Chemistry of C-2 Glyceryl Radicals: Indications for a New Mechanism of Lipid Damage

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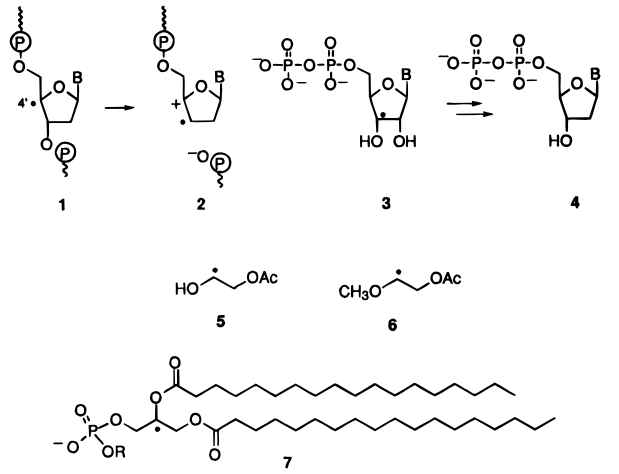
Abstract: Precursors for the selective generation of C-2 glyceryl radicals were synthesized, and the chemical behavior of the corresponding radicals was investigated by ESR spectroscopy, product analysis, and kinetic measurements. It was found that cleavage of the β-C,O bond proceeds rapidly, if a hydroxyl group is present at the radical carbon center. The rate constant for the elimination of a β-acetoxy group from radical **30** was dependent on the solvent ($k_E = 4 \times 10^5$ s^{-1} in methanol, $k_E = 2 \times 10^7$ s^{-1} in toluene). With these results and *ab initio* calculations a concerted elimination mechanism is suggested. α-Methoxy-substituted C-2 glyceryl radicals **42** and **43** showed heterolytic β-C,O bond cleavage under formation of radical cations. With ester-substituted radicals **24** and **35** no elimination could be observed. To demonstrate the biological significance of these findings, C-2 lysolecithin radical **53** was generated, which led to fast β-elimination.

Introduction

Free radicals play an important role in several biological processes, where they are either beneficial or damaging to the organism.[1] Damage occurs for example through hydrogen abstraction from DNA, proteins, or lipids.[2] The attack of DNA by reactive radicals such as HO• yields a mixture of DNA radicals of which the 4′-DNA radical **1** can undergo spontaneous heterolytic β-C,O bond scission, resulting in the cleavage of the DNA strand (**1** → **2**, Scheme 1).[3] Radical-induced cleavage also takes place in the enzymatic deoxygenation of ribonucleotides by ribonucleotide reductase[4] (**3** → **4**, Scheme 1). The β-C,O bond scission of substituted glycol radicals, generated by γ-radiolysis in aqueous solution, was studied as a model for the radical-induced cleavage of DNA. Schulte-Frohlinde and co-workers[5] observed fast β-elimination for 2-acetoxy-1-*hydroxy*-1-ethyl radical (**5**) whereas Gilbert, Larkin, and Norman,[6] found slower elimination of the β-acetate group from 2-acetoxy-1-*methoxy*-1-ethyl radical (**6**).

The C-2 glyceryl radical **7** has a substitution pattern similar to those of radicals **1**, **3**, **5**, and **6** since it bears oxygen substituents in the α- and β-positions. Therefore, an analogous reactivity of **7** toward β-elimination can be expected. However, until now, the radical-induced damage of lipids in biological systems has been discussed to occur only by radical attack at the allylic or bisallylic position of unsaturated fatty acids, leading to autoxidation products.[7] Thus, we were motivated by the question of why phospholipids in cell membranes should only be damaged by attack at the lipophilic fatty acid chains. The polar glycerol moieties are more exposed to "water-born" radicals (e.g., HO•) than the fatty acid chains, and they are susceptible to H abstraction.[8] Therefore, damage of membrane lipids *via* glyceryl radicals such as **7** might also occur. Only a few examples of glyceryl radicals are described in the literature.[9] In this work we describe the synthesis of C-2 glyceryl radical precursors, the selective generation of the corresponding radicals, and their chemical behavior in the absence of O_2.

Scheme 1



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(3) For reviews see: (a) Giese, B.; Beyrich-Graf, X.; Erdmann, P.; Petretta, M.; Schwitter, U. *Chem. Biol.* **1995**, *2*, 367−375. (b) Breen, A. P.; Murphy, J. A. *Free Radical Biol. Med.* **1995**, *18*, 1033−1077. (c) Von Sonntag, C.; Hagen, U.; Schön-Bopp, A.; Schulte-Frohlinde, D. *Adv. Radical Biol.* **1981**, *9*, 109−142.
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(8) Buxton, G. V.; Greenstock, C. L.; Helman, W. P.; Ross, A. B. *J. Phys. Chem. Ref. Data* **1988**, *17*, 513−886.
(9) (a) Steenken, S.; Behrens, B.; Schulte-Frohlinde, D. *Int. J. Radiat. Biol.* **1974**, *25*, 205−210. (b) Samuni, A.; Neta, P. *J. Phys. Chem.* **1973**, *77*, 2425−2429.

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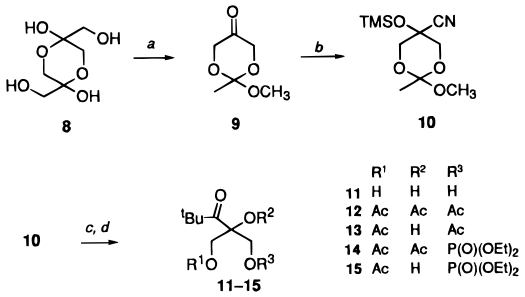
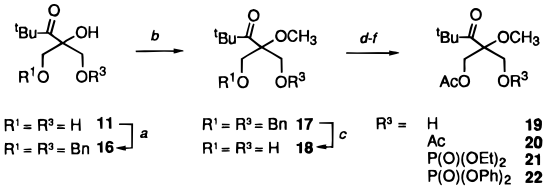
Scheme 2



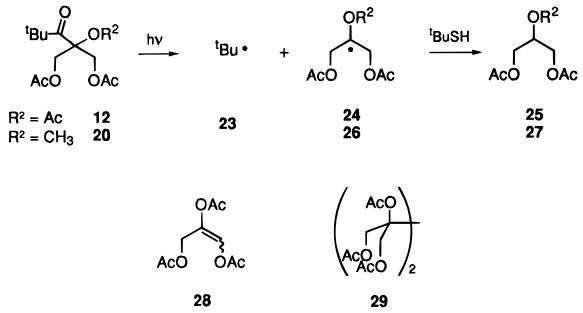
[a] $CH_3C(OCH_3)_3$, CSA, dioxane, 81%. [b] TMSCN, catalytic 18-crown-6/KCN, 90%. [c] ^{t}BuLi, catalytic CuI, 63%. [d] Hydrolysis or esterification, 70−99%.

Scheme 3



[a] $BnOC(=NH)CCl_3$, catalytic Tf_2O, 82%. [b] KOH, CH_3I, DMSO, 61%. [c] H_2, Pd/C, EtOH, 82%. [d] (1) $CH_3C(OCH_3)_3$, CSA, (2) AcOH/H_2O (**18** → **19**), 93%. [e] Ac_2O, NEt_3, DMAP (**18** → **20**), 90%. [f] R^3Cl, NMI, (**19** → **21**, 76%), (**19** → **22**, 83%).

Scheme 4



Results and Discussion

A. Model Compounds. Synthesis of the Radical Precursors. *tert*-Butyl ketones were synthesized as photolabile precursors[10] for C-2 glyceryl radicals bearing acetate or phosphate groups in the β-position. The *tert*-butyl ketones **11**−**15** were prepared starting from the dimer of 1,3-dihydroxyacetone **8** which was transformed *via* orthoester **9** into the protected cyanohydrin **10** (Scheme 2). Copper-catalyzed nucleophilic attack of *tert*-butyllithium, subsequent hydrolysis, and esterification yielded the *tert*-butyl ketones **11**−**15**.

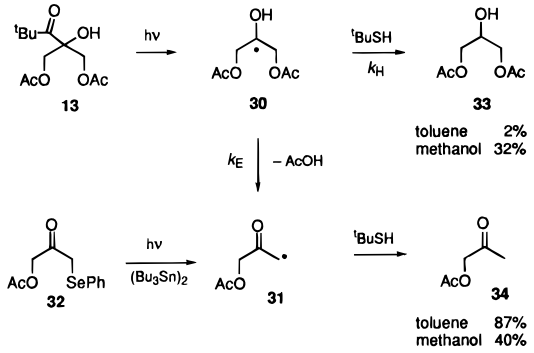
We also synthesized radical precursors bearing different α-substituents in order to study their influence on the reactivity of the glyceryl radicals (OR^2 = OH, OAc, OMe). The α-methoxy *tert*-butyl ketones **19**−**22** were obtained from **11** by benzylation of the primary hydroxyl groups (**11** → **16**), followed by methylation of the tertiary OH group (**16** → **17**), and deprotection of the primary alcohols (**17** → **18**). Subsequent esterifications yielded the radical precursors **19**−**22** (Scheme 3).

Acetate as a Potential Leaving Group. The α-acetoxy precursor **12** bearing β-acetates as potential leaving groups was irradiated in the cavity of the ESR spectrometer (benzene as solvent). The ESR spectrum showed signals corresponding to the *tert*-butyl radical (**23**) and the glyceryl radical **24**. No additional radicals could be observed (Scheme 4 and Table 1, entries 1 and 6).

