrolysis of the acetate afforded alcohol **13** in virtually quantitative yield. Insertion of the phosphocholine moiety by using phosphorus oxychloride followed by choline tosylate¹⁶ gave product **2a**. Aryl iodide **2a** was subjected to Stille coupling¹⁷ with hexa-*n*-butylditin (bis(tributyltin)) and catalytic Pd(MeCN)₂Cl₂ at room temperature, affording aryltin intermediate **2b** in 51% yield.¹⁸ The latter is stable for months at -20 °C. To illustrate the utility of **2a** as a photoactivatable label, radioiodine was incorporated in Professor Gilbert Arthur's laboratory (University of Manitoba, Winnipeg, Canada) by *ipso* destannylation of **2b** using Na¹²⁵I with chloramine-T in buffered aqueous ethanol solution. Fractionation on solid-phase extraction cartridges (aminopropyl and reversed phase silica) provided product **2c**.

Aryl iodide **17** was synthesized as follows. As shown in Scheme 2, trifluoroacetylation of the Grignard reagent of **14** with *N*-(trifluoroacetyl)piperidine provided trifluoroacetophenone analog **15** in high yield. Electrophilic aromatic

iodination of **15** took place with very high regioselectivity; the methoxy and trifluoroacetyl groups both direct the iodo group to the desired position. *O*-Demethylation of anisole **16** with lithium chloride in DMF at reflux provided precursor **17** in high yield. Phenol **17** was coupled to alcohol **6** via Mitsunobu reaction (Scheme 1); it can also be coupled to primary alkyl halides via its phenolate group.

In this synthetic procedure, an ether linkage was chosen as the means of coupling the aryl trifluoromethyldiazirine moiety to the *rac*-1 *O*-alkyl chain of the probe. An advantage of using an ether linkage to tether the carbene precursor to the lipid is that this linkage affords resistance to chemical and enzymatic hydrolysis. However, this linkage has the disadvantage of introducing a polar atom into the hydrophobic region of the molecule, which is accommodated in the interior region of the membrane bilayer. In Part 2 of this chapter, an improved C-C linker is described.

Scheme 2. Synthesis of Compound 17



The synthesis of the new photoactivatable analog of **1** features several noteworthy steps: (a) regioselective introduction of an iodine atom into the aromatic ring of compound **15** (Scheme 2) in the presence of excess HgO, affording aryl iodide **16** in high yield;¹⁹ (b) *O*-demethylation of **16**, followed by Mitsunobu coupling of phenol **17** with ω -alcohol **6** (Scheme 1); (c) Stille coupling to replace the nonradiolabeled iodide in **2a** with a tin-containing group, affording the stable intermediate **2b**; and (d) chloramine-T mediated *ipso* iododestannylation of **2b**, giving radioiodinated **2c**.

The synthetic sequence used to prepare **2a** can be readily modified to prepare a range of photoactivatable compounds carrying linkers with different chain lengths at the *sn*-1 position coupled to the aryldiazirine moiety.

The biological studies were carried out in Professor Gilbert Arthur's laboratory, University of Manitoba, Winnipeg, Manitoba, Canada.

Epithelial cancer cells derived from breast (MCF-7, MDA-MB-231, MDA-MB-468, BT549), lung (A549), and prostate (DU145) were incubated with **2a** for 96 h. As shown in Figure 1, the order of decreasing susceptibility to the cytotoxic effects was: MDA-MB-231 > MCF-7 > DU145 = MDA-MB-468. Under the same experimental conditions, **2a** was not cytotoxic toward BT549 and A549, but the proliferation of the cells was reduced to approximately 20% of the controls. Table 1 presents the IC₅₀ values (drug concentrations required to inhibit growth by 50%) found for incubation of the cells with **2a** for 48 h and 96 h. Also shown are the IC₅₀ values obtained when the cells were incubated with ET-18-OCH₃ (**1**) for 48 h. An incubation period of 96 h with **2a** was needed to obtain IC₅₀ values approaching those of the parent compound (**1**). While the reason for this is unclear, we speculated that the presence of polar atoms in the

hydrophobic region of **2a** and/or the shorter *sn*-1 chain length in **2a** than in **1** may reduce the rate of uptake of the compound and its accumulation in the cells; thus compound **21** was synthesized (described in Part 2). Nevertheless, the above results indicate that **2a** is an effective probe since it retains the antiproliferative and cytotoxic activity of the parent compound (**1**).²⁰

Figure 2 shows the results of radioiodination of MCF-7 cytosolic proteins in the presence of **2c** (lane 1). Analysis by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) showed a prominent band of labeled protein with a molecular mass (*Mr*) of about 47 kDa. Another protein with a *Mr* of about 170 kDa was also present. Both of these bands were markedly reduced in intensity when an excess of **2a** was present (lanes 2 and 3), indicating that the interaction of **2c** with these proteins is specific. A band having *Mr* of about 62-kDa is also apparent in the presence of excess **2a**.

TABLE 1. Growth Inhibitory Properties of ET-18-OCH₃ (1) and 2a on Epithelial Tumor Cells^a

Cell line	IC ₅₀ (μM)		
	ET-18-OCH ₃ (incubation period, 48 h)	2a (incubation period, 48 h)	2a (incubation period, 96 h)
MCF-7	2	17.5	7
MDA-MB-231	3	9	6
BT549	3.5	37	15
A549	8	37	23
MDA-MB-468	19	48	18
DU145	15	>50	9

^aCells were incubated with 1 or 2a for 48 h or 96 h, as indicated in the table. For details of the in vitro assay, see the Experimental Section and ref 23.

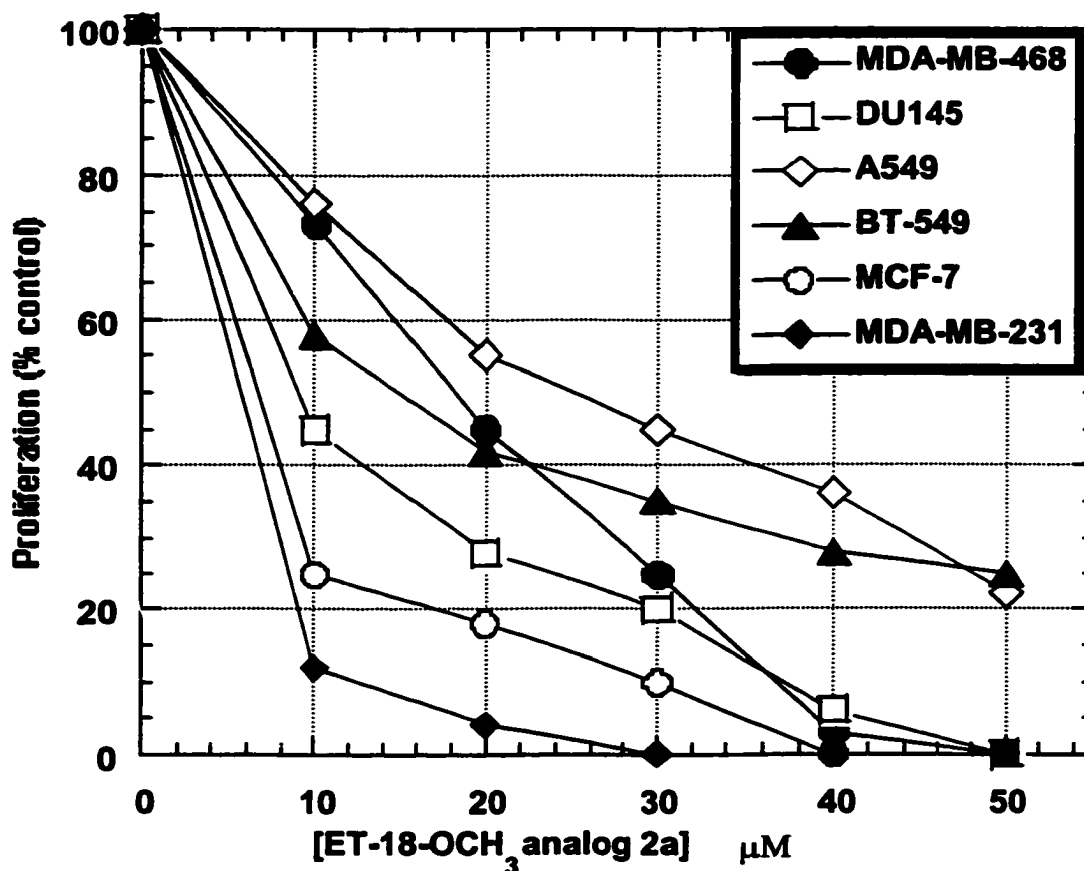


Figure 1. Effect of **2a** on the proliferation of epithelial cancer cell lines. Proliferating MCF-7, MDA-MB-468, MDA-MB-231, BT-549, A549, and DU145 cells were incubated in 24-well plates with medium containing **2a** (0-50 μ M). Cells in representative wells were counted on day 0 prior to the addition of **2a**. After 48 h, the medium was replaced with freshly prepared drug-containing media for another 48 h. The cell numbers were determined in each well, and the increase over day 0 for each concentration was expressed as a % of that in control wells with no drug. The results are the means of two different experiments with quadruplicate wells per experiment.

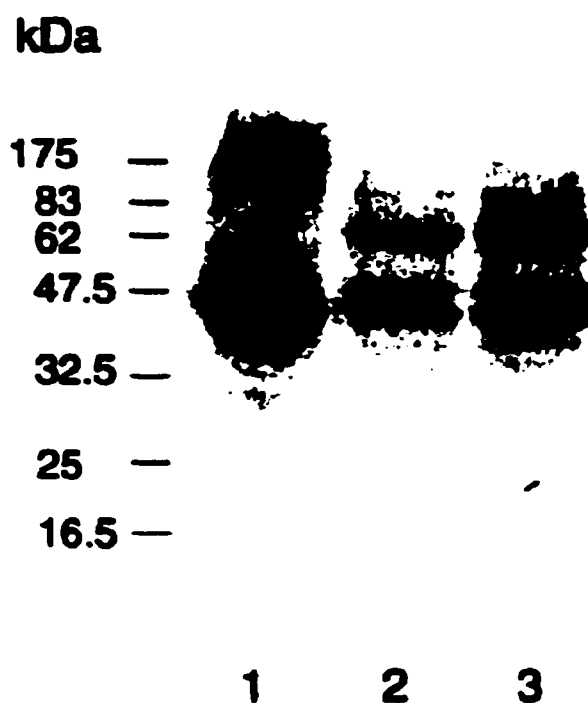


Figure 2. Radioiodination of MCF-7 Cytosolic Proteins by 2c. Cytosolic proteins were photolyzed with 2c (lane 1) and with 2c in the presence of a 200-fold excess (lane 2) and a 100-fold excess (lane 3) of 2a as described in the Experimental Section. Proteins were separated on 10% SDS gels and subjected to autoradiography.

Conclusions

The synthesis of 2a, the first photoactivatable probe of ET-18-OCH₃ (1), in 30% overall yield is described here. This model photoactivatable probe was found to qualitatively mimic the antiproliferative properties of the parent compound (1) against a variety of epithelial cancer cell lines in the dark. The ability of 2c to label cytosolic

proteins obtained from MCF-7 cells was also demonstrated. This study suggests that intracellular proteins that interact with AELs can be identified by photoactivatable labeling.

Experimental Section

General. See Chapter 1 for detailed information. TLC visualization was with 10% sulfuric acid solution in ethanol. Na^{125}I (17 Ci/mg) in 10^{-5} M NaOH (pH 8-11) was purchased from New England Nuclear Life Sciences. Aluminum foil was used to exclude light from the reaction flasks containing the TID probe. Exponentially growing MCF-7 cells were harvested, lysed by sonication, and the cytosol was obtained by centrifugation.⁵ The cytosolic proteins were irradiated for 30 min at room temperature in plastic trays (Accutran purchased from Schleicher & Schuell) with long-wavelength (365 nm) UV light (Mineralight lamp model UVGL-58, UVP, Inc.) at a distance of 1 cm from the lamp.

rac-1-(4'-Methoxyphenyl)glycerol 2,3-cyclic sulfate (**3**) was synthesized from *rac*-1-(4'-methoxyphenyl)glycerol as previously reported.¹²

1-O-(11'-Undecenyl)-3-O-(4'-methoxyphenyl)-*rac*-glycerol (4). To an ice-cooled solution of 11-undecenol (10.2 g, 60.0 mmol) in 250 mL of THF, 24 mL (60 mmol) of *n*-butyllithium (a 2.5 M solution in hexane) was added by a syringe under argon. The mixture was stirred for about 30 min until no more bubbles formed. A solution of 7.81 g (30 mmol) of cyclic sulfate **3** in 50 mL of THF was added dropwise at 0 °C. The mixture was stirred overnight at room temperature. To facilitate the workup of the product (product **4** and 11-undecenol have very similar R_f values), methyl iodide (8.5

g, 60 mmol) was added to convert the unreacted 11-undecenol into the corresponding methyl ether. After the reaction mixture was stirred for 10 h, a solution of 80 mL of concentrated H₂SO₄ in 400 mL of water was added. After the solution was stirred overnight, the product was extracted with Et₂O. The ether layer was washed with water, saturated aqueous sodium bicarbonate solution, saturated aqueous sodium thiosulfate solution, brine, and then dried (MgSO₄). The residue obtained after concentration was purified by chromatography (elution with hexane/EtOAc 6:1) to provide 9.8 g (94%) of **4** as a clear oil; *R_f* 0.35 (hexane/EtOAc 6:1). ¹H NMR: δ 6.85-6.79 (m, 4H), 5.82-5.76 (m, 1H), 5.00-5.89 (m, 2H), 4.13-4.10 (m, 1H), 3.96-3.94 (m, 2H), 3.75 (s, 3H), 3.57-3.44 (m, 4H), 2.04-1.99 (m, 2H), 1.61-1.54 (m, 2H), 1.35-1.25 (m, 12H). ¹³C NMR: δ 154.0, 152.7, 139.2, 115.5, 114.6, 114.1, 71.7, 71.4, 69.7, 69.1, 55.7, 33.8, 29.6, 29.5, 29.4, 29.1, 28.9, 26.1. DEI-MS 350 (M)⁺; HRMS calcd for (C₂₁H₃₄O₄)⁺ 350.2457, found 350.2461.

1-*O*-(11'-Undecenyl)-2-*O*-methyl-3-*O*-(4'-methoxyphenyl)-*rac*-glycerol (5).

