Nitroxides Derived from 3,4-Dihydro-2,5-dimethyl-2*H*-pyrrole 1-Oxide: A New Series of Minimum Steric Perturbation Lipid Spin Labels

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The synthesis and stereochemical assignments of a series of *cis*- and *trans*-2,5-dialkyl-2,5-dimethylpyrrolidine (azethoxyl) nitroxides are described. A significant reduction in steric bulk compared to other nitroxide labeled lipids results from integration of the nitrogen atom and two of the pyrrolidine ring carbon atoms into the lipid chain. Models suggest the cis isomers resemble the geometry about a cis carbon–carbon double bond, while the trans isomers are reasonably good analogues of a saturated chain. In the synthetic route, nitrone **1** was converted (**1** → **2** → **3** → **8** + **9**) into a mixture of nitroxide alcohols **8** and **9**. These were separated as diacetates **6** and **7** (trans/cis, 75:25). The isomer ratio in the series could be altered by the choice of reaction pathways. Thus, nitrone **13** was converted via **14** and **15** into a mixture of **8** and **9** (trans/cis, 35:65). Cis,trans structure assignments were made as follows. The isomer identities of nitroxides **16** and **17** (prepared from nitrone **2**) were established by ^{19}F NMR spectroscopy of (+)-methoxy(trifluoromethyl)phenylacetate derivatives **20** and **21**. The two series were linked by converting **9** to cis nitroxide **17** via **12** and **11**. In another series of experiments a mixture of **8** and **9** was converted to separable isomeric iodides **10** and **11**, which were then converted separately into azethoxyl acids **25** and **26** via **23** and **24**. Phospholipid **31** was prepared by coupling **25** to lysophosphatidylcholine.

Nitroxide[2] labeled lipids and phospholipids have been used extensively in ESR studies[3] of the structure and function of biological membranes. Despite the proven usefulness of the technique, key studies are sometimes precluded by the unavailability of labels having suitable spectral, physical, and/or chemical properties. For example, nitroxide spin label studies in certain systems are plagued by ESR signal loss due to in situ reduction[2] of the nitroxide. Spin labeled analogues of bent chain fatty acids such as those which contain a cis double bond or a cyclopropane ring have not been readily available. Finally, there is the constant concern that the spin labeled molecule does not accurately reflect the behavior of its naturally occurring analogue owing to the steric bulk of the nitroxide moiety.

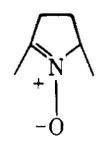
In a recent communication[4] we described a new series of minimum steric perturbation nitroxide lipid spin labels with unique features making them especially attractive probes for the study of biological membranes. These nitroxides are derivatives of the pyrrolidine ring system in which the alkyl side chains are attached to the C_2 and C_5 ring atoms. Thus, the nitroxide moiety is an integral part of the hydrocarbon chain, significantly reducing the steric bulk over that found in other classes of nitroxide labels. The existence of cis and trans isomers, moreover, provides for a series of either bent or straight chain structures. These labels show resistance to reduction by sodium ascorbate superior to that of proxyl and doxyl nitroxides.[2,4] For convenience, we have called these spin labels azethoxyl[5] nitroxides in order to distinguish them from the chemically similar but structurally different proxyl nitroxides.[6]

We now describe in detail the synthesis and stereochemical assignments of the *cis*- and *trans*-azethoxyl nitroxides. The ESR spectral characteristics of these labels in a number of systems will be the subject of a subsequent paper.[7]

Results and Discussion

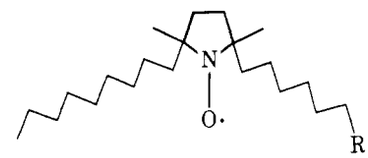
The method of synthesis of the azethoxyl nitroxides takes advantage of much of the chemistry developed in the synthesis of the proxyl nitroxides.[6] Starting with nitrone **1**[8] (Chart I), two successive Grignard addition–air oxidation sequences (e.g., **1** → **2** → **3**) would yield a nitroxide with side chains attached at positions 2 and 5 of the pyrrolidine ring. In order that the cis isomer would resemble naturally occuring oleic acid, it was decided to build a nitroxide in which the pyrrolidine ring was near the center of the chain, roughly at the 9,10 position. Thus, nonylmagnesium bromide was added to nitrone **1**.[8] The intermediate *N*-hydroxy compound was oxidized to give in 45% yield nitrone **2**. The addition of the Grignard reagent derived from the tetrahydropyranyl ether of 6-chlorohexanol followed by Cu^{2+}-catalyzed air oxidation[9] gave nitroxide **3**. Although the yield for this reaction is low (~20%), much of the starting nitrone could easily be recovered for reuse. A major side reaction thus appeared to be the generation of the anion of the nitrone which yields the starting nitrone upon aqueous workup. The yield of **3** could be improved by doing the Grignard addition in refluxing THF.