Table 1. ESR Spectral Data at 7 °C in Benzene

entry	radical precursor	radical	*g*-value	β-coupling constant (G)	other coupling constants (G)
1	**12**	**24**	2.002 73	11.7 (4 H_β)	
2	**20**	**26**	2.002 85	8.1 (4 H_β)	2.7 (3 H_γ)
3	**13**	**30**	2.002 88	8.4 (4 H_β)	1.1 (1 H_β)
4	**14**	**35**	2.002 75	11.8 (4 H_β)	2.5 (1 P_γ)
5	**13,32**	**31**	2.004 50		19.6 (2 H_α)
6	**12**−**15,20**	**23**	2.002 7	22.7 (9 H_β)	

Scheme 5



Irradiation of α-acetoxy ketone **12** in the presence of the hydrogen donor 2-methylpropane-2-thiol in toluene or methanol gave glyceryl triacetate (**25**) in 93% and 82% yield, respectively. In the absence of the thiol, the yield of **25** decreased to less than 40% and the formation of disproportionation product **28** and recombination product **29** was observed. No products resulting from β-elimination of the acetate group could be detected.

Photolysis of the α-methoxyglyceride **20** in benzene generated radical **26** which was observed by ESR spectroscopy (Scheme 4 and Table 1, entry 2). Product studies in the presence of ^{t}BuSH in toluene and methanol yielded the glyceride **27** in 72% and 68% yield, respectively, with no detectable amount of products resulting from β-C,O bond cleavage. However, upon photolysis of α-hydroxyglyceride **13** in benzene three radicals could be observed. The ESR spectra showed not only the signals of *tert*-butyl radical (**23**) and the quintet of doublets of radical **30**, but also signals of enolate radical **31** (Scheme 5 and Table 1, entries 3 and 5). The identity of the latter radical was proven by its independent generation from the phenylselenide **32**. Evidence for the assignment of the barely resolved quintet of doublets of **30** could be obtained by substitution of the hydroxyl proton with deuterium. Because the *D* coupling constant was well below the resolution of 0.5 G, it simplified the signal of **30**-d_1 to a quintet. Photolysis of **13** in toluene in the presence of 10 equiv of 2-methylpropane-2-thiol (30 mM) yielded ketone **34**, the product of acetic acid elimination, as the major product (87%). Only 2% of the reduction product **33** could be observed. In methanol, 1-acetoxyacetone (**34**) and glyceride **33** were formed in 40% and 32% yield, respectively (Scheme 5).

The formation of both the reduction and the elimination products from the intermediate radical **30** enabled us to measure the relative rate constant $k_{rel} = k_E/k_H$ by competitive kinetic experiments (k_E = rate constant for β-elimination; k_H = rate constant for H-transfer). Using pseudo-first-order kinetics (see the Experimental Section) with ^{t}BuSH as hydrogen atom donor, the relative rate constants k_{rel}(25 °C) were determined to be 0.16 M in methanol, 1.4 M in dioxane, and 13 M in toluene. The

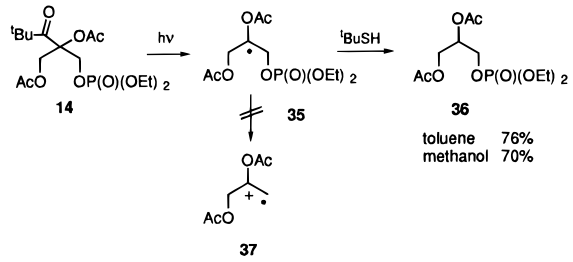
(10) Bamford, C. H.; Norrish, R. G. W. *J. Chem. Soc.* **1935**, 1504−1511.

Table 2. Kinetic Data at 25 °C

entry	radical precursor	radical	solvent	k_{rel} [M]	k_E [s^{-1} M^{-1}]
1	13	30	methanol	0.16	4.0×10^5 [a]
2	13	30	dioxane-d_8	1.4	2.1×10^6 [a]
3	13	30	toluene-d_8	13	2.0×10^7 [b]
4	21	42	methanol	14.8[c]	3.7×10^7 [a,c]
5	22	43	methanol	540[c]	1.4×10^9 [a,c]
6	52	53	methanol	1.4[c]	3.5×10^6 [a,c]
7	52	53	methanol	0.15[d]	3.8×10^5 [a,d]

[a] The rate $k_H(CH_3OH, 30\ °C) = 2.5 \times 10^6\ s^{-1}\ M^{-1}$ was applied. [b] The rate k_H(hexane, 30 °C) $= 1.5 \times 10^6\ s^{-1}\ M^{-1}$ was applied. [c] Phosphate as leaving group. [d] Palmitate as leaving group.

Scheme 6

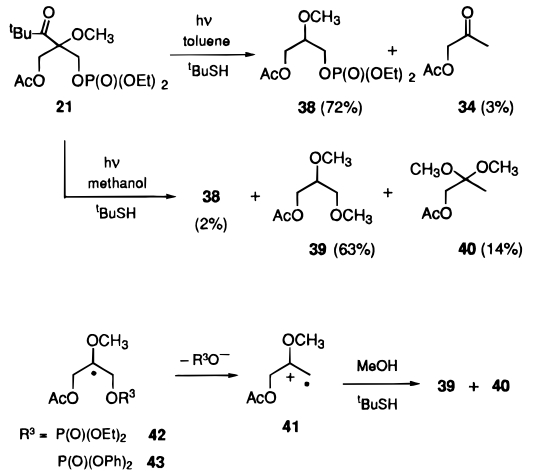


absolute rate of elimination could be obtained from the absolute rate of H-transfer (k_H). The coefficient k_H for the H-atom transfer from thiols to carbon-centered radicals is known to be dependent upon the polarity of the solvent. Contrary to phenols[11] the rate constant k_H for thiols is increased in polar solvents, due to a polarized transition state during hydrogen atom transfer.[12] The rate constant for H-transfer from ^{t}BuSH to a tertiary carbon-centered radical k_H is $2.5 \times 10^6\ s^{-1}\ M^{-1}$ in methanol and $1.5 \times 10^6\ s^{-1}\ M^{-1}$ in hexane at 30 °C.[13] Since the rate of hydrogen atom transfer in toluene was not known, it was approximated by the rate constant k_H in hexane. The value of k_H in methanol was also used for experiments in dioxane solution. With these data the rate constants of acetate elimination k_E from radical **30** were determined to be 4.0×10^5 (methanol), 2.1×10^6 (dioxane), and 2.0×10^7 (toluene) s^{-1} M^{-1} (Table 2, entries 1−3). Thus, the rate of β-elimination increased by a factor of 50 in going from methanol to toluene.

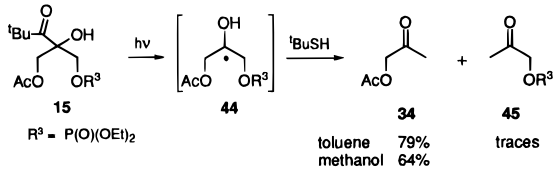
Phosphate as a Potential Leaving Group. A simple model for C-2 phospholipid radicals is glyceryl radical **35** which is formed upon photolysis of precursor **14**. It bears a β-phosphate and a β-acetate substituent (Scheme 6). The ESR spectrum of radical **35** showed a quintet coupling of 11.8 G for the methylene protons and doublet coupling of 2.5 G for the phosphorus nucleus (Table 1, entry 4). Photolysis of the α-acetoxy precursor **14** in the presence of 2-methylpropane-2-thiol gave glyceride **36** in 76% (toluene) and 70% (methanol) yield. No elimination products were formed in toluene or in methanol, thereby indicating a slow rate of β-elimination.

Photolysis of the α-methoxy-β-(diethoxyphosphoryl)oxy precursor **21** in toluene in the presence of 10 equiv of 2-methylpropane-2-thiol (15 mM) gave reduction product **38** in 72% yield, together with 3% of the elimination product **34**. In methanol, however, the substitution products **39** (63%) and **40** (14%) were formed together with only 2% of the reduction product **38** (Scheme 7). The formation of **39** and **40** can be explained by trapping of the intermediate radical cation **41**. The

Scheme 7



Scheme 8



strong solvent dependence of the ratio of reduction *versus* substitution products (∼25:1 in toluene and 1:40 in methanol) is in agreement with the heterolytic β-C,O bond cleavage and the formation of charged species (phosphate anion, radical cation). Similar effects have been observed in model studies on the β-bond scission of 4′-DNA radicals.[14] From competitive kinetic experiments in methanol we could determine the rate of β-elimination $k_E[(EtO)_2P(O)O^-] = 3.7 \times 10^7\ s^{-1}$ (Table 2, entry 4) and $k_E[(PhO)_2P(O)O^-] = 1.4 \times 10^9\ s^{-1}$. Thus, diphenyl phosphate is eliminated 75 times faster than diethyl phosphate, which can be explained by the higher acidity of $(PhO)_2P(O)$-OH compared to $(EtO)_2P(O)OH$.[15]

Photolysis of the α-hydroxy *tert*-butyl ketone **15** led to the formation of the hydroxyalkyl radical **44** bearing a diethyl phosphate and an acetate as leaving groups (Scheme 8). 1-Acetoxyacetone (**34**) which is formed through phosphate elimination was obtained in the presence of ^{t}BuSH as the major product in 79% (toluene) and 64% (methanol) yield. Ketone **45**, resulting from the elimination of acetate, was only detected in trace amounts. Since no reduced glyceride was found, we can conclude that the rate of diethyl phosphate elimination is dramatically increased if the α-methoxy substituent of **42** is changed to the α-hydroxy substituent of radical **44**. Since no methanol trapping products could be detected, the diethyl phosphate elimination of the α-hydroxy-substituted radical **44** must occur *via* a different mechanism compared to the α-methoxy-substituted radical **42**.

The rate of heterolytic elimination of a β-substituent in α-oxygen-substituted radicals depends upon the pK_a of the conjugate acid of the leaving group.[14] This is in agreement with the fast elimination of diphenyl and diethyl phosphate from radicals **42** and **43** compared to that of acetate from radical **26**, where the elimination was too slow to be detected. In addition, the rate is influenced by the nature of the α-substituent. Fast

(11) Valgimigli, L.; Banks, J. T.; Ingold, K. U.; Lusztyk, J. *J. Am. Chem. Soc.* **1995**, *117*, 9966−9971.