To an ice-cooled solution of 7.65 g (21.8 mmol) of alcohol **4** in 200 mL of THF, 1.05 g (43.8 mmol) of sodium hydride was added. After about 40 min (when no bubbles were still formed), a solution of 9.29 g (66 mmol) of methyl iodide in 10 mL of THF and 0.5 g (1.6 mmol) of tetra-*n*-butylammonium bromide were added. The mixture was stirred for 20 h at room temperature, and then concentrated. After Et₂O (200 mL) and water (100 mL) were added, the aqueous layer was extracted with Et₂O (150 mL). The combined ether layer was washed with water and brine, and dried (MgSO₄). The product (5.92 g, 74%) was purified by chromatography (elution with hexane/EtOAc 20:1, then 10:1); *R_f* 0.65 (hexane/EtOAc 3:1). ¹H NMR: δ 6.86-6.79 (m, 4H), 5.82-5.75 (m, 1H), 4.99-4.89 (m, 2H), 4.03-3.07 (m, 2H), 3.74 (s, 3H), 3.74-3.67 (m, 1H), 3.60-3.56 (m, 2H), 3.49 (s,

3H), 3.45-3.42 (m, 2H), 2.04-1.98 (m, 2H), 1.56-1.51 (m, 2H), 1.36-1.24 (m, 12H). ^{13}C NMR: δ 153.8, 153.0, 139.2, 115.5, 114.5, 114.1, 78.8, 71.8, 69.9, 68.2, 58.2, 55.7, 33.8, 29.6, 29.5, 29.4, 29.1, 28.9, 26.1. DEI-MS 364 (M) $^{+}$; HRMS calcd for $(\text{C}_{22}\text{H}_{36}\text{O}_4)^{+}$ 364.2614, found 364.2612.

1-*O*-(11'-Hydroxyundecyl)-2-*O*-methyl-3-*O*-(4'-methoxyphenyl)-*rac*-glycerol

(6). To a solution of 1.82 g (5.0 mmol) of alkene **5** in 40 mL of THF, 15 mL (7.5 mmol) of a 0.5 M solution of 9-BBN in THF was added dropwise under argon. After the solution was stirred for 8 h at room temperature, the reaction was quenched with 1 mL of MeOH. A solution of 0.45 g of NaOH in 4 mL of water was added, and the mixture was cooled in an ice bath; then 2 mL of 30% H_2O_2 (18 mmol) was added dropwise. After the mixture was stirred overnight, THF was removed under reduced pressure, and the residue was extracted with Et_2O twice. The ether layer was washed with brine twice, dried (MgSO_4), and concentrated. Product **6** (1.84 g, 91%) was obtained by chromatography (elution with hexane/EtOAc 2:1) as a clear oil; R_f 0.10 (hexane/EtOAc 3:1). ^1H NMR: δ 6.90-6.83 (m, 4H), 4.07-4.17 (m, 2H), 3.79 (s, 3H), 3.79-3.61 (m, 5H), 3.54 (s, 3H), 3.50-3.46 (m, 2H), 1.61-1.55 (m, 4H), 1.47 (br s, 1H), 1.35-1.28 (m, 12H). ^{13}C NMR: δ 153.9, 153.0, 115.5, 114.6, 78.8, 71.8, 69.9, 68.3, 63.0, 58.2, 55.7, 32.8, 29.6, 29.5, 29.40, 29.38, 26.1, 25.7. DEI-MS 382 (M) $^{+}$; HRMS calcd for $(\text{C}_{22}\text{H}_{38}\text{O}_5)^{+}$ 382.2719, found 382.2707.

4-Methoxytrifluoroacetophenone (15) (Scheme 2) was synthesized as previously reported^{19b} in 91% yield.

3-Iodo-4-methoxy-trifluoroacetophenone (16). A mixture of 1.03 g (50 mmol) of **15**, 2.16 g (100 mmol) of HgO , 0.5 mL of concentrated H_2SO_4 , and 1.27 g (50 mmol) of iodine in 25 mL of CCl_4 was heated to reflux with vigorous stirring for 5 h. The

reaction mixture was cooled to room temperature, and then filtered through a pad of Celite. The filtrate was washed with 2 N aqueous sodium thiosulfate solution and brine, and then dried (Na_2SO_4). The residue obtained after evaporation was purified by column chromatography (elution with hexane/EtOAc 9:1), affording 1.32 g (80%) of the product as a white solid; mp 52-54 °C; R_f 0.22 (hexane/EtOAc 9:1). ^1H NMR: δ 8.47 (s, 1H), 8.04 (d, J = 8.8 Hz, 1H), 6.89 (d, J = 8.8 Hz, 1H), 3.98 (s, 1H). ^{13}C NMR: δ 177.9 (q, $J_{\text{C-F}}$ = 35 Hz), 163.6, 141.7, 132.7 (d, J = 2.1 Hz), 124.3, 116.6 (q, $J_{\text{C-F}}$ = 145 Hz), 110.3, 86.5, 56.9.

4-Hydroxy-3-iodo-trifluoroacetophenone (17). *O*-Demethylation of **16** was carried out as follows. A mixture of 3.35 g (10.0 mmol) of **16** and 1.3 g (31 mmol) of LiCl in 25 mL of DMF was heated at reflux under argon for 2 h. The reaction mixture was cooled to room temperature, poured into water (100 mL), and acidified with 10% HCl. The product was extracted with Et_2O (2 x 75 mL). The ethereal layer was washed with brine twice, dried (MgSO_4), and concentrated. The residue was dried under high vacuum, affording 2.8 g (87%) of **17** (R_f 0.45, CH_2Cl_2), which was used directly in the next step (see Scheme 1, step d). A small amount of the product was purified by chromatography (elution with CH_2Cl_2) to give a white solid; mp 87-88 °C. ^1H NMR: δ 8.40 (d, J = 0.4 Hz, 1H), 7.98 (d, J = 8.6 Hz, 1H), 7.09 (d, J = 8.8 Hz, 1H), 6.13 (s, 1H). ^{13}C NMR: δ 178.4 (q, $J_{\text{C-F}}$ = 35 Hz), 160.8, 141.2 (d, J = 1.6 Hz), 132.9 (d, J = 1.0 Hz), 125.9, 124.6, 118.0 (q, $J_{\text{C-F}}$ = 290 Hz), 115.3, 86.3. DEI-MS 316 (M^+); HRMS calcd for $(\text{C}_8\text{H}_4\text{F}_3\text{IO}_2)^+$ 315.9208, found 315.9208.

1-*O*-[11'-(2-(Iodo-4-(trifluoroacetyl)phenoxy)undecyl)]-2-*O*-methyl-3-*O*-(4'-methoxyphenyl)-*rac*-glycerol (7). To a mixture of 627 mg (1.64 mmol) of **6** was added

a solution of 588 mg (1.84 mmol) of 4-hydroxy-3-iodo-trifluoroacetophenone (17) and 504 mg (1.92 mmol) of Ph_3P in 12 mL of THF, followed by a solution of 400 mg (1.88 mmol, 95% purity) of diisopropyl azodicarboxylate (DIAD) in 3 mL of THF. The mixture was stirred for 18 h at room temperature. After concentration, the residue was purified by chromatography (elution with hexane/EtOAc 6:1) to afford 0.99 g (88%) of 7 as an oil; R_f 0.40 (hexane/EtOAc 3:1). ^1H NMR: δ 8.49 (d, $J = 1.7$ Hz, 1H), 8.03 (dd, $J = 7.6$ Hz, 0.8 Hz, 1H), 6.88-6.80 (m, 5H), 4.12 (t, $J = 6.4$ Hz, 2H), 4.05-4.00 (m, 2H), 3.76 (s, 3H), 3.69-3.59 (m, 3H), 3.51 (s, 3H), 3.48-3.44 (m, 2H), 1.89-1.86 (m, 2H), 1.61-1.51 (m, 4H), 1.38-1.26 (br m, 12H). ^{13}C NMR: δ 177.9 (q, $J_{\text{C-F}} = 35$ Hz), 163.2, 153.9, 153.0, 141.7, 132.7, 124.0, 115.5, 114.6, 111.0, 87.0, 78.8, 71.8, 69.91, 69.89, 68.2, 58.2, 55.7, 29.7, 29.6, 29.53, 29.45, 29.43, 29.2, 28.7, 26.1, 25.9. FAB-MS 680 (M) $^+$; HRMS calcd for $(\text{C}_{30}\text{H}_{40}\text{F}_3\text{IO}_6)^+$ 680.1822, found 680.1808.

1-*O*-[11'-(2-Iodo-4-(trifluoroacetyl)phenoxy)undecyl]-2-*O*-methyl-3-*O*-acetyl-*rac*-glycerol (8). To an ice-cooled mixture of 1.89 g (2.76 mmol) of PMP ether 7 in 40 mL of MeCN and 20 mL of water was added 4.76 g (8.69 mmol) of ammonium ceric(IV) nitrate (CAN). The mixture was stirred for 2.5 h at room temperature, and then stirred with aqueous 1 N $\text{Na}_2\text{S}_2\text{O}_3$ for 30 min. The mixture was extracted with Et_2O (2 x 150 mL), washed with brine, and dried (MgSO_4). After concentration, the residue was dissolved in 50 mL of anhydrous Et_2O . To the solution of the crude alcohol were added 1.5 g (14.9 mmol) of Et_3N , 25 mg (0.20 mmol) of DMAP, and 1.0 g (9.8 mmol) of acetic anhydride. The mixture was stirred overnight and then acidified with 2 N HCl. After the mixture was extracted with Et_2O (twice), the combined organic layers were washed with saturated aqueous sodium bicarbonate solution and brine, and dried (MgSO_4). The

product (1.46 g, 86% for two steps) was obtained as an oil by chromatography (elution with hexane/EtOAc 6:1, then 2:1); R_f 0.30 (hexane/EtOAc 3:1). ^1H NMR: δ 8.49 (d, J = 1.6 Hz, 1H), 8.03 (d, J = 8.6 Hz, 1H), 6.86 (d, J = 8.9 Hz, 1H), 4.25 (dd, J = 11.7 Hz, 3.8 Hz, 1H), 4.14-4.09 (m, 3 H), 3.53-3.50 (m, 1H), 3.48-3.46 (m, 2H), 3.43 (s, 3H), 3.43-3.39 (m, 2H), 2.05 (s, 3H), 1.87-1.82 (m, 2H), 1.55-1.48 (m, 4H), 1.36-1.23 (m, 12H). ^{13}C NMR: δ 177.9 (q, $J_{\text{C-F}}$ = 35 Hz), 170.9, 163.2, 141.6, 132.6, 124.0, 116.6 (q, $J_{\text{C-F}}$ = 290 Hz), 111.0, 87.0, 78.0, 71.8, 69.9, 69.7, 63.7, 58.0, 29.52, 29.49, 29.43, 29.41, 29.2, 29.1, 28.7, 26.0, 25.9, 20.9.

1-*O*-[11'-(2-Iodo-4-(trifluoroacetyloxime)phenoxy)undecyl]-2-*O*-methyl-3-*O*-acetyl-*rac*-glycerol (9). A mixture of 240 mg (0.39 mmol) of **8** and 32 mg (0.47 mmol) of hydroxylamine hydrochloride in 3 mL of EtOH and 0.5 mL of pyridine was heated at 50-60 °C for 30 h. The mixture was concentrated and partitioned between Et₂O and water. The ether layer was washed with 2 N HCl, saturated aqueous sodium bicarbonate solution, brine, and dried (MgSO₄). On concentration and drying under vacuum, 239 mg (97%) of **9** was obtained as an oil, which was pure enough to be used in the next step. ^1H NMR: (as a mixture of the anti and syn stereoisomers) δ 8.96-8.67 (two broad peaks, 1H), 7.95-7.89 (2d, 1H), 7.47-7.40 (2dd, 1H), 6.83-6.76 (2d, 1H), 4.22-4.01 (m, 4H), 3.71-3.40 (m, 8H), 2.06 (s, 3H), 1.81-1.80 (m, 2H), 1.59-1.49 (m, 4H), 1.33-1.17 (m, 12H).

1-*O*-[11'-(2-Iodo-4-(trifluoroacetyl)phenoxy)undecyl]-2-*O*-methyl-3-*O*-acetyl-*rac*-glycerol *O*-Tosyl Oxime (10). To an ice-cooled solution of 1.31 g (2.07 mmol) of syn and anti oximes **9**, 530 mg (5.3 mmol) of Et₃N, and 20 mg (0.16 mmol) of DMAP in 40 mL of dry CH₂Cl₂, *p*-TsCl (434 mg, 2.3 mmol) was added slowly. The

mixture was stirred at room temperature for about 1 h. After CH₂Cl₂ was added, the mixture was washed with saturated aqueous sodium bicarbonate solution, brine, and dried (MgSO₄). After concentration, 1.61 g (99%) of *O*-tosyl oxime **10** was obtained; *R*_f 0.80 (CH₂Cl₂/MeOH 10:1). ¹H NMR (CDCl₃) (two stereoisomers): δ 7.98-7.83 (m, 2H), 7.77-7.74 (m, 1H), 7.41-7.29 (m, 3H), 6.81-6.55 (2d, 1H), 4.24-4.20 (m, 1H), 4.10-4.00 (m, 3H), 3.51-3.38 (m, 8H), 2.44-2.43 (2s, 3H), 2.04 (s, 3H), 1.81-1.79 (m, 2H), 1.52-1.48 (m, 4H), 1.36-1.21 (m, 12H). FAB-MS 786 (MH)⁺; HRMS calcd for (C₃₂H₄₃F₃INO₈S+H)⁺ 786.1786, found 786.1738.

1-*O*-[11'-(2'-Iodo-4'-(3"-trifluoromethyldiaziridinyl)phenoxy)undecyl]-2-*O*-methyl-3-*O*-acetyl-*rac*-glycerol (11). To a solution of 1.50 g (1.91 mmol) of **10** in 35 mL of CH₂Cl₂ in a pressure tube was added liquid ammonia (30 mL) at -78 °C. After the mixture was stirred at room temperature for 2.5 h, the excess ammonia was evaporated, and the product was extracted with CH₂Cl₂. The organic layer was washed with water and brine, and dried (MgSO₄). The residue was purified by chromatography (elution with CH₂Cl₂/MeOH 20:1), providing 1.13 g (93%) of **11** as a viscous oil; *R*_f 0.60 (CH₂Cl₂/MeOH 10:1). ¹H NMR: δ 7.97 (d, *J* = 2.0 Hz, 1H), 7.51 (dd, *J* = 8.5 Hz, 1.8 Hz, 1H), 6.76 (d, *J* = 8.6 Hz, 1H), 4.25-4.21 (m, 1H), 4.10-4.06 (m, 1H), 4.00 (t, *J* = 6.3 Hz, 2H), 3.53-3.51 (m, 1H), 3.48-3.46 (m, 2H), 3.43 (s, 3H), 3.42-3.39 (m, 2H), 2.73 (d, *J* = 8.7 Hz, 1H), 2.16 (d, *J* = 8.7 Hz, 1H), 2.05 (s, 3H), 1.82-1.79 (m, 2H), 1.55-1.46 (m, 4H), 1.25 (br m, 12H). ¹³C NMR: δ 171.0, 158.9, 138.9, 129.5, 125.2, 123.4 (q, *J*_{C-F} = 277 Hz), 111.4, 86.5, 78.0, 71.8, 69.7, 69.4, 63.7, 58.0, 56.9 (q, *J*_{C-F} = 145 Hz), 29.53, 29.51, 29.47, 29.46, 29.42, 29.2, 28.9, 26.02, 25.97, 20.9.