Chart I

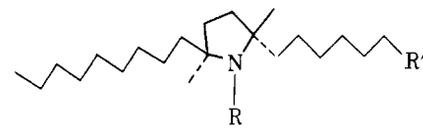


1

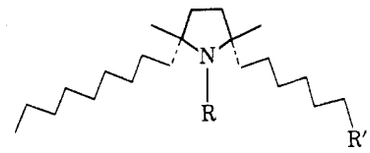
2



3, R = OTHP
4, R = OH
5, R = OSO_2CH_3



6, R = OAc; R′ = OAc
8, R = O·; R′ = OH
10, R = O·; R′ = I



7, R = OAc; R′ = OAc
9, R = O·; R′ = OH
11, R = O·; R′ = I
12, R = O·; R′ = OSO_2CH_3

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Chart II

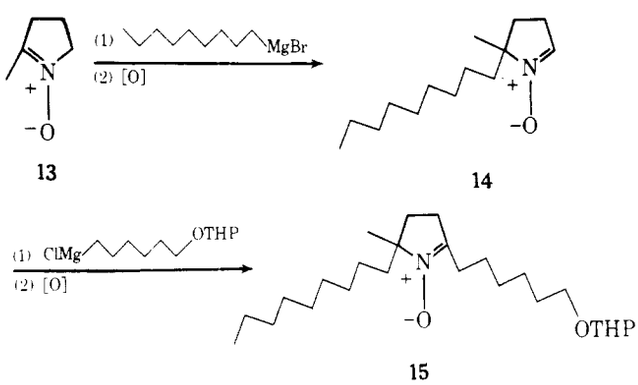
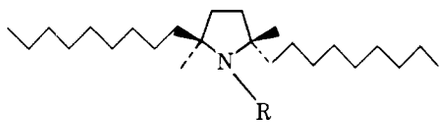
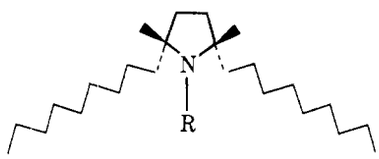


Chart III



16, R = O·
18, R = OAc
20, R = $O_2CC(OCH_3)(CF_3)C_6H_5$



17, R = O·
19, R = OAc
21, R = $O_2CC(OCH_3)(CF_3)C_6H_5$
22, R = $OCH_2CH_2CH_3$

However, the purification of **3** was complicated by the formation of several higher molecular weight nitroxides formed under these conditions, presumably via self-condensation of the nitrone.

Cleavage of the THP protecting group with *p*-toluenesulfonic acid in methanol gave the nitroxide alcohol **4** as a mixture of cis,trans isomers. It was not possible to separate the two alcohol isomers, probably because the hydroxyl group, which governs the behavior during chromatography, is remote from that portion of the molecule which gives rise to the different isomers. However, when the nitroxides were reduced (Pd/C, H_2) to the corresponding *N*-hydroxy compounds and then converted to the diacetates **6** and **7**, it was possible to separate these latter isomers by simple column chromatography on silica gel.

Since it was expected that the Grignard reagent would prefer to attack nitrone **2** from the less hindered side, the major isomer **6** (75%) was assigned the trans geometry and the minor isomer **7** (25%) the cis geometry. These assignments later proved to be correct based on the work described below. One would also expect that the addition of methyllithium to a nitrone in which the two long chain alkyl groups are already in place should give a product mixture that favors the cis isomer. This proved to be correct. Nitrone **13**[10] (Chart II) was treated with nonylmagnesium bromide followed by copper-catalyzed air oxidation to give nitrone **14**. The Grignard reagent derived from 6-chlorohexanol tetrahydropyranyl ether was then added to nitrone **14** followed by copper-catalyzed air oxidation to give nitrone **15**. Methyllithium addition to nitrone **15** and subsequent copper-catalyzed air oxidation gave a cis,trans mixture of nitroxide **3**. Conversion to the diacetates **6** and **7** followed by chromatographic separation gave a