(12) Tronche, C.; Martinez, F. N.; Horner, J. H.; Newcomb, M.; Senn, M.; Giese, B. *Tetrahedron Lett.* **1996**, *37*, 5845−5848.

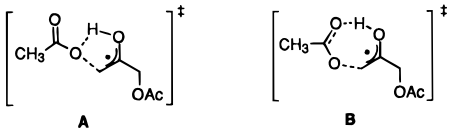
(13) The constants were determined according to ref 12. The data are collected in the Supporting Information.

(14) Giese, B.; Beyrich-Graf, X.; Burger, J.; Kesselheim, C.; Senn, M.; Schäfer, T. *Angew. Chem.* **1993**, *105*, 1850−1852; *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1742−1744.

(15) p$K_a[(EtO)_2P(O)OH] = 3.15$ and p$K_a[(PhO)_2P(O)OH] = 2.71$ in 80% ethanol: Mastryukova, T. A.; Melent'eva, T. A.; Shipov, A. E.; Kabachnik, M. *Zh. Obshch. Khim.* **1959**, *29*, 2178−2182.

elimination of acetate or phosphate groups was observed from radicals bearing an α-hydroxy substituent. With a methoxy group at the radical center only good leaving groups like diethyl or diphenyl phosphates underwent fast β-elimination. No elimination could be observed from α-acetoxy-substituted radicals. We rationalize the rate-reducing effect of the acetate group by its electron-withdrawing properties, which destabilizes a potential radical cation such as **37**.

The rate of β-elimination strongly increases in going from α-methoxy radicals **42** and **26** to α-hydroxy radicals **44** and **30**. From the solvent dependence of the rate of β-elimination of the hydroxyalkyl radical **30**, we deduce that a new reaction mechanism is operative.[16] Because hydrogen-bond-breaking solvents such as dioxane and especially methanol reduced the rate of β-elimination, we suggest that elimination occurs through a concerted mechanism, where glyceryl radical **30** is directly converted into enolate radical **31** *via* a cyclic, hydrogen-bridged transition state. Two possible cyclic transition states can be envisaged: a five-membered transition state, **A**, where the hydroxyl proton is transferred to the β-oxygen, or a seven-membered transition state, **B**, with a proton shift to the oxygen



A B

of the ester carbonyl group. The small β-coupling constant of 8.4 G (Table 1, entry 3) shows that the C-2 glyceryl radical **30** adopts a conformation where the β-C,O bond is in the same plane as the singly occupied orbital of the radical. This conformation is stabilized by an orbital overlap between the nonbonding electron pair of α-oxygen, the σ^* orbital of the C,O bond, and the radical orbital.[17] In the five-membered transition state **A** this overlap energy is lost due to an almost orthogonal arrangement of the σ^* and the radical orbital. In the seven-membered transition state **B**, however, the σ^* orbital and the SOMO can align and thus maintain the σ^*-SOMO overlap.

Ab initio ground state calculations of **30** suggest the existence of a hydrogen bridge between the hydroxyl group and the carbonyl oxygen and a parallel arrangement of the radical orbital with the β-C,O bond (Figure 1).[18] These calculations, together with the ground state conformation deduced from ESR measurements, support the hypothesis of a cyclic transition state. *Ab initio* calculations on the transition state of the simplified radical **46** confirm a cyclic transition structure. The observed solvent effect on the rate of β-elimination of **30** is in agreement with this mechanism. In toluene the intramolecular hydrogen bridge is likely to hold the radical in a cyclic conformation. In methanol, on the other hand, H-bridging between the OH group of **30** and solvent molecules disfavors the seven-membered ring and stabilizes an open-chain conformation, thus reducing the elimination rate. A similar mechanism should also explain the phosphate elimination from radical **44**.

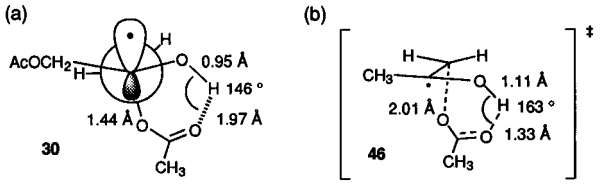
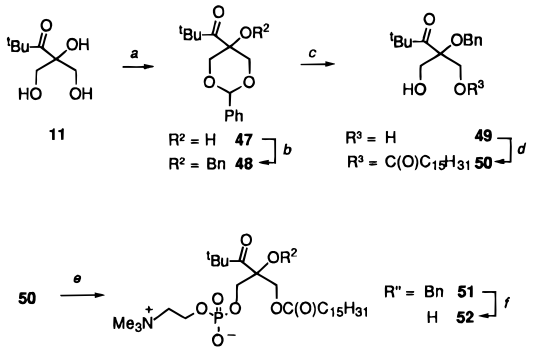


Figure 1. *Ab initio* calculations of (a) the ground state conformation of radical **30** and (b) the transition structure of the acetic acid elimination of radical **46**.

Scheme 9



[a] PhCHO, TsOH, toluene, Δ, 45%. [b] BnBr, NaH, Bu_4NI, THF, >99%. [c] 80% AcOH, 60 °C, 88%. [d] $C_{15}H_{31}COCl$, pyr, 66%. [e] (1) $POCl_3$, pyr, CH_2Cl_2, (2) choline tosylate, CH_3CN, pyr, (3) H_2O, 77%. [f] H_2, Pd, EtOH, 50 °C, 87%.

B. Phospholipids. In contrast to the model glyceride **36** most natural phospholipids (e.g., lecithin) are phospho*di*- or phospho*mono*esters. Because we have found that β-elimination in phospho*tri*ester radical **35** is too slow to be detected, it seems unlikely that radicals of phospholipids which are phospho*di*- and phospho*mono*esters undergo β-elimination in substantial amounts.[19] However, lysolipid radicals with a free C-2 hydroxyl group can be expected to eliminate phosphate, forming enolate radicals analogous to **31**.

We therefore synthesized a lysolecithin analog, **52**, bearing a *tert*-butyl ketone at C-2 as radical precursor. The synthesis of **52** started from triol **11** which was converted into its 1,3-benzylidene acetal **47** (Scheme 9). The corresponding 1,2-benzylidene acetal was formed as side product and could be hydrolyzed to recover the starting triol **11**. The tertiary hydroxyl group of **47** was then benzylated (**47** → **48**), the benzylidene acetal cleaved under acidic conditions, and the resulting diol **49** acylated with palmitoyl chloride (**49** → **50**). The phosphocholine moiety was introduced using phosphorus oxychloride

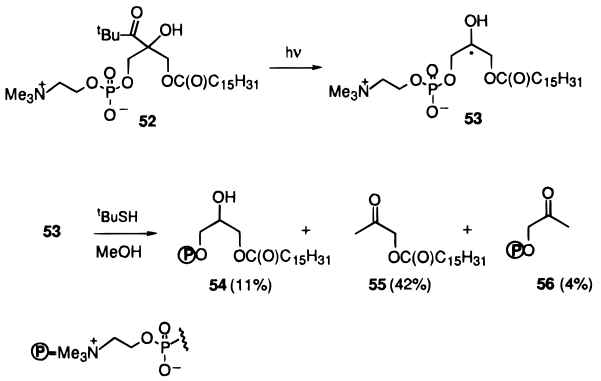
(16) *Ab initio* calculations in the gas phase showed that the homolytic β-cleavage reaction of **30** is endothermic by ~36 kcal mol^{-1}. This high reaction enthalpy cannot account for the fast β-elimination observed in solution (> 10^5 s^{-1}). The calculations were carried out at the PMP2/6-31G*//UHF/3-21G* level of theory with Gaussian 94 (revision B.2: Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Gill, P. M. W.; Johnson, B. G.; Robb, M. A.; Cheeseman, J. R.; Keith, T.; Petersson, G. A.; Montgomery, J. A.; Raghavachari, K.; Al-Laham, M. A.; Zakrzewski, V. G.; Ortiz, J. V.; Foresman, J. B.; Cioslowski, J.; Stefanov, B. B.; Nanayakkara, A.; Challacombe, M.; Peng, C. Y.; Ayala, P. Y.; Chen, W.; Wong, M. W.; Andres, J. L.; Replogle, E. S.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Binkley, J. S.; Defrees, D. J.; Baker, J.; Stewart, J. P.; Head-Gordon, M.; Gonzalez, C.; Pople, J. A. (Gaussian, Inc., Pittsburgh, PA, 1995)). Furthermore, no intermediate enolate radical **31** (ESR) is formed *via* a homolytical pathway. A heterolytic cleavage mechanism is quite unlikely to occur since the formation of charged species (anion, radical cation) is favored in polar solvents as observed for radicals **42** and **43**. For the hydroxyalkyl radical **30**, however, the rates of elimination in methanol and dioxane are up to 50 times slower than in toluene. A synchronous transition state of H_2O elimination in 1,2-dihydroxy radicals was discussed in Zipse, H. *J. Am. Chem. Soc.* **1995**, *117*, 11798−11806.

(17) Giese, B.; Dupuis, J.; Gröninger, K.; Hasskerl, T; Nix, M.; Witzel, T. In *Substituent Effects in Radical Chemistry*; Viehe, H. G., Janousek, Z., Merényi, R., Eds.; D. Reidel: Dordrecht, The Netherlands, 1986; pp 283−296.

(18) The calculations were carried out at the PMP2/6-31G*//UHF/3-21G* level of theory with GAUSSIAN 94 (revision B.2). The transition state **46** (11 kcal mol^{-1} higher than the ground state) was checked by frequency calculation, and only one imaginary frequency was found. An IRC calculation was performed to verify to which educt and products the transition state leads.

(19) According to investigations of Schulte-Frohlinde and co-workers phospho*di*- and phospho*mono*esters undergo slower heterolytic β-elimination than phospho*tri*esters: Behrens, G.; Koltzenburg, G.; Ritter, A.; Schulte-Frohlinde, D. *Int. J. Radiat. Biol.* **1978**, *33*, 163−171.