1-*O*-[11'-(2'-Iodo-4'-(3"-trifluoromethyldiaziriny)phenoxy)undecyl]-2-*O*-methyl-3-*O*-acetyl-*rac*-glycerol (12). A mixture of 1.03 g (1.63 mmol) of 11, 457 mg (1.79 mmol) of iodine, and 412 mg (4.08 mmol) of Et₃N in 15 mL of MeOH was stirred at room temperature for 0.5 h. After 150 mL of Et₂O was added, the mixture was poured into 1 N Na₂S₂O₃ solution. The organic layer was washed with brine and dried (MgSO₄). The product (1.0 g, 98%) was obtained by chromatography (elution with CH₂Cl₂); R_f 0.70 (CH₂Cl₂/MeOH 50:1). ¹H NMR: δ 7.55 (d, *J* = 2.2 Hz, 1H), 7.16 (dd, *J* = 2.0 Hz, 8.6 Hz, 1H), 6.74 (d, *J* = 8.7 Hz, 1H), 4.25-4.21 (m, 1H), 4.10-4.07 (m, 1H), 3.98 (t, *J* = 6.4 Hz, 2H), 3.52-3.40 (m, 8H), 2.06 (s, 3H), 1.81-1.76 (m, 2H), 1.55-1.46 (m, 4H), 1.30-1.26 (m, 12H). ¹³C NMR: δ 170.9, 158.8, 137.6, 128.2, 122.4, 22.0 (q, *J* = 273 Hz), 111.5, 86.9, 78.1, 71.8, 69.8, 69.4, 63.7, 58.0, 29.55, 29.52, 29.46, 29.43, 29.2, 28.9, 27.4 (q, *J* = 40.5 Hz), 26.03, 25.96, 20.9.

1-*O*-[11'-(2'-Iodo-4'-(3"-trifluoromethyldiaziriny)phenoxy)undecyl]-2-*O*-methyl-*rac*-glycerol (13). To a solution of 1.0 g (1.59 mmol) of 12 in 25 mL of MeOH was added ~8 mg (0.33 mmol) of NaH. The mixture was stirred at room temperature for 2 h. After 3 drops of 10% HCl were added, the mixture was concentrated and the residue was extracted with Et₂O (2 x 75 mL). The ether layer was washed with brine and dried (MgSO₄). The product (0.92 g, 99%) was purified by chromatography (elution with CH₂Cl₂/MeOH 100:2.5); R_f 0.40 (CH₂Cl₂/MeOH 50:1). ¹H NMR: δ 7.54 (d, *J* = 2.3 Hz, 1H), 7.16 (dd, *J* = 2.2 Hz, *J* = 8.8 Hz, 1H), 6.74 (d, *J* = 8.6 Hz, 1H), 3.98 (t, *J* = 6.4 Hz, 2H), 3.73-3.63 (m, 2H), 3.53-3.50 (m, 2H), 3.44 (s, 3H), 3.437-3.39 (m, 3H), 1.83-1.76 (m, 2H), 1.56-1.44 (m, 4H), 1.44-1.23 (m, 12H). ¹³C NMR: δ 158.8, 137.6, 128.3, 122.4,

122.0 (q, $J_{\text{C-F}} = 273$ Hz), 111.5, 86.9, 79.8, 71.9, 70.6, 69.9, 69.4, 62.7, 57.7, 29.56, 29.51, 29.47, 29.46, 29.42, 29.2, 28.9, 27.5 (q, $J_{\text{C-F}} = 40.5$ Hz), 26.1, 26.0.

1-*O*-[11'-(2'-Iodo-4'-(3"-trifluoromethyldiaziriny)phenoxy)undecyl]-2-*O*-methyl-*rac*-glycero-3-phosphocholine (2a). A 50-mL flask was flame-dried and flushed with argon. A solution of 117 mg (0.76 mmol) of POCl₃ and 77 mg (0.76 mmol) of Et₃N in 0.5 mL of alcohol-free CHCl₃ was cooled to -10 °C. A solution of 360 mg (0.61 mmol) of 13 in 3 mL of CHCl₃ was added. After the mixture was stirred for 1 h, choline tosylate (250 mg, 0.91 mmol) and 0.5 mL of pyridine were added. The reaction mixture, which became orange, was stirred overnight at room temperature. After water (0.2 mL) was added to quench the reaction, the mixture was stirred for 30 min. The residue obtained after concentration was passed through a TMD-8 resin (elution with THF/H₂O 9:1). The fractions containing the product were combined, concentrated, and purified by chromatography (elution with CHCl₃/MeOH 4:1, then with CHCl₃/MeOH/H₂O 65:25:4). The product (330 mg, 72%) was obtained after lyophilization from benzene and filtration of a chloroform solution of the product through a 0.2-μm Millipore filter to remove suspended silica gel; R_f 0.18 (CHCl₃/MeOH/H₂O 65:25:4). ¹H NMR: δ 7.53 (d, $J = 2.1$ Hz, 1H), 7.16 (dd, $J = 8.5$ Hz, 2.2 Hz, 1H), 6.72 (d, $J = 8.6$ Hz, 1H), 4.23-3.74 (m, 9H), 3.45-3.30 (m, 16H), 1.78-1.73 (m, 2H), 1.46-1.40 (m, 4H), 1.27-1.20 (m, 12H). ¹³C NMR: δ 158.8, 137.5, 128.2, 122.4, 122.0 (q, $J_{\text{C-F}} = 273$ Hz), 111.5, 86.8, 79.6, 71.7, 70.2, 69.4, 66.3, 64.9, 59.2, 57.7, 54.3, 29.7, 29.6, 29.5, 29.2, 29.0, 28.9, 28.7, 27.5 (q, $J_{\text{C-F}} = 40.5$ Hz), 26.1, 26.0. FAB-MS 752 (MH)⁺, 724 (MH-N₂)⁺; HRMS calcd for (C₃₂H₄₆F₃IN₃PO₇+H)⁺ 752.2150, found 752.2189; UV (MeOH) λ_{max} (ϵ , M⁻¹cm⁻¹) 295 nm (1975), 375 nm (329) (See Figure 3.).

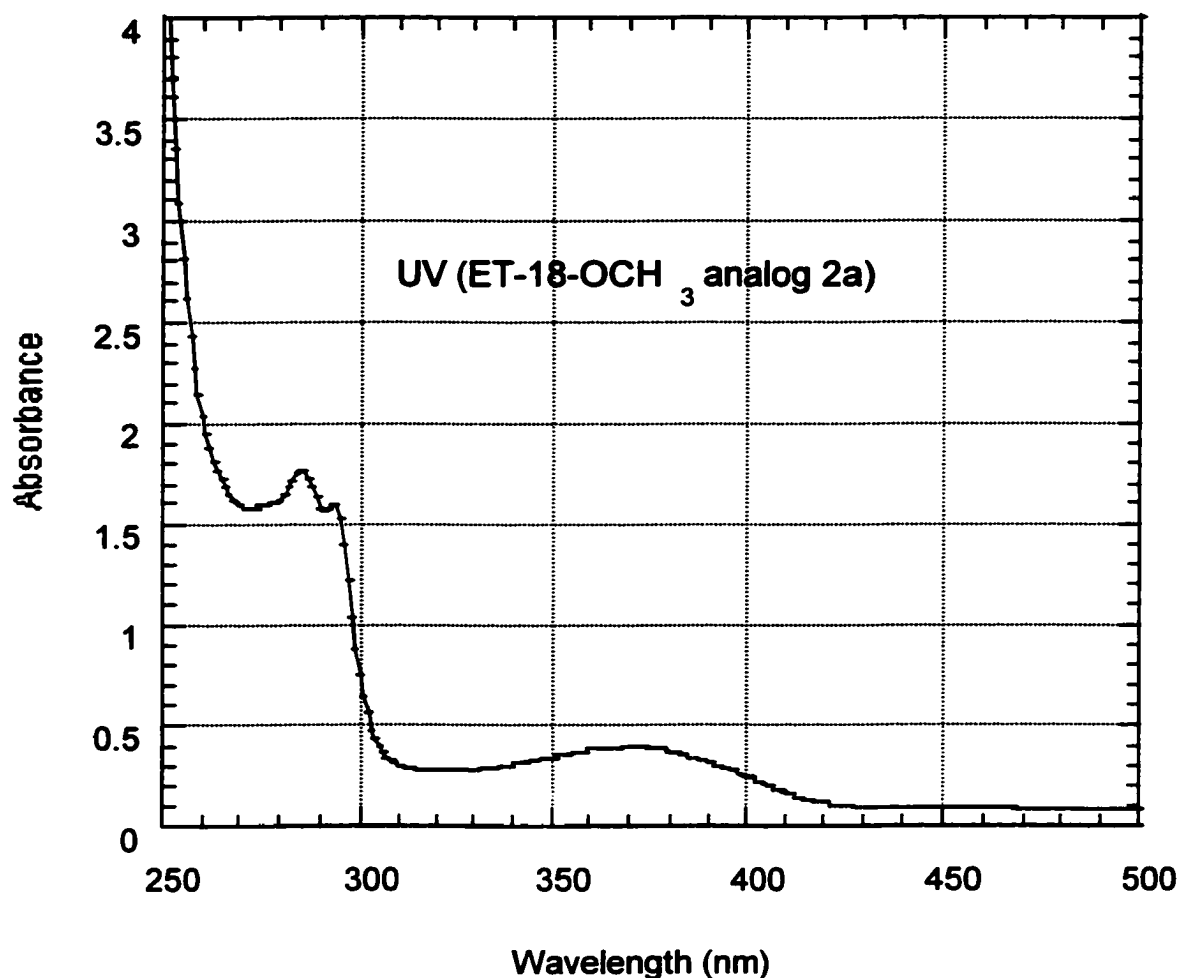


Figure 3. UV Spectrum of ET-18-OCH₃ Analog 2a in Absolute EtOH

1-*O*-[11'-(2'-(Tri-*n*-butylstannyl)-4'-(3''-trifluoromethyldiaziriny)phenoxy)-undecyl]-2-*O*-methyl-*rac*-glycero-3-phosphocholine (2b). A mixture of 48 mg (0.064 mmol) of **2a**, 371 mg (0.64 mmol) of hexa-*n*-butylditin, and 8.0 mg (0.031 mmol) of Pd(CH₃CN)₂Cl₂ in 3 mL of HMPA was stirred for 18 h at room temperature under argon. The reaction mixture was diluted with 5 mL of CHCl₃, and then passed through an

aminopropyl silica gel solid-phase extraction cartridge (500 mg) (elution with CHCl_3 to remove the solvent and unreacted hexabutylditin, then with $\text{CHCl}_3/\text{MeOH}$ 4:1). The filtrate was passed through another aminopropyl cartridge (elution with $\text{CHCl}_3/\text{MeOH}$ 4:1). After concentration, the residue was dissolved in a small volume of MeOH and loaded onto a C-18 cartridge (2 g) (elution with MeOH). The first part of the eluant consisted of starting material **2a** (9 mg), whereas the last part afforded 24.2 mg (51%) of the desired product. ^1H NMR: δ 7.14 (s, 1H), 7.07 (dd, $J = 2.0$ Hz, 8.7 Hz, 1H), 6.73 (d, $J = 8.6$ Hz, 1H), 4.28 (m, 2H), 3.89-3.77 (m, 6H), 3.50-3.34 (m, 17H), 1.73 (m, 2H), 1.51-1.25 (m, 28H), 1.03-0.87 (m, 6H), 0.85 (t, $J = 7.3$ Hz, 3H). ^{13}C NMR: δ 164.3, 135.0, 131.5, 128.5, 122.4 (q, $J_{\text{C-F}} = 275$ Hz), 120.8, 109.3, 79.7, 71.8, 70.4, 67.9, 66.4, 64.9, 59.3, 57.8, 54.4, 29.7, 29.63, 29.56, 29.46, 29.3, 29.2, 29.1, 29.0, 27.3, 26.14, 26.10, 13.6, 9.9.

1-*O*-[11'-(2'-[^{125}I]-4'-(3''-Trifluoromethyldiaziriny)phenoxy)undecyl]-2-*O*-methyl-*rac*-glycero-3-phosphocholine (2c). The following iododestannylation procedure was used to incorporate radioiodine. (This was carried out at the University of Manitoba.) To a 1.5-mL cone-shaped vial were added 100 μL (0.093 μmol) of a solution of arylstannane **2b** (1.7 mg of **2b** in 2 mL of absolute EtOH), 50 μL (10 μmol) of a solution of NaH_2PO_4 (276 mg of $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ in 10 mL of H_2O), 40 μL (1.3 μmol) of unlabeled NaI, and 10 μL of Na^{125}I (1 mCi). Then 50 μL (0.23 μmol) of chloramine-T (from a freshly prepared stock solution of 13 mg (0.046 mmol) of chloramine-T trihydrate in 10 mL of absolute EtOH) was added with stirring. The reaction mixture, which became pale yellow, was stirred for 30 min. The reaction was quenched with 50 μL of 30% aqueous NaHSO_3 solution. After 200 μL of chloroform was added, the

mixture was shaken and the bottom layer was collected and concentrated to dryness by using a stream of nitrogen. The residue was dissolved in 100 μL of CHCl_3 and loaded onto an aminopropyl solid-phase extraction cartridge (500 mg). The vial was washed with an additional 100 μL of CHCl_3 . The latter solution was also loaded onto the cartridge. Elution with 4 mL of CHCl_3 removed the byproducts (presumably tributyltin iodide and *p*-toluenesulfonamide); then elution with 4 mL of 1:1 $\text{CHCl}_3/\text{MeOH}$ afforded product **2c** in a single fraction.

