2-(Tributylstannyl)-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzyl Alcohol: A Building Block for Photolabeling and Cross-Linking Reagents of Very High Specific Radioactivity

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Abstract: A general approach for the synthesis of novel, radioiodinated photolabeling and cross-linking reagents at no-carrier-added (nca) specific radioactivity (>2000 Ci/mmol) is described. In this approach, 2-(tributylstannyl)-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzyl alcohol **12** serves as a module which, through acylation of its alcohol function, is connected to other structural/functional elements to make the tin-containing precursor forms of the reagents. The final, radioactive compounds are then conveniently prepared in yields varying from 15−50% by electrophilic aromatic substitution of the tributylstannyl group by $^{125}I^+$ generated *in situ* from $^{125}I^-$ by peracetic acid. Using this approach, we have synthesized two analogues of phosphatidylcholine (PC) (**20** and **21**), an analogue of ceramide (**36**), as well as two heterobifunctional label-transfer cross-linkers (**29** and **33**). PC **21**, examined more closely, is a substrate of the PC-specific phospholipid exchange protein from beef liver and of phospholipase D from cabbage. The latter was utilized to enzymatically convert PC **21** into two additional phospholipids, phosphatidylserine (PS) **25** and phosphatidic acid (PA) **26**. Reagents that contain this iodinated photophor also combine desirable photochemical properties. Thus, evidence is presented that they undergo efficient photolysis to generate a (singlet) carbene intermediate which inserts efficiently into hydrocarbon CH bonds. In addition, we demonstrate that the diazirine can be photolyzed in a selective manner, that is, without photodeiodination to occur to a significant extent.

Introduction

Photo(affinity) labeling and cross-linking refer to a variety of important biochemical techniques to investigate structural and functional properties of biological systems.[1] These techniques make use of reagents which, after targeting to a biological system or component, can be activated with UV light to generate highly reactive intermediates capable of forming covalent bonds with adjacent molecules. Labeling reagents can be divided into three main classes: first, probes designed to report on general properties of a system (for example, small apolar molecules and lipids which partition into membranes and, upon activation, label selectively integral proteins); second, affinity labeling reagents intended to interact with and label components (receptors) in a specific, functionally relevant manner; and, third, heterobifunctional (photo)cross-linkers, containing a photoactivatable and a conventional (thermal) reactive function, preferentially connected via a cleavable linker. These latter reagents are primarily used to study the spatial relationship between components in complex systems, or to make macromolecular affinity probes.

Most of the photoreagents developed to date are based on aryl azide or nitroaryl azide chemistry. However, unlike what had been assumed earlier, aryl azides or nitroaryl azides do not react via short-lived nitrene intermediates, but instead rapidly undergo ring expansion to dehydroazepines. The latter tend to be trapped by nucleophiles such as amines, or, in the absence of nucleophiles, undergo poorly defined polymerization reactions.[2] More suitable in this respect are fluoro- and chlorophenyl azides.[3] When activated in hydrocarbon solvents, the intermediate nitrenes insert intermolecularly into CH bonds, the most desired type of reaction also for establishing a covalent bond with a target biopolymer. Another class of reagents are those which upon activation generate a carbene intermediate. Among these, 3-(trifluoromethyl)-3-aryldiazirines appear to come closest to satisfying the chemical and biological criteria required for most photoreagents.[4] This functionality is easily synthesized in high yield and, in the absence of UV light, is remarkably stable under a range of different physical and chemical conditions, including heat (80 °C), strong bases, strong acids, oxidizing conditions and mild reducing agents. Upon irradiation with light at around 350 nm, the diazirine is rapidly photolyzed to generate a carbene capable of reacting with the full range of functional groups occurring in biomolecules, including paraffinic

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CH bonds. While most of the covalent adducts thereby formed are stable under a wide range of conditions, insertion into NH and OH bonds may lead, in some cases, to less stable products, a point to consider in the analysis of labeled target molecules.[4b,d]

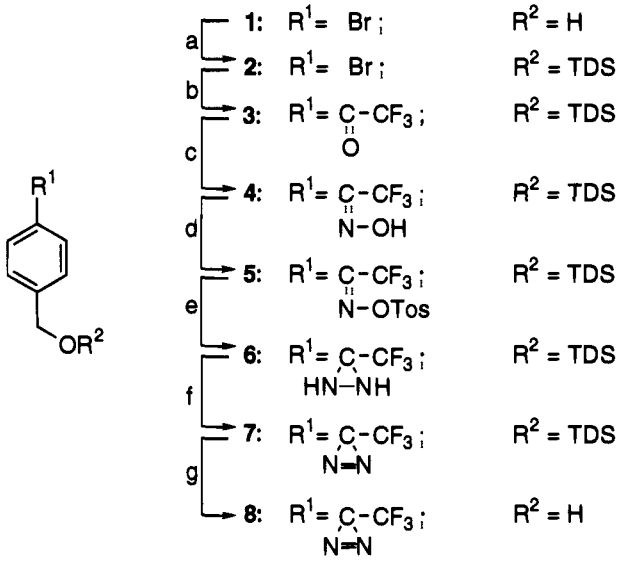
For most applications, photolabeling reagents must be available in an isotopically labeled form of (very) high specific radioactivity. A widely employed strategy to realize this task is to introduce one of the common radioisotopes of iodine, ^{125}I or ^{131}I, a γ- and a strong β-emitter, respectively. Unfortunately, however, UV-irradition of most of these reagents is accompanied by photolytic liberation of iodine. This is a severe shortcoming, since iodine (radicals), in turn, can be trapped by biological targets resulting in their unspecific labeling.[5] Toward the development of photophors with improved properties, iodinated azidoperfluorobenzoates and 4-azido-3,5,6-trifluorobenzoates have recently been examined.[3b] When photolyzed in cyclohexane, methyl 4-azido-2-iodo-3,5,6-trifluorobenzoate gave the expected CH insertion product in 12% yield, together with substantial amounts (8%) of the deiodinated counterpart and other products. Placement of the iodine atom into a ring different from the phenyl azide ring has not led to a significant improvement. Another recent class of potentially useful reagents are iodinated 3-(trifluoromethyl)-3-(alkoxy)phenyldiazirines.[6] However, their photochemical properties have not yet been determined. A further question concerning both iodinated perfluorophenyl azides and (trifluoromethyl)-3-(alkoxy)phenyldiazirines is whether the iodination reactions/conditions can be adopted to prepare reagents of high specific radioactivity. Radioiodine can hardly be obtained and manipulated at those (iodine) concentrations (molar range) used to prepare the nonradioactive materials.

In this article, we now report the synthesis of several members of a novel familiy of radioiodinated photolabeling and cross-linking reagents which can easily be prepared at the nca specific radioactivity of ^{125}I (>2000 Ci/mmol) and which also display favorable photochemical properties. Central in this work was the development of the building block **12**. Tin-containing photoreagents synthesized from this module can be converted into the final reagents by electrophilic displacement of the tributylstannyl group by $^{125}I^+$. In contrast to the photochemical performance of iodinated aryl azides, the diazirine can be photolyzed without significant loss of iodine.

Results and Discussion

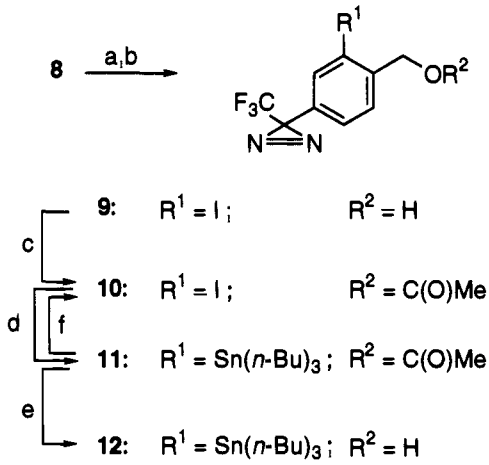
Design and Synthesis of 2-(Tributylstannyl)-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzyl Alcohol (12). Our original idea was to incorporate radioiodine through aromatic thallation/iodination of an appropriately functionalized 3-phenyl-3-(trifluoromethyl)diazirine, an approach analogous to that taken recently for the preparation of (nonradioactive) 3-(alkoxy-iodophenyl)-3-(trifluoromethyl)diazirines.[6] Because of undesirable quenching reactions expected for *ortho*-substituted arylcarbenes,[7] we planned to position the substituents *meta* or/and *para* to the 3-(trifluoromethyl)-3*H*-diazirinyl group. As the parent molecule we have chosen 4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzyl alcohol **8**. Acylation of the alcohol function was viewed to offer a convenient way to introduce additional structural or functional elements and, specifically, to provide a basis for the preparation of alkali-cleavable label-transfer reagents (see below). Diazirine **8** was prepared by means of a combination of reported procedures (Scheme 1). For transient protection of the alcohol the (1,1,2-trimethylpropyl)dimethylsilyl (TDS), rather than the previously used dimethyl-*tert*-butylsilyl (DTBS) group, was employed. This provided a significant advantage during purification of diazirinylbenzyl alcohol **8**, which, following the deprotection reaction (Scheme 1, step g), can be more easily separated from TDS−OH than from DTBS-OH.

Scheme 1[a]



[a] (a) TDS chloride/imidazole/DMF; (b) 1. Mg/THF/reflux, 2. (trifluoracetyl)piperidine/0 °C, 3. saturated NH_4Cl; (c) hydroxylamine/pyridine/reflux; (d) tosyl chloride/DMAP/Et_3N/CH_2Cl_2; (e) NH_3 under pressure/Et_2O; (f) I_2/triethylamine/MeOH; (g) HCl/MeOH.

Scheme 2[a]



[a] (a) $Tl(CF_3SO_3)_3$/trifluoroacetic acid/80 °C; (b) aqueous NaI; (c) Ac_2O/DMAP/THF; (d) hexabutylditin/*n*-Bu_4NF/KH_2PO_4/THF/50 °C; (e) NH_3/MeOH/Et_2O; (f) $Na^{125}I$/peracetic acid/AcOH.

Functionalization of benzyl alcohol **8** was achieved through reaction with thallium(III) -(trifluoromethane)sulfonate, an agent applied previously to thallate strongly deactivated perfluoroarenes.[8] Treatment of the reaction product mixture with an excess of iodide then afforded 2-iodo-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzyl alcohol **9** in 63% yield (Scheme 2). The position of the iodine atom *meta* to the diazirine group was confirmed by NMR spectroscopy. The ^{19}F NMR spectrum showed a triplet with a coupling constant of J = 0.7 Hz,

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consistent with *two* protons *ortho* to the diazirinyl group. In addition, the HCC-correlation–2D-NMR spectrum of acetate **10** showed coupling between the benzylic protons and three aromatic carbons, of which only one bears a H atom. In turn, only one aromatic proton shows long-range coupling with the benzylic carbon atom. Although the above pathway could, in principle, be used to introduce ^{125}I or ^{131}I, at the low concentrations (<0.1 mM iodine) at which these isotopes are accessible, the reaction turned out to be too sluggish to be of practical utility. Moreover, this approach would not permit the radioisotope to be introduced in the very last step in the synthesis of actual cross-linking reagents (see below).

The above results prompted us to examine an alternative approach, iododestannylation. The use of organotin chemistry for the preparation of radiohalogenated (and tritiated) organic compounds is well established, and for a variety of situations, in which high specific radioactivity, rapidity of incorporation and site-specificity of labeling are required, electrophilic destannylation is the method of choice.[9] The requisite [(trialkylstannyl)aryl]diazirine (**12**) was synthesized in 36% yield by reaction in THF of aryl iodide **10** with hexabutylditin in the presence of *n*-Bu_4NF as a catalyst.[10] To prevent (extensive) cleavage of benzyl ester **10** during the stannylation reaction, solid KH_2PO_4 was added to the reaction mixture.[11] Treatment of **11** with methanolic ammonia then afforded the free alcohol **12** in 97% yield.

2-Iodo-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzyl Acetate: Radioisotopic Labeling. To evaluate the suitability of **12** as a building block for photoreagents, acetate **11** was used as a model compound. In the presence of peracetic acid as an oxidant, (radio)iododestannylation of **11** proved both efficient and experimentally very simple. In fact, 2 min after addition of peracetic acid to a dilute solution of **11** (1 mM) and [^{125}I]-NaI (0.05 mM) in AcOH, the reaction was essentially complete and gave **10** in 50–60% yield (Scheme 2, step f). This suggested that a broad diversity of photoreagents can be synthesized as stable, tin-containing precursor forms, which, prior to use, are then easily and rapidly converted into the final radioiodinated reagents.

Photochemical Properties. Consistent with the spectral properties of other 3-(trifluoromethyl)-3-phenyldiazirines,[4a,b,e] the iodinated diazirines described here show a characteristic absorption at around 350 nm. This is depicted in Figure 1 for model compound **10** (Scheme 3), which was chosen for some photolysis studies.