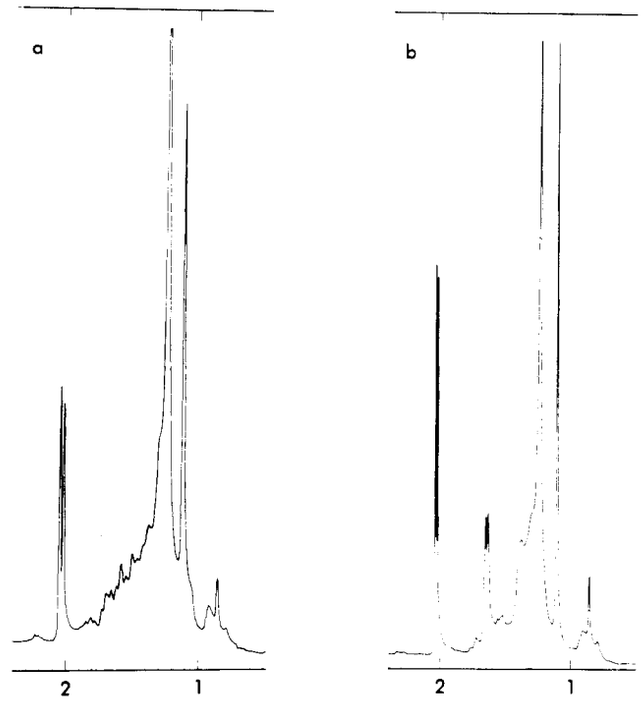


Figure 1. 100 MHz NMR spectra ($CDCl_3$) of (a) trans diacetate **6** and (b) cis diacetate **7**.

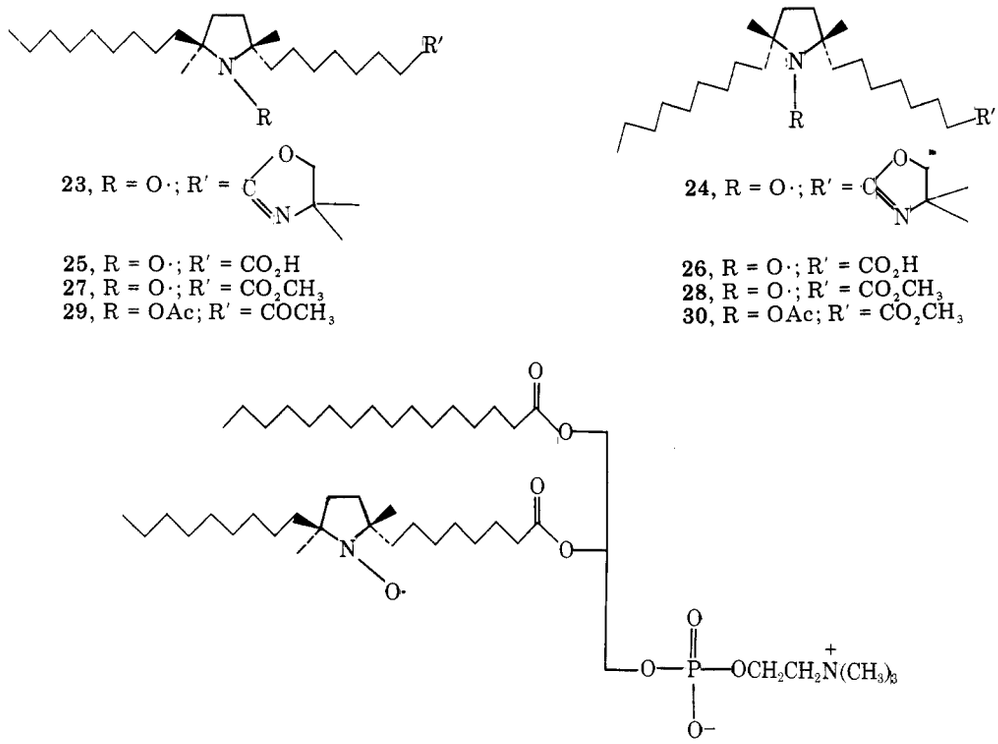
trans/cis ratio of 35:65. Thus, it is possible to control the isomer ratio by appropriate choice of reaction pathways.