Effects of Compounds 1 and 2a on Cell Proliferation.* A 50 mM stock concentration of **2a** was prepared in absolute EtOH and stored at $-80\text{ }^\circ\text{C}$ in the dark. The cells were maintained under subdued light to avoid carbene generation. The in vitro assays have been described previously.²¹⁻²⁴ Briefly, cells were subcultured into 24-well plates and allowed to grow to the exponential phase. The medium was changed and replaced with one containing **2a** (0-50 μM) or **1** (0-20 μM) in 10% fetal bovine serum supplemented medium. At the end of the incubation period (48 or 96 h), the cells were detached with trypsin and the cell numbers were determined with a Coulter counter. The increase in cell numbers was expressed as a percentage relative to those in wells without any drug. When the cells were incubated with **2a** for 96 h, the medium in all wells was replaced after 48 h with fresh medium. All manipulations were carried out in a partially darkened room.

Gel Electrophoresis and Autoradiography.* For radioiodination of MCF-7 cytosolic proteins, 2 μL of a solution of **2c** in EtOH (10 nmol, 2.2×10^6 cpm) was added to 500 μL of cytosolic proteins (1.2 mg of protein) in a 1.5-mL microfuge tube. Competition experiments were carried out in the presence of a 100- and a 200-fold excess

of **2a** in 4 μL of EtOH. The tubes were rotated in the dark at room temperature prior to photolysis. After photolysis, the samples were boiled at 100 °C for 5 min in 300 μL of Laemmli SDS sample buffer.⁵ The proteins were separated by SDS-PAGE on 10% gels, which were dried and exposed to film (Kodak X-OMAT AR) for 3 d.

* These studies were carried out in Professor Gilbert Arthur's laboratory, University of Manitoba, Winnipeg, Manitoba, Canada.

References and Notes

- (1). Lohmeyer, M.; Bittman, R. *Drugs Future* **1994**, *19*, 1021-1037.
- (2). Houlihan, W. J.; Lohmeyer, M.; Workman, P.; Chen, S. H. *Med. Res. Rev.* **1995**, *15*, 157-223.
- (3). Bittman, R.; Arthur, G. "Antitumor Ether Lipids: Biological and Biochemical Effects" In *Liposomes: Rational Design*; Janoff, A. S., Ed.; Marcel Dekker: New York; 1999, pp 125-144.
- (4). Arthur, G.; Bittman, R. *Biochim. Biophys. Acta* **1998**, *1390*, 85-102.
- (5). Zhou, X.; Lu, X.; Richard, C.; Xiong, W.; Lichtfield, D. W.; Bittman, R.; Arthur, G. *J. Clin. Invest.* **1996**, *98*, 937-944.
- (6). Samadder, P.; Arthur, G. *Cancer Res.* **1999**, *59*, 4808-4815.
- (7). Gajate, C.; Santos-Beneit, A.; Modolell, M.; Mollinedo, F. *Mol. Pharmacol.* **1998**, *53*, 602-612.
- (8). (a) Bayley, H. *Photogenerated Reagents in Biochemistry and Molecular Biology*; North-Holland/Elsevier: Amsterdam, 1983. (b) Brunner, J. *Annu. Rev. Biochem.* **1993**, *62*, 483-514.
- (9). Pérochon, E.; Leray, C.; Crémel, G.; Hubert, P. *Anal. Biochem.* **1997**, *254*, 109-118.
- (10). Brunner, J. *Methods Enzymol.* **1989**, *172*, 628-687.
- (11). The trifluoromethyl group suppresses photo-rearrangement to a linear diazo compound. Trifluoromethylaryldiazirines offer many advantages, e.g., high chemical

stability in subdued ambient light toward various mild reducing agents, oxidizing agents, acidic and basic conditions, and elevated temperatures.⁸ They are activated in the near UV ($\lambda > 360$ nm), avoiding protein-damaging wavelengths.

- (12). He, L.; Byun, H.-S.; Bittman, R. *J. Org. Chem.* **1998**, *63*, 5696-5699.
- (13). For a recent review of cyclic sulfate chemistry, see: Byun, H.-S.; He, L.; Bittman, R. *Tetrahedron* **2000**, *56*, 7051-7091.
- (14). For a review of the Mitsunobu reaction, see: Hughes, D. L. *Org. React.* **1992**, *42*, 335-656.
- (15). (a) Harter, C.; Bächli, T.; Semenza, G.; Brunner, J. *Biochemistry* **1988**, *27*, 1856-1864. (b) Weber, T.; Brunner, J. *J. Am. Chem. Soc.* **1995**, *117*, 3084-3095.
- (16). Deroo, P. W.; Rosenthal, A. F.; Isaacson, Y. A.; Vargas, L. A.; Bittman, R. *Chem. Phys. Lipids* **1976**, *16*, 60-70.
- (17). For a review of the Stille coupling reaction, see: Farina, V.; Krishnamurthy, V.; Scott, W. J. *Org. React.* **1997**, *50*, 1-652.
- (18). The bromo analog of **2a** failed to undergo the Stille reaction with hexaalkylditin (step 1 of Scheme 1) even at 60 °C.
- (19). In contrast, the use of arylthallation chemistry to prepare aryldiazirinyliodides proceeded in very low yield: (a) Brunner, J.; Senn, H.; Richards, F. M. *J. Biol. Chem.* **1980**, *255*, 3313-3318. (b) Hatanaka, Y.; Hashimoto, M.; Kurihara, H.; Nakayama, H.; Kanaoka, Y. *J. Org. Chem.* **1994**, *59*, 383-387.
- (20). Photoprobes having as much as 10³-fold lower bioactivity than the parent compound have been reported to still be useful: Dormán, G.; Prestwich, G. D. *Trends Biotechnol.* **2000**, *18*, 64-77.
- (21). Lu, X.; Arthur, G. *Cancer Res.* **1992**, *52*, 2806-2812.
- (22). Xi, Z.; Lu, X.; Arthur, G. *Anticancer Res.* **1992**, *12*, 1659-1662.
- (23). Ashagbley, A.; Samadder, P.; Bittman, R.; Erukulla, R. K.; Byun, H.-S.; Arthur, G. *Anticancer Res.* **1996**, *16*, 1813-1818.
- (24). Samadder, P.; Byun, H.-S.; Bittman, R.; Arthur, G. *Anticancer Res.* **1998**, *18*, 465-470.

Part 2. Synthesis of Photoactivatable ET-18-OCH₃ Analog 21

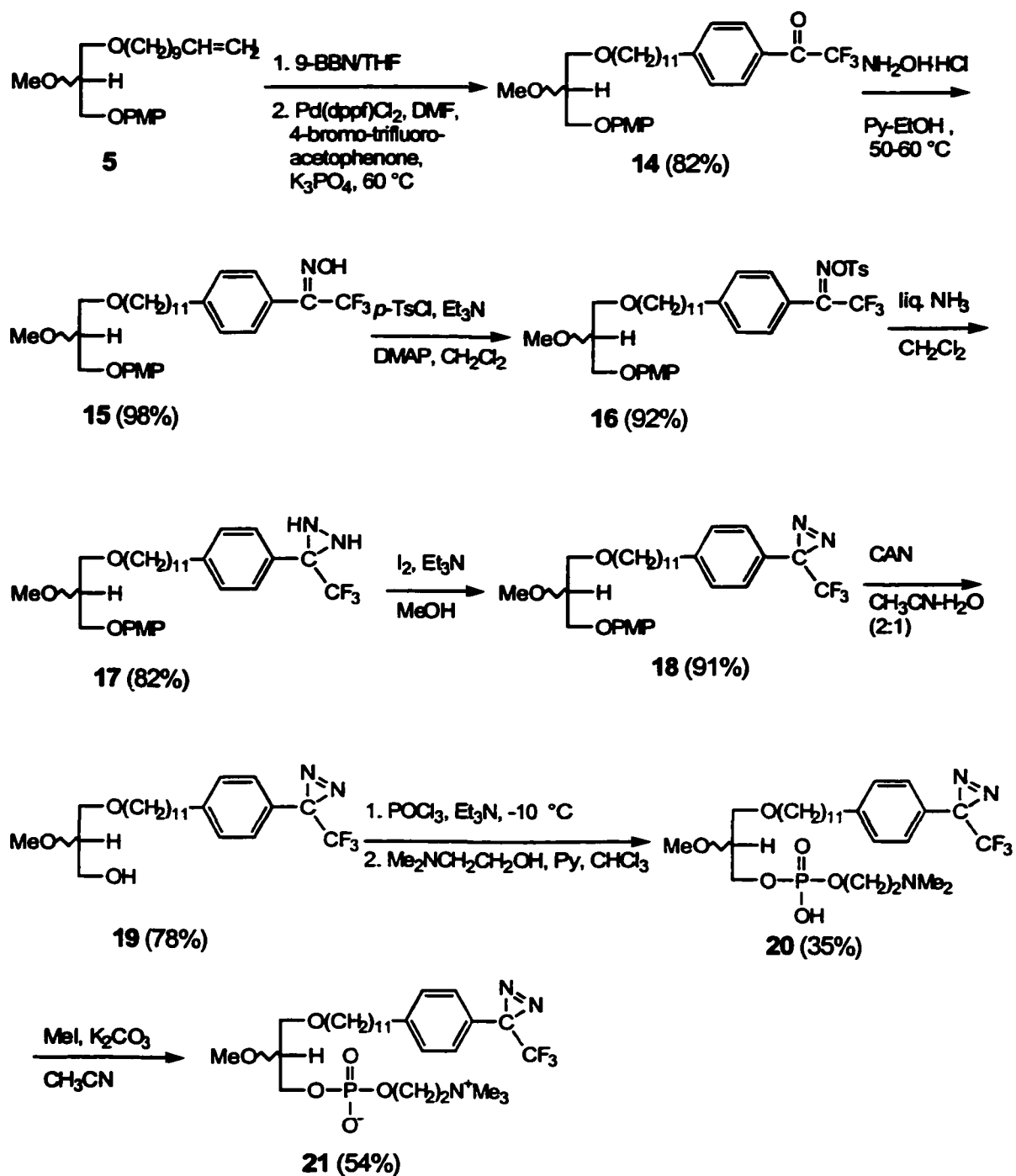
Abstract

A very potent ($IC_{50} = 5 \mu M$) antiproliferative ET-18-OCH₃ analog **21** was synthesized in a concise sequence. The following modifications were made in efforts to enhance the biological activity of analog **2a** (Part 1 of this chapter): (1) the phenoxy ether linkage was replaced by the less polar C-C bond linkage; (2) the iodo group was removed, and ³H rather than ¹²⁵I will be used as the radiolabel. Formation of ω -aryl-substituted glycerol derivative **14** by hydroboration of **5** with 9-BBN followed by Suzuki coupling with 4-bromo-2',2',2'-trifluoroacetophenone was the key step in the synthesis. Another improvement is that the *p*-methoxyphenyl ether protecting group was removed after formation of the trifluoromethyldiazirine group.

Results and Discussion

Part 1 of this chapter showed that **2a**, a photoactivatable analog of ET-18-OCH₃, had a lower potency in the dark (avoiding carbene generation) than that of the parent compound **1** with respect to inhibiting the proliferation of a panel of epithelial cancer cell lines.¹ The presence of an oxygen atom near the end of the *sn*-1 chain and/or the shorter length of the *sn*-1 chain in the analog may reduce the amount of the analog that is taken up into the cell membrane. In an effort to improve the antiproliferative potency of **2a**, and thus obtain a more suitable photoactivatable analog with which to study the mechanism of action of ET-18-OCH₃, the following modifications have been made:

Scheme 3. Synthesis of ET-18-OCH₃ analog 21



(1) the oxygen atom in the linker was deleted and (2) the iodine atom in the *m*-iodophenyl moiety was replaced with a hydrogen atom. (In work in progress, ^3H is being introduced into the choline group.) The antiproliferative data (Figure 4; data obtained in Prof. Arthur's laboratory at the University of Manitoba) show that the new analog **21** is nearly as potent against MCF-7 cells ($\text{IC}_{50} = 5 \mu\text{M}$, incubation for 48 h) in the dark as ET-18-OCH₃ ($\text{IC}_{50} \sim 2.0\text{--}2.5 \mu\text{M}$).

As shown in Scheme 3, the alkene precursor **5** was coupled with 4-bromo-2',2',2'-trifluoroacetophenone by Suzuki reaction^{2,3} to afford intermediate **14** in 82% yield. Through this key reaction, a diazirine precursor was attached at the end of the *sn*-1 chain by a C-C bond. The following standard steps^{4,5} were used to introduce the diazirine group into **18**: (a) formation of oxime **15** with hydroxylamine hydrochloride (98%); (b) tosylation of the oxime with *p*-TsCl and Et₃N, affording **16** in 92% yield; (c) formation of diaziridine **17** (82% yield) in liquid ammonia; and (d) oxidation with iodine to form diazirine **18** in 91% yield.

In the synthesis of the previous analog **2a** (Part 1), we were concerned about the possibility that the use of a radical oxidation reaction (CAN) could lead to decomposition of the diazirine. Therefore, the PMP group was converted to an acetyl group before formation of the diazirine functionality. In the preparation of analog **21**, an improvement is the deprotection of the PMP group with CAN after the formation of diazirine **18**. The good yield of **19** (78%) suggests that the diazirine group is able to tolerate CAN oxidation. In order to introduce the radioactive label (which is required for autoradiography), the phosphocholine moiety was installed in two steps:^{6,7} (a) dimethylamino phospholipid derivative **20** (35%) was prepared from alcohol **19** and (b)