When a 2 mM solution of **10** in cyclohexane was irradiated with the filtered light from a high-pressure mercury lamp, the diazirine absorption at 351 nm decreased with a half-life period of ca. 14.6 s following apparent first-order kinetics (Figure 1). Upon photolysis, the solution turned slightly orange (absorbance maximum at around 460 nm), a result of the well-documented partial rearrangement of the diazirine to the diazo isomer.[4a,b,e] After photolysis for 60 s, the solution was concentrated and subjected to TLC and GC/MS analyses. The most prominent, fastest migrating compound on TLC was identified as the product from insertion of the carbene into a CH bond of

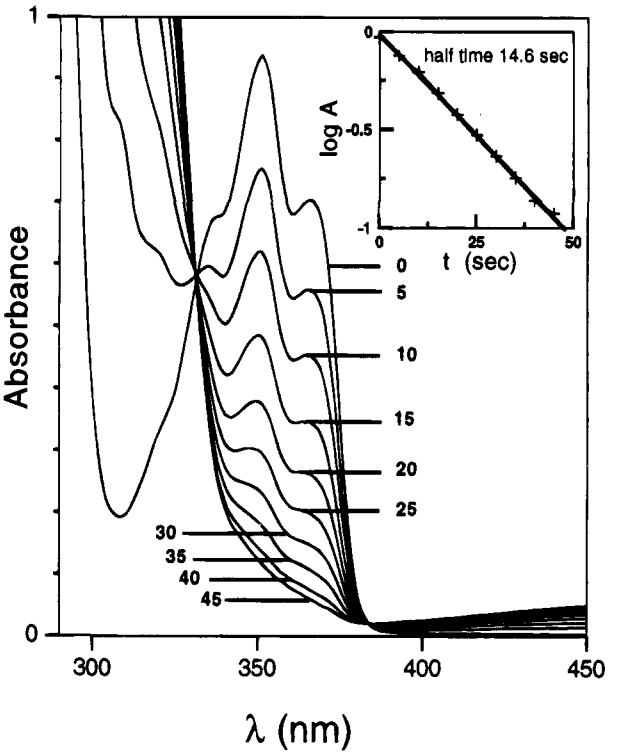
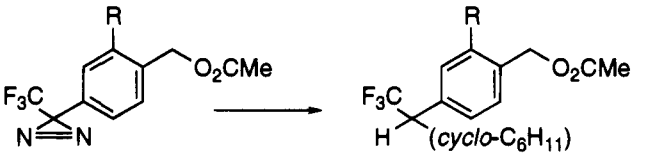


Figure 1. UV–vis spectra of the reaction products from photolysis of 2-iodo-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzyl acetate (**10**). A solution (2 mM) in cyclohexane was irradiated with filtered UV light (λ > 320 nm) for increasing periods of time (in seconds) as indicated with numbers. The spectra show the decay of the diazirine characteristic absorption with a maximum at 351 nm. The inset shows the decay as a function of time of photolysis in a semilogarithmic plot.

Scheme 3[a]



10: R = I **13:** R = H **14:** R = I **15:** R = H

[a] Cyclohexane/*hν* (λ > 320 nm).

cyclohexane (**14**) (Scheme 3). The isolated yield of **14** was approximately 25%. This product also corresponds to the largest peak (estimated 48%) in the gas chromatogram (Figure 2A).

To examine whether photolysis of diazirine **10** is accompanied by loss of iodine, the reaction products were compared with *noniodinated* reference compounds prepared by photolysis in cyclohexane of a 2 mM solution of the (*noniodinated*) diazirine benzyl acetate **13**. As shown (Figure 2B), photolysis of **13** also gave rise to several components, among which the CH (cyclohexane) insertion product **15**, being the most prominent, accounted for approximately 58%.[12] As revealed by the gas chromatograms (Figures 2A and B), no common products were formed from **10**, and **13** (the minor component at 15.32 min (Figure 2B)) has not been identified, but must be distinct from the (iodinated) CH-insertion product **14** having nearly the same retention time (15.40 min). It should be noted that these results do not rule out the possibility that loss of iodine from **10** leads to the destruction along pathways resulting in products different

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(11) Initial attempts to prepare **12** through stannylation of free benzyl alchol **9** resulted in the formation of **8** as the main product. Reduction of arylhalogenides during stannylation with hexaalkylditin is a side reaction reported in the literature; see ref 10.

(12) At an initial concentration of **13** of 30 mM, the CH insertion product **15** was produced in 28±5% yield, as determined by quantitative GC analysis.

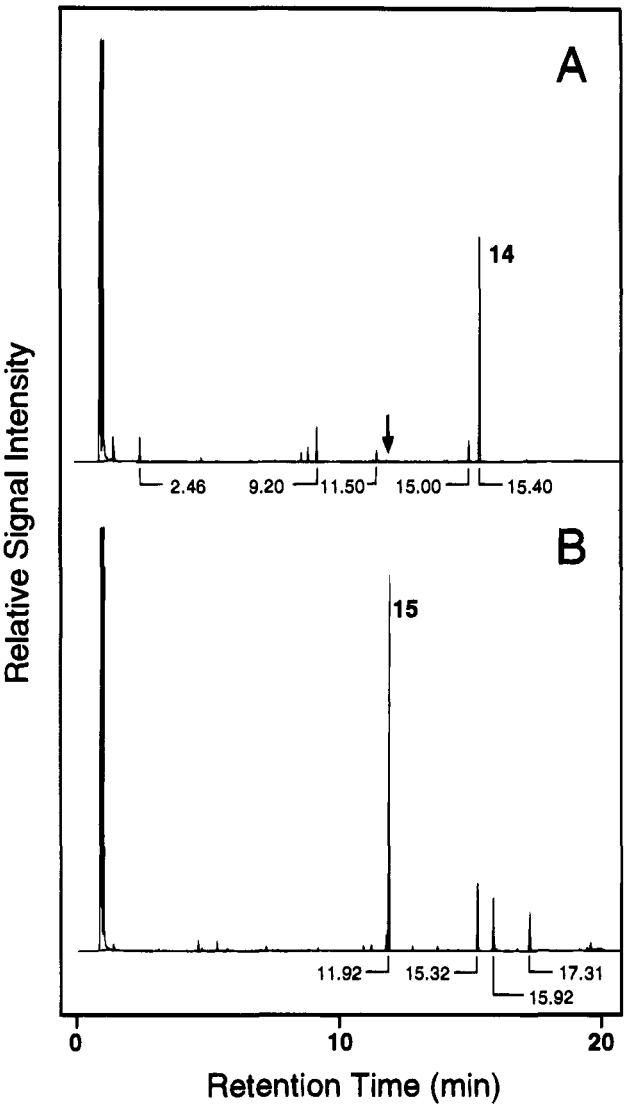

Figure 2. Gas chromatograms of reaction products derived from photolysis of 2 mM solutions of (A) 2-iodo-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzyl acetate (**10**) and (B) 4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzyl acetate (**14**) in cyclohexane. After irradiation with filtered UV light (λ > 320 nm) for 60 s, the solutions were concentrated to approximately one third of the original volume and 1 μL each was analyzed by GC (SPB-S Supelco 2-4034 column (30 m, 0.25 mm), injection block at 250 °C, 1:10 split mode) using FI detection. The main product peaks (retention times 15.40 min (A) and 11.92 min (B)) correspond to the CH insertion products **14** and **15**, respectively. The arrow marks the retention time (11.92 min) for compound **15**. For more details see main text and Experimental Section.

from those in Figure 2B.[13] To further examine this point, we also investigated whether iodine is liberated upon photolysis of **10**. Following photolysis of a solution (5 mM) of trace-labeled (^{125}I) **10** in cyclohexane, any free iodine was reduced and extracted into aqueous $Na_2S_2O_5$. Less than 0.5% (nonphotolyzed control samples: <0.2%) of the original radioactivity was recovered in the aqueous phase, a result which further corroborates the view that the diazirine function of **10** can be photolyzed without significant loss of iodine. This behavior is in marked contrast to what is observed for iodinated (fluoro)-phenyl azides and may reflect the fact that diazirines absorb at longer wavelengths and are more efficiently photolyzed than phenyl azides. In addition, the electron-withdrawing trifluoroethyl group may lead to an increased stability of the C−I bond.

(13) We are grateful to one of the referees for pointing to this fact.

That this type of iodinated phenyldiazirines make promising photolabeling reagents is inferred also from a number of studies with 3-(trifluoromethyl)-3-(3-[^{125}I]iodophenyl)diazirine ([^{125}I]-TID), a reagent widely used in the past for labeling of the apolar phase of membranes.[14] The superb selectivity of this reagent in labeling integral (versus peripheral) proteins may be a direct reflection of the C−I bond stability during diazirine photolysis.

The lower CH insertion efficiency measured by TLC as compared to GC can be attributed, in part, to losses during product isolation by TLC and is likely to be due to thermal decomposition of the diazo isomer present in the photolysis reaction mixture upon injection of the sample into the GC system, resulting in a larger proportion of the CH insertion product.[15] Although further studies are needed to elucidate the details of the photochemistry of iodinated diazirines, we, nonetheless, note that the efficiency of **13** and **10** to insert into cyclohexane CH bonds, as determined by GC analysis, is in a range comparable to that reported for the nonsubstituted 3-(trifluoromethyl)-3-phenyldiazirine (≥50%)[4a] or for *N*-BOC-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]phenylalanine methyl ester (73%).[4b] The high efficiency of CH insertion reactions is suggestive of a mechanism involving a singlet carbene, implicating that the heavy atom iodine has, if any at all, only a moderate effect in promoting triplet photochemical reactions.

Design, Synthesis, and Biochemical Properties of Phospholipid Photolabels. Over the last 20 years, numerous photoactivatable lipids have been synthesized and applied as general labeling reagents[16] or as photoaffinity probes.[17] Most of these lipids are PCs carrying the photoactivatable group linked through an amide, ester, or ether group to the ω-position of the *sn*-2 fatty acyl chain. Accumulating evidence suggesting that lipids, in addition to being structural components of membranes, also act as second messengers,[18] is expected to increase the demands for appropriate photoaffinity probes generating short-lived intermediates and accessible at high specific radioactivity. For this reason, two new photoreactive PCs meeting the above criteria have been synthesized and some of their properties examined.

We decided to synthesize two PC analogues containing either a dodecanoyl or a hexadecanoyl *sn*-1 fatty acyl residue. These

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(15) GC-analysis of an *unphotolyzed* solution of **10** (1%) in cyclohexane gave a pattern of compounds similar to that in Figure 2A; presumably these compounds were formed upon thermal decomposition of **10** and gas-phase reactions of the carbene.

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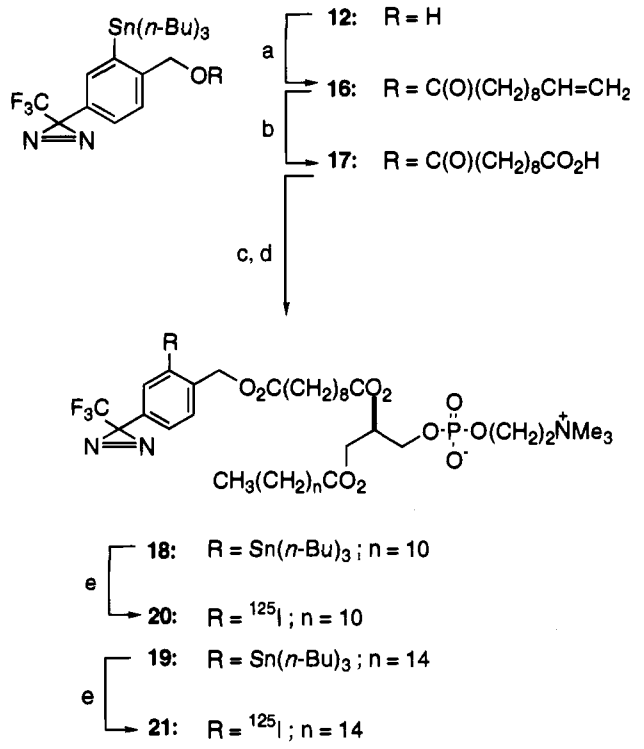
(18) Liscovitch, M.; Cantley, L. C.; *Cell* **1994**, *77*, 329−334

Scheme 4[a]



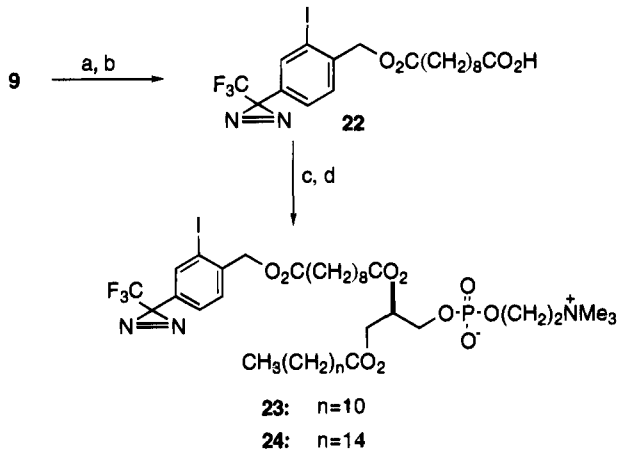
[a] (a) 10-Undecenoic anhydride/DMAP/Et_2O; (b) $KMnO_4$/18-crown-6/CH_2Cl_2/AcOH/water; (c) DCC/CCl_4; (d) 1-*O*-dodecanoyl- and 1-*O*-hexadecanoyl-lyso-PC, respectively/DMAP/pyridine/$CHCl_3$; (e) $Na^{125}I$/peracetic acid/AcOH.

lipids could be expected to exhibit different monomer solubility in water, one of the factors determining spontaneous intermembrane exchange/transfer rates which are of interest from an application point of view.[19] First, the ω-substituted fatty acid **17** was synthesized (Scheme 4). Following established procedures, the anhydride of **17** was formed and used to acylate 1-dodecanoyl- and 1-hexadecanoyl-*sn*-glycero-3-phosphocholine (lyso-PC) to afford the asymmetrical 1,2-*O*-diacyl-*sn*-glycero-3-phosphocholines **18** and **19** (Scheme 4). Conversion into the final radioactive lipids **20** and **21** was accomplished by using a 20-fold stoichiometric excess of the tin precursor over $Na^{125}I$ and peracetic acid as the oxidant (Scheme 4). In both cases, the principal (organic) radioactive product formed was indistinguishable (TLC and HPLC analysis) from the corresponding nonradioactive reference compounds **23** and **24**, prepared by the more direct route depicted in Scheme 5. Although TLC can be applied to separate the radioiodinated lipids from excess of tin precursors, reverse-phase HPLC proved more convenient and effective. Both iodinated PCs are eluted as distinct peaks, well-separated from the excess of the tin counterparts which were retained significantly longer (for details, see Experimental Section). In this way, PCs **20** and **21** could be obtained at nca specific radioactivity (>2000 Ci/mmol) and in 35–50% yields with respect to the starting $Na^{125}I$.