Since the essentially quantitative catalytic reduction and acetylation of the nitroxide function yields diamagnetic compounds, this procedure constitutes a useful alternative to the in situ phenylhydrazine reduction of nitroxides[11] for obtaining NMR spectral information. The *N*-acetoxy derivatives have the advantage that they can be stored indefinitely, although some conversion to the nitroxide is observed when they are chromatographed on silica gel. NMR spectra of **6** and **7** are shown in Figure 1. Note the characteristic pattern at δ 1.6–1.75 for the cis isomer which is absent in the spectrum for the trans compound. These patterns were observed for every cis–trans combination that has thus far been examined.

Alkaline hydrolysis of diacetates **6** and **7** with concurrent air oxidation gave the nitroxide alcohols **8** and **9**. The identity of the two geometrical isomers was established as follows. Nitroxides **16** and **17** (Chart III) were synthesized by the reaction of nonylmagnesium bromide with nitrone **2** followed by air oxidation. In this instance, where the primary absorption site is located in the same portion of the molecule that gives rise to the different isomers, the isomers were readily separated by silica gel chromatography using $CHCl_3$ (cis/trans ratio, 20:80). The nitroxides were then in turn converted to the acetates **18** and **19**. The hope was that the trans isomer would show two different acetate absorptions in the NMR spectrum when complexed with an optically active NMR shift reagent[12] while the cis isomer (a meso compound) would show only one. However, even with a large excess of tris[(3-trifluoromethyl)hydroxymethylene-*d*-camphorato]europium(III) in CCl_4, only minor differences in the chemical shift values were observed, and there was no evidence of two acetate absorptions for either isomer.

Turning to another approach, catalytic reduction of both **16** and **17** followed by reaction of the resulting N–OH compounds with (+)-methoxy(trifluoromethyl)phenylacetyl chloride (MTPACl)[13] using the procedure of Mosher[13] gave the corresponding MTPA esters **20** and **21** in ~60% yield. The reaction of the trans isomer with an optically active acid chloride must give rise to two diasteriomeric esters, whereas the cis isomer can only yield one compound. The ^{19}F NMR

Chart IV



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spectrum for **20** showed two absorptions (see Experimental Section). By contrast, the ^{19}F NMR spectrum for **21** showed only one.

To complete the isomer assignment of nitroxide alcohols **8** and **9**, **9** was converted to iodide **11** by way of mesylate **12**. The coupling of **11** with propylmagnesium chloride was readily accomplished using Li_2CuCl_4[14] as a catalyst. The nitroxide moiety was reduced to the N–OH compound under the conditions of the reaction and had to be reoxidized, affording **17**. There was also a sizable amount of the O-alkylated product **22** isolated. The nitroxide obtained from the coupling reaction was identical chromatographically to cis nitroxide **17**. Upon reduction followed by acetylation, both nitroxides gave the same acetate (by NMR).

The presence of a hydroxyl group at the end of the *cis*- and *trans*-azethoxyl nitroxides made possible their conversion into spin labeled analogues of the biologically important fatty acids. The method chosen for this transformation was that of Meyers et al.,[15] in which an alkyl halide is converted into a carboxylic acid having a chain two carbons longer.

Fortuitously, when the cis,trans mixture of alcohols **4** was converted to the mixture of mesylates **5** and thence to iodides **10** and **11**, it was found that these latter isomers could be readily separated by silica gel chromatography. Thus, it was no longer necessary to separate the isomers as the diacetates **6** and **7**. The minor (18%) isomer **11** was identical with that obtained from nitroxide alcohol **9**. Iodides **10** and **11** were separetely converted to the corresponding acids **25** and **26** via the oxazoline derivatives **23** and **24** (Chart IV). The acids could be esterified with diazomethane to give **27** and **28**. Catalytic reduction of these latter substances followed by acetylation as described above gave esters **29** and **30**. The NMR spectra of **29** and **30** showed the pattern at δ 1.6–1.8 which was characteristic of other cis,trans pairs (see above).