Scheme 4. Proposed Introduction of [³H] into Probe 21

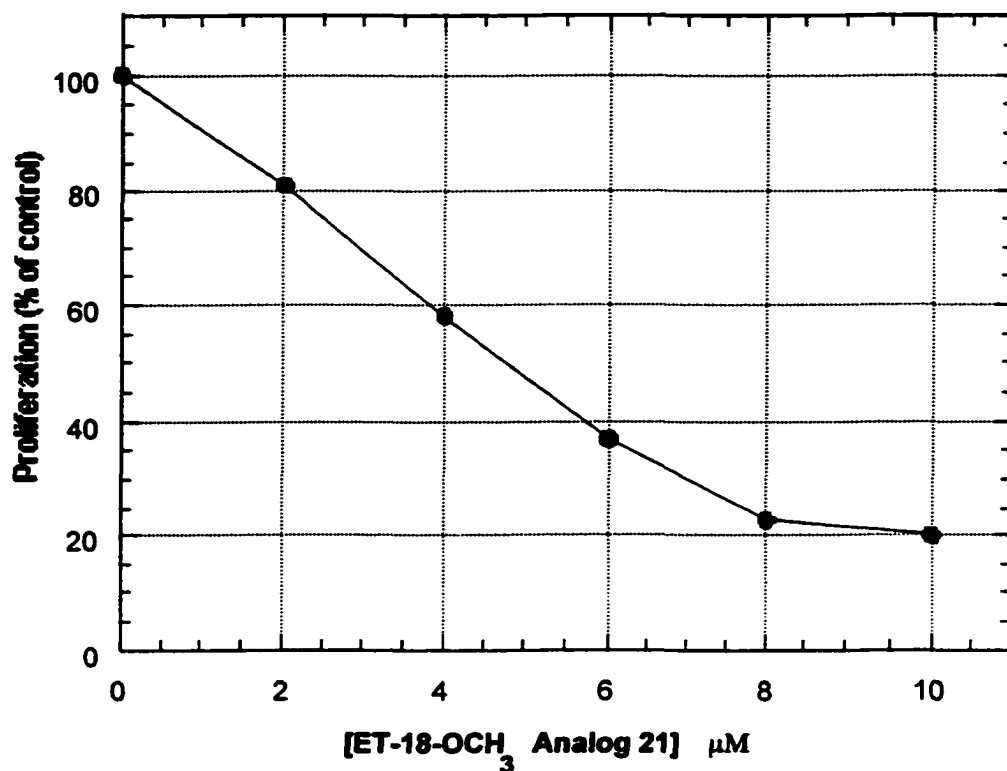
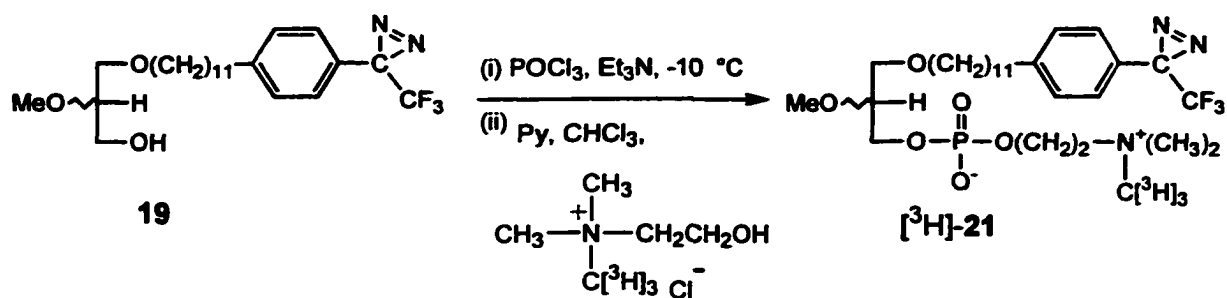


Figure 4. Effect of Photoactivatable Analog 21 on MCF-7 Cell Proliferation.

Nonlabeled **21** was used. The MCF-7 cells were incubated with **21** in the dark for 48 h.

N-methylation (MeI, K₂CO₃ in acetonitrile) of **20** afforded phosphocholine **21** in 54% yield. As stated above, nonradioactive **21** was found in Professor Arthur's laboratory to closely mimic the antiproliferative activity of ET-18-OCH₃ against MCF-7 cells in the dark. Therefore, radioactivity can be incorporated into **21** by carrying out the *N*-methylation with C³H₃I. Another proposed procedure to introduce the radiolabel is shown in Scheme 4. The phosphocholine moiety will be installed by using phosphorus oxychloride followed by [³H]-choline chloride to form ³H-labeled probe **21**. This radioactive photoactivatable probe will be used in efforts to identify putative ET-18-OCH₃-binding proteins in MCF-7 cells.

Experimental Section

General procedures. See Chapter 1 for detailed information. Pd(dppf)Cl₂ and 4-bromo-2',2',2'-trifluoroacetophenone were purchased from Aldrich. Low and high resolution MS data were recorded at the Schering-Plough Research Institute Mass Spectrometry Facility.

1-*O*-[11'-(4"-Trifluoroacetylphenyl)undecyl]-2-*O*-methyl-3-*O*-(4'-methoxyphenyl)-*rac*-glycerol (14). To a solution of 1-*O*-(11'-undecenyl)-2-*O*-methyl-3-*O*-(4'-methoxyphenyl)-*rac*-glycerol **5** (1.09 g, 3.0 mmol) in 20 mL of THF was added 10 mL (5.0 mmol) of 9-BBN (a 0.5 M solution in THF) at 0 °C. The mixture was stirred overnight at room temperature. To the reaction mixture was added an aqueous solution of K₃PO₄ (1.27 g, 6.0 mmol in 2 mL of H₂O), followed by a solution of 4-bromo-2',2',2'-trifluoroacetophenone (1.14 g, 4.5 mmol) in 30 mL of DMF and Pd(dppf)Cl₂ (150 mg, 0.21 mmol). The mixture was heated at 60 °C for 2 h. After the reaction mixture was

concentrated under reduced pressure, the residue was partitioned between CH_2Cl_2 (150 mL) and 100 mL of saturated aqueous NaHCO_3 solution. The organic layer was washed with H_2O and brine, and dried (MgSO_4). The desired product **14** (1.33 g, 82%) was obtained by chromatography (elution with hexane/ CH_2Cl_2 1:1, 1:2, then 1:4); R_f 0.50 (CH_2Cl_2). ^1H NMR (CDCl_3): δ 7.99 (d, $J = 8.8$ Hz, 2H), 7.34 (d, $J = 8.8$ Hz, 2H), 6.87-6.80 (m, 4H), 4.04-3.99 (m, 2H), 3.76 (s, 3H), 3.68-3.58 (m, 3H), 3.51 (s, 3H), 3.47-3.44 (m, 2H), 2.69 (t, $J = 7.9$ Hz, 2H), 1.63-1.55 (m, 4H), 1.30-1.24 (m, 14H). ^{13}C NMR: δ 153.7, 152.7, 151.7, 130.1, 129.0, 127.4, 115.4, 114.4, 78.7, 71.7, 69.9, 68.2, 58.1, 55.7, 36.2, 30.9, 29.64, 29.61, 29.56, 29.50, 29.47, 29.3, 26.2. MS calcd for $(\text{C}_{30}\text{H}_{41}\text{F}_3\text{O}_5+\text{H})^+$ 539.29, found 539.1.

1-*O*-[11'-(4"-Trifluoroacetyloximephenyl)undecyl]-2-*O*-methyl-3-*O*-(4'-methoxyphenyl)-*rac*-glycerol (15). A mixture of **14** (1.28 g, 2.38 mmol) and hydroxylamine-HCl (330 mg, 4.76 mmol) in 40 mL of absolute EtOH and 8 mL of pyridine was heated at 60 °C overnight. After concentration in vacuo, the residue was partitioned between CH_2Cl_2 (150 mL) and 1 N HCl (100 mL). The organic layer was washed with brine and dried (MgSO_4). Product **15** (1.29 g, 98%) was obtained by chromatography (elution with CH_2Cl_2 , then with $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 98:2); R_f 0.15 (CH_2Cl_2). ^1H NMR: δ (two stereoisomers) 9.05 and 8.78 (s, 1H), 7.44-7.39 (m, 2H), 7.28-7.20 (m, 2H), 6.88-6.81 (m, 4H), 4.07-3.98 (m, 2H), 3.76 (s, 3H), 3.74-3.69 (m, 1H), 3.67-3.60 (m, 2H), 3.52 (s, 3H), 3.50-3.45 (m, 2H), 2.65-2.61 (m, 2H), 1.63-1.57 (m, 4H), 1.29-1.24 (m, 14H). MS calcd for $(\text{C}_{30}\text{H}_{42}\text{F}_3\text{NO}_5+\text{H})^+$ 554.30, found 554.1.

1-*O*-[11'-(4-(Trifluoroacetylphenyl)undecyl)]-2-*O*-methyl-3-*O*-(4'-methoxyphenyl)-*rac*-glycerol *O*-Tosyl Oxime (16). To an ice-cooled solution of **15** (1.26 g, 2.28

mmol), Et₃N (0.77 mL, 7.6 mmol), and DMAP (20 mg, 0.16 mmol) in anhydrous CH₂Cl₂ (40 mL) was added *p*-TsCl (0.52 g, 2.74 mmol) slowly. The mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with 100 mL of CH₂Cl₂ and washed with 1 N HCl (50 mL), saturated aqueous NaHCO₃ solution (50 mL), brine, and dried (MgSO₄). The product (1.48 g, 92%) was obtained by chromatography (elution with hexane/CH₂Cl₂ 2:1, then 1:1); *R_f* 0.55 (CH₂Cl₂). ¹H NMR (CDCl₃): δ (two stereoisomers) 7.89 (m, 2H), 7.39-7.33 (m, 4H), 7.28-7.21 (m, 2H), 6.88-6.81 (m, 4H), 4.06-3.97 (m, 2H), 3.76 (s, 3H), 3.73-3.56 (m, 3H), 3.52 (s, 3H), 3.49-3.45 (m, 2H), 2.65-2.60 (m, 2H), 2.47 and 2.65 (s, 3H), 1.64-1.55 (m, 4H), 1.31-1.26 (m, 14H). MS calcd for (C₃₇H₄₈F₃NO₅+H)⁺ 708.31, found 708.1.

1-*O*-[11'-[4'-[(3"-Trifluoromethyl)-diaziridinyl]phenyl]undecyl]-2-*O*-methyl-3-*O*-(4'-methoxyphenyl)-*rac*-glycerol (17). In a pressure tube, a solution of **16** (1.40 g, 1.98 mmol) in CH₂Cl₂ (30 mL) was cooled to -78 C. After addition of liquid ammonia (30 mL), the tube was sealed and its contents were warmed to room temperature and stirred at room temperature overnight. After the excess NH₃ was released slowly, the product was extracted with CH₂Cl₂ (100 mL). The solution was washed with water and brine, and dried (MgSO₄). The residue was purified by chromatography (elution with CH₂Cl₂/MeOH 20:1), providing 900 mg (82 %) of **17**; *R_f* 0.75 (hexane/EtOAc 2:1). ¹H NMR: δ 7.51 (d, *J* = 8.0 Hz, 2H), 7.22 (d, *J* = 8.0 Hz, 2H), 6.88-6.80 (m, 4H), 4.07-3.97 (m, 2H), 3.76 (s, 3H), 3.70-3.56 (m, 3H), 3.51 (s, 3H), 3.47-3.44 (m, 2H), 2.77 (br s, 1H), 2.62 (t, *J* = 8.0 Hz, 2H), 2.20 (br s, 1H), 1.63-1.53 (m, 4H), 1.30-1.25 (m, 14H). MS calcd for (C₃₀H₄₃F₃N₂O₄+H)⁺ 553.32, found 553.1.

1-*O*-[11'-[4'-[(3''-Trifluoromethyl)-diazirinyl]phenyl]undecyl]-2-*O*-methyl-3-*O*-(4'-methoxyphenyl)-*rac*-glycerol (18). A mixture of **17** (860 mg, 1.56 mmol), I₂ (420 mg, 1.65 mmol), and Et₃N (394 mg, 3.9 mmol) in MeOH (15 mL) was stirred for 0.5 h at room temperature. After dilution with 100 mL of CH₂Cl₂, the mixture was poured into 50 mL of 1 N Na₂S₂O₃ solution. The organic layer was washed with brine and dried (MgSO₄). The product (780 mg, 91%) was obtained by chromatography (elution with CH₂Cl₂); R_f 0.90 (hexane/EtOAc 2:1). ¹H NMR: δ 7.20 (d, *J* = 8.4 Hz, 1H), 7.09 (d, *J* = 8.4 Hz, 1H), 6.88-6.81 (m, 4H), 4.07-3.97 (m, 2H), 3.76 (s, 3H), 3.70-3.56 (m, 2H), 3.52 (s, 3H), 3.48-3.44 (m, 2H), 2.60 (t, *J* = 8.0 Hz, 2H), 1.60-1.55 (m, 4H), 1.28-1.24 (m, 14H). MS calcd for (C₃₀H₄₁F₃N₂O₄+H)⁺ 551.30, found 551.1.

1-*O*-[11'-[4'-[(3''-Trifluoromethyl)-diazirinyl]phenyl]undecyl]-2-*O*-methyl-*rac*-glycerol (19). To an ice-cooled mixture of **18** (220 mg, 0.40 mmol) in 5 mL of MeCN and 2.5 mL of water was added CAN (660 mg, 1.20 mmol). The mixture was stirred at room temperature for 2.5 h, and then was stirred with 1 N aqueous Na₂S₂O₃ solution (20 mL) for 30 min. The product was extracted with CH₂Cl₂ (2 x 50 mL), washed with brine, and dried (MgSO₄). Product **19** (138 mg, 78%) was obtained by chromatography (elution with hexane/EtOAc 10:1); R_f 0.90 (hexane/EtOAc 2:1). ¹H NMR: δ 7.19 (d, *J* = 8.0 Hz, 2H), 7.08 (d, *J* = 8.0 Hz, 2H), 3.77-3.73 (m, 1H), 3.66-3.62 (m, 1H), 3.57-3.39 (m, 8.0 H), 2.59 (t, *J* = 8.0 Hz, 2H), 1.59-1.52 (m, 4H), 1.28-1.25 (m, 14H). ¹³C NMR: δ 144.6, 128.3, 126.6, 125.8, 124.7 (q, *J*_{C-F} = 272 Hz), 79.9, 71.9, 70.5, 62.6, 57.7, 35.8, 35.6, 35.53, 31.50, 31.3, 29.9, 29.6, 29.5, 29.3, 26.1, 25.8. MS calcd for (C₂₃H₃₅F₃N₂O₃+H)⁺ 445.26, found 445.1.

1-*O*-[11'-[4'-[(3''-Trifluoromethyl)-diazirinyl]phenyl]undecyl]-2-*O*-methyl-*rac*-glycero-3-phosphoethanoldimethylamine (20). A 25-mL flask was flame-dried and flushed with N₂. A solution of POCl₃ (56 mg, 0.37 mmol) and Et₃N (38 mg, 0.37 mmol) in 0.5 mL of CHCl₃ was cooled to -10 °C. To the mixture was added a solution of **19** (130 mg, 0.29 mmol) in 3 mL of CHCl₃. After the mixture was stirred for 1 h, 2-(*N,N*-dimethylamino)ethanol (39 mg, 0.44 mmol) and 0.5 mL of pyridine were added. The reaction mixture was stirred overnight at room temperature. The mixture was stirred for 30 min after water (0.2 mL) was added. The residue obtained after concentration was purified by chromatography on silica gel (elution with CHCl₃/MeOH 4:1, then CHCl₃/MeOH/H₂O 65:25:4). Product **20** (60 mg, 35%) was obtained; *R*_f 0.45 (CHCl₃/MeOH/H₂O 65:25:4). ¹H NMR: δ 7.19 (d, *J* = 8.0 Hz, 2H), 7.09 (d, *J* = 8.0 Hz, 2H), 4.17-4.13 (m, 2H), 3.99-3.91 (m, 2H), 3.55-3.39 (m, 8H), 3.04 (br s, 2H), 2.71 (s, 6H), 2.59 (t, *J* = 7.6 Hz, 2H), 1.57-1.52 (m, 4H), 1.27-1.24 (m, 14H). MS calcd for (C₂₇H₄₅F₃N₃O₆P+H)⁺ 596.30, found 596.1.