Scheme 10



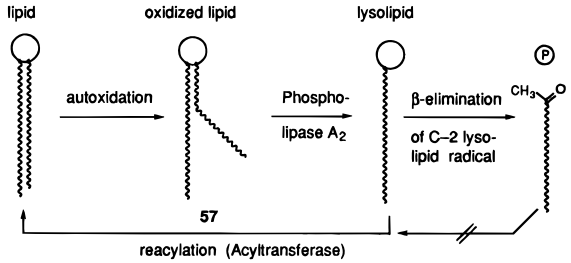
followed by the addition of choline tosylate with subsequent hydrolysis (**50** → **51**).[20] Deprotection of the tertiary C-2 hydroxyl group yielded the lysolecithin precursor **52**.

The radical precursor **52** contains a hydrophilic part (phosphocholine) and a hydrophobic part (fatty acid chain) and is therefore amphiphilic. In aqueous solution amphiphiles tend to self-associate, forming aggregates such as micelles or liposomes, depending on their geometry.[21] From light scattering experiments the aggregate structure of **52** in water was determined to be cylindrical micelles, the predominant aggregate type of lysolecithins.[22]

In methanol solution amphiphilic molecules are known to exist as monomers.[23] Photolysis of precursor **52** in methanol solution in the presence of 40 equiv of 2-methylpropane-2-thiol (12 mM) yielded the phosphate elimination products **55** in 42% yield, the palmitate elimination product **56** in 4% yield and the reduction product **54** in 11% yield (Scheme 10). In competitive kinetic experiments analogous to that of radical **30** we determined the rate constant k_E for phosphocholine elimination from radical **53** as 3.5×10^6 s^{-1} (Table 2, entry 6). The palmitate elimination, leading to ketone **56**, was found to have the rate constant $k_E = 3.8 \times 10^5$ s^{-1} (Table 2, entry 7). Thus, fast β-elimination of C-2 lysolecithin radicals in methanol occurred with both phosphate and carboxylate as leaving groups. Photolysis of micellar aggregates of **52** in water resulted in the formation of the phosphate elimination product **55** in 24% yield along with the glyceride **54** and ketone **56** in 1−2% yield. By addition of 60 equiv of the radical scavenger thioglycolic acid, the overall yield of products could be improved (∼50%) and the ratio of reduction (**54**) *versus* phosphate elimination product (**55**) increased to 1:1.

Despite their tendency to form micelles, lysolecithin molecules can be incorporated into the membrane of liposomes.[24] We therefore investigated liposomes made out of egg yolk lecithins and cephalins and 10% of the radical precursor **52** in saline buffer (pH 7.4). The liposomes were made by the extrusion method[25] and were approximatively 100 nm in diameter. It was found that upon photolysis up to 19% of elimination product **55** was formed in the liposome membrane. In the presence of 70 equiv of the radical scavenger thioglycolic acid in the extravesicular buffer, the yield of **55** dropped to less than 3%, indicating an increased amount of reduction of the intermediate lysolipid radical **53**. These experiments clearly demonstrate the tendency of lysolipid radical **53** to undergo β-elimination even in a lipid double layer. However, photolysis, generation of the radicals, and formation of the elimination products had no detectable influence on the stability of the membrane. Vesicle fusion and fission (influencing the size distribution) are known indicators of membrane disruption. The size distributions of the liposomes containing precursor **52** were determined by the dynamic light scattering method and found to be identical within experimental error before and after photolysis (87 ± 10 nm versus 89 ± 12 nm).[22b]

Scheme 11



γ-Radiolysis. In order to demonstrate that C-2 lysolipid radicals can be generated by unselective attack of hydroxyl radicals, micellar solutions of lysolipid **54** were γ-irradiated with a ^{137}Cs source. If lysolipid radicals were generated, β-elimination should lead to ketone **55**. Indeed, we observed the elimination product **55**, a non amphiphilic ketone, with a radiochemical yield of 4%.[26] This is a remarkable amount if one considers the number of possible reaction sites of hydroxyl radicals with the lysolipid.

The actual amount of lysolipids in biological membranes rarely exceeds a few percent.[27] Nevertheless, damage through lysolipid radicals could become significant in tissues that have been affected by autoxidation. It is known that oxidized phospholipids **57** show an increased rate of hydrolysis of the C-2 fatty ester, due to selective hydrolysis by phospholipase A_2.[28] The resulting lysolipids could subsequently be damaged by C-2 radical formation and β-elimination (Scheme 11). Enzymatic repair of the autoxidation damage by reacylation of the lysolipids would then be prevented.

Conclusion

We have shown by selective radical generation that lysolecithins can be damaged through C-2 lysolecithin radicals. These radicals undergo fast β-elimination of the phosphocholine and the carboxylate group ($k_E \approx 10^6$ s^{-1}), forming non-amphiphilic ketones.[29] The β-elimination was observed both in solution and in the aggregated state. From radiolysis experiments the formation of C-2 lysolecithin radicals *via* H-abstraction could be demonstrated. Although little damage of diacylated phospholipids can be expected *via* C-2 radicals

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(21) Israelachvili, J. N.; Marcelja, S.; Horn, R. G. *Q. Rev. Biophys.* **1980**, *13*, 121−200.
(22) (a) The measured average radius of precursor **52** (0.017 M in water) was 37 nm, which is well beyond the maximum radius of spherical micelles (<10 nm). (b) The measurements were done on a ALV-125 laser light scattering spectrometer/goniometer with a ALV-5000IE correlator at 20 °C.
(23) Lawrence, M. J. *Chem. Soc. Rev.* **1994**, 416−424.
(24) Bent, E. D.; Bell, J. D. *Biochim. Biophys. Acta* **1995**, *1254*, 349−360.
(25) Mayer, L. D.; Hope, M. J.; Cullis, P. R. *Biochim. Biophys. Acta* **1986**, *858*, 161−168.
(26) The ketone **55** was formed with the radiochemical yield $G = 0.015$ μmol J^{-1}. G is defined as the number of moles of product formed per mole of reactive species generated from water (e.g. •OH, •H). The radiolytic yields of hydroxyl and hydrogen radicals are $G = 0.28$ and 0.06 μmol J^{-1}, respectively. The concentration of **55** depended linearly on the dose of γ-irradiation.
(27) Weltzien, H. U. *Biophys. Biochem. Acta* **1979**, *559*, 259−287.
(28) Salgo, M. G.; Corongiu, F. P.; Sevanian, A. *Arch. Biochem. Biophys.* **1993**, *304*, 123−132.
(29) The first experiments in the presence of O_2 have demonstrated that radical **53** can be trapped by O_2 in competition with the spontaneous cleavage. The reaction with O_2 leads to a substituted 2-keto-1,2-propanediol.

under oxygen-free conditions, β-elimination of C-2 glyceryl radicals might become significant in lipids damaged by autoxidation.

Experimental Section

General Procedures. Melting points were recorded on a Kofler block and are uncorrected. ^{1}H, ^{13}C, and ^{31}P NMR spectra were run in $CDCl_3$ (or, where indicated, in benzene-d_6, toluene-d_8, methanol-d_4, or dioxane-d_8) at 300, 75, and 121 MHz, respectively. ^{1}H and ^{13}C chemical shifts are downfield from tetramethylsilane as internal standard. ^{31}P chemical shifts are quoted with respect to $(PhO)_3PO$ ($\delta = -18$ ppm).

2-Methyl-2-methoxy-1,3-dioxan-5-one (9). Dimeric dihydroxyacetone **8** (4.04 g, 22.4 mmol) and camphor-10-sulfonic acid (52.5 mg, 226 μmol, 0.01 equiv) were heated to 60 °C in dioxane (200 mL) under argon. Ten minutes after the dimer was dissolved, trimethyl orthoacetate (60 mL, 57.0 g, 474 mmol, 10 equiv) was added. The solution was stirred overnight at 60 °C and concentrated to 20 mL and the residue distilled in a Kugelrohr oven (at 140 °C at 20 mbar). Orthoester **9** (5.31 g, 36.3 mmol, 81%) was obtained as a clear, colorless liquid: ^{1}H NMR δ 4.34 (2H, d, $J = 17.9$ Hz), 4.18 (2H, d, $J = 17.9$ Hz), 3.39 (3H, s), 1.59 (3H, s); ^{13}C NMR δ 204.5, 112.4, 67.5, 51.3, 20.5. Anal. Calcd for $C_6H_{10}O_4$: C, 49.31; H, 6.90. Found: C, 49.13; H, 7.07.

2-Methyl-2-methoxy-5-cyano-5-[(trimethylsilyl)oxy]-1,3-dioxane (10). To a stirred solution of orthoester **9** (12.7 g, 87.0 mmol) dissolved in DMF (75 mL) were added a few crystals of potassium cyanide and 18-crown-6, and the mixture was cooled to 0 °C under argon. Trimethylsilyl cyanide (12.5 mL, 9.91 g, 100 mmol) was added slowly, the solvent removed after 40 min, and the residue distilled in a Kugelrohr oven (at 80 °C at 0.08 mbar). A diastereomeric mixture of **10a** and **10b** (19.2 g, 78.3 mmol, 90%) in a ratio of 9:1 was obtained as a clear oil. Data for **10a**: ^{1}H NMR (C_6D_6) δ 3.82 (2H, d, $J = 10.6$ Hz), 3.73 (2H, d, $J = 10.6$ Hz), 2.85 (3H, s), 1.40 (3H, s), 0.07 (9H, s); ^{13}C NMR (C_6D_6) δ 120.5, 111.9, 66.4, 63.9, 50.5, 21.3, 1.0. Data for **10b**: ^{1}H NMR (C_6D_6) δ 4.04 (2H, d, $J = 11.5$ Hz), 3.58 (2H, d, $J = 11.5$ Hz), 2.88 (3H, s), 1.31 (3H, s), 0.08 (9H, s); ^{13}C NMR (C_6D_6) δ 118.5, 111.7, 66.3, 64.7, 50.4, 21.3, 1.0. Anal. Calcd for $C_{10}H_{19}NO_4Si$: C, 48.96; H, 5.71. Found: C, 48.76; H, 5.76.