A representative phospholipid containing an azethoxyl nitroxide was synthesized by coupling *trans*-10-azethoxyleicosanoic acid (**25**) with lysopalmitoylglycerolphosphatidylcholine using carbonyldiimidazole[16,17] to give the spin labeled phosphatidylcholine derivative **31**.[18]

The synthetic route to azethoxyl labeled lipids and phospholipids is sufficiently straightforward that these nitroxides should prove useful in spin labeling studies. Experiments are currently underway to determine their value in probing biological membranes.

Experimental Section[19]

3,4-Dihydro-2,5-dimethyl-2-nonyl-2*H*-pyrrole 1-Oxide (2). To 100 mL of a 1.0 M nonylmagnesium bromide solution in ether was added with stirring 5.65 g (50.0 mmol) of nitrone **1**[8] in 30 mL of ether at a rate sufficient to maintain gentle reflux. The solution was stirred for an additional 30 min at 21 °C and then treated with an amount of saturated aqueous NH_4Cl sufficient to collect the precipitated aqueous salts in a mass at the bottom of the flask. The ether layer was decanted and combined with two ether washings of the aqueous residue. The solvent was evaporated to yield a yellow oil which was taken up in 50 mL of CH_3OH and 5 mL of concentrated aqueous NH_4OH and stirred with 1 g of $Cu(OAc)_2 \cdot H_2O$ under O_2 until the solution developed a deep blue color. The solution was diluted with ether and H_2O. The usual workup gave a brown oil which was distilled to give 5.43 g (45%) of **2**: bp 100–109 °C (0.005 mm); IR (CCl_4) 1595 cm^{-1} (C=N); NMR δ 0.88 (3 H, m, term Me), 1.38 (3 H, s, ring Me), 2.02 (3 H, t, J = 2 Hz, N=CMe), 2.57 (2 H, m, N=CCH_2); MS *m/e* 239.223 (31) (calcd for $C_{15}H_{29}NO$, 239.225), 222 (34), 113 (68), 96 (75), 73 (27), 55 (28), 45 (53), 43 (100), 41 (36).

2,5-Dimethyl-5-nonyl-2-(6′-tetrahydropyranyloxyhexyl)tetrahydropyrrole-1-oxyl (3) (Mixture of Cis and Trans Isomers). To 25 mL of a 1 M THF solution of the Grignard reagent derived from the tetrahydropyranyl ether of 6-chlorohexanol was added at 21 °C with stirring over a period of 15 min 10 mL of a THF solution of 2.39 g (0.010 mmol) of nitrone **2**. After the addition was complete (~15 min), saturated aqueous NH_4Cl was added. The ether was decanted and combined with an ether washing of the residue. The ether was evaporated, the residue was taken up in 100 mL of CH_3OH and stirred vigorously with 30 mg of $Cu(OAc)_2 \cdot H_2O$ for 30 min, and the solvent was then evaporated. Silica gel chromatography of the residue ($CHCl_3$ elution) gave 0.929 g (22%) of **3** as a yellow oil sufficiently pure for the next reaction. An analytical sample was prepared by preparative TLC (ether, R_f 0.7): MS *m/e* 424.380 (6) (calcd for $C_{26}H_{50}NO_3$, 424.379),

352 (6), 340 (10), 282 (22), 240 (100), 224 (68), 214 (66), 198 (22), 85 (100), 73 (20), 69 (26), 57 (24), 55 (33), 43 (26), 41 (30).

2,5-Dimethyl-5-nonyl-2-(6′-hydroxyhexyl)tetrahydropyrrole-1-oxyl (4) (Mixture of Cis and Trans Isomers). To a 50 mL CH_3OH solution of 0.929 g (2.19 mmol) of **3** was added 70 mg of *p*-toluenesulfonic acid monohydrate. The solution was allowed to stand for 2 h at 21 °C and then was diluted with ether and H_2O. The ether phase was washed with H_2O and brine, dried over Na_2SO_4, filtered, and then evaporated to give a yellow oil. This was taken up in $CHCl_3$ and put on a 2 × 20 cm dry silica gel column. The column was eluted with 200 mL of $CHCl_3$ followed by 200 mL of ether. The ether portion was evaporated to give 0.569 g (76%) of **4** as a mixture of the cis and trans isomers which was pure by TLC (ether, R_f 0.4).