1-*O*-[11'-[4'-[(3''-Trifluoromethyl)-diazirinyl]phenyl]undecyl]-2-*O*-methyl-*sn*-glycerol-*rac*-3-phosphocholine (21). To a mixture of **20** (20 mg, 0.034 mmol), anhydrous K₂CO₃ (46 mg, 0.34 mmol) and 18-crown-6 (2 mg, 7.5 μmol) in 1.5 mL of CH₃CN was added 23.8 mg (0.168 mmol) of iodomethane. The mixture was stirred at room temperature for 4 h. After concentration in vacuo, the residue was partitioned between 4 mL of CHCl₃ and 1 mL of H₂O. The organic layer was concentrated, and the product was purified by preparative TLC (elution with CHCl₃/MeOH/H₂O 65:25:4). The desired band was scraped from the plate, and the product was extracted with CHCl₃/MeOH/H₂O 65:25:4. After the solvents were evaporated, the residue was

dissolved in 3 mL of CHCl_3 , which was filtered through a Gelman 0.25- μm filter to remove suspended silica gel. Product **21** (11 mg, 54%) was obtained; R_f 0.30 ($\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$ 65:25:4). ^1H NMR: δ 7.18 (d, $J = 8.0$ Hz, 2H), 7.08 (d, $J = 8.0$ Hz, 2H), 4.39 (br s, 1H), 4.03 (m, 2H), 3.88 (m, 1H), 3.59-3.40 (m, 16H), 2.82 (br s, 3H), 2.59 (t, $J = 7.6$ Hz, 2H), 1.58-1.53 (m, 4H), 1.25 (br s, 14H). MS calcd for $(\text{C}_{28}\text{H}_{48}\text{F}_3\text{N}_3\text{O}_6\text{P}+\text{H})^+$ 610.32, found 610.1. HR-MS (FAB) calcd for $(\text{C}_{28}\text{H}_{48}\text{F}_3\text{N}_3\text{O}_6\text{P}+\text{H})^+$ 610.3233, found 610.3239.

Reference

- (1). Li, G.; Samadder, P.; Arthur, G.; Bittman, R. *Tetrahedron* **2001**, *57*, 8925-8932.
- (2). Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457-2483.
- (3). Ishiyama, T.; Miyaura, N.; Suzuki, A. *Synlett* **1991** (10), 687-688.
- (4). Weber, T.; Brunner, J. *J. Am. Chem. Soc.* **1995**, *117*, 3084-3095.
- (5). Li, G.; Bittman, R. *Tetrahedron Lett.* **2000**, *41*, 6737-6741.
- (6). Bittman, R. In *Phospholipids Handbook*; Cevc, G., Ed.; Marcel Dekker: New York, 1993; pp 141-232.
- (7). Abdelmageed, O. H.; Duclos, Jr. R. I.; Abushanab, E.; Makriyannis, A. *Chem. Phys. Lipids* **1990**, *54*, 49-59.

Chapter 4

Synthesis of Novel Fluorescent Labeled Cholesterol, Cholesteryl Palmitate, and Cholesteryl Oleate

Abstract

This chapter describes the syntheses of novel fluorescent cholesterol **5** and cholesteryl ester analogs **6a,b**. A trifluoroacetyl-substituted naphthalene moiety was employed as the fluorophore in these probes. The fluorophore was installed through an ether linkage by Mitsunobu reaction in 88% yield.

Introduction

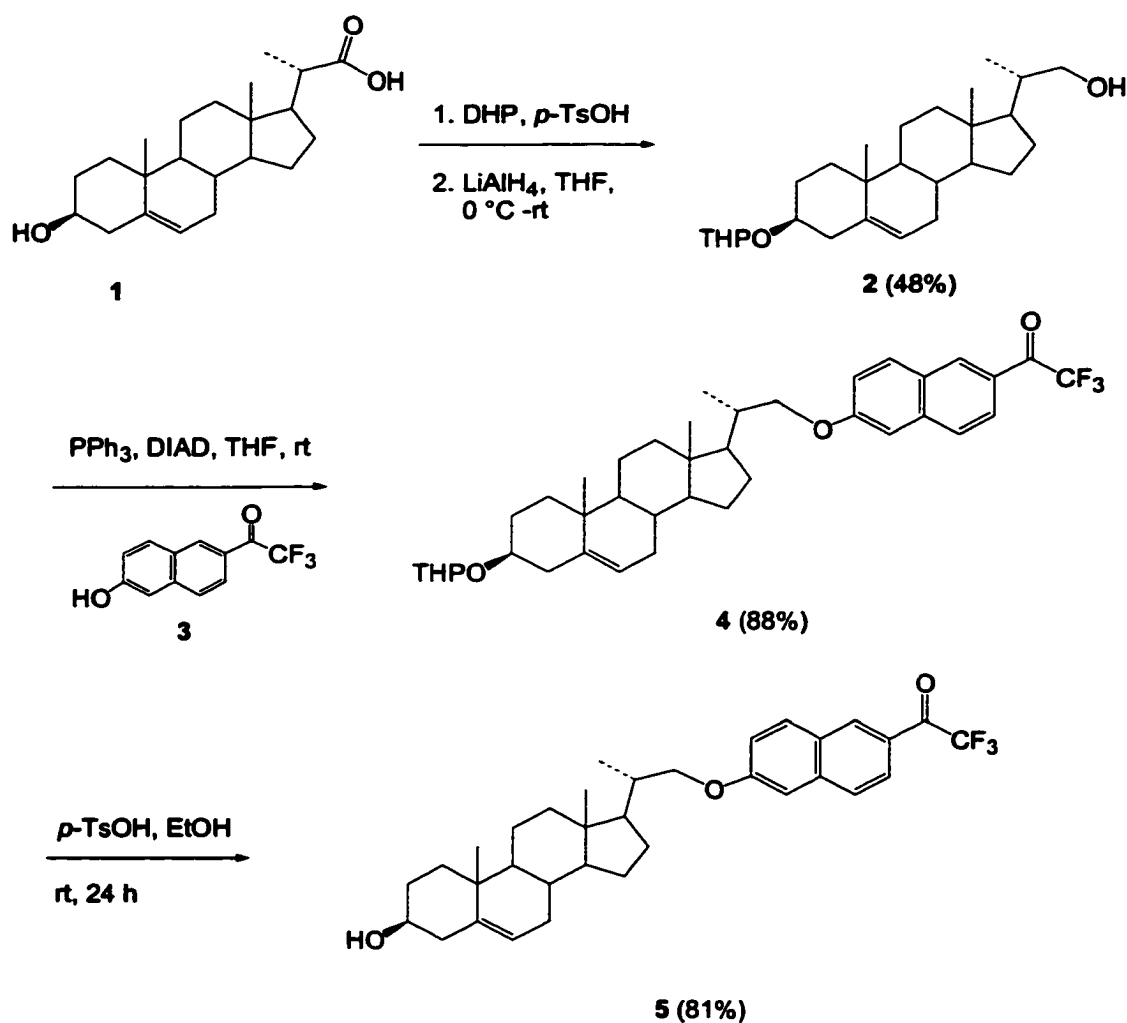
Fluorescent cholesterol analogs have a multitude of uses, including studies on cholesterol absorption,¹ membrane lipid distribution,^{2,3} and cholesterol metabolism.⁴

In this chapter, the synthesis of a new fluorescent cholesterol probe **5** is described. This analog was found by our collaborators, Dr. George Rothblat and Dr. Ginny Kellner-Weibel, Children's Hospital of Philadelphia, to be extremely useful because, unlike most of the other fluorescent analogs currently used, this compound was absorbed and metabolized practically identically to radiolabeled cholesterol in J774 macrophage cells. Therefore, compound **5** could be used instead of radiolabeled cholesterol for metabolic studies or to track the movement of cholesterol in the cell using fluorescence microscopy.

Fluorescent cholesteryl esters are also useful tools since the majority of cholesterol that enters the cell via lipoproteins is in the esterified form. Fluorescent cholesteryl ester analogs may be incorporated into phospholipid droplets, as well as into reconstituted lipoproteins or native lipoproteins, through the action of cholesteryl ester

transfer protein, in order to measure the uptake, movement, distribution, and metabolism of cholesteryl esters in cells in tissue cultures. This chapter also describes the esterification of sterol **5** with two fatty acids, affording fluorescent cholesteryl esters **6a** and **6b**.

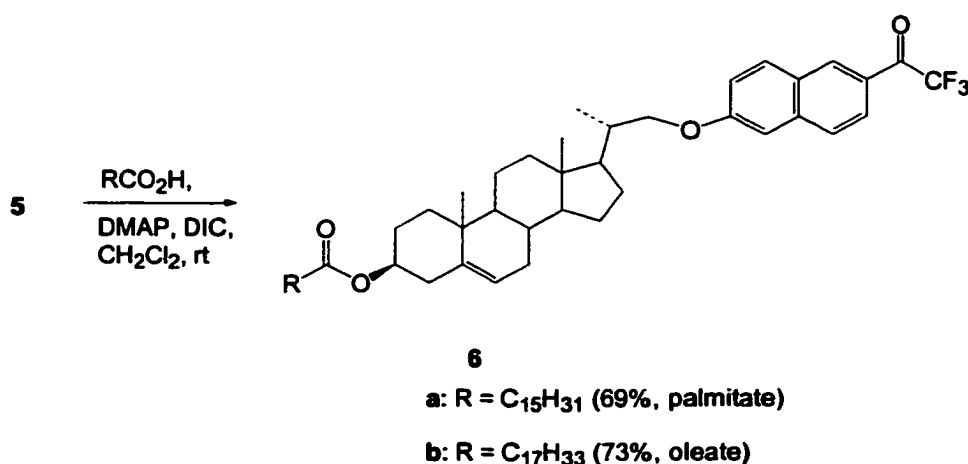
Scheme 1. Synthesis of Fluorescent Cholesterol Analog **5**



Results and Discussion

The 3 β -tetrahydropyranyl (THP) protected alcohol **2** was prepared from 23,24-bisnor-5-cholenic acid-3 β -ol (**1**) by protecting the 3 β -hydroxy group with a THP group followed by reduction with LiAlH₄ in 48% yield.⁵ Mitsunobu reaction of **2** with 6-trifluoroacetyl-2-naphthol (**3**) afforded fluorescent intermediate **4**, which was treated with catalytic *p*-TsOH in ethanol, affording cholesterol probe **5** in 81% yield. Cholesteryl esters **6a,b** were also synthesized by acylation of **5** with palmitic acid and oleic acid, respectively, via their anhydrides in 69% and 73% yield.

Scheme 2. Synthesis of Fluorescent Cholesteryl Palmitate **6a** and Cholesteryl Oleate **6b**



Experimental Section

General procedures: See chapter 1 for detailed information. 23,24-Bisnor-5-cholenic acid-3 β -ol was purchased from Steraloids (Newport, RI).

23,24-Bisnorcholest-5-en-3 β ,24-diol 3-Tetrahydropyranyl Ether (2). This compound was prepared by the procedure described previously.⁵ A mixture of 23,24-bisnor-5-cholenic acid-3 β -ol (1) (3.47 g, 10.0 mmol), 3,4-dihydro-2*H*-pyran (DHP, 4.20 g, 50 mmol), and *p*-TsOH (20 mg, 0.10 mmol) in 150 mL of benzene was stirred for 5 h at room temperature. Then a solution of LiAlH₄ (1.52 g, 40 mmol) in 50 mL of THF was added dropwise at 0 °C. The reaction mixture was stirred overnight at room temperature, quenched slowly with H₂O, and extracted with CHCl₃ (2 x 100 mL). The organic layer was dried (MgSO₄) and concentrated. The product (2.0 g, 48%) was obtained as a white solid by chromatography (elution with hexane/EtOAc 20:1, then 10:1), mp 131.0-131.5 °C.

2-Hydroxy-6-(trifluoroacetyl)naphthalene (3). See compound 3 in Chapter 2.

22-[6'-(Trifluoroacetyl)naphthyl-2'-oxy]-23,24-bisnorcholest-5-en-3 β -ol 3-Tetrahydropyranyl Ether (4). A mixture of 2 (258 mg, 0.62 mmol), 3 (156 mg, 0.65 mmol), PPh₃ (179 mg, 0.68 mmol), and DIAD (145 mg, 0.68 mmol) in 12 mL of THF was stirred for 5.5 h at room temperature under argon. The mixture was concentrated and purified by chromatography (elution with hexane/EtOAc 10:1), affording the desired product (350 mg, 88%); *R_f* 0.50 (hexane/EtOAc 5:1). ¹H NMR (CDCl₃): δ 8.53 (s, 1H), 8.02 (d, *J* = 8.6 Hz, 1H), 7.89 (d, *J* = 9.0 Hz, 1H), 7.79 (d, *J* = 8.8 Hz, 1H), 7.24 (d, *J* = 2.3 Hz, 1H), 7.13 (d, *J* = 2.1 Hz, 1H), 5.37 (br s, 1H), 4.72 (br s, 1H), 4.08-4.06 (m, 1H), 3.92-3.83 (m, 2H), 3.52-3.49 (m, 2H), 2.37 (m, 2H), 2.08-1.03 (m, 31H), 0.76 (s, 3H). ¹³C NMR (CDCl₃): δ 180.1 (q, *J*_{C-F} = 34 Hz), 160.9, 141.1, 140.9, 138.6, 133.0, 131.7, 127.6, 127.4, 125.0, 124.9, 121.3, 120.7, 117.0 (q, *J*_{C-F} = 290 Hz), 106.5, 97.0, 96.8, 75.9,

73.4, 62.9, 62.8, 52.7, 50.15, 50.11, 42.6, 40.2, 39.6, 38.7, 37.4, 36.79, 36.75, 36.4, 31.92, 31.89, 31.3, 29.7, 28.0, 27.84, 25.5, 24.4, 21.0, 20.0, 19.4, 17.4, 11.9.

22-[6'-(Trifluoroacetyl)naphthyl-2'-oxy]-23,24-bisnorcholest-5-en-3 β -ol (5).