1-Acetoxy-3-hydroxy-2-pivaloyl-2-[(trimethylsilyl)oxy]propane (58). Cyanohydrin **10a,b** (7.20 g, 29.3 mmol), CuI (40.0 mg, 210 μmol, 0.01 equiv), and dry diethyl ether (500 mL) were stirred and cooled to −78 °C under argon. *tert*-Butyllithium (80 mL, 128 mmol, 1.6 M in hexane, 4.4 equiv) was added in 30 min, upon which the reaction mixture turned dark. After 3 h at −78 °C acetic acid (100 mL, 50% in water) was rapidly added *via* syringe. The reaction mixture was allowed to warm, additional acetic acid (200 mL) and water (100 mL) were added, and the mixture was vigorously stirred overnight. The phases were separated, the aqueous phase was extracted with diethyl ether (3 × 150 mL) and the combined organic phases were dried over magnesium sulfate. The extract was concentrated *in vacuo* at room temperature and extracted with concentrated sodium hydrogen carbonate (100 mL) against diethyl ether (750 mL). Chromatography (silica gel, pentane/*tert*-butyl methyl ether (5:1) → *tert*-butyl methyl ether) gave **58** (5.32 g, 18.3 mmol, 63%) as a clear oil: ^{1}H NMR δ 4.29 (1H, d, $J = 11.6$ Hz), 4.23 (1H, d, $J = 11.6$ Hz), 3.76 (1H, dd, $J = 11.2$ Hz, 9.4 Hz), 3.63 (1H, dd, $J = 11.2$ Hz, 3.6 Hz), 2.24 (1H, dd, $J = 9.4$ Hz, 3.6 Hz), 2.06 (3H, s), 1.26 (9H, s), 0.23 (9H, s); ^{13}C NMR δ 217.6, 170.3, 86.4, 67.6, 66.4, 45.2, 26.4, 20.8, 2.0. Anal. Calcd for $C_{13}H_{26}O_5Si$: C, 53.76; H, 9.02. Found: C, 53.78; H, 8.80. Additionally 1.3 g (5.96 mmol, 20%) of **59** could be isolated.

1-Acetoxy-2,3-dihydroxy-2-pivaloylpropane (59). To a solution of silyl ether **58** (87.4 mg, 300 μmol) in dry tetrahydrofuran (10 mL) was added dropwise HF·pyridine (65% in pyridine, 500 μL), and the reaction mixture was stirred at room temperature for 5 h. Purification of the reaction mixture on silica gel (pentane/ethyl acetate, 1:1) yielded **59** (50.3 mg, 230 μmol, 77%) as a colorless solid: mp 54.5−55.5 °C; ^{1}H NMR δ 4.38 (1H, d, $J = 11.7$ Hz), 4.33 (1H, d, $J = 11.7$ Hz), 3.87 (1H, s), 3.81 (1H, dd, $J = 11.3$, 7.7 Hz), 3.68 (1H, dd, $J = 11.3$, 5.5 Hz), 2.16 (1H, dd, $J = 5.5$ Hz, 5.7 Hz), 2.08 (3H, s), 1.28 (9H, s); ^{13}C NMR δ 215.8, 171.0, 83.6, 67.5, 65.2, 44.5, 25.9, 20.3; UV 296 ($\epsilon = 24.5$, n → π^*, C═O). Anal. Calcd for $C_{10}H_{18}O_5$: C, 55.03; H, 8.31. Found: C, 55.01; H, 8.62.

1,3-Diacetoxy-2-hydroxy-2-pivaloylpropane (13). To a solution of diol **59** (1.01 g, 4.63 mmol) and 4-(dimethylamino)pyridine (120 mg, 982 μmol, 0.2 equiv) in dry dichloromethane (30 mL) were added under argon pyridine (0.45 mL, 5.55 mmol, 1.2 equiv) and acetic anhydride (0.52 mL, 5.55 mmol, 1.2 equiv). After 20 min the reaction was quenched with concentrated sodium hydrogen carbonate (20 mL) and the aqueous phase extracted with dichloromethane (10 mL). The combined organic phases were dried over magnesium sulfate, concentrated *in vacuo*, and chromatographed (silica gel, pentane/diethyl ether, 1:1). Diacetate **13** (1.10 g, 4.23 mmol, 91%) was obtained as a colorless oil, which crystallized in the cold: mp 40.5−42 °C; ^{1}H NMR δ 4.34 (2H, d, $J = 11.4$ Hz), 4.27 (2H, d, $J = 11.4$ Hz), 3.44 (1H, s), 2.08 (6H, s), 1.28 (9H, s); ^{13}C NMR δ 214.0, 170.8, 82.8, 67.4, 44.9, 26.2, 20.5. Anal. Calcd for $C_{12}H_{20}O_6$: C, 55.38; H, 7.74. Found: C, 55.33; H, 7.90.

1,2,3-Triacetoxy-2-pivaloylpropane (12). To a solution of diol **59** (117 mg, 536 μmol) and 4-(dimethylamino)pyridine (136 mg, 1.07 mmol, 2 equiv) in dry dichloromethane (5 mL) was added under argon acetic anhydride (0.2 mL, 2.16 mmol, 4 equiv), and the reaction mixture was stirred at room temperature for 12 h. After addition of water (2 mL) and diethyl ether (15 mL) the reaction mixture was washed with concentrated sodium hydrogen carbonate (2 × 2 mL). The organic phases were dried over magnesium sulfate, the solvent was removed *in vacuo*, and the residue was chromatographed (silica gel, pentane/diethyl ether, 1:1) to yield **12** (114 mg, 377 μmol, 70%) as a colorless oil, which crystallized in the cold: mp 36.5−37 °C; ^{1}H NMR δ 4.76 (2H, d, $J = 11.7$ Hz), 4.47 (2H, d, $J = 11.7$ Hz), 2.13 (3H, s), 2.04 (6H, s), 1.25 (9H, s); ^{13}C NMR δ 210.2, 169.8 (2C), 169.2, 87.3, 62.8, 45.1, 27.1, 21.5, 20.6 (2C); UV 291 ($\epsilon = 30.5$, n → π^*, C═O). Anal. Calcd for $C_{14}H_{22}O_7$: C, 55.62; H, 7.33. Found: C, 55.48; H, 7.53.

3-Acetoxy-1-[(diethoxyphosphoryl)oxy]-2-hydroxy-2-pivaloylpropane (15). To a cooled (0 °C) solution of diol **59** (505 mg, 2.31 mmol) in dry dichloromethane (10 mL) were simultaneously added under argon *N*-methylimidazole (0.55 mL, 568 mg, 7 mmol, 3 equiv) and diethoxyphosphoryl chloride (1 mL, 1.20 g, 7 mmol, 3 equiv), and the resulting mixture was stirred for 30 min at 0 °C and 40 min at room temperature. Chromatography (silica gel, pentane/ethyl acetate, 1:1) gave **15** (683 mg, 1.93 mmol, 83%) as a colorless oil: ^{1}H NMR δ 4.5 (1H, br s), 4.34 (1H, d, $J = 11.4$ Hz), 4.30 (1H, dd, $J = 8.4$ Hz, 10.9 Hz), 4.22 (1H, d, $J = 11.4$ Hz), 4.14 (1H, dd, $J = 8.4$ Hz, 10.9 Hz), 4.13 (2H, quint, 7.1 Hz), 4.12 (2H, quint, $J = 7.1$ Hz), 2.07 (3H, s), 1.35 (6H, td, $J = 7.1$ Hz, 1.0 Hz), 1.34 (6H, td, 7.1 Hz, 1.0 Hz), 1.29 (9H, s); ^{13}C NMR δ 214.3, 170.6, 83.0 (d, $J = 5$ Hz), 70.5 (d, $J = 6$ Hz), 67.0, 64.4 (d, $J = 6$ Hz), 45.2, 26.3, 20.7, 16.0 (d, $J = 7$ Hz); UV 301 ($\epsilon = 23$, n → π^*, C═O). Anal. Calcd for $C_{14}H_{27}O_8P$: C, 47.46; H, 7.68. Found: C, 47.25; H, 7.50.

2,3-Diacetoxy-1-[(diethoxyphosphoryl)oxy]-2-pivaloylpropane (14). To a cooled (0 °C) solution of alcohol **15** (320 mg, 903 μmol) and 4-(dimethylamino)pyridine (33 mg, 0.3 mmol, 0.3 equiv) in dry pyridine (3.6 mL, 45 mmol, 50 equiv) was added under argon acetic anhydride (4.2 mL, 4.56 g, 45 mmol, 50 equiv), and the reaction mixture was stirred for 15 min at 0 °C and 20 h at room temperature. The solvent was removed *in vacuo* and the residue chromatographed (silica gel, pentane/*tert*-butyl methyl ether/ethanol, 80:40:3). Phosphate **14** (332 mg, 838 μmol, 93%) was obtained as a yellow oil: ^{1}H NMR δ 4.71 (1H, d, $J = 11.8$ Hz), 4.67 (1H, dd, $J = 5.5$ Hz, 10.9 Hz), 4.43 (1H, d, $J = 11.8$ Hz), 4.41 (1H, dd, $J = 5.7$ Hz, 10.9 Hz), 4.09 (2H, quint, $J = 7.8$ Hz), 4.085 (2H, quint, $J = 7.8$ Hz), 2.16 (3H, s), 2.04 (3H, s), 1.32 (6H, td, $J = 7.1$ Hz, 1.0 Hz), 1.25 (9H, s); ^{13}C NMR δ 210.2, 169.7, 169.4, 87.1 (d, $J = 9$ Hz), 65.3 (d, $J = 5$ Hz), 64.0 (d, $J = 6$ Hz), 62.3, 45.1, 27.1, 21.5, 20.6, 16.0 (d, $J = 7$ Hz); ^{31}P NMR δ −1.83 (m). Anal. Calcd for $C_{16}H_{29}O_9P$: C, 48.48; H, 7.37. Found: C, 48.9; H, 7.23.