Trans Diacetate 6 and Cis Diacetate 7. The mixture of cis and trans nitroxide alcohols **4** (121 mg, 0.355 mmol) in 3 mL of dry THF was hydrogenated in a Brown microhydrogenator[20] using 10 mg of 10% Pd/C catalyst. The hydrogen uptake stopped cleanly at 0.5 mol equiv of H_2. The mixture was filtered directly into a N_2-flushed flask and cooled to 0 °C under N_2. To the stirred solution was added 0.2 mL of Et_3N followed by 0.1 mL of acetyl chloride. The mixture was warmed to 21 °C, diluted with a four-fold volume of cyclohexane, and filtered, and the solvent was evaporated. The residue was put on a 2 × 35 cm silica gel column which was eluted with CH_2Cl_2. A total of 200 6-mL fractions were collected with the aid of a fraction collector. Fractions 30–50 gave 2.2 mg (14%) of the cis isomer **7**: single spot by TLC ($CHCl_3$, R_f 0.5); IR (CCl_4) 1770 (NOC=O), 1740 (COC=O) cm^{-1}; NMR δ 0.88 (3 H, m, term Me), 1.12 (6 H, s, ring Me), 1.66 (4 H, m, ring Me), 2.03 (3 H, s, acetate), 2.05 (3 H, s, acetate), 4.06 (2 H, t, J = 6.5 Hz, CH_2O); MS *m/e* 425.352 (2) (calcd for $C_{25}H_{47}NO_4$, 425.350), 410 (3), 383 (20), 368 (11), 298 (43), 282 (49), 256 (81), 240 (100), 224 (22), 55 (12), 43 (16). Fractions 60–120 gave 65.7 mg (44%) of the trans isomer **6**: single spot by TLC ($CHCl_4$, R_f 0.4); IR (CCl_4) 1770, 1740 cm^{-1}; NMR δ 0.88 (3 H, m, term Me), 1.14 (6 H, s, ring Me), 2.04 (3 H, s, acetate), 2.06 (3 H, s, acetate), 4.06 (2 H, t, J = 6.5 Hz, CH_2O); MS *m/e* 425.349 (3) (calcd for $C_{25}H_{47}NO_4$, 425.350), 410 (3), 383 (15), 368 (7), 298 (34), 256 (75), 240 (67), 220 (36), 205 (66), 61 (86), 57 (45), 55 (44), 43 (100). Later fractions off the column gave nitroxide-containing material. It was found that if pure **6** or **7** was subjected to silica gel chromatography, a certain amount of it was converted to the nitroxide, indicating that slow hydrolysis of the acetyl group and subsequent oxidation occurred during the chromatography.

3,4-Dihydro-2-methyl-2-nonyl-2*H*-pyrrole 1-Oxide (14). Following the procedure for the synthesis of **2**, nitrone **14** was obtained from nitrone **13**[10] and nonylmagnesium bromide in 20% yield as a yellow oil: bp 90–100 °C (0.005 mm); NMR δ 0.89 (3 H, m, term Me), 1.42 (3 H, s, ring Me), 1.6–1.9 (2 H, m, CH_2–C–N), 1.9–2.3 (2 H, m, ring CH_2), 2.4–2.7 (2 H, m, CH_2C=NO), 6.80 (1 H, t, J = 3 Hz, CH=N).

3,4-Dihydro-2-methyl-2-nonyl-5-(6′-tetrahydropyranyloxyhexyl)-2*H*-pyrrole 1-Oxide (15). To a solution of 612 mg (2.54 mmol) of **14** in 1 mL of THF was added 2.6 mL of a 1.0 M THF solution of the Grignard reagent derived from the tetrahydropyranyl ether of 6-chlorohexanol. After a 15-min stir at 21 °C, the solution was diluted with ether, saturated aqueous NH_4Cl was added, and the ether was decanted and evaporated. The residue was taken up in 25 mL of CH_3OH and 1 mL of concentrated aqueous NH_4OH, and then 336 mg of $Cu(OAc)_2·H_2O$ was added. The solution was stirred under air for 30 min. The solvent volume was reduced by half, and then ether was added. The ether layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over K_2CO_3, and evaporated to give a yellow oil. Chromatography on a silica gel column eluting with acetone gave 403 mg (39%) of the nitrone **15** as a yellow oil: single spot by TLC (acetone, R_f 0.3); NMR δ 0.88 (3 H, m, term Me), 1.38 (3 H, s, ring Me), 2.3–2.7 (4 H, m, CH_2C=N), 3.2–4.0 (6 H, m, CH_2O), 4.58 (1 H, m, O–CH–O); MS *m/e* 409.353 (0.6) (calcd for $C_{25}H_{47}NO_3$, 409.356), 325 (30), 308 (28), 252 (24), 239 (27), 198 (27), 182 (28), 114 (100), 85 (49), 55 (29), 41 (35).