When liposomes, prepared from egg-PC and traces of either **20** or **21**, were incubated with biological "acceptor" membranes (human erythrocyte membrane or dog pancreas microsomes), >10% of PC **20** was spontaneously transferred within 15 min at 37 °C, whereas the long-chain PC **21** did not undergo significant transfer (<3% in 4 h).[20] Nevertheless, it was also possible to incorporate **21** into preformed membranes. This was

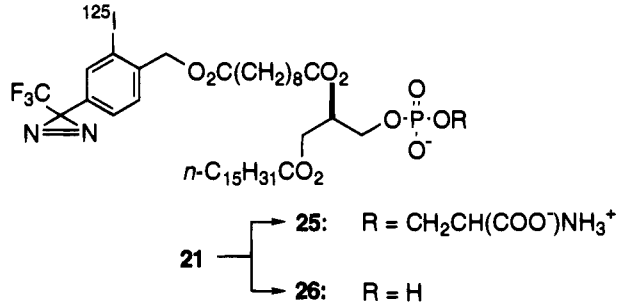
(19) (a) Smith, R.; Tanford, C. *J. Mol. Biol.* **1972**, *67*, 75–83. (b) Massey, J. B.; Gotto, A. M.; Pownall, H. J. *Biochemistry* **1982**, *21*, 3630–3636.
(20) Weber, T.; Martoglio, B. Unpublished results.

Scheme 5[a]



[a] (a) 10-Undecenoic anhydride/DMAP/ether; (b) H_2CrO_4/OsO_4/water; (c) DCC/CCl_4; (d) 1-*O*-dodecanoyl- and 1-*O*-hexadecanoyl-lyso-PC, respectively/DMAP/pyridine/$CHCl_3$.

Scheme 6[a]



[a] Serine/Phospholipase D/aqueous buffer, pH 5.6/Et_2O.

achieved by catalyzing the exchange/transfer process with the PC-specific phospholipid exchange protein from beef liver. This technique has been applied recently to incorporate radioiodinated **21** into the envelope of influenza virus and to label the membrane-embedded segments of the virus' fusion protein, hemagglutinin.[21]

Phosphatidylcholine **21** was also found to be a substrate of phospholipase D from cabbage (and presumably of other phospholipases as well). Thus, following standard biochemical protocols, in a biphasic system (Et_2O/aqueous L-serine) in the presence of phospholipase D, **21** was converted into the corresponding (radioactive) PS **25** and the product of hydrolysis, PA **26** (Scheme 6). On TLC the radioactivity co-migrates with authentic samples of dipalmitoyl–PS and dipalmitoyl–PA, respectively. In addition, relatively apolar components were formed (data not shown), one of which may correspond to diacylglycerol which apparently is also produced during this reaction.[16l] The various phospholipids could easily be separated by TLC and, upon rechromatography, gave single spots as visualized by autoradiography (Figure 3). In all likelihood, these lipids can be transformed further and, therefore, represent valuable starting materials for the enzymatic generation of a variety of additional photoactivatable, radioiodinated analogues of lipids and lipid metabolites, molecules with considerable scope and potential as photoaffinity probes.

^{125}I-Label-Transfer Photocross-linking Reagents. In the past years, much attention has been focused on the development of reagents commonly referred to as label-transfer photocross-linkers.[22] These are cleavable, radiolabeled heterobifunctional

(21) Weber, T.; Paesold, G.; Galli, C.; Mischler, R.; Semenza, G.; Brunner, J. *J. Biol. Chem.* **1994**, *269*, 18353–18358.

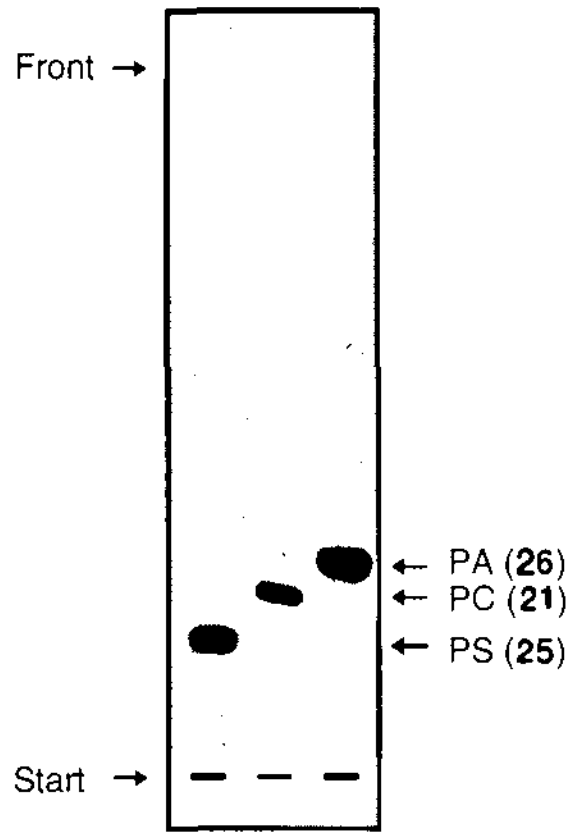


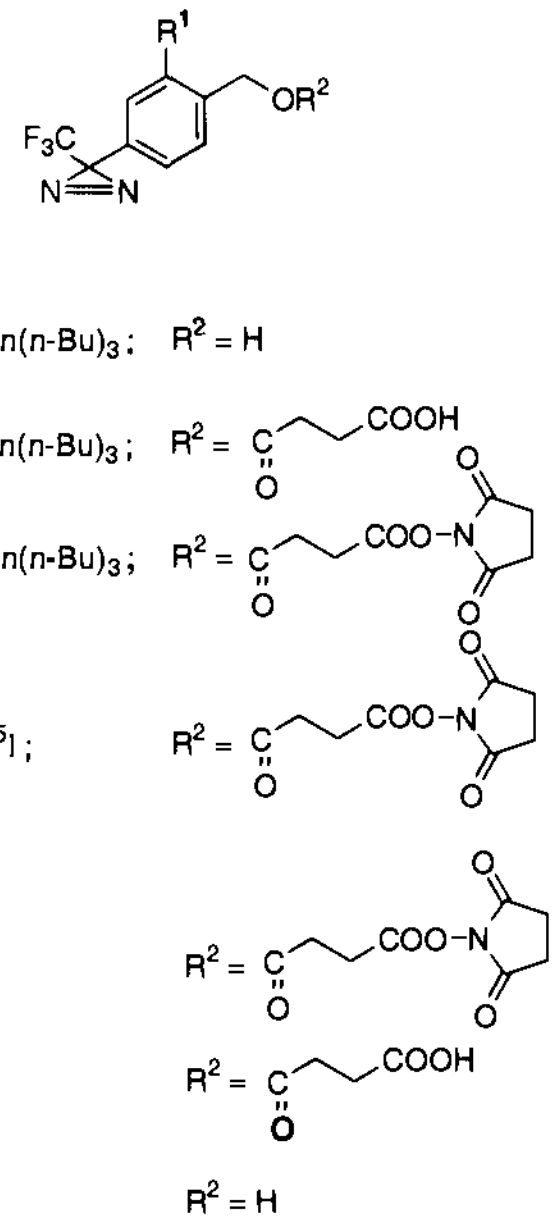
Figure 3. Autoradiography of the thin-layer chromatogram of three phospholipids, isolated from the mixture of the phospholipase D-catalyzed reaction of PC **21** and L-serine in buffer/Et_2O emulsion. The phospholipids were extracted from the reaction mixture with $CHCl_3$/MeOH and separated by TLC. The corresponding silica gel bands, visualized by autoradiography, were scraped off and the radioactive lipids were eluted with $CHCl_3$/MeOH. Rechromatography (this figure) gave clean, well-separated bands of PC (**21**), PS (**25**), and PA (**26**).

reagents and are mainly used for studying the neighborhood relationship (protein−protein contacts) in macromolecular complexes. Label-transfer cross-linking includes targeting to (a) nucleophilic residue(s) in the primary protein component, photoinduced cross-linking to the secondary component, and transfer of the radioactivity to the site of insertion by cleaving the susceptible bond. Because of the shortcomings of previous reagents noted above, two new cross-linkers, **29** and **33**, have now been synthesized. While *N*-hydroxysuccinimide ester **29** can be coupled to primary or secondary amines, maleimide **33** reacts preferentially with sulfhydryl groups.

The corresponding tin precursors **28** and **32** were obtained in a straightforward manner starting from **12**. *O*-Acylation of **12** with succinic anhydride yielded carboxylic acid **27** which then was converted to the activated *N*-hydroxysuccinimide ester **28**, the tin precursor of **29** (Scheme 7). Likewise, O-acylation of **12** with with maleimidopropionic anhydride gave precursor **32** (Scheme 8). While the standard radioiodination protocol could be applied to convert **28** into **29**, radioiododestannylation of **32** required somewhat milder conditions to prevent oxidative damage of the *N*-alkylmaleimide moiety. Under these conditions less of the aryl iodide was formed (15−20% as compared to 35−40% for the other compounds). Cross-linkers **29** and **33** prepared in this manner showed R_f values on TLC identical to those of corresponding nonradioactive reference compounds **31** and **34** synthesized through acylations of **9** (Schemes 7 and 8). Since both cross-linkers contain an alkali-labile ester linkage, treatment of cross-linked biomolecules with aqueous hydroxylamine or ammonia will lead to rapid cleavage of this functionality, resulting in a formal transfer of the radioisotope from the primary component to the target molecule.

(22) (a) Schwartz, M. A.; Das, O. P.; Hynes, R. O. *J. Biol. Chem.* **1982**, *257*, 2343−2349. (b) Denny, J. B.; Blobel, G. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 5286−5290. (c) Resek, J. F.; Bhattacharya, S.; Khorana, H. G. *J. Org. Chem.* **1993**, *58*, 7598−7601.

Scheme 7[a]



[a] (a) Succinic anhydride/DMAP/THF; (b) DCC/*N*-hydroxysuccinimide/THF; (c) Na^{125}I/peracetic acid/AcOH; (d) succinic anhydride/DMAP/THF; (e) DCC/*N*-hydroxysuccinimide/THF.

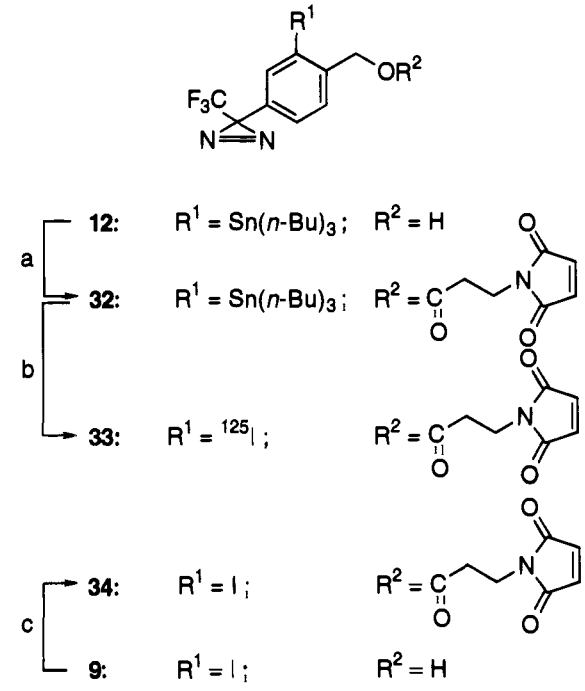
Synthesis of a Ceramide Analogue. Ceramide, a product of *de novo* synthesis and sphingolipid degradation, is comprised of a fatty acid moiety linked through an amide to a long-chain sphingoid base (predominantly sphingosine). It serves as a biosynthetic precursor of sphingomyeline and complex glycosphingolipids, implicated to play a regulatory role in membrane trafficking and protein sorting.[23] Moreover, ceramide, or other sphingolipids, are required in Semliki Forest virus-induced membrane fusion, possibly by activating the viral fusion protein in a specific manner.[24] Perhaps most importantly, ceramide also acts as a second messenger, stimulating a serine/threonine ceramide-activated protein kinase to transduce the cytokine signal used in inflammation, immune responses, and apoptosis.[25] With the ultimate goal of identifying and structurally mapping the various cellular targets for ceramide, the ceramide analogue **36** was synthesized. This was accomplished through *N*-acylation of D-*erythro*-sphingosine with *N*-hydroxysuccinimide ester **28** and subsequent conversion of the tin precursor **35** to the final product **36** (Scheme 9). Ceramide **36** co-chromatographed (TLC and reverse-phase HPLC) with reference compound **37** obtained by *N*-acylation of D-*erythro*-sphingosine with **31**, a result demonstrating that the conditions (peracetic acid) employed for the iododestannylation reaction

(23) Van Meer, G.; Burger, K. N. J. *Trends Cell Biol.* **1992**, *2*, 332−337.