1,2,3-Trihydroxy-2-pivaloylpropane (11). A solution of acetate **59** (149 mg, 683 μmol) in ammonia (15 mL, 50% in methanol) was stirred for 90 min at 0 °C and the solvent removed to yield **11** (120 mg, 683 μmol, >99%) as a colorless solid: mp 75−76 °C; ^{1}H NMR δ 3,96 (1H, s), 3.84 (2H, dd, $J = 11.4$ Hz, 7.4 Hz), 3.78 (2H, dd, $J = 11.4$ Hz, 5.3 Hz), 2.14 (2H, dd, J = 7.4 Hz, 5.3 Hz), 1.29 (9H, s); ^{13}C NMR δ 217.6, 84.4, 65.5, 44.8, 26.6. Anal. Calcd for $C_8H_{16}O_4$: C, 54.53; H, 9.15. Found: C, 54.69; H, 8.93.

1,3-(Dibenzyloxy)-2-hydroxy-2-pivaloylpropane (16). To a suspension of triol **11** (457 mg, 2.60 mmol) in dichloromethane (12 mL) and cyclohexane (6 mL) was added benzyl 2,2,2-trichloroacetimidate (1.45 mL, 1.96 g, 7.78 mmol, 1.5 equiv), followed by trifluoromethanesulfonic anhydride (15 drops). After 45 min the reaction mixture was diluted with cyclohexane (10 mL) and filtered over Celite. The filtrate was washed with concentrated sodium hydrogen carbonate (50 mL), dried over magnesium sulfate, and chromatographed (silica gel, pentane/diethyl ether, 9:1) after removal of the solvent. Compound **16** (758 mg, 2.13 mmol, 82%) was obtained as an oil: ^{1}H NMR δ 7.4−7.2 (10H, m), 4.52 (4H, s), 3.74 (2H, d, J = 9.4Hz), 3.53 (2H, d, J = 9.4Hz), 3.48 (1H, s), 1.25 (9H, s); ^{13}C NMR δ 216.1, 137.6, 128.3, 127.7, 127.6, 84.7, 73.7, 73.6, 44.8, 26.7. Anal. Calcd for $C_{22}H_{28}O_4$: C, 74.13; H, 7.92. Found: C, 73.73; H, 7.78. The oil, which was used in the next step, contained some trichloroamidate .

1,3-(Dibenzyloxy)-2-methoxy-2-pivaloylpropane (17). To a solution of alcohol **16** (734 mg, 2.06 mmol) in dry dimethyl sulfoxide (2 mL) was added a suspension of pulverized potassium hydroxide (688 mg, 12.4 mmol, 6 equiv) in dimethyl sulfoxide (4 mL), followed by methyl iodide (0.77 mL, 1.75 g, 12.4 mmol, 6 equiv). The reaction mixture was stirred under argon for 75 min and excess methyl iodide removed *in vacuo*. After addition of dichloromethane (7 mL) and water (40 mL) the aqueous phase was extracted with dichloromethane (100 mL) and the organic phase washed with concentrated sodium hydrogen carbonate (10 mL) and dried over magnesium sulfate. Chromatography (silica gel, pentane/dichloromethane, 1:1) yielded **17** (469 mg, 1.27 mmol, 61%) as a clear oil: ^{1}H NMR δ 7.35−7.2 (10H, m), 4.48 (2H, d, J = 12.2 Hz), 4.43 (2H, d, J = 12.2 Hz), 3.70 (2H, d, J = 10.0 Hz), 3.66 (2H, d, J = 10.0 Hz), 3.40 (3H, s), 1.23 (9H, s); ^{13}C NMR δ 215.9, 138.0, 128.3, 127.5, 127.5, 89.5, 73.5, 70.3, 51.2, 45.2, 26.8.

1,3-Dihydroxy-2-methoxy-2-pivaloylpropane (18). Benzyl ether **17** (464 mg, 1.25 mmol) and palladium (10% on charcoal, 150 mg, 0.141 mmol, 0.11 equiv) were suspended in ethanol (50 mL) and shaken at 3 bar of hydrogen in a Parr apparatus for 2 h at room temperature. The reaction mixture was filtered over Celite to remove the catalyst and concentrated *in vacuo*. Chromatography (silica gel, pentane/*tert*-butyl methyl ether, 1:1) yielded **18** (195 mg, 1.03 mmol, 82%) as a white powder: mp 82−83.5 °C; ^{1}H NMR δ 3.89 (2H, dd, J = 11.8 Hz, 7.9Hz), 3.83 (2H, dd, J = 11.8 Hz, 5 Hz), 3.45 (3H, s) 2.52 (2H, dd, J = 7.9 Hz, 5 Hz), 1.24 (9H, s); ^{13}C NMR δ 219.1, 89.2, 63.3, 51.4, 45.3, 26.6.

1-Acetoxy-3-hydroxy-2-methoxy-2-pivaloylpropane (19). To a solution of diol **18** (423 mg, 2.22 mmol) and camphor-10-sulfonic acid (9.65 mg, 41.5 μmol, 0.02 equiv) in dry dichloromethane (10 mL) was added trimethyl orthoacetate (1.1 mL, 1.05 g, 8.90 mmol, 4 equiv), and the resulting mixture was stirred for 8 h at room temperature under argon. Hydrolysis was accomplished with acetic acid (10 mL, 50% in water) with vigorous stirring for 2.5 h and was followed by the addition of *tert*-butyl methyl ether (200 mL) and extraction with concentrated sodium hydrogen carbonate (4 × 15 mL). The aqueous phases were extracted with *tert*-butyl methyl ether (70 mL) and the combined organic layers dried over magnesium sulfate. Removal of the solvent *in vacuo* yielded **19** (480 mg, 2.06 mmol, 93%) as a colorless oil: ^{1}H NMR δ 4.49 (1H, d, J = 12.1 Hz), 4.19 (1H, d, J = 12.1 Hz), 3.82 (1H, dd, J = 11.6 Hz, 7.8 Hz), 3.74 (1H, dd, J = 11.6 Hz, 3.2 Hz), 3.39 (3H, s), 2.05 (3H, s), 2.05 (1H, dd, J = 7.8 Hz, 3.2 Hz), 1.25 (9H, s); ^{13}C NMR δ 216.2, 170.3, 88.5, 62.9, 62.4, 50.7, 45.3, 26.5, 20.8.

3-Acetoxy-2-methoxy-1-[(diethoxyphosphoryl)oxy]-2-pivaloylpropane (21). A solution of alcohol **19** (50.0 mg, 215 μmol) in dry dichloromethane (2 mL) was cooled to 0 °C under argon. After addition of N-methylimidazole (51 μL, 52.7 mg, 642 μmol, 3 equiv) and diethoxyphosphoryl chloride (77 μL, 92.4 mg, 535 μmol, 2.5 equiv), the reaction mixture was stirred for 1.5 h at 0 °C and 30 min at room temperature and was then separated on silica gel (pentane/*tert*-butyl methyl ether, 2:1). Phosphate **21** (60.1 mg, 163 μmol, 76%) was obtained as a clear oil: ^{1}H NMR δ 4.49 (1H, d, J = 11.8 Hz), 4.26 (1H, dd, J = 10.8 Hz, 4.6 Hz), 4.20 (1H, dd, J = 10.8 Hz, 4.2 Hz), 4.15−4.05 (4H, m), 4.10 (1H, d, J = 11.8 Hz), 3.43 (3H, s), 2.05 (3H, s), 1.4−1.3 (6H, m), 1.25 (9H, s); ^{13}C NMR δ 213.8, 170.0, 87.7 (d, J = 9 Hz), 65.8 (d, J = 6 Hz), 64.0 (M), 62.5, 50.9, 45.3, 26.5, 20.7, 16.0 (d, J = 7 Hz); ^{31}P NMR δ -1.73 (m). Anal. Calcd for $C_{15}H_{29}O_8P$: C, 48.91; H, 7.94. Found: C, 48.99; H, 7.84.

3-Acetoxy-2-methoxy-1-[(diphenoxyphosphoryl)oxy]-2-pivaloylpropane (22). A solution of alcohol **19** (118 mg, 510 μmol) in dry dichloromethane (5 mL) was cooled to 0 °C under argon. After addition of *N*-methylimidazole (0.16 mL, 165 mg, 2.01 mmol, 4 equiv) and diphenoxyphosphoryl chloride (0.32 mL, 416 mg, 1.55 mmol, 3 equiv), the reaction mixture was stirred for 1 h at 0 °C and 1 h at room temperature and then separated on silica gel (pentane/diethyl ether, 1:1). Phosphate **22** (196 mg, 422 μmol, 83%) was obtained as a yellow oil: ^{1}H NMR δ 7.4−7.3 (2H, m), 7.25−7.15 (3H, m), 4.46 (1H, d, J = 11.8 Hz), 4.45 (2H, d, J = 4.5 Hz), 4.09 (1H, d, J = 11.8 Hz), 3.31 (3H, s), 2.02 (3H, s), 1.19 (9H, s); ^{13}C NMR δ 213.3, 169.7, 150.1 (d, J = 7 Hz), 129.6 (2C, d, J = 4 Hz), 125.4, 119.8 (d, J = 5 Hz), 87.4 (d, J = 9 Hz), 66.9 (d, J = 6 Hz), 62.4, 50.7, 45.2, 26.3, 20.5; ^{31}P NMR δ −12.61 (m). Anal. Calcd for $C_{23}H_{29}O_8P$: C, 59.48; H, 6.29. Found: C, 59.35; H, 6.41.