To a solution of **4** (150 mg, 0.235 mmol) in 5 mL of EtOH (95%), *p*-TsOH (5 mg, 0.026 mmol) was added. The mixture was stirred for 24 h at room temperature. The reaction mixture was concentrated and purified by chromatography (elution with hexane/EtOAc 4:1), affording the desired product (105 mg, 81%); R_f 0.35 (hexane/EtOAc 2:1). The emission spectrum was measured in EtOH (5.4×10^{-4} M). With an excitation wavelength of 338 nm, the $\lambda_{\max}$ of emission was 472 nm. ^1H NMR (CDCl_3): δ 8.52 (s, 1H), 8.02 (dd, $J = 8.8$ Hz, 0.9 Hz, 1H), 7.88 (d, $J = 9.0$ Hz, 1H), 7.79 (d, $J = 8.8$ Hz, 1H), 7.25 (d, $J = 2.3$ Hz, 1H), 7.13 (d, $J = 2.3$ Hz, 1H), 5.35 (br s, 1H), 4.08-4.05 (m, 1H), 3.87-3.83 (m, 1H), 3.55-3.52 (m, 1H), 2.28-1.02 (m, 27H), 0.76 (s, 3H). ^{13}C NMR (CDCl_3): δ 180.1 (q, $J_{\text{C-F}} = 34$ Hz), 160.9, 140.7, 138.6, 132.97, 132.95, 131.7, 127.6, 127.4, 125.0, 124.9, 121.5, 120.7, 117.0 (q, $J_{\text{C-F}} = 290$ Hz), 106.5, 73.4, 71.8, 56.5, 52.7, 50.1, 42.5, 42.2, 39.6, 37.2, 36.5, 36.4, 31.9, 31.8, 31.6, 27.8, 24.4, 21.0, 19.4, 17.4, 11.9.

22-[6'-(Trifluoroacetyl)naphthyl-2'-oxy]-23,24-bisnorcholest-5-en-3 β -ol

Palmitate (6a). To a mixture of **5** (46 mg, 0.092 mmol), palmitic acid (59 mg, 0.23 mmol), and DMAP (34 mg, 0.28 mmol) in 5 mL of CH_2Cl_2 was added DIC (36 μL , 0.23 mmol) by syringe under argon. The mixture was stirred for 5 h at room temperature. Product **6a** (50 mg, 69%) was obtained by chromatography (elution with hexane/EtOAc 100:3); R_f 0.45 (hexane/EtOAc 10:1). ^1H NMR (CDCl_3): δ 8.52 (s, 1H), 8.02 (dd, $J = 8.8$ Hz, 0.9 Hz, 1H), 7.88 (d, $J = 9.0$ Hz, 1H), 7.79 (d, $J = 8.8$ Hz, 1H), 7.25 (d, $J = 2.3$ Hz, 1H), 7.13 (d, $J = 2.3$ Hz, 1H), 5.38 (br s, 1H), 4.64-4.61 (m, 1H), 4.07 (dd, $J = 3.2$ Hz, 8.5

Hz, 1H), 3.85 (dd, $J = 8.8$ Hz, 7.4 Hz, 1H), 2.33-2.25 (m, 4H), 1.88 (m, 6H), 1.63-1.03 (m, 45H), 0.88 (t, $J = 7.2$ Hz, 3H), 0.76 (s, 3H). ^{13}C NMR (CDCl_3): δ 180.1 (q, $J_{\text{C-F}} = 34$ Hz), 173.3, 160.9, 139.8, 138.6, 133.0, 131.8, 127.6, 127.4, 125.04, 124.98, 122.4, 120.7, 117.1 (q, $J_{\text{C-F}} = 290$ Hz), 106.6, 73.6, 73.4, 56.4, 52.7, 50.0, 42.6, 38.2, 36.6, 36.5, 34.7, 31.94, 31.92, 31.88, 29.71, 29.68, 29.67, 29.61, 29.5, 29.4, 29.3, 29.13, 27.9, 25.1, 22.7, 19.3, 17.5, 14.1, 12.0.

22-[6'-(Trifluoroacetyl)naphthyl-2'-oxy]-23,24-bisnorcholest-5-en-3 β -ol

Oleate (6b). To a mixture of **7** (50 mg, 0.10 mmol), oleic acid (71 mg, 0.25 mmol), and DMAP (34 mg, 0.28 mmol) in 5 mL of CH_2Cl_2 was added DIC (40 μL , 0.25 mmol) by syringe under argon. The mixture was stirred overnight at room temperature. Product **6b** (60 mg, 73%) was obtained by chromatography (elution with hexane/EtOAc 100:3); R_f 0.45 (hexane/EtOAc 10:1). ^1H NMR (CDCl_3): δ 8.52 (s, 1H), 8.02 (d, $J = 8.8$ Hz, 1H), 7.88 (d, $J = 9.0$ Hz, 1H), 7.79 (d, $J = 8.8$ Hz, 1H), 7.25 (d, $J = 2.3$ Hz, 1H), 7.13 (d, $J = 2.2$ Hz, 1H), 5.39-5.32 (m, 3H), 4.65-4.58 (m, 1H), 4.07 (dd, $J = 3.2$ Hz, 8.5 Hz, 1H), 3.85 (m, 1H), 2.33-2.25 (m, 4H), 2.01-1.86 (m, 10H), 1.63-1.03 (m, 41H), 0.89 (t, $J = 6.5$ Hz, 3H), 0.76 (s, 3H). ^{13}C NMR (CDCl_3): δ 180.1 (q, $J_{\text{C-F}} = 34$ Hz), 173.3, 160.9, 139.8, 138.6, 133.0, 131.8, 130.0, 129.8, 127.6, 127.4, 125.0, 124.9, 122.4, 120.8, 117.0 (q, $J_{\text{C-F}} = 290$ Hz), 106.5, 73.6, 73.4, 56.4, 52.7, 50.0, 42.6, 38.2, 37.0, 36.6, 36.5, 34.7, 31.92, 31.90, 31.87, 29.8, 29.7, 29.6, 29.3, 29.2, 29.1, 27.9, 27.8, 27.2, 25.1, 22.7, 19.3, 17.5, 14.1, 12.0.

References

- (1). Sparrow, C. P.; Patel, S.; Baffic, J.; Chao, Y.; Hernandez, M.; Lam, M.; Montenegro, J.; Wright, S. D.; Dermers, P. A. *J. Lipid Res.* **1999**, *40*, 1747-1757.

- (2). Boesze-Battaglia, K.; Clayton, S. T.; Schimmel, R. J. *Biochemistry* **1996**, *35*, 6664-6673.
- (3). Mukherjee, S.; Zha, X.; Tabas, I.; Maxfield, F. R. *Biophys. J.* **1998**, *75*, 1915-1925.
- (4). Lusa, S.; Tanhuanpaa, K.; Ezra, T.; Somerharju, P. *Biochem. J.* **1998**, *332*, 451-457.
- (5). Morisaki, M.; Shibata, M.; Duque, C.; Imamura, N.; Ikekawa, N. *Chem. Pharm. Bull.* **1980**, *2*, 606-611.

Chapter 5

A Concise Route to an Enone Precursor of *D-erythro*-Sphingosine via a Sulfoxide Intermediate

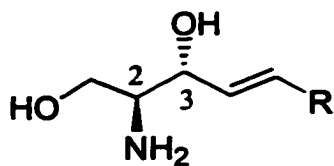
Abstract

Efficient procedures for preparing enone **2**, a precursor of *D-erythro*-sphingosine **1a** and its short-chain analog **1b**, are described in this chapter. (*E*)-Enone **2** was synthesized via dehydrosulfenylation of sulfoxide intermediate **5**.

Introduction

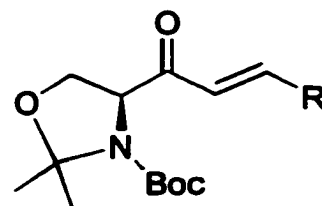
Sphingolipids have been implicated in a vast variety of physiological functions but are not readily obtained from natural sources in homogeneous form.¹ Therefore, there is a great demand for chemical methods that provide sphingolipids in sufficient amounts and high chiral purity for use in biological and biochemical studies. Since the first report of the synthesis of racemic sphingosine in 1951,² many different methods have been employed for the chemical synthesis of naturally occurring (2*S*,3*R*)-sphingosine (**1a**, Chart 1).³ However, because of the need to generate two stereogenic centers with high stereoselectivity, as well as an (*E*)-C(4)-C(5) double bond, most of the synthetic procedures are quite lengthy. An efficient synthetic route to enone intermediates of *D-erythro*-sphingosine (**1a**) and its short-chain analog **1b** in only four steps (two of which take place in one pot, with no intermediate being isolated) is presented here that involves the use of β -ketosulfoxide intermediate for C=C bond formation. A dehydrosulfenylation reaction of the sulfoxide intermediate (Scheme 1) affords (*E*)-alkene exclusively, as demonstrated previously.⁴

Chart 1. Structures of Sphingosine 1 and Enone 2



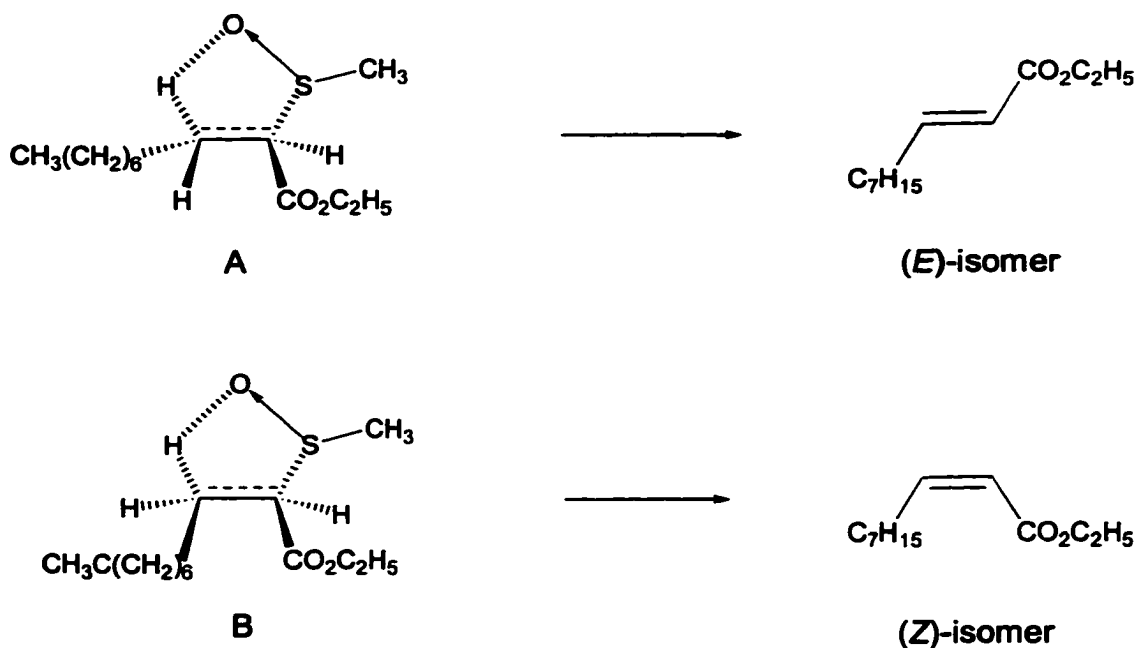
1a R = C₁₃H_{27-n}

1b R = C₅H_{11-n}



2a,b

Scheme 1. Possible Transition States of Dehydrosulfenylation



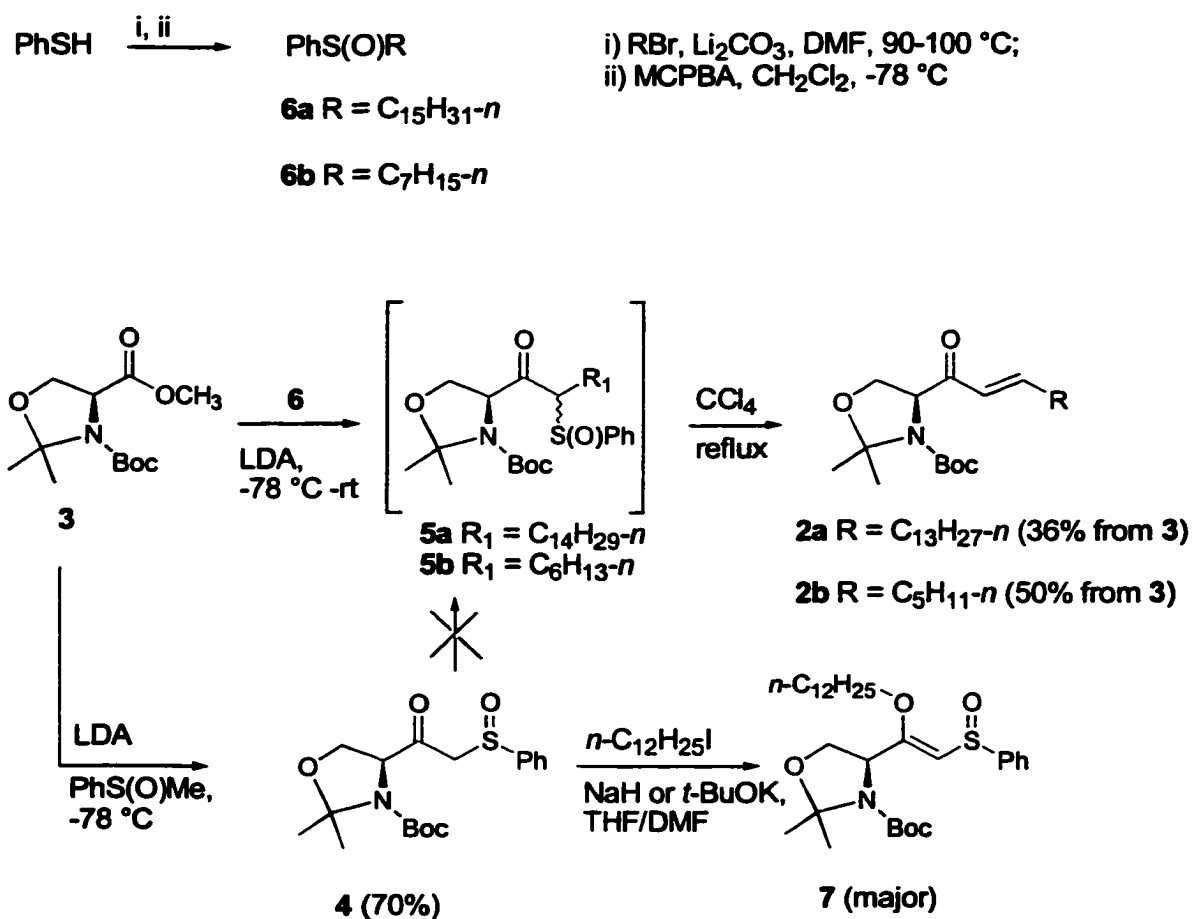
Sphingolipids have not been made previously by dehydrosulfenylation of a sulfoxide, although reactions of sulfur-containing carbanions have been used extensively in organic synthesis.⁵ This method provides facile access to synthon 2 from *N*-Boc-L-

serine methyl ester derivative **3**. It has previously been shown that synthon **2** affords naturally occurring *D-erythro*-sphingosine **1a**.^{6,7}

Results and Discussion

The synthesis of **1a** began with the reaction of *L*-serine-derived *N*-Boc-

Scheme 2. Synthesis of Enones **2 from Garner Ester **3** and Sulfoxides **6****



oxazolidine methyl ester **3**⁸ with an alkyl phenyl sulfoxide (**6**) (Scheme 2). The latter was prepared in high yield by the reaction of thiophenol with (a) *n*-pentadecyl bromide or (b) *n*-heptyl bromide in the presence of Li₂CO₃ in DMF followed by brief treatment of the resulting sulfide with MCPBA in CH₂Cl₂ at -78 °C.⁹ Nucleophilic addition of the anion of sulfoxides **6a,b** (2 equiv) to ester **3** gave sulfoxide intermediates **5a,b**,¹⁰ which were not isolated. On heating in CCl₄ overnight, **5a,b** afforded enones **2a,b** in 36% and 50% overall yield, respectively. The higher yield obtained for **2b** presumably arises from the lower degree of steric hindrance in the reaction of *N*-Boc derivative **3** with the short-chain α -sulfinyl carbanion derived from **6b**. Short-chain sphingoid bases such as **1b** may be useful in cell studies because they are more water soluble than natural sphingolipids.