(24) Nieva, J. L.; Bron, R.; Corver, J.; Wilschut, J. *EMBO J.* **1994**, *13*, 2798−2804.

(25) (a) Hannun, Y. A. *J. Biol. Chem.* **1994**, *269*, 3125−3128. (b) Kolesnick, R.; Golde, D. W. *Cell* **1994**, *77*, 325−328. (c) Schütze, S.; Potthoff, K.; Machleidt, T.; Bercovic, D.; Wiegmann, K.; Krönke, M. *Cell* **1992**, *71*, 765−776.

Scheme 8[a]



[a] (a) 3-Maleimidopropionic acid/DCC/DMAP/$NaHCO_3$; (b) $Na^{125}I$/peracetic acid (*in situ* generated)/aqueous buffer, pH 4.5; (c) maleimidopropionic acid/DCC/$NaHCO_3$/DMAP.

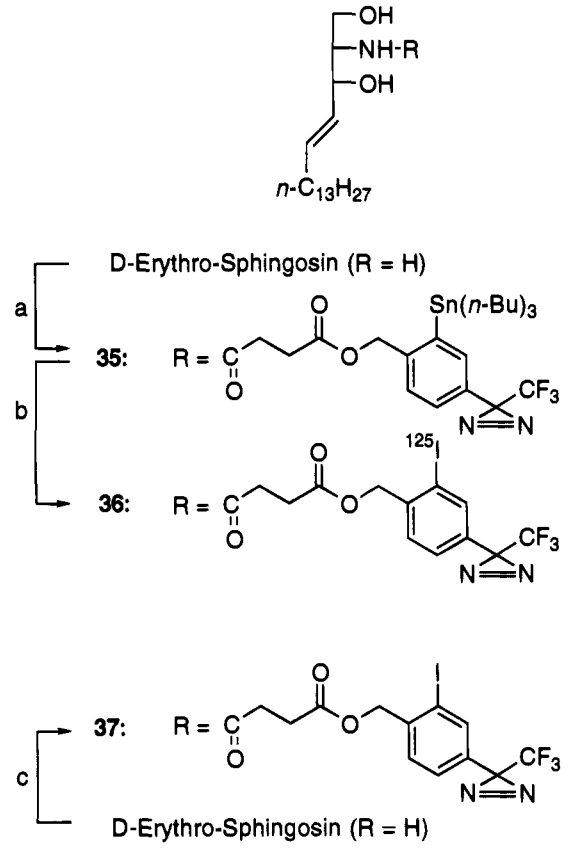
did not result in oxidative damage of the allylic or the primary alcohol function of ceramide. We have recently been informed that the ceramide analogue **37** is as effective as natural ceramide in mediating activation of the transcription factor NF-κB in permeabilized Jurkat cells.[26]

Concluding Remarks. In view of the increasing demands for the identification of the molecular targets for a wide structural diversity of ligands, the detailed analyses of ligand–receptor binding sites and the elucidation of the structural and dynamic organization of multicomponent systems, we have undertaken a significant effort to develop a novel building block for the synthesis of photo(affinity)labeling and cross-linking reagents of extremely high specific radioactivity. This module was used to prepare several members of different classes of photoreagents, which combine properties that had not been met before with other reagents. All compounds were easily obtained at nca ^{125}I-iodine specific radioactivity (>2000 Ci/mmol), and initial studies demonstrate that the photogenerated radioiodinated arylcarbene undergoes intermolecular CH insertion reactions with no significant loss of radioiodine. We expect this new class of photolabeling reagents to find successful applications in many areas of biochemistry and molecular cell biology.

Experimental Section

General Methods. Melting points (mp) and boiling points (bp) are uncorrected. ^{1}H, ^{13}C, ^{19}F and ^{31}P NMR spectra were recorded on Bruker instruments (200 to 500 MHz). Chemical shifts (δ) are reported in ppm relative to internal ($(CH_3)_4Si$, $\delta = 0.00$ (^{1}H and ^{13}C NMR)) and external standards ($CFCl_3$, $\delta = 0.00$ (^{19}F NMR) and 85% H_3PO_4 ($\delta = 0.00$)), respectively. IR spectra were recorded on a Perkin Elmer 1600 Series FT-IR spectrometer. Low-resolution mass spectra were recorded on a VG-TRIBRID and FAB mass spectra on a VG-SABSEQ instrument. Gas chromatography (GC) was carried out using an SPB-S Supelco 2-4034 column (30 m; 0.25 mm); injection block at 250 °C (1:10 split mode). The column was heated from 100 to 250 °C at 10 °C/min; detection was by flame-ionization. Thin-layer chromatography (TLC) was performed on precoated silica gel 60 F_{254} plates (Merck, Darmstadt, FRG); column chromatography, unless stated otherwise, was performed with silica gel 60 (230–400 mesh) from Merck. High-pressure liquid chromatography (HPLC) was perfomed on a system of Pharmacia using either an RP18-AB (250 × 4 mm) or, for purification of the radiolabeled phospholipids, a Nucleosil 100-5 C8 (125 × 4 mm) column (both from Machery-Nagel). The following solvent mixtures were used: (A) CH_2Cl_2/MeOH, 1:1; (B) Et_2O/AcOH, 100:1; (C) CH_2Cl_2/MeOH,100:15; (D) Et_2O/hexane, 2:1; (E) Et_2O/hexane, 1:2; (F) Et_2O/pentane, 1:9; (G) Et_2O/hexane/AcOH, 200:100:3; (H) $CHCl_3$/MeOH/water, 65:25:4; (I) Et_2O/hexane/AcOH, 100:100:2; (J) CH_2Cl_2/MeOH/AcOH, 100:2:5; (K) $CHCl_3$/MeOH/water/Ac_2O, 65:25:5:5; (L) CH_2Cl_2/hexane, 1:1. $Na^{125}I$ (350–600 mCi/mL in NaOH) was purchased from Amersham International, Amersham, UK. All work with radioactivity (>1 μCi) was performed in a C-type laboratory. For quantitation of ^{125}I, a γ-counting system (GAMMAmatic) from Kontron was used. Autoradiographies were done with RX Fuji Medical X-ray films.

Scheme 9[a]



[a] (a) **28**/triethylamine/THF; (b) $Na^{125}I$/peracetic acid/AcOH (c) **31**/triethylamine/THF.

4-Bromobenzyl (1,1,2-Trimethylpropyl)dimethylsilyl Ether (2). 4-Bromobenzyl alcohol (100 g, 0535 mol) was added to a solution of 97.5 g (0.545 mol) of TDS–Cl and 51.6 g (0.787 mol) of imidazole in 270 mL of *N,N*-dimethylformamide (DMF). After stirring at room temperature for 2 h, the reaction mixture was partitioned between water and hexane. The organic phase was washed with water and dried over $MgSO_4$. After evaporation of the solvent, the crude silyl ether was purified by distillation to give 147.7 g (83.8%) of a colorless liquid with bp 117–118 °C (0.1 Torr). ^{1}H NMR ($CDCl_3$): $\delta = 0.13$ (s, 3 H), 0.89 (s, 6 H), 0.91 (d, 6 H, $J = 7$ Hz), 1.66 (sept, 1 H, $J = 7$ Hz), 4.66 (s, 2 H), 7.18 (d, 2 H, $J = 9$ Hz), 7.44 (d, 2 H, $J = 9$Hz). MS (EI): *m/z* (relative intensity) 330/328 (0.1/0.1) (M^+), 245/243 (91/65) ($M^+ - C_6H_{13}$), 171/169 (98/100) ($M^+ - C_8H_{19}OSi$). Anal. Calcd for $C_{15}H_{25}BrOSi$ (329.35): C, 54.70; H, 7.65. Found: C, 54.99; H, 7.39.

4-Trifluoroacetylbenzyl (1,1,2-Trimethylpropyl)dimethylsilyl Ether (3). Mg turnings (11.1 g, 0.456 g-atom) and **2** (147.3 g, 0.447 mol) in 600 mL anhydrous tetrahydrofuran (THF) were cautiously heated until

(26) Schütze; S.; Krönke, M. (Institut für Medizinische Mikrobiologie und Hygiene Technische Universität München.) Unpublished results, 1994.

a vigorous reaction took place. After 2 h of refluxing almost all of the Mg turnings had disappeared. The reaction mixture was then cooled in an ice/MeOH bath. A solution of *N*-(trifluoroacetyl)piperidine (84.4 g, 0.465 mol) was added to the Grignard reagent over a period of 50 min with stirring at 0−2 °C. After an additional hour of stirring at room temperature, 1 vol of saturated NH_4Cl was slowly added such that the temperature did not exceed 30 °C. The mixture was extracted twice with Et_2O, and the combined organic phases were washed with saturated NH_4Cl and brine. After drying over $MgSO_4$, the solvent was removed under reduced pressure and the crude ketone purified by distillation to give 105.2 g (66.6%) of a colorless liquid with bp 108−114 °C (15 Torr). For analytical purposes, a sample was further purified by column chromatography (L) and subsequent bulb-to-bulb distillation. TLC: R_f (F) = 0.66. ^{1}H NMR ($CDCl_3$): δ = 0.16 (s, 6 H), 0.92 (s, 6 H), 0.93 (d, 6 H, J = 7 Hz), 1.68 (sept, 1 H, J = 7 H), 4.81 (s, 2 H), 7.28 (d, 2 H, J = 9 Hz), 7.5 (d, 2 H, J = 9 Hz). ^{19}F NMR ($CDCl_3$): δ = −71.6. IR (neat): ν = 1717 (C=O) cm^{-1}. MS (EI): *m/z* (relative intensity) 346 (0.03) (M^+), 261 (100) ($M^+ - C_6H_{13}$). Anal. Calcd for $C_{17}H_{25}F_3O_2Si$ (346.46): C, 58.94; H, 7.27. Found: C, 58.93; H, 7.38.

1-[4-[[[(1,1,2-Trimethylpropyl)dimethylsilyl]oxy]methyl]phenyl]-2,2,2-trifluoro-1-ethanone Oxime (4). A solution of **3** (105.2 g, 0.303 mol) and hydroxylamine hydrochloride (63.3 g, 0.911 mol) in pyridine (200 mL) was refluxed for 1 h and 45 min. After evaporation of the pyridine under reduced pressure, the residue was partitioned between EtOAc and 0.2 M citric acid. The organic phase was washed with water and dried over $MgSO_4$. Evaporation of the solvent yielded 103.3 g of the crude oxime as a pale, viscous oil containing residual solvent. For analytical purposes, a sample was purified by column chromatography (D). TLC: R_f($CHCl_3$) = 0.37. ^{1}H NMR of a mixture of of the *E*- and Z-isomers ($CDCl_3$): δ = 0.15 (two s, 6 H), 0.90−0.94 (m, 12 H), 1.65 (m, 1 H), 4.76 and 4.77 (two s, 2 H), 7.39−7.51 (m, 4 H), 8.36 (s, 1 H). IR ($CHCl_3$): ν = 3557 (O−H), 3307 (O−H) (*E*- and Z-isomers of the oxime) cm^{-1}. ^{19}F NMR ($CDCl_3$): δ = −66.9, −62.6. MS (EI): *m/z* (relative intensity) 362 (1.6) ($M^+ + 1$); 276 (100) ($M^+ - C_6H_{13}$). Anal. Calcd for $C_{17}H_{26}NO_2F_3Si$ (361.48): C, 56.49; H, 7.25; N, 3.87. Found: C, 56.71, H, 7.43; N, 3.93.