1,3-Diacetoxy-2-methoxy-2-pivaloylpropane (20). Diol **18** (9.71 mg, 51.0 μmol), acetic anhydride (13.9 μL, 15.1 mg, 148 μmol, 3 equiv), triethylamine (21.0 μL, 15.2 mg, 150 μmol, 3 equiv), and 4-(dimethylamino)pyridine (0.15 mg, 1.22 μmol, 0.02 equiv) were stirred under argon at room temperature for 1 h. Removal of solvent and reagents at high vacuum yielded **20** (12.6 mg, 45.9 μmol, 90%) as a slightly yellow oil, which crystallized at −20 °C: mp 37.5−40 °C; ^{1}H NMR δ 4.50 (2H, d, J = 11.9 Hz), 4.12 (1H, d, J = 11.9 Hz), 3,34 (3H, s), 2.05 (6H, s), 1.25 (9H, s); ^{13}C NMR δ 214.2, 170.1, 87.5, 62.8, 50.7, 45.4, 26.5, 20.8. Anal. Calcd for $C_{13}H_{22}O_6$: C, 56.92; H, 8.08. Found: C, 57.19; H, 7.96.

Benzylidene Acetals 47 and 60 of 1,2,3-Trihydroxy-2-pivaloylpropane (11). Triol **11** (870 mg, 4.94 mmol), benzaldehyde (1 mL, 1.05 g, 9.87 mmol, 2 equiv), and a few crystals of *p*-toluenesulfonic acid were suspended in 75 mL of toluene and heated to reflux under argon with a Dean-Stark trap for 3 h. After cooling, triethylamine (2 mL) was added and the reaction mixture separated on silica gel (pentane, 100% → pentane/*tert*-butyl methyl ether, 1:1), yielding 1,3-acetal **47** (585 mg, 2.21 mmol, 45%) as a white solid and 1,2-acetal **60a,b** (649 mg, 2.46 mmol, 50%) as a yellow oil. Data for **47**: mp 122−125 °C; ^{1}H NMR δ 7.55−7.45 (2H, m), 7.45−7.35 (3H, m), 5.57 (1, s), 4.26 (2H, dm, J = 11.0 Hz), 3.99 (2H, dm, J = 11.0 Hz), 3.57 (1H, s), 1.30 (9H, s); ^{13}C NMR δ 213.8, 137.2, 129.3, 128.4, 125.9, 101.5, 78.3, 74.7, 45.6, 25.8. Anal. Calcd for $C_{15}H_{20}O_4$: C, 68.16; H, 7.63. Found: C, 68.14; H, 7.55. The diastereomeric 2:1 mixture of **60** could be separated on silica gel (pentane/*tert*-butyl methyl ether, 5:1). Data for **60a**: mp 63−65.5 °C; ^{1}H NMR δ 7.55−7.45 (2H, m), 7.45−7.35 (3H, m), 6.02 (1H, s), 4.33 (1H, d, J = 9.3 Hz), 3.91 (1H, d, J = 9.3 Hz), 3.87 (1H, dd, J = 11.2 Hz, 7.6 Hz), 3.63 (1H, dd, J = 11.2 Hz, 4.5 Hz), 2.29 (1H, dd, J = 7.6 Hz, 4.5 Hz), 1.30 (9H, s); ^{13}C NMR δ 217.1, 136.5, 129.6, 129.0, 126.3, 105.5, 90.4, 71.4, 67.4, 44.8, 25.0. Data for **60b**: mp 43−45.5 °C; ^{1}H NMR δ 7.6−7.5 (2H, m), 7.45−7.35 (3H, m), 5.98 (1H, s), 4.22 (1H, d, J = 9.0 Hz), 4,11 (1H, d, J = 9.0 Hz), 3.95 (1H, dd, J = 11.5 Hz, 8.5 Hz), 3.82 (1H, dd, J = 11.5 Hz, 5.0 Hz), 2.25 (1H, dd, J = 8.5 Hz, 5.0 Hz), 1.19 (9H, s); ^{13}C NMR δ 216.5, 135.7, 129.3,128.3, 126.3, 104.4, 90.2, 71.8, 65.5, 44.9, 25.8. Anal. Calcd for $C_{15}H_{20}O_4$: C, 68.16; H, 7.63. Found: C, 68.46; H, 7.54.

Hydrolysis of Benzylidene Acetal 60. Acetal **60a,b** (578 mg, 2.19 mmol) was dissolved in acetic acid (32 mL) and water (8 mL) and heated to 60 °C for 3 h under argon. Removal of the solvents and subsequent chromatography (silica gel, pentane/*tert*-butyl methyl ether, 1:1) yielded triol **11** (334 mg, 1.90 mmol, 87%).

5-(Benzyloxy)-2-phenyl-5-pivaloyl-1,3-dioxane (48). To a mixture of alcohol **47** (585 mg, 2.21 mmol), sodium hydride (55% in oil, 195 mg, 4.42 mmol, 2 equiv), and tetrabutylammonium iodide (81.6 mg, 221 μmol, 0.1 equiv), cooled to −78 °C under argon, was added dry tetrahydrofuran (20 mL), and the cooling bath was removed. After the alcohol had completely dissolved, benzyl bromide (525 μL, 758 mg, 4.42 mmol, 2 equiv) was added dropwise, and the resulting mixture was stirred for 16 h in the dark at room temperature. Quenching of the reaction with methanol (1 mL) was followed by the addition of concentrated sodium hydrogen carbonate (75 mL) and extraction with *tert*-butyl methyl ether (5 × 100 mL). The combined organic phases were dried over magnesium sulfate and chromatographed with 2 mL of trimethylamine (silica gel, pentane/*tert*-butyl methyl ether, 20:1). Removal of residual benzyl bromide in high vacuum at 120 °C yielded

48 (783 mg, 2.21 mmol, >99%) as a white powder: mp 110−113 °C; ^{1}H NMR δ 7.55−7.45 (4H, m), 7.4−7.3 (6H, m), 5.63 (1H, s), 5.18 (2H, s), 4.51 (2H, dm, J = 12.3 Hz), 4.41 (2H, dm, J = 12.3 Hz), 1.21 (9H, s); ^{13}C NMR δ 214.4, 138.5, 137.7, 129.2, 128.3, 128.3, 127.8, 127.4, 126.2, 101.1, 82.0, 73.3, 69.3, 45.5, 26.7. Anal. Calcd for $C_{22}H_{26}O_4$: C, 74.55; H, 7.39. Found: C, 74.47; H, 7.57.

2-(Benzyloxy)-1,3-dihydroxy-2-pivaloylpropane (49). Benzylidene acetal **48** (519 mg, 1.46 mmol) was suspended in acetic acid (32 mL) and water (8 mL) and heated to 60 °C for 4 h under argon. Removal of the solvent *in vacuo* and chromatography (silica gel, pentane/*tert*-butyl methyl ether, 1:1) yielded **49** (341 mg, 1.28 mmol, 88%) as a white powder: mp 99−102 °C; ^{1}H NMR δ 7.4−7.3 (5H, m), 4.75 (2H, s), 4.01 (2H, dd, J = 11.9 Hz, J = 8.5 Hz), 3.94 (2H, dd, J = 11.9 Hz, J = 4.7 Hz), 2.27 (2H, dd, J = 8.5 Hz, J = 4.7 Hz), 1.26 (9H, s); ^{13}C NMR δ 218.9, 138.0, 128.6, 127.8, 127.5, 89.8, 66.2, 64.0, 45.4, 26.6. Anal. Calcd for $C_{15}H_{22}O_4$: C, 67.64; H, 8.33. Found: C, 67.68; H, 8.41.

Palmitoylation of 2-(Benzyloxy)-1,3-dihydroxy-2-pivaloylpropane To Give 50. To a solution of diol **49** (284 mg, 1.07 mmol) in dry pyridine (3 mL) was added under argon palmitoyl chloride (354 μL, 320 mg, 1.17 mmol, 1.1 equiv), and the mixture was stirred for 14 h at room temperature. Subsequent chromatography (silica gel, pentane/*tert*-butyl methyl ether, 40:1) yielded **50** (355 mg, 703 μmol, 66%) as a colorless oil and the dipalmitate **61** (165 mg, 222 μmol, 20%) as well as starting material **49** (40.6 mg, 152 μmol, 14%). Data for **50**: ^{1}H NMR δ 7.4−7.3 (5H, m), 4.65 (2H, s), 4.62 (1H, d, J = 12.1 Hz), 4.29 (1H, d, J = 12.1 Hz), 3.92 (1H, dd, J = 11.8 Hz, 8.3 Hz), 3.84 (2H, dd, J = 11.8 Hz, 4.5 Hz), 2.27 (2H, t, J = 7.4 Hz), 2.01 (1H, dd, J = 8.3 Hz, 4.5 Hz), 1.65−1.55 (2H, m), 1.35−1.20 (33H, m, CH_2), 0.88 (3H, m); ^{13}C NMR δ 215.9, 173.2, 137.6, 128.5, 127.8, 127.6, 89.1, 65.8, 63.4, 63.2, 45.4, 34.2, 31.9, 29.66, 29.63, 29.58, 29.4, 29.3, 29.2, 29.1, 26.6, 24.8, 22.7, 14.1. Anal. Calcd for $C_{31}H_{52}O_5$: C, 73.77; H, 10.38. Found: C, 73.64; H, 10.26. Data for **61**: ^{1}H NMR δ 7.35−7.25 (5H, m), 4.63 (2H, d, J = 11.9 Hz), 4.58 (2H, s), 4.22 (2H, d, J = 11.9 Hz), 2.27 (4H, t, J = 7.5 Hz), 1.58 (4H, m), 1.35−1.15 (57H, m), 0.88 (6H, m); ^{13}C NMR δ 213.8, 173.0, 137.4, 128.3, 127.8, 127.7, 87.8, 65.9, 63.3, 45.4, 34.1, 31.9, 29.68, 29.65, 29.62, 29.58, 29.4, 29.3, 29.2, 29.1, 26.6, 24.8, 22.7, 14.1.