Diacetates 6 and 7 via Methyllithium Addition to Nitrone 15. To a solution of 181 mg (0;22 mmol) of **15** in 6 mL of dry ether was added dropwise with stirring under N_2 1.0 mL of a 1.6 M ether solution of methyllithium. The solution was treated with saturated aqueous NH_4Cl 2 min after the addition was complete. The ether phase was washed with H_2O and brine and evaporated, and the residue was taken up in CH_3OH and stirred with 10 mg of $Cu(OAc)_2·H_2O$ under air for 15 min. The solvent was evaporated and the resulting green oil chromatographed on a silica gel column ($CHCl_3$ elution) to yield 61.4 mg (34%) of **3** as a mixture of cis and trans isomers. Hydrolysis of the THP ether, hydrogenation, acylation, and chromatography as described above gave 19.0 mg (29% from **3**) of **7** and 10.6 mg (16.4% from **3**) of **6**.

Base Hydrolysis of Trans and Cis Diacetates 6 and 7 to Trans and Cis Alcohol Nitroxides 8 and 9. A solution of 40 mg of KOH in 5 mL of CH_3OH was added to a solution of 65 mg (0.15 mmol) of **6** and 2 mg of $Cu(OAc)_2·H_2O$ in 5 mL of CH_3OH, and the mixture was stirred under air at 21 °C for 6 h. The solution was diluted with ether, washed with H_2O and brine, and evaporated to give a yellow oil which after silica gel chromatography gave 45.5 mg (88%) of **8** as a yellow oil: single spot by TLC (ether, R_f 0.4); IR (CCl_4) 3200–3600 cm^{-1} (OH); MS *m/e* 340.321 (8) (calcd for $C_{21}H_{42}NO_2$, 340.320), 326 (7), 310 (6), 240 (100), 224 (37), 214 (86), 198 (49), 69 (20), 55 (41), 43 (17), 41 (20). Anal. Calcd for $C_{21}H_{42}NO_2$: C, 74.06; H, 12.43; N, 4.11. Found: C, 73.81; H, 12.48; N, 3.78.

In an analogous fashion, **9** was obtained from **7** (76%) as a yellow oil: single spot by TLC (ether, R_f 0.4); IR (CCl_4) 3200–3600 cm^{-1} (OH); MS *m/e* 340 (12), 326 (7), 310 (3), 240 (100), 224 (14), 214 (89), 198 (16), 55 (23), 43 (10), 41 (11). Anal. Calcd for $C_{21}H_{42}NO_2$: C, 74.06; H, 12.43; N, 4.11. Found: C, 73.58; H, 12.03; N, 4.32.

***trans*-2,5-Dimethyl-2,5-dinonyltetrahydropyrrole-1-oxyl (16) and *cis*-2,5-Dimethyl-2,5-dinonyltetrahydropyrrole-1-oxyl (17).** Following the procedure for the preparation of **3**, treatment of 122 mg (0.51 mmol) of **2** with nonylmagnesium bromide followed by $Cu(OAc)_2·H_2O$ gave a mixture of **16** and **17** which was chromatographed on a dry silica gel column using $CHCl_3$ to yield 26 mg of **16** (14.2%) as a yellow waxy solid [single spot by TLC ($CHCl_3$, R_f 0.4); MS *m/e* 366.372 (6) (calcd for $C_{24}H_{48}NO$, 366.374), 352 (2), 350 (3), 336 (4), 240 (100), 224 (61), 55 (21), 43 (20), 41 (18)]. Anal. Calcd for $C_{24}H_{48}NO$: C, 78.62; H, 13.19; N, 3.82. Found: C, 78.74; N, 13.31; N, 3.66. Also obtained was 6.2 mg of **17** (3.4%) as a yellow oil [single spot by TLC ($CHCl_3$, R_f 0.3); MS *m/e* 366 (8), 352 (3), 350 (2), 336 (2), 240 (100), 224 (35), 69 (15), 55 (15), 43 (22), 41 (10)]. Anal. Calcd for $C_{24}H_{48}NO$: C, 78.62; H, 13.19; N, 3.82. Found: C, 78.14; H, 13.05; N, 3.29.