1-[4-[[[(1,1,2-Trimethylpropyl)dimethylsilyl]oxy]methyl]phenyl]-2,2,2-trifluoro-1-ethanone *O*-Tosyl Oxime (5). To a solution of oxime **4** (103.3 g, 0.285 mol), triethylamine (59.7 mL, 0.429 mol), and 4-(dimethylamino)pyridine (DMAP) (698 mg, 5.70 mmol) in CH_2Cl_2 (150 mL) at room temperature was added with stirring over 30 min a solution of *p*-toluenesulfonyl chloride (54.5 g, 0.285 mol) in CH_2Cl_2 (150 mL). After additional stirring at room temperature for 30 min, a 0.2 M solution of citric acid was added, and the organic layer was washed with water and dried over $MgSO_4$. Evaporation of the solvent yielded 128.2 g (87%) of the the crude *O*-tosyl oxime as a highly viscous oil. For analytical purposes, a sample was purified by column chromatography (CH_2Cl_2). TLC: R_f ($CHCl_3$) = 0.76. ^{1}H NMR of a mixture of the *E*- and Z-isomers ($CDCl_3$): δ = 0.14 and 0.16 (two s, 6 H), 0.88−0.94 (m, 12 H), 1.68 (m, 1 H), 2.46 and 2.48 (two s, 3H), 4.75 and 4.77 (two s, 2 H), 7.36−7.45 (m, 6 H), 7.86−7.91 (m, 2 H). ^{19}F NMR ($CDCl_3$): δ = −66.8, −61.7. MS (EI): *m/z* (relative intensity) 515 (0.04) (M^+); 430 (100) ($M^+ - C_6H_{13}$). Anal. Calcd for $C_{24}H_{32}F_3NO_4SSi$ (515.67): C, 55.90; H, 6.25; N, 2.72. Found: C, 56.07; H, 6.35; N, 2.95.

3-[4-[[[(1,1,2-Trimethylpropyl)dimethylsilyl]oxy]methyl]phenyl]-3-(trifluoromethyl)-3*H*-diaziridine (6). A solution of 10−15 g of the crude tosyl oxime **5** in 100 mL of Et_2O was cooled to −78 °C. Liquid ammonia (10 mL) was added, and the mixture was stirred at room temperature for 15 h in a sealed glass tube. After the resulting two-phase system was cooled to −78 °C, 50 mL of water was added, and the mixture was allowed to reach room temperature. The organic layer was separated, washed with water, and dried over $MgSO_4$. Evaporation of the solvent yieled the crude diaziridine (total 71.3 g (79%) starting from 128.2 g (0.254 mol) of **5**). Crystallization from pentane at −20 °C afforded 57.7 g of pure **6**. An additional 11.1 g was recovered from the concentrated filtrate by column chromatography (E). Total of 68.8 g (76%) of **6** as white crystals with mp 20−22 °C were obtained. TLC: R_f ($CHCl_3$) = 0.48. ^{1}H NMR ($CDCl_3$): δ = 0.14 (s, 6 H), 0.90 (s, 6 H), 0.92 (d, 6 H, J = 7 Hz), 1.67 (sept, 1 H, J = 7 Hz), 2.20 (d, 1 H, J = 9 Hz), 2.77 (d, 1 H, J = 9 Hz), 4.74 (s, 2 H), 7.38 (d, 2 H, J = 8 Hz), 7.57 (d, 2 H, J = 8 Hz). ^{19}F NMR ($CDCl_3$): δ = −75.82. IR (neat): ν = 3228 (N−H) cm^{-1}, 3251 (N−H) cm^{-1}. MS (EI): *m/z* (relative intensity) 359 (0.4) ($M^+ - 1$); 275 (100) ($M^+ - C_6H_{13}$). Anal. Calcd for $C_{17}H_{27}F_3N_2OSi$ (360.49): C, 56.64; H, 7.55; N, 7.77. Found: C, 56.82; H, 7.31, N, 7.80.

3-[4-[[[(1,1,2-Trimethylpropyl)dimethylsilyl]oxy]methyl]phenyl]-3-(trifluoromethyl)-3-diazirine (7). To a vigorously stirred solution of **6** (68.8 g, 0.19 mol) and triethylamine (38.4 g, 0.38 mol) in 150 mL MeOH was added portionwise I_2 (total 48.4 g, 0.191 mol). After being stirred for 30 min at room temperature, the dark brown reaction mixture was partitioned between Et_2O and saturated aqueous citric acid. The Et_2O phase was washed with solutions of citric acid, sodium hydrogensulfite, and, finally, with water. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to yield 66.7 g (97.4%) of crude **7** as a pale yellow oil, which crystallized at −20 °C. For analytical purposes, a sample was purified by column chromatography (hexane). TLC: R_f ($CHCl_3$) = 0.70. ^{1}H NMR ($CDCl_3$): δ = 0.13 (s, 6 H), 0.89 (s, 6 H), 0.91 (d, 6 H, J = 7 Hz), 1.67 (sept, 1 H, J = 7 Hz), 4.72 (s, 2 H), 7.16 (d, 2 H, J = 8 Hz), 7.35 (d, 2 H, J = 8 Hz). ^{19}F NMR ($CDCl_3$): δ = −65.6. MS (EI): *m/z* (relative intensity) 358 (0.8) (M^+); 330 (8) ($M^+ - N_2$); 273 (100) ($M^+ - C_6H_{13}$). Anal. Calcd for $C_{17}H_{25}F_3N_2OSi$ (358.48): C, 56.96; H, 7.03; N, 7.81. Found: C, 57.16; H, 6.85; N, 7.75.

4-[3-(Trifluoromethyl)-3*H*-diazirin-3-yl]benzyl Alcohol (8). A solution of crude diazirine **7** (26.25 g, 0.073 mol) in 150 mL MeOH and 10 mL of concentrated HCl was stirred at room temperature for 1.5 h. After removal of most of the MeOH, the residue was diluted with Et_2O and extracted with aqueous $NaHCO_3$ and water. The organic phase was dried over $MgSO_4$ and concentrated under reduced pressure. The yellow residue was subjected to column chromatography (G) to give 14.7 g (93%) of **8**. TLC: R_f ($CHCl_3$) = 0.37. ^{1}H NMR ($CDCl_3$): δ = 1.80 (s, 1 H); 4.71 (d, 2 H, J = 4 Hz), 7.19 (d, 2 H, J = 8 Hz), 7.39 (d, 2 H, J = 8 Hz). ^{19}F NMR ($CDCl_3$): δ = −65.6. MS (EI): *m/z* (relative intensity) 216 (0.8) (M^+); 188 (100) ($M^+ - N_2$). Anal. Calcd for $C_9H_7F_3N_2O$ (216.16): C 50.01; H, 3.26; N, 12.96. Found: C, 50.07; H, 3.44; N, 12.71.

Special Attention for the Thallation of Diazirines. Thallium compounds are very toxic. However, they can be safely handled if prudent laboratory procedures are practiced. Rubber gloves should be worn, and reactions should be carried out in a well-ventilated hood (see ref 27 for more information).

2-Iodo-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzyl Alcohol (9). The title compound was synthesized by thallation/iodination of **8**, essentially as described for the preparation of iodoperfluoro arenes.[8] Thallium(III) trifluoroacetate (59.5 g, 0.11 mol) was dissolved in 109 mL of trifluoroacetic acid. After addition of trifluoromethanesulfonic acid (57.6 g, 0.384 mol), the white precipitate was dissolved by dropwise addition of water (4.4 mL). Alcohol **8** (8.2 g, 0.038 mol) was added to 151 mL of this solution, and the mixture was kept at 80 °C for 2 h in a sealed glass tube. The reaction mixture was allowed to cool to room temperature, and a solution of NaI (82.4 g, 0.55 mol) in 380 mL water was added. After being stirred for 45 min in the dark, the elemental iodine formed was reduced with sodium hydrogen sulfite and the solution made alkaline with potassium hydroxide platelets. One volume of THF was added, and the yellow thallium(I) iodide precipitate was removed by filtration through cellite. The filtrate was extracted twice with Et_2O, and the pooled organic phases were washed with water and dried over $MgSO_4$. After removal of the solvent, the residue was crystallized twice from hexane. Additional product was recovered following column chromatography (D) of the combined filtrates to yield 8.18 g (63%) of **9** as white needles with mp 71−72 °C. TLC: R_f (C) = 0.47. ^{1}H NMR (DMSO-d_6): δ = 4.41 (d, 2 H, J = Hz), 5.61 (t, 1 H, J = 5 Hz), 7.42 (dd, 1 H, J = 2 Hz, 8 Hz), 7.59 (d, 1 H, J = 8 Hz), 7.61 (d, 1 H, J = 2 Hz).^{19}F NMR (DMSO-d_6): δ = −64.4. ^{19}F NMR ($CDCl_3$): δ = −65.5 (t, $^5J_{H-F}$ = 0.7 Hz). MS (EI): *m/z* (relative intensity) 342 (0.4) (M^+); 314 (100) ($M^+ - N_2$); 187 (89) ($M^+ - N_2$, − I). Anal. Calcd for $C_9H_6F_3IN_2O$ (342.06): C, 31.60; H, 1.77; N, 8.19. Found: C, 31.52; H, 1.87; N, 8.06.

2-Iodo-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzyl Acetate (10). A solution of benzyl alcohol **9** (7.5 g, 0.0219 mol), Ac_2O (14.6 g, 0.143

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mol), and DMAP (13.56 g, 0.111 mol) in 112 mL of THF was stirred for 30 min. After concentrating, the solution was diluted with Et_2O, extracted with 2 M HCl, and washed with water. The organic phase was dried over $MgSO_4$, and the solvents were removed under reduced pressure. Excess Ac_2O and AcOH were removed by co-evaporation with toluene to yield 7.6 g (90%) of crude **10** as a yellowish oil. For analytical purposes, a sample was purified by column chromatography (E). TLC: R_f (E) = 0.46. ^{1}H NMR ($CDCl_3$): δ = 2.15 (s, 3 H), 5.11 (s, 2 H), 7.25 (dd, 1 H, J = 2 Hz, 8 Hz), 7.39 (d, 1H, J = 8 Hz), 7.61 (d, 1 H, J = 2 Hz). ^{13}C NMR ($CDCl_3$): δ = 20.81, 27.53 (q, $^2J_{C-F}$ = 41 Hz), 69.39, 98.90, 121.81 (q, $^1J_{C-F}$ = 275 Hz), 126.61, 129.91, 130.65, 137.27, 140.38, 170.35. ^{19}F NMR ($CDCl_3$) δ = −65.4. IR ($CHCl_3$): ν =1742 (C=O) cm^{-1}. MS (EI): *m/z* (relative intensity) 384 (0.8) (M^+); 356 (35) ($M^+ - N_2$); 229 (65) ($M^+ - N_2, - I$), 187 (100) ($M^+ - N_2, - I, - C_2H_2O$). Anal. Calcd for $C_{11}H_8F_3IN_2O_2$ (384.10): C, 34.40; H, 2.10; N, 7.29. Found: C, 34.22; H, 2.15; N, 7.53.

2-(Tributylstannyl)-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzyl Acetate (11). The substitution of the iodine by a tributylstannyl group was accomplished using a modified version of a published procedure.[10] Iodobenzyl ester **10** (7.0 g, 0.0185 mol) was added to a mixture consisting of KH_2PO_4 (24.8 g, 0.182 mol), 1.1 M *n*-Bu_4NF (49.7 mL) in dry THF, and hexabutylditin (13.7 g, 0.0236 mol). After reaction for 2 h at 50 °C, the mixture was diluted with Et_2O, extracted with water, and dried over $MgSO_4$. Following evaporation of the solvents, the crude product was purified by column chromatography (E) to yield 3.6 g (36%) of **11**. TLC: R_f (F) = 0.71. ^{1}H NMR ($CDCl_3$): δ = 0.89 (t, 9 H, J = 7 Hz), 1.11 (t, 6 H, J = 8 Hz), 1.36 (m, 6 H), 1.48 (m, 6 H), 2.09 (s, 3 H), 5.02 (s, 2 H), 7.11 (d, 1 H, J = 8 Hz), 7.28 (s, 1 H), 7.40 (d, 1 H, J = 8 Hz). ^{19}F NMR ($CDCl_3$): δ = −65.3. IR ($CHCl_3$): ν = 1737 (C=O) cm^{-1}. MS (EI): *m/z* (relative intensity) 547/545/543 (0.2/0.15/0.1) (^{120}Sn/^{118}Sn/^{116}Sn: $M^+ - 1$); 491/489/487 (100/75/42)(^{120}Sn/^{118}Sn/^{116}Sn: $M^+ - C_4H_9$). Anal. Calcd for $C_{23}H_{35}F_3N_2O_2Sn$ (547.25): C, 50.48; H, 6.45; N, 5.12. Found: C, 50.39; H, 6.19; N, 5.08.

2-(Tributylstannyl)-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzyl Alcohol (12). Benzyl ester **11** (3.6 g, 6.56 mmol) was dissolved in 120 mL of saturated methanolic ammonia and 30 mL of Et_2O. After being stirred overnight at 4 °C, the reaction mixture was partitioned between Et_2O and water, and the organic phase was washed once with water and dried over $MgSO_4$. Removal of the solvent yielded 3.20 g (97%) of the crude alcohol **12**. A sample of the yellowish oil was purified by column chromatography (L). TLC: R_f (F) = 0.40. ^{1}H NMR ($CDCl_3$): δ = 0.89 (t, 9 H), J = 7 Hz), 1.00 (t, 6 H, J = 8 Hz), 1.28 (m, 6 H), 1.46 (m, 6 H), 4.65 (d, 2 H, J = 4 Hz), 7.10 (d, 1 H, J = 8 Hz), 7.30 (s, 1 H), 7.37 (d, 1 H, J = 8 Hz). ^{19}F NMR ($CDCl_3$): δ = −65.4. MS (EI): *m/z* (relative intensity) 508/506/504 (8/7/4) (^{120}Sn/^{118}Sn/^{116}Sn: $M^+ + 2$); 449/447/445 (51/40/23) (^{120}Sn/^{118}Sn/^{116}Sn: $M^+ - C_4H_9$). Anal. Calcd for $C_{21}H_{33}F_3N_2OSn$ (505.21): C, 49.93; H, 6.58; N, 5.54. Found: C, 49.65; H, 6.63; N, 5.80.