2-(Benzyloxy)-1-[(cholinylphosphoryl)oxy]-3-(palmitoyloxy)-2-pivaloylpropane (51). To a cooled (−15 °C) solution of pyridine (0.7 mL) in dry dichloromethane (25 mL) was added under argon phosphoryl chloride (93 μL, 156 mg, 1.02 mmol, 2 equiv), followed by the dropwise addition during 15 min of alcohol **50** (256 mg, 507 μmol) dissolved in dichloromethane (20 mL). After stirring for 15 min, pyridine (6.3 mL) was added, and stirring was maintained for 1 h at −15 °C and 40 min at room temperature. Then, a solution of choline tosylate (1.40 g, 5.08 mmol, 10 equiv) in pyridine (3 mL) and acetonitrile (15 mL) was added, upon which an intermediate precipitate was formed. The reaction was quenched after 90 min by addition of water (15 mL). Removal of the solvents *in vacuo* and chromatography (silica gel, dichloromethane/methanol/water, 15:15:1 → 5:5:1) yielded compound **51** (261 mg, 390 μmol, 77%): ^{1}H NMR (CD_3OD/$CDCl_3$ 6:1) δ 7.45−7.35 (2H, m), 7.35−7.25 (3H, m), 4.82 (1H, d, J = 10.2 Hz), 4.69 (1H, d, J = 11.8 Hz), 4.67 (1H, d, J = 10.2 Hz), 4.26 (1H, dd, J = 11.0 Hz, 3.6 Hz), 4.24−4.14 (2H, m), 4.21 (1H, d, J = 11.8 Hz), 4.13 (1H, dd, J = 11.0 Hz, 4.1 Hz), 3.60 (2H, t, J = 4.7 Hz), 3.20 (9H, s), 2.26 (2H, t, J = 7.3 Hz), 1.55 (2H, m), 1.4−1.15 (33H, m), 0.88 (3H, m); ^{13}C NMR (CD_3OD/$CDCl_3$, 6:1) δ 216.1, 174.4, 139.2, 129.2, 128.9, 128.5, 89.9 (d, J = 10 Hz), 67.3 (M), 66.9 (d, J = 9 Hz), 66., 64.1, 60.2 (d, J = 5 Hz), 54.6 (t, J = 4 Hz), 46.2, 34.9, 32.9, 30.62, 30.59, 30.57, 30.52, 30.34, 30.31, 30.2, 30.0, 27.2, 25.8, 23.6, 14.4; ^{31}P NMR (CD_3OD/$CDCl_3$, 6:1) δ −1.96 (m). Anal. Calcd for $C_{36}H_{64}NO_8P{\cdot}H_2O$: C, 62.86; H, 9.67; N, 2.04. Found: C, 62.94; H, 9.65; N, 2.21.

1-[(Cholinylphosphoryl)oxy]-2-hydroxy-3-(palmitoyloxy)-2-pivaloylpropane (52). A suspension of benzyl ether **51** (260 mg, 398 μmol) and palladium black (91 mg, 0.869 mmol) in ethanol (20 mL) was stirred at 50 °C under hydrogen atmosphere for 45 h. Filtration of the reaction mixture through Celite to remove the catalyst and subsequent chromatography (silica gel, dichloromethane/methanol/water, 10:10:1), followed by coevaporation in acetone, yielded **52** (201 mg, 347 μmol, 87%) as a white powder: mp 154−157.5 °C; ^{1}H NMR (CD_3OD) δ 4.3−4.2 (2H, m), 4.26 (1H, d, J = 11.1 Hz), 4.20 (1H, d, J = 11.1 Hz), 4.08 (1H, dd, J = 10.8 Hz, 6.6 Hz), 3.92 (1H, dd, J = 10.8 Hz, 6.4 Hz), 3.64 (2H, m), 3.22 (9H, s), 2.30 (2H, t, J = 7.4 Hz), 1.58 (2H, m), 1.28 (33H, br s), 0.89 (3H, m); ^{13}C NMR (CD_3OD) δ 217.2, 174.7, 84.2 (d, J = 7 Hz), 70.3 (d, J = 6 Hz), 68.5, 67.2 (M), 60.2 (d, J = 5 Hz), 54.6 (t, J = 4 Hz), 46.0, 34.8, 32.8, 30.55, 30.52, 30.47, 30.3, 30.2, 30.1, 29.9, 26.9, 25.7, 23.5, 14.5; ^{31}P NMR (CD_3OD) δ -0.59 (quint, J = 6.5 Hz). Anal. Calcd for $C_{29}H_{58}NO_8P$: C, 60.08; H, 10.08; N, 2.42. Found: C, 60.00; H, 9.98; N, 2.44.

3-Acetoxy-1-(phenylselenyl)-2-propanone (32). Orthoester **9** (438 mg, 3 mmol, 3 equiv compared to selenophenol) was dissolved in dry diethyl ether (1 mL) and cooled to 0 °C under argon. Selenophenol (0.1 mL, 148 mg, 943 μmol) and boron trifluoroetherate (125 μL, 141 mg, 1 mmol, 1 equiv) were then added dropwise, and the reaction was quenched after 15 min by addition of concentrated sodium hydrogen carbonate (1 mL). Extraction with dichloromethane (10 mL) and chromatography (silica gel, pentane/diethyl ether, 1:1) yielded selenide **32** (72.0 mg, 266 μmol, 28%) as a yellow oil: ^{1}H NMR δ 7.5−7.6 (2H, m), 7.3−7.25 (3H, m), 4.81 (2H, s), 3.61 (2H, s), 2.13 (3H, s); ^{13}C NMR δ 198.8, 169.9, 133.6, 129.4, 128.3, 128.2, 66.3, 32.1, 20.3. Anal. Calcd for $C_{11}H_{12}O_3Se$: C, 48.73; H, 4.46. Found: C, 49.34; H, 4.75.

General Procedure for ESR Measurements. A solution of the *tert*-butyl ketone (1 mL, 0.1 M in benzene) was treated with argon for 15 min in a Suprasil quartz tube and irradiated at 7 °C in the cavity of a Bruker ESP-300 spectrometer with the filtered light (water-cooled Schott UG-5) of a Hanovia 977-B1, 1 kW Hg−Xe high-pressure lamp. Instrument settings: 2 mW microwave power, 8×10^5 receiver gain, 1 G modulation amplitude, 82 ms time constant, 5−10 scans. The g values were measured with a Bruker ER 035M NMR-gaussmeter and a Hewlett-Packard microwave counter.

Independant Generation of Radical 31. A solution of selenide **32** (70 mg in 1 mL of benzene) was treated with argon for 15 min and irradiated after addition of hexabutylditin (0.1 mL).

General Procedure for Product Studies. A solution of the radical precursor (ca. 15 μmol) in deuterated solvent (0.6 mL) was treated for 10 min with argon in a borosilicate NMR tube and the tube sealed with a septum after addition of thiol. The solution was irradiated with a Heraeus TQ150 high-pressure lamp, temperature controlled at 25 ± 5 °C, and the conversion followed periodically by NMR. The yields were determined by integration of GC−MS spectra with decane (5 μL) as internal standard. All experiments were additionally conducted in nondeuterated solvents and analyzed by GC−MS only. The yields of the nonvolatile compounds in connection with precursor **52** were determined by comparison of the integrals of characteristic ^{1}H NMR signals with the integrals of the methylene groups of choline. Those two sets of signals (∼4.25 and ∼3.65 ppm in CD_3OD) can be expected to have the same chemical shift, regardless of the chemical reactions of radical **53**, and do integrate as 100%.

Preparation and Analysis of Liposomes. A mixture of 30 μmol of lecithine and 15 μmol of cephalin from egg yolk (Avanti Polar Lipids) was used as a liposome matrix for the incorporation of 5 μmol of the radical precursor **52**. The lipid/precursor mixture was vortexed in 5 mL of buffer containing 145 mM sodium chloride, 2.5 mM HEPES, and 0.02% sodium azide at pH 7.4. After five freeze−thaw cycles the resulting vesicles were extruded 5 times through a doubly layered Costar Nucleaopore 400 nm polycarbonate filter membrane and 10 times through a doubly layered 100 nm filter at 25 bar of nitrogen. The vesicles were photolyzed for 1 h at 25 °C, the reaction mixture was then lyophylized, and the residue was suspended in 4 mL of pentane and filtered through a 450 nm disposable filter. The eluate was concentrated and the residue dissolved in 8 mL of diethyl ether and purified over a 4 cm (∅ = 5 mm) silica gel column to give the phosphate elimination product **55** which was analyzed by NMR and GC−MS.

Competition Kinetic Experiments. An approximatively 10 mM solution of the radical precursor was treated for 10 min with argon in a NMR tube followed by the addition of 2-methylpropane-2-thiol through a septum. The sample was then photolyzed to 80−100% conversion at 25 ± 0.5 °C and the ratio of elimination to reduction products determined by NMR (if deuterated solvents were used) or by GC−FID. The rate constant k_{rel} was obtained as the slope of a plot of

[**E**]/[**R**] against the inverse thiol concentration (e.g., for radical **30**: **E** = **34** and **R** = **33**). The thiol was used in at least 10-fold excess at concentations of 0.2−2 M so that pseudo-first-order conditions could be applied.

γ-Irradiation Experiments. Solutions of **54** (20 mM in water) were γ-irradiated at a dose rate of $I = 0.35$ Gy s^{-1} using ^{137}Cs with doses of 5−30 kGy. The chemical yields of **55** at the corresponding doses were determined by GC.

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Supporting Information Available: ESR spectra of **24**, **26**, **30**, **30-d_1**, **31**, and **35**, physical data of new reference compounds (**28**, **29**, **38**, **40**, **45**, **55**, **56**, **62**), and a table showing the kinetic data (10 pages). See any current masthead page for ordering and Internet access instructions.

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