β -Ketosulfoxide **4** was prepared in 70% yield from **3** by using 2 equiv of the carbanion of methyl phenyl sulfoxide (Scheme 2).¹¹ However, attempts to carry out the C-alkylation of **4** with long-chain alkyl halides mediated by different bases have not been successful. When a strong base as NaH or *t*-BuOK in THF/DMF was used, *O*-alkylation was the major reaction, providing enol ether **7**. When a relatively weak base such Cs₂CO₃ or K₂CO₃ in DMF was used, the reaction was very slow and the yield of **5a** was low.

In summary, enone **2**, a precursor of **1a,b**, was synthesized from commercially available L-serine-derived synthon **3** by employing β -ketosulfoxide intermediate **5**. The (*E*)-C(4)-C(5) double bond was generated in **1** by dehydrosulfinylation of **5**. This practical method can be applied to the construction of sphingoid bases containing a modified aliphatic chain. For example, it has afforded a concise strategy to synthesize novel sphingosine analogs.¹²

Experimental Section

General procedures: See Chapter 1 for detailed information.

Hexadecyl Phenyl Sulfoxide (6a). A mixture of thiophenol (1.10 g, 10.0 mmol), *n*-hexadecyl bromide (2.91 g, 10.0 mmol), and Li_2CO_3 (1.48 g, 20.0 mmol) in 20 mL of DMF was heated at overnight 90-100 °C. After cooling to room temperature, the mixture was poured into 50 mL of H_2O and extracted with EtOAc (2 x 75 mL). The organic layer was dried (MgSO_4) and concentrated. The residue was dissolved in 100 mL of CH_2Cl_2 and cooled at -78 °C. Then a solution of *m*-chloroperbenzoic acid (2.65 g, 65% purity, 10.0 mmol) in 60 mL of CH_2Cl_2 was added. The reaction was monitored by TLC (developed with hexane). After the starting material disappeared, the reaction mixture was poured into 150 mL of 15% aqueous NaHSO_3 solution. The aqueous layer was extracted with CH_2Cl_2 and washed twice with aqueous NaHCO_3 solution. The product was purified by chromatography (elution with hexane, then with hexane/EtOAc 20:1, and finally with hexane/EtOAc 3:1). The product **6a** (2.73 g, 81.3% for two steps) was obtained as a colorless solid. ^1H NMR (CDCl_3) δ 7.63-7.61 (m, 2H), 7.54-7.49 (m, 3H), 2.80-2.76 (m, 2H), 1.76-1.60 (m, 2H), 1.43-1.24 (m, 24H), 0.88 (t, J = 6.8 Hz, 3H). ^{13}C NMR (CDCl_3) δ 144.0, 130.8, 129.1, 124.0, 57.3, 31.9, 29.64, 29.62, 29.60, 29.54, 29.46, 29.31, 29.29, 29.11, 28.6, 22.6, 22.1, 14.1. MS calcd for $(\text{C}_{21}\text{H}_{36}\text{OS}+\text{H})^+$ 337.25, found 337.1.

Heptyl Phenyl Sulfoxide (6b). ^1H NMR (CDCl_3) δ 7.59-7.41 (m, 5H), 2.71 (t, J = 7.2 Hz, 2H), 1.70-1.18 (m, 10H), 0.79 (t, J = 7.0 Hz, 3H). ^{13}C NMR (CDCl_3) δ 143.9, 130.7, 129.0, 57.2, 31.3, 28.6, 28.4, 22.3, 22.0, 13.9. MS calcd for $(\text{C}_{13}\text{H}_{20}\text{OS}+\text{H})^+$ 225.12, found 224.1.

***N*-tert-Butoxycarbonyl (4*S*)-4-[(Phenylsulfinyl)acetyl]-2,2-dimethyl-1,3-oxazolidine (4).** To a solution of diisopropylamine (930 μ L, 6.6 mmol) in 8 mL of THF was added 2.64 mL of *n*-butyllithium (2.5 M solution in hexane, 6.6 mmol) at -15 °C under nitrogen. After the mixture was stirred at this temperature for 30 min, a solution of methyl phenyl sulfoxide (841 mg, 6.0 mmol) in 5 mL of THF was added dropwise. The mixture was stirred at -15 °C for 30 min, and then chilled to -78 °C. A solution of ester 3 (778 mg, 3.0 mmol) in 5 mL of THF was added dropwise. The solution was stirred at -78 °C for 2 h and allowed to warm to room temperature overnight. After 10 mL of saturated aqueous NH₄Cl solution was added, the product was extracted with EtOAc, washed with brine, and dried (MgSO₄). Purification by column chromatography (hexane/EtOAc 1:4, *R_f* 0.72) gave 771 mg (70%) of 4 as a yellow solid: mp 82.5-85.0 °C; ¹H NMR (C₆H₆, 70 °C) δ 7.49 (m, 2H), 7.10 (m, 3H), 4.26-4.40 (two set of s, 1H), ¹³C 3.75 (m, 4H), 1.67 (s, 3H), 1.40 (s, 3H), 1.35 (s, 9H). HRMS (FAB, MH⁺) calcd for *m/z* C₁₈H₂₆NO₅S 368.1532, found 368.1516.

***N*-tert-Butoxycarbonyl (4*S*)-4-[1'-Oxa-(2'E)-hexadecenyl]-2,2-dimethyl-1,3-oxazolidine (2a).** To a solution of diisopropylamine (470 μ L, 3.3 mmol) in 4 mL of dry THF was added 1.3 mL (3.3 mmol) of *n*-BuLi (a 2.5 M solution in hexane) at -15 °C under N₂. After the mixture was stirred for 30 min, a solution of phenyl sulfoxide 6a (1.0 g, 3.0 mmol) in 5 mL of THF was added dropwise. After 30 min at -15 °C, a solution of ester 3 (389 mg, 1.5 mmol) in 5 mL of THF was slowly added. The reaction mixture was stirred at -15 °C for 2 h and allowed to warm to room temperature overnight, then quenched with saturated aqueous NH₄Cl solution (10 mL). The product was extracted with EtOAc, washed with brine, and dried (MgSO₄). Concentration gave crude β -

ketosulfoxide **7a**, which was dissolved in 25 mL of CCl₄ and heated at reflux for 12 h. Concentration and purification by flash chromatography (hexane/EtOAc 4:1, R_f 0.80) gave 236 mg (36%, two steps) of **3a** as a colorless oil; $[\alpha]^{25}_{\text{D}} -30.0^{\circ}$ (*c* 0.85, CHCl₃) (lit.⁶ $[\alpha]^{25}_{\text{D}} -27.3^{\circ}$ (*c* 0.54, CHCl₃); lit.^{3c} $[\alpha]^{20}_{\text{D}} -21.0^{\circ}$ (*c* 0.85, CHCl₃)). ¹H NMR (C₆H₆, 70 °C) δ 6.88-6.81 (m, 1H), 6.21 (d, *J* = 15.7 Hz, 1H), 4.41 (br s, 1H), 3.83-3.76 (m, 2H), 1.92 (m, 2H), 1.80 (s, 3H), 1.54 (s, 3H), 1.39-1.18 (m, 34H), 0.88 (t, *J* = 6.8 Hz, 3H). ¹³C NMR (C₆H₆) δ 195.4, 195.0, 152.2, 151.6, 148.5, 148.3, 126.6, 125.9, 95.3, 94.4, 80.0, 79.8, 65.9, 65.5, 64.5, 64.4, 32.7, 32.3, 30.10, 30.08, 30.06, 29.93, 29.78, 29.75, 29.55, 29.47, 28.52, 28.33, 28.29, 28.18, 26.5, 25.5, 24.4, 23.1, 14.3.

***N*-tert-Butoxycarbonyl (4*S*)-4-[1'-Oxa-(2'*E*)-heptenyl]-2,2-dimethyl-1,3-oxazolidine (2b).** This compound was prepared by the procedure described above for **2a** in 50% yield (two steps from ester **3**); $[\alpha]^{25}_{\text{D}} -7.4^{\circ}$ (*c* 1.5, CHCl₃). ¹H NMR (C₆H₆, 70 °C) δ 6.84-6.77 (m, 1H), 6.18 (d, *J* = 15.7 Hz, 1H), 4.41 (br s, 1H), 3.86-3.75 (m, 2H), 1.90 (m, 2H), 1.75 (s, 3H), 1.51 (s, 3H), 1.38-1.10 (m, 18H), 0.79 (t, *J* = 6.8 Hz, 3H). ¹³C NMR (C₆H₆) δ 195.4, 195.0, 152.1, 151.5, 148.6, 148.3, 126.5, 126.0, 95.2, 94.3, 80.0, 79.7, 65.9, 65.5, 64.9, 64.4, 32.6, 32.5, 30.9, 30.8, 28.3, 28.1, 26.5, 26.4, 22.7, 22.4, 14.1.

***N*-tert-Butoxycarbonyl (4*S*)-4-[(1'-*n*-Dodecyloxy-2'-phenylsulfinyl)vinyl]-2,2-dimethyl-1,3-oxazolidine (7).** To a solution of **3** (37 mg, 0.10 mmol), potassium *tert*-butoxide (16.8 mg, 0.15 mmol), and 18-crown-6 (10 mg, 0.038 mmol) in 2 mL of DMF was added a solution of *n*-C₁₂H₂₅I (45 mg, 0.15 mmol) in 2 mL of THF. The mixture was stirred at room temperature overnight and quenched with H₂O. The mixture was extracted with EtOAc (2 x 50 mL) and washed with brine and dried (MgSO₄). After concentration, the residue was subjected to chromatography (elution with hexane/EtOAc

10:1, 5:1, then 2:1). A major product **7** (12 mg, 22%) was obtained; R_f 0.30 (hexane/EtOAc 3:1). ^1H NMR (CDCl_3) δ 7.82-7.39 (m, 5H), 5.50-5.35 (m, 2H), 4.39-4.27 (m, 1H), 4.04-4.00 (m, 1H), 3.76-3.60 (m, 2H), 1.70-1.21 (m, 35H), 0.85 (t, $J = 6.8$ Hz, 3H). MS calcd for $(\text{C}_{30}\text{H}_{49}\text{NO}_5\text{S}+\text{H})^+$ 536.33, found 536.1.

References and Notes

- (1). (a) Hannun, Y. A. *Science* **1996**, *274*, 1855-1859. (b) Ariga, T.; Jarvis, W. D.; Yu, R. K. *J. Lipid Res.* **1998**, *39*, 1-16. (c) Perry, D. K.; Hannun, Y. A. *Biochim. Biophys. Acta* **1998**, *1436*, 233-243.
- (2). Grob, C. A.; Jenny, E. F.; Utzinger, H. *Helv. Chim. Acta* **1951**, *34*, 2249-2254.
- (3). For reviews, see: (a) Byun, H.-S.; Bittman, R. In *Phospholipids Handbook*; Cevc, G., Ed.; Marcel Dekker: New York, 1993; pp 97-140. (b) Koskinen, P. M.; Koskinen, A. M. P. *Synthesis* **1998**, *8*, 1075-1091. (c) Jung, K.-H.; Schmidt, R. R. In *Lipid Synthesis and Manufacture*; Gunstone, F. D., Ed.; Sheffield Academic Press: Sheffield, U. K.; CRC Press: Boca Raton, FL, 1999; pp 208-249. (d) Curfman, C.; Liotta, D. *Methods Enzymol.* **1999**, *311*, 391-457. (e) Koskinen, P. M.; Koskinen, A. M. P. *Methods Enzymol.* **1999**, *311*, 458-479.
- (4). Trost, M. T.; Salzmann, T. N.; Hiroi, K. *J. Am. Chem. Soc.* **1976**, *98*, 4887-4902.
- (5). (a) Magnus, P. D. *Tetrahedron* **1977**, *33*, 2019-2045. (b) Block, E. *Reactions of Organosulfur Compounds*; Academic: New York, 1978. (c) Trost, B. M. *Chem. Rev.* **1978**, *78*, 363-382. (d) Simpkins, N. S. *Sulphones in Organic Synthesis*; Pergamon: Oxford, 1993.
- (6). Tao, J.; Hoffman, R. V. *J. Org. Chem.* **1998**, *63*, 3979-3985.
- (7). Koskinen, A. M. P.; Koskinen, P. M. *Synlett* **1993**, 501-502.
- (8). L-Oxazolidine methyl ester **5** and aldehyde **12**, which are commercially available, are readily prepared from L-serine: Garner, P.; Park, J. M. *Org. Synth.* **1991**, *70*, 18-28.
- (9). Dodson, R. M.; Srinivasan, V.; Sharma, K. S.; Sauers, R. F. *J. Org. Chem.* **1972**, *37*, 2367-2372.
- (10). For the formation of a β -ketosulfoxide by reaction of an ester with an α -sulfinyl carbanion, see: Corey, E. J.; Chaykovsky, M. *J. Am. Chem. Soc.* **1965**, *87*, 1345-1353.

(11). The sulfoxide **4** was synthesized by using a similar procedure as in the following paper: Khiar, N.; Singh, K.; García, M.; Martín-Lomas, M. *Tetrahedron Lett.* **1999**, *40*, 5779-5782.

(12). Chun, J.; Li, G.; Byun, H.-S.; Bittman, R. *J. Org. Chem.* **2002**, *67*, in press.

(13). The pairs of signals arise from the conformers present in the compound containing the oxazolidine ring system.