4-[3-(Trifluoromethyl)-3*H*-diazirin-3-yl]benzyl Acetate (13). Benzyl alcohol **8** (1.06 g, 4.5 mmol) was reacted with Ac_2O (0.432 g, 4.3 mmol) in the presence of DMAP (27.5 mg, 0.22 mmol) and triethylamine (1 mL) in CH_2Cl_2 (5 mL). After 1 h (room temperature), the solvent was evaporated, the residue dissolved in Et_2O, extracted with aqueous citric acid, and then washed with water. The organic phase was dried over $MgSO_4$, and the solvent was removed under reduced pressure. The oily residue was subjected to bulb-to-bulb distillation at 70 °C (0.1 Torr) to yield 0.6 g of **13**. ^{1}H NMR ($CDCl_3$): δ = 2.10 (s, 3 H), 5.10 (s, 2 H), 7.19 (d, 2 H, J = 8 Hz), 7.38 (d, 2 H J = 8.2 Hz). MS (EI): *m/z* (relative intensity) 230 (22) ($M^+ - N_2$), 151 (16), 43 (100). Anal. Calcd for $C_{11}H_9F_3N_2O_2$ (258.20): C, 51.17; H, 3.51; N, 10.85. Found: C, 50.92; H, 3.72; N, 10.88.

10-Undecenoic Acid, 2-(Tributylstannyl)-4-[(trifluoromethyl)-3*H*-diazirin-3-yl]benzyl Ester (16). 10-Undecenoic anhydride was prepared following the general procedure of Selinger and Lapidot[28] (mp 14 °C; lit.[29] 13−13.5 °C). A solution of 10-undecenoic anhydride (1.05 g, 3 mmol), alcohol **12** (1.01 g, 2 mmol), and DMAP (488 mg) in 10 mL of Et_2O was stirred at room temperature for 1 h. After dilution with 5 mL of hexane, the product was purified by column chromatography (D) to yield 1.32 g (98.4%) of a pale oil. ^{1}H NMR ($CDCl_3$): δ = 0.89 (t, 9 H, J = 7 Hz), 1.10 (t, 6 H, J = 8 Hz), 1.24−1.38 (m, 15 H), 1.42−1.67 (m, 9 H), 2.03 (m, 2 H), 2.33 (t, 2 H, J = 8 Hz), 4.89−4.96 (m, 2 H), 5.03 (s, 2 H), 5.80 (m, 1 H), 7.10 (d, 1 H, J = 8 Hz), 7.28 (s, 1 H), 7.40 (d, 1 H, J = 8 Hz). ^{19}F NMR ($CDCl_3$): δ = −65.4. IR (neat): ν = 1742 (C=O) cm^{-1}. MS (FAB): *m/z* (relative intensity) 671/669/667 (1/1.5/2) (^{120}Sn/^{118}Sn/^{116}Sn: $M^+ - 1$); 615/613/611 (100/81/49) (^{120}Sn/^{118}Sn/^{116}Sn: $M^+ - C_4H_9$). Anal. Calcd for $C_{32}H_{51}F_3N_2O_2Sn$ (671.47): C, 57.24; H, 7.66; N, 4.17. Found: C, 57.47; H, 7.55; N, 3.98.

1,10-Decanedioic Acid, Mono-2-(tributylstannyl)-4-[(trifluoromethyl)-3*H*-diazirin-3-yl]benzyl Ester (17). This compound was prepared by oxidative cleavage of olefin **16** using a modified procedure of Lee and Chang.[30] To an emulsion of decenoic acid ester **16** (1.22 g, 1.82 mmol) and 18-crown-6 (0.185 g) in 37 mL of CH_2Cl_2 and 18.5 mL of 10% (v/v) aqueous AcOH was added portionwise $KMnO_4$ (858 mg). After vigorous stirring for 3 h, AcOH (3.7 mL) was added, and excess of $KMnO_4$ and MnO_2 reduced with $NaHSO_3$. After dilution with 1 M aqueous citric acid, the product was extracted with Et_2O to yield 1.3 g of the crude ester as a pale yellowish oil. Purification by silica gel column chromatography (G) gave 755 mg (60%) of **17**. ^{1}H NMR ($CDCl_3$): δ = 0.89 (t, 9 H, J = 7 Hz), 1.10 (t, 6 H, J = 8 Hz), 1.20−1.38 (m, 15 H), 1.41−1.62 (m, 9 H), 2.33 (t, 4 H, J = 8 Hz), 5.02 (s, 2 H), 7.11 (d, 1 H, J = 8 Hz), 7.28 (s, 1 H), 7.40 (d, 1 H, J = 8 Hz), 10.5 (s, 1 H). ^{19}F NMR ($CDCl_3$): δ = −65.4. IR (neat): ν = 1740 (C=O, ester), 1710 (C=O, acid) cm^{-1}. MS (FAB): *m/z* (relative intensity) 689 (2) (^{120}Sn: $M^+ - 1$); 633/631/629 (82/66/38) (^{120}Sn/^{118}Sn/^{116}Sn: $M^+ - C_4H_9$). Anal. Calcd for $C_{31}H_{49}F_3N_2O_4Sn$ (689.45): C, 54.01; H, 7.16; N, 4.06. Found: C, 54.03; H, 7.19; N, 3.79.

1-*O*-Dodecanoyl-2-*O*-[9-[[[2-(tributylstannyl)-4-[(trifluoromethyl)-3*H*-diazirin-3-yl]benzyl]oxy]carbonyl]nonanoyl]-*sn*-glycero-3-phosphocholine (18). This compound was synthesized following the general procedure of Gupta et al.[16b] Briefly, fatty acid **17** (345 mg, 0.50 mmol) was converted to the anhydride by reaction with 0.55 equiv (57 mg, 0.275 mmol) of dicyclohexylcarbodiimide (DCC) in 3.8 mL of CCl_4 (5 h, room temperature). After removal of the precipitated dicyclohexylurea, the solvent was evaporated and the crude anhydride used to acylate 1-*O*-dodecanoyl-*sn*-glycero-3-phosphocholine (1-*O*-dodecanoyl-lyso-PC) (88 mg, 0.20 mmol). The reaction was performed in 5 mL of $CHCl_3$/pyridine (4:1, v/v) and was catalyzed by DMAP (36.6 mg, 0.30 mmol). The mixture was stirred magnetically, using a Teflon-coated stir bar, for 14 h at room temperature under an argon atmosphere. Evaporation of the solvent under reduced pressure yielded crude product that was redissolved in a minimal volume of solvent A and subjected to Sephadex LH-20 gel filtration (3 × 20 cm column) using the same solvent. Product-containing fractions (monitored by TLC) were pooled and concentrated under reduced pressure. Further purification by silica gel column chromatography (H) afforded 74 mg (32%) of pure **18**. TLC: R_f (K) = 0.37. ^{1}H NMR ($CDCl_3$): δ = 0.87 (t, 3 H, J = 7 Hz), 0.89 (t, 9 H, J = 7 Hz), 1.07−1.12 (m, 6 H), 1,23−1.37 (m, 32 H), 1.45−1.67 (m, 10 H), 2.24−2.36 (m, 6 H), 3.37 (s, 9 H), 3.76−3.83 (m, 2 H), 3.90−4.03 (m, 2 H), 4.14 (dd, 1 H, J = 12 Hz, J = 7 Hz), 4.30−4.38 (br, 2 H), 4.41 (dd, 1 H, J = 12 Hz, J = 3 Hz), 5.02 (s, 2 H), 5.16−5.24 (m, 1 H), 7.10 (dd, 1 H, J = 8 Hz, J = 1 Hz), 7.28 (d, 1 H, J = 1 Hz), 7.39 (d, 1 H, 8 Hz). ^{19}F NMR ($CDCl_3$): δ = −65.30. ^{31}P NMR ($CDCl_3$): δ = −(0.053−0.050). MS (FAB): *m/z* (relative intensity) 1114/1112/1110 (1/1.2/0.4) (^{120}Sn/^{118}Sn/^{116}Sn: M^+); 1086/1084/1082 (3.0/13/6.1) (^{120}Sn/^{118}Sn/^{116}Sn: $M^+ - N_2$). Anal. Calcd for $C_{51}H_{89}F_3N_3PO_{10}Sn{\cdot}2H_2O$ (1146.99): C, 53.41; H, 8.17; N, 3.66. Found: C, 53.32; H, 8.23 N, 3.66.

1-*O*-Hexadecanoyl-2-*O*-[9-[[[2-(tributylstannyl)-4-[(trifluoromethyl)-3*H*-diazirin-3-yl]benzyl]oxy]carbonyl]nonanoyl]-*sn*-glycero-3-phosphocholine (19). The procedures used for the preparation of **19** were similar to those used to synthesize **18**. Starting from fatty acid **17** (345 mg, 0.5 mmol) and 1-hexadecanoyl-lyso-PC (99 mg, 0.2 mmol), 54 mg (23%) of pure **19** was obtained. TLC: R_f (K) = 0.39. ^{1}H NMR ($CDCl_3$): δ = 0.89 (m, 12 H), 1.10 (t, 3 H, J = 8 Hz), 1.25 (m, 42 H), 1.41−1.59 (m, 12 H), 2.23−2.36 (m, 6 H), 3.36 (s, 9 H), 3.81−3.93

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(m, 4 H), 4.06–4.42 (m, 3 H), 5.02 (s, 2 H), 5.18 (s, 1 H), 7.10 (d, 1 H, J = 8 Hz), 7.28 (s, 1 H), 7.39 (d, 1 H, J = 8 Hz). ^{19}F NMR ($CDCl_3$): δ = −65.4. ^{31}P NMR ($CDCl_3$): δ = −0.31. MS (FAB): m/z (relative intensity) 1168/1166/1164 (0.5/0.4/0.2) (^{120}Sn/^{118}Sn/^{116}Sn: M^+); 1140/1138/1136 (3/2/1) (^{120}Sn/^{118}Sn/^{116}Sn: $M^+ - N_2$). IR ($CHCl_3$): ν = 3359 (O–H), 1732 (C=O) cm^{-1}. Anal. Calcd for $C_{55}H_{97}F_3N_3O_{10}PSn{\cdot}H_2O$ (1185.08): C, 55.74; H, 8.42; N, 3.55; P, 2.61. Found: C, 55.44; H, 8.18; N, 3.26; P, 2.35.

1-*O*-Dodecanoyl-2-*O*-[9-[[[2-(^{125}I)Iodo-4-[(trifluoromethyl)-3*H*-diazirin-3-yl]benzyl]oxy]carbonyl]nonanoyl]-*sn*-glycero-3-phosphocholine (20). Tin precursor **18** (~25 μg; ~20 nmol), dried (15 min, 10^{-2} Torr) in the tip of a 1 mL Reacti-Vial (Pierce, Rockford, IL), was dissolved in 20 μL of AcOH. Na^{125}I (2 mCi) was added, and the iodination reaction started by the addition of 10 μL of a 32% solution of peracetic acid in AcOH. After 2 min at room temperature, the reaction was quenched with 100 μL of 10% $Na_2S_2O_5$. Then, 100 μL of $CHCl_3$/MeOH (2:1) was added, and, after vortexing, the organic phase was collected and concentrated (volatile radioactivity was adsorbed onto a charcoal filter). The residue was dissolved in 50 μL of MeOH/$CHCl_3$/H_2O (9:1:1) and subjected to reverse-phase HPLC using the same solvent. The flow rate was 1 mL/min, and fractions (0.5 mL) were collected. PC **20** was eluted at approximately 2.3 min, excess tin precursor **18** at 5.2 min (UV detection at 254 nm; 0.1 OD = 100%). The pooled product fractions were concentrated and freed of water by co-evaporation with toluene/ethanol (1:1) under reduced pressure to yield 1.0 mCi of radiochemically pure **20**, which was stored as a solution in ethanol/toluene (1:1) at approximately 1 mCi/mL and −20 °C.

1-*O*-Hexadecanoyl-2-*O*-[9-[[[2-(^{125}I)Iodo-4-((trifluoromethyl)-3*H*-diazirin-3-yl)benzyl]oxy]carbonyl]nonanoyl]-*sn*-glycero-3-phosphocholine (21). This compound was prepared in a manner similar to that described for **20**. Starting from **19** (~20 nmol) and 2 mCi Na^{125}I, approximately 0.8 mCi (40%) of HPLC-purified **21** was obtained. The retention times for ^{125}I–TID–PC/16 and excess tin precursor were 3.0 and 6.6 min, respectively. The compound was stored as a solution (ca. 1 mCi/mL) in ethanol/toluene (1:1) at −20 °C. The radiochemical purity of a sample analyzed after one month was >98%.

1,10-Decanedioic Acid, Mono-2-iodo-4-[(trifluoromethyl)-3H-diazirin-3-yl]benzyl Ester (22). This compound was prepared by acylation of **9** (2.0 g, 5.8 mmol) with 10-undecenoic anhydride (2.9 g, 8.3 mmol) and subsequent oxidative cleavage of the alkene with Jones reagent/osmium tetroxide.[31] Purification of the main product by column chromatography (G) gave 2.1 g (83%) of **22**, which crystallized on storage at 4 °C. An analytical sample was recrystallized three times from hexane to yield white needles, mp 56 °C. TLC: R_f (G) = 0.46. ^{1}H NMR ($CDCl_3$): δ = 1.28–1.43 (m, 8 H), 1.56–1.73 (m, 4 H), 2.30–2.44 (m, 4 H), 5.10 (s, 2 H), 7.26 (dd, 1 H, J = 2 Hz, J = 8 Hz), 7.38 (d, 1 H , J = 8 Hz), 7.61 (d, 1 H, J = 2 Hz). MS (FAB): m/z (relative intensity) 527 (28) (M^+ + 1), 509 (62) (M^+ − OH), 325 (20), 297 (42), 170 (100). Anal. Calcd for $C_{19}H_{22}N_2F_3IO_4$ (526.29): C, 43.36; H, 4.21, N, 5.32. Found: C, 43.41; H, 4.30; N, 5.32.

1-*O*-Dodecanoyl-2-*O*-[9-[[[2-iodo-4-[(trifluoromethyl)-3*H*-diazirin-3-yl]benzyl]oxy]carbonyl]nonanoyl]-*sn*-glycero-3-phosphocholine (23). This compound was prepared using a procedure similar to that used for **18**. Starting from fatty acid **22** (137 mg, 0.22 mmol) and 1-*O*-dodecanoyl-lyso-PC (44 mg, 0.10 mmol), 55 mg (53%) of pure **23** was obtained. TLC: R_f (K) = 0.33. ^{1}H NMR ($CDCl_3$): δ = 0.87 (t, 3 H, J = 7 Hz), 1.22–1.38 (m, 24 H), 1.50–1.70 (m, 6 H), 2.12 (br s, 1 H), 2.23–2.35 (m, 4 H), 2.9 (t, 2 H, J = 7 Hz), 3.37 (s, 9 H), 3.78–3.88 (m, 2 H), 3.92–4.04 (m, 2 H), 4.13 (dd, 1 H, J = 12 Hz, J = 7 Hz), 4.41 (dd, 1 H, J = 12 Hz, J = 3 Hz), 5.10 (s, 2 H), 5.18–5.26 (m, 1 H), 7.24 (dd, 1 H, J = 8 Hz, J = 2 Hz), 7.38 (d, J = 8 Hz), 7.60 (d, J = 2 Hz).^{31}P NMR ($CDCl_3$): δ = −0.13. MS (FAB): m/z (relative intensity) 948 (24) (M^+), 920 (17) ($M^+ - N_2$), 793 (1.7) ($M^+ - N_2, - I$), 184 (100), 86 (55). Anal. Calcd for $C_{39}H_{62}N_3F_3IO_{10}P{\cdot}H_2O$ (965.82): C, 48.50; H, 6.68; N, 4.35. Found: C, 48.43; H, 6.76; N, 4.47.

1-*O*-Hexadecanoyl-2-*O*-[9-[[[2-iodo-4-[(trifluoromethyl)-3*H*-diazirin-3-yl]benzyl]oxy]carbonyl]nonanoyl]-*sn*-glycero-3-phosphocholine (24). This compound was prepared using a procedure similar to that used for **19**. Starting from fatty acid **22** (561 mg, 0.93 mmol) and 1-*O*-hexadecanoyl-lyso-PC (149 mg, 0.30 mmol), 307 mg (26%) of TLC- and HPLC-pure **24** was obtained. TLC: R_f (K) = 0.35. ^{1}H NMR ($CDCl_3$): δ = 0.87 (t, 3 H, J = 7 Hz), 1.23–1.35 (m, 32 H), 1.5–1.7 (m, 6 H), 1.97 (br s, 2 H), 2.29 (m, 4 H), 2.39 (t, 2 H, J = 7 Hz), 3.37 (s, 9 H), 3.78–3.86 (m, 2 H), 3.94–4.04 (m, 2 H), 4.13 (dd, 1 H, J = 12 Hz, J = 7 Hz), 4.41 (dd, 1 H, J = 12 Hz, J = 3 Hz), 5.10 (s, 2 H), 5.18–5.26 (m, 1 H), 7.24 (dd, 1 H, J = 8 Hz, J = 2 Hz), 7.38 (d, J = 8 Hz), 7.60 (d, J = 2 Hz). ^{31}P NMR ($CDCl_3$): δ = −0.04. MS (FAB): m/z (relative intensity) 1004 (2) (M^+), 976 (5) ($M^+ - N_2$), 848 (1.4) ($M^+ + 1, - N_2, - I$), 184 (100), 86 (55). Anal. Calcd for $C_{43}H_{70}N_3F_3IO_{10}P{\cdot}H_2O$ (1021.93): C, 50.54, H, 7.10; N, 4.11. Found: C, 50.41, H, 6.69; N, 4.05.

Succinic Acid, Mono-2-(tributylstannyl)-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzyl Ester (27). A solution of alcohol **12** (505 mg, 1 mmol), succinic anhydride (200 mg, 2 mmol), and DMAP (270 mg, 2.2 mmol) in 4 mL of THF was stirred at room temperature overnight. After evaporation of the solvent, the residue was partitioned between Et_2O and 1 M aqueous citric acid. The organic phase was washed with water, dried over $MgSO_4$, and concentrated under reduced pressure. The crude acid was purified by column chromatography (I) to yield 465 mg of a yellowish oil. TLC: R_f (M) = 0.28. ^{1}H NMR ($CDCl_3$): δ = 0.89 (t, 9 H, J = 7 Hz), 1.14 (t, 6 H, J = 8 Hz), 1.30 (m, 6 H), 1.50 (m, 6 H), 2.68 (m, 4 H), 5.07 (s, 2 H), 7.10 (d, 1 H, J = 8 Hz), 7.28 (s, 1 H), 7.40 (d, 1 H, J = 8 Hz). ^{19}F NMR ($CDCl_3$): δ = −65.3. IR (neat) ν = 1744 (C=O, ester), 1716 (C=O, acid) cm^{-1}. Although **27** was pure as judged by TLC and reverse-phase HPLC, the elemental composition was not completely satisfactory. Anal. Calcd for $C_{25}H_{37}N_2O_4F_3Sn$ (605.28): C, 49.61;, H, 6.18; N, 4.63. Found: C, 50.09; H, 6.25, N, 4.62.

3-[[[2-(Tributylstannyl)-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzyl]oxy]carbonyl]propanoic Acid, *N*-Hydroxysuccinimide Ester (28). 422 mg (0.697 mmol) of **27** was dissolved in 3.7 mL of THF. *N*-Hydroxysuccinimide (89 mg, 0.771 mmol) and DCC (158 mg, 0.771 mmol) were added, and the mixture was allowed to stirr at room temperature overnight. Precipitated dicyclohexylurea was removed by filtration, and the filtrate was concentrated under reduced pressure. Crude **28** was purified by silica gel column chromatography (D) to yield 259 mg (53%) of a yellowish oil. For analytical purposes, a sample was further purified by column chromatography ($CHCl_3$). TLC: R_f (B) = 0.74. ^{1}H NMR ($CDCl_3$): δ = 0.89 (t, 9 H, J = 7 Hz), 1.10 (t, 6 H, J = 8 Hz), 1.30 (m, 6 H), 1.50 (m, 6 H), 2.77 (t, 2 H, J = 7 Hz), 2.82 (s, 4 H), 2.97 (t, 2 H, J = 7 Hz), 5.09 (s, 2 H), 7.11 (d, 1 H, J = 8 Hz), 7.28 (s, 1 H), 7.40 (d, 1 H, J = 8 Hz). ^{19}F NMR ($CDCl_3$): δ = −65.3. IR (neat): ν = 1818, 1790, 1744 (C=O) cm^{-1}. MS (FAB): m/z (relative intensity) 704 (2) (^{120}Sn: M^+ + 1); 646/644/642 (100/79/48) (^{120}Sn/^{118}Sn/^{116}Sn:$M^+ - C_4H_9$). Anal. Calcd for $C_{29}H_{40}F_3N_3O_6Sn$ (702.36): C, 49.59; H, 5.74; N, 5.98. Found: C, 49.77; H, 5.78; N, 5.94.

3-[[[2-(^{125}I)Iodo-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzyl]oxy]carbonyl]propanoic Acid, *N*-Hydroxysuccinimide Ester (29). Tin precursor **28** (20 nmol) was dissolved in 10 μL of AcOH in the tip of a 1 mL Reacti-Vial equipped with a Mininert valve (Pierce, Rockford, IL). Na^{125}I (2 mCi) was added, and the iodination reaction started by the addition of 5 μL of a 30% solution of peracetic acid in AcOH. After 2 min at room temperature, the reaction was quenched with 50 μL of 10% $NaHSO_3$. Subsequently, 0.1 mL of EtOAc was added and, after vigorous vortexing, the organic phase was collected, concentrated by means of a stream of nitrogen (volatile radioactivity was adsorbed to a charcoal filter), and applied onto a 5–7 mm starting line on a silica gel TLC plate. The chromatogram was developed in solvent B. The radioactive product band was localized either by co-chromatography of the nonradioactive reference compound **31** or by brief (1 min) exposure of the TLC plate to an X-ray film. The product band was scraped out and extracted with 100–200 μL Et_2O. After concentration, the purified product was dissolved in EtOAc/toluene (1:1) at ~1 mCi/mL and stored at −20 °C. Typical yields of **29** were 35–40% with respect to the initial amount of radioactivity.

Succinic Acid, Mono-2-iodo-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzyl Ester (30). A solution of alcohol **9** (698 mg, 2.04 mmol), succinic anhydride (408 mg, 4.08 mmol), and DMAP (551 mg, 4.4 mmol) in 9 mL of THF was stirred overnight (room temperature). After

(31) Henry, J. R.; Weinreb, S. M. *J. Org. Chem.* **1993**, *58*, 4745.

dilution with Et_2O, the mixture was extracted with aqueous citric acid, washed with water, and dried over $MgSO_4$. Evaporation of the solvent yielded the crude product which was purified by column chromatography (I) to yield 697 mg (77%) of a crystalline solid. The compound was further purified by recrystallization from ethanol; mp 84–85 °C. ^{1}H NMR ($CDCl_3$): δ = 2.13 (s, 4 H), 5.15 (s, 2 H), 7.24 (dd, 1 H, J = 8 Hz, J = 2 Hz; partially hidden by $CHCl_3$), 7.38 (d, 1 H, J = 8 Hz), 7.60 (d, 1 H, J = 2 Hz). MS (EI): m/z (relative intensity) 442 (0.8) ($M^+ - N_2$), 287 (4) ($M^+ - N_2$, –I), 187 (37), 101 (100). Anal. Calcd for $C_{13}H_{10}N_2F_3IO_4$ (442.13): C, 35.32; H, 2.28; N, 6.34. Found: C, 35.36; H, 2.39; N, 6.28.

3-[[[2-Iodo-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzyl]oxy]carbonyl]propanoic Acid, *N*-Hydroxysuccinimide Ester (31). Acid **30** (580 mg, 1.31 mmol), *N*-hydroxysuccinimide (140 mg, 1.22 mmol), and DCC (254 mg, 1.22 mmol) in 8 mL of THF were stirred overnight. After removal of the precipitated dicyclohexyl urea, the filtrate was concentrated under reduced pressure and the residue subjected to column chromatography (EtOAc). The product-containing fractions were pooled, and the solvent was removed under reduced pressure to yield 472 mg (72%) of **31** as a white solid. An analytical sample was recrystallized twice from ethanol to afford white needles with mp 105 °C. TLC: R_f (B) = 0.40. ^{1}H NMR ($CDCl_3$): δ = 2.73 (t, 2 H, J = 7 Hz), 3.86 (t, 2 H, J = 7 Hz), 5.11 (s, 2 H), 6.68 (s, 2 H), 7.26 (dd, 1 H, J = 2 Hz, J = 8 Hz), 7.68 (d, 1 H, J = 2 Hz). MS (FAB): m/z (relative intensity) 540 (73) (M^+), 325 (38), 297 (42), 170 (100), 77 (51). Anal. Calcd for $C_{17}H_{13}F_3N_3IO_6$ (539.21): C, 37.87; H, 2.43; N, 7.79. Found: C, 37.99; H, 2.54; N, 7.77.

3-Maleimidopropionic Acid, 2-(Tributylstannyl)-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzyl Ester (32). To alcohol **12** (505 mg, 1 mmol) in THF (10 mL) were added 3-maleimidopropionic acid (676 mg, 4 mmol), DCC (453 mg, 2.2 mmol), $NaHCO_3$ (1 g) and 0.2 mL of a 0.1 M solution of DMAP in THF. After being stirred at room temperature, for 1 h, the reaction mixture was partitioned between Et_2O and 1 M aqueous citric acid. The organic phase was washed once with water and dried over $MgSO_4$. After evaporation of the solvent the crude product was purified by silica gel column chromatography (D) to afford 377 mg (58%) of **32** as a pale yellowish oil. TLC: R_f (D) = 0.47. ^{1}H NMR ($CDCl_3$): δ = 0.89 (t, 9 H, J = 7 Hz), 1.1 (t, 6 H, J = 8 Hz), 1.30 (m, 6 H), 1.55 (m, 6 H), 2.67 (t, 2 H, J = 7 Hz), 3.84 (t, 2 H, J = 7 Hz), 5.03 (s, 2 H), 6.68 (s, 2 H), 7.12 (d, 1 H, J = 8 Hz), 7.26 (br s; 1 H), 7.41 (d, 1 H, J = 8 Hz). ^{19}F NMR ($CDCl_3$): δ = –65.3. IR ($CHCl_3$): ν = 1737 (C=O), 1713 (C=O) cm^{-1}. MS (FAB): m/z (relative intensity) 600/598/596 (29/22/12) (^{120}Sn/^{118}Sn/^{116}Sn: $M^+ - C_4H_9$). Anal. Calcd for $C_{28}H_{38}F_3N_3O_4Sn$ (656.33): C, 51.24; H, 5.84; N, 6.40. Found: C, 51.42; H, 5.69; N, 6.29.

3-Maleimidopropionic Acid, 2-(^{125}I)Iodo-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzyl Ester (33). Tin precursor **32** (approximately 20 nmol) was dissolved in 2 μL of ethanol. A 5 μL aliquot of 0.1 M NaOAc buffer, pH 4.5, and Na^{125}I (2 mCi) was added. The iodination reaction was initiated by the addition of 5 μL of oxidant (peracetic acid generated *in situ* by mixing 11.4 M (~35%) aqueous H_2O_2 and AcOH (2:1, v/v) to which H_2SO_4 was added to a final concentration of 0.2 M; this mixture was allowed to stand at room temperature for 2 h and, prior to use, was diluted 100 times with 0.1 M NaOAc, pH 4.5). The reaction was allowed to proceed for 15 min (room temperature) with occasional vortexing and then was quenched with 50 μL of an aqueous solution containing ascorbic acid (1 M) and NaOH (0.5 M). Excess educt and product were extracted into 100 μL of CH_2Cl_2. After concentrating with a stream of nitrogen (volatile radioactivity was adsorbed on charcoal filter) product **33** was purified by TLC (D) in a manner as described for compound **29** and dissolved in EtOAc/toluene (1:1) (1 mCi/mL). Typical yields of **33** were 15–20% with respect to the initial amount of Na^{125}I.

3-Maleimidopropionic Acid, 2-Iodo-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzyl Ester (34). The procedure used to prepare this compound was similar to that used for the synthesis of **32**. Starting from alcohol **9** (513 mg, 1.50 mmol), 571 mg (79%) of column chromatographically (H) purified **34** was obtained. An analytical sample was crystallized twice from ethanol. White crystals with mp 74–75 °C. TLC: R_f (D) = 0.38. ^{1}H NMR ($CDCl_3$): δ = 2.73 (t, 2 H, J = 7 Hz); 3.86 (t, 2 H, J = 7 Hz), 5.11 (s, 2 H), 6.68 (s, 2 H), 7.26 (dd, 1 H, J = 8 Hz, J = 2 Hz), 7.39 (d, 1 H, J = 8 Hz), 7.61 (d, 1 H, J = 2 Hz). MS (FAB): m/z (relative intensity) 494 (27) (M^+ + 1), 325 (38), 297 (17), 170 (100), 77 (45). Anal. Calcd for $C_{16}H_{11}F_3N_3IO_4$ (493.18): C, 38.97; H, 2.25; N, 8.52. Found: C, 39.02; N, 2.37; N, 8.48.

***N*-[3-[[[2-(Tributylstannyl)-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzyl]oxy]carbonyl]propanoyl]-D-*erythro*-sphingosine (35).** A solution of D-*erythro*-sphingosine (Fluka) (25 mg, 0.033 mmol), succinimide ester **28** (58 mg, 0.082 mmol), and triethylamine (17 mg, 0.17 mmol) in THF was stirred overnight. The reaction mixture was partitioned between Et_2O and 1 M aqueous citric acid. After washing with brine, the organic phase was dried over $MgSO_4$, and the solvent was removed under reduced pressure to give the crude product as a colorless oil. Purification by column chromatography (J) yielded 47.7 mg (57.2%) of **35**. A sample (10 mg) was further purified by reverse-phase HPLC. ^{1}H NMR ($CDCl_3$): δ = 0.88 (s, 3 H), 0.89 (t, 9 H, J = 7 Hz), 1.06–1.14 (m, 6 H), 1.26–1.58 (m, 34 H), 2.01–2.08 (m, 2 H), 2.48–2.55 (m, 2 H), 2.68–2.78 (m, 4 H), 3.72 (m, 1 H), 3.81–3.90 (m, 1 H), 3.94–3.99 (m, 1 H), 4.35 (s, 1 H), 5.05 (s, 2 H), 5.47–5.58 (m, 1 H), 5.69–5.87 (m, 1 H), 6.39 (d, 1 H, J = 8 Hz), 7.10 (d, 1 H, J = 8 Hz), 7.28 (s, 1 H), 7.40 (d, 1 H, J = 8 Hz). ^{19}F NMR ($CDCl_3$): δ = –65.4. MS (FAB): m/z (relative intensity) 888/886/884 (4/3/2) (^{120}Sn/^{118}Sn/^{116}Sn: M^+ + 1); 830/828/826 (11/8/4) (^{120}Sn/^{118}Sn/^{116}Sn: $M^+ - C_4H_9$). Although **35** was pure as judged by TLC and HPLC, the elemental composition was not satisfactory. Anal. Calcd for $C_{43}H_{72}N_3O_5F_3Sn$ (886.77): C, 58.24; H, 8.18; N, 4.74. Found: C, 59.77; H, 9.03; N, 4.81.

***N*-[3-[[[2-(^{125}I)Iodo-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzyl]oxy]carbonyl]propanoyl]-D-*erythro*-sphingosine (36).** Tin ceramide **35** (approximately 20 nmol) was dissolved in 20 μL of AcOH, and 1.9 mCi of Na^{125}I and 5 μL of peracetic acid (32% in AcOH) were added. After 2 min the reaction was quenched by the addition of 0.1 mL of 10% $NaHSO_3$ and 70 μL of 5 M NaOH. The mixture was extracted with $CHCl_3$/MeOH (2:1, v/v, total 300 μL) and the organic phase concentrated by means of a stream of nitrogen (volatile radioactivity was adsorbed on charcoal filter). The residue was then subjected to TLC (J). The main radioactive band (localized by autoradiography) was extracted with 0.2 mL of THF. After evaporation of the solvent the residue was dissolved in toluene/EtOH (1:1, v/v ca. 1 mCi/mL) and the solution stored at –20 °C. The yield of ^{125}I–TID–ceramide (**30**) was 0.47 mCi (24%).

***N*-[3-[[[2-Iodo-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzyl]oxy]carbonyl]propanoyl]-D-*erythro*-sphingosine (37).** The procedure was similar to that used for the synthesis of **35**. Starting from D-*erythro*-sphingosine (18 mg, 0.06 mmol; obtained by alkaline deprotection of (2′*E*,4*S*,1′*R*)-4-(1′-hydroxyhexadec-2′-enyl)-1,3-oxazolidin-2-one[32] (gift from Dr. B. Bernet, ETH Zürich)) and succinimide ester **31** (42 mg, 0.077 mmol), 29 mg (66%) of **37** was obtained. ^{1}H NMR ($CDCl_3$): δ = 1.26–1.40, (m, 22 H), 2.55 (t, 2 H, J = 7 Hz), 2.80 (t, 2 H, J = 7 Hz), 2.64 (br dd, J = 11.5 Hz, 3.2 Hz, H-C(1)), 3.87 (dq, J = 7.7 Hz, 3.5 Hz, H-C(2)), 3.97 (dd, J = 11.5, 3.2, H-C(1)), 4.35–4.59 (m, H-C(3)), 5.16 (s, 2H), 5.52 (br dd, J = 15.3, 6.2, H-C(4)), 5.79 (dtd, J = 15.4, 7.0, 1.0), 6.40 (d, J = 7.1, H-N), 7.24 (dd, 1 H, J = 8 Hz, 2 Hz; partially hidden by $CHCl_3$), 7.39 (d, 1 H, J = 8.2 Hz), 7.60 (d, 1 H, J = 2 Hz). MS (FAB): m/z (relative intensity) 724 (11) (M^+), 706 (100) ($M^+ - H_2O$), 264 (47), 170 (83). Although the product was pure as judged by TLC and reverse-phase HPLC, the elemental composition was not completely satisfactory. Anal. Calcd for $C_{31}H_{45}N_3F_3IO_5$ (723.61): C, 51.46; H, 6.27; N, 5.81. Found: C, 52.05; H, 6.32; N, 5.46.

Photolysis of 13 and 14. All photolysis experiments were carried out employing a high-pressure mercury lamp (Osram HBO 350 W) mounted in a SUSS LH 1000 lamp house equipped with a shutter to control exposure times. The light was filtered through a 1.5 cm thick saturated aqueous $CuSO_4$ sleeve. This filter cuts off essentially all the UV emission below 320 nm. Samples to be photolyzed were placed in Pyrex glass tubes (<10 mL), or round-shaped Pyrex vessels (up to 100 mL). The solutions were degassed by three freeze–pump–thaw cycles, layered with argon, and tightly capped with a rubber septum. Photolyses were done at ambient temperature (20–25 °C).

(32) Julina, R.; Herzig, T.; Bernet, B.; Vasella, A. *Helv. Chim. Acta* **1986**, *69*, 368–373.

Upon photolysis of **10** (40 mg, 0.104 mmol) in 100 mL of cyclohexane, the reaction mixture was concentrated under reduced pressure, the residue was applied on two TLC (20 × 20 cm) plates, and the chromatogram was developed in CH_2Cl_2. The main band migrating just above the orange diazo compound was scraped off and eluted with CH_2Cl_2 to yield approximately 17 mg of **14**. Using a similar protocol, photolysis of a solution of **13** (97 mg, 0.37 mmol) in 100 mL of cyclohexane and separation of the product by TLC yielded approximately 20 mg of the slightly volatile compound **15**. Analytical data for **14**: ^{1}H NMR ($CDCl_3$): δ = 0.95–2.05 (m, 11 H), 2.16 (s, 3 H), 2.99 (dq, 1 H, $^3J_{H-F}$ = 10 Hz, $^3J_{H-H}$ = 8 Hz), 5.12 (s, 2 H), 7.24 (d, 1 H, J = 2 Hz, J = 8 Hz), 7.34 (d, 1 H, J = 8 Hz), 7.72 (d, 1 H, J = 2 Hz). ^{19}F NMR ($CDCl_3$): δ = −63.51 (d, $^3J_{F-H}$ = 10 Hz). MS (EI): *m/z* (relative intensity) 440 (0.4) (M^+), 313 (52) ($M^+ - I$), 271 (100) ($M^+ + 1 - I, - CH_3COO$), 189 (19) ($M^+ + 1, - I, - C_6H_{11}$), 83 (61), 55 (60), 43 (49). Analytical data for **15**: ^{1}H NMR ($CDCl_3$): δ = 0.75–2.10 (m, 11 H), 2.12 (s, 3 H), 3.04 (dq, 1 H, $^3J_{H-F}$ = 10 Hz, $^3J_{H-H}$ = 8 Hz), 5.10 (s, 2 H), 7.23 (d, 2 H, J = 8 Hz), 7.33 (d, 2 H, J = 8 Hz). ^{19}F NMR ($CDCl_3$): δ −63.56 (d, $^3J_{F-H} \approx$ 10 Hz). MS (EI): *m/z* (relative intensity) 314 (10) (M^+), 172 (100) ($M^+, - C_6H_{11}, - CH_3COO$).

Phospholipase D-Catalyzed Reactions of 21. To radioactive **21** (100 μCi; 2000 Ci/mmol), dried (15 min, <0.05 Torr) in a 0.5 mL Reacti-Vial, were added L-serine (23 mg), 40 μL of 100 mM NaOAc, and 100 mM $CaCl_2$, pH 5.6. After being stirred for 5 min at 45 °C (to dissolve L-serine), 10 μL of phospholipase D (Sigma, Type I; 3 units/μL) dissolved in the above buffer, 100 μL of Et_2O was added, and the vial was tightly sealed. After vigorous stirring for 15 min at 45 °C, the reaction was stopped by cooling on ice and addition of 100 μL of water. Et_2O was evaporated by means of a stream of nitrogen, and the reaction mixture was extracted twice with $CHCl_3$/MeOH (2:1). The combined organic phases were concentrated and subjected to TLC (solvent H). The radioactive products (PS **25** and PA **26**), visualized by autoradiography and identified on the basis of their R_f values (cochromatography of commercial samples of 1,2-dipalmitoyl-PS and 1,2-dipalmitoyl-PA), were isolated by extraction with solvent H from the corresponding silica gel bands.

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