Trans Acetate 18 and Cis Acetate 19. Nitroxide **16** (10 mg, 0.027 mmol) in 2 mL of dry THF was hydrogenated in a Brown microhydrogenator[20] using 5 mg of 10% Pd/C catalyst. The mixture was filtered directly into a N_2-flushed flask and cooled to 0 °C under N_2. To the stirred solution was added 50 mg of Et_3N followed by 35 mg of acetyl chloride. The stirred mixture was allowed to warm to room temperature, diluted with cyclohexane, and filtered. The solvent was evaporated to give 9.8 mg (89%) of acetate **18** as a yellow waxy solid: single spot by TLC (CH_2Cl_2, R_f 0.7); IR (CCl_4) 1770 (NOC=O), 1200 (C–O) cm^{-1}; MS *m/e* 409.391 (0.5) (calcd for $C_{26}H_{51}NO_2$, 409.392), 282 (50), 240 (100), 224 (25), 55 (11), 43 (12), 41 (9); NMR δ 0.88 (6 H, m, term Me), 1.15 (6 H, s, ring Me), 2.08 (3 H, s, acetate).

Similarly prepared was 4.2 mg of acetate **19** from 4.3 mg (0.012 mmol) of **17** as a yellow oil: single spot by TLC (CH_2Cl_2, R_f 0.8); IR (CCl_4) 1770 (NOC=O), 1200 (C–O) cm^{-1}; MS *m/e* 409.391 (1) (calcd for $C_{26}H_{51}NO_2$, 409.392), 367 (7), 352 (6), 282 (75), 240 (100), 224 (10), 71 (15), 69 (14), 57 (20), 55 (18), 43 (24), 41 (14); NMR δ 0.89 (6 H, m, term Me), 1.14 (6 H, s, ring Me), 1.68 (4 H, m, ring CH_2), 2.08 (3 H, s, acetate).

(+)-Methoxy(trifluoromethyl)phenylacetate Esters of 16 and 17. A solution of 16.8 mg (0.0458 mmol) of **16** in 3 mL of THF was hydrogenated in a Brown microhydrogenator using 5 mg of 10% Pd/C; 0.0226 mmol of H_2 was absorbed. The mixture was filtered into a N_2-flushed flask, and the solvent was evaporated with a slow stream of N_2. Following the procedure of Mosher,[13] a solution of 32.3 mg (0.127 mmol) of (+)-methoxy(trifluoromethyl)phenylacetyl chloride[13] in 0.1 mL of CCl_4 was added followed by 0.1 mL of dry pyridine. After an 18-h stir at 21 °C, 1 mL of H_2O and 20 mL of ether were added. The ether phase was separated, washed with 10% aqueous HCl, saturated aqueous $NaHCO_3$, and brine, and then dried over $MgSO_4$. Evaporation of the solvent and chromatography on silica gel gave 16.4 mg (61%) of **20** as a colorless oil: single spot by TLC (CH_2Cl_2, R_f 0.8); IR (CCl_4) 1775 (NOC=O), 1190 (C–O) cm^{-1}; MS *m/e* 583.419 (0.4) (calcd for $C_{34}H_{56}NO_3F_3$, 583.421), 456 (100), 366 (23), 254 (30), 240 (23), 224 (27), 189 (36); NMR δ 0.88 (6 H, m, term Me), 1.10 (3 H, s, ring Me), 1.12 (3 H, s, ring Me), 3.58 (3 H, m, OCH_3).

Similarly prepared using the above procedure was **21** as a colorless oil: single spot by TLC (CH_2Cl_2, R_f 0.8); IR (CCl_4) 1775 (NOC=O), 1190 (C–O) cm^{-1}; MS *m/e* 583.420 (0.2) (calcd for $C_{34}H_{56}NO_3F_3$, 583.421), 456 (36), 366 (11), 316 (43), 308 (13), 254 (24), 240 (13), 224 (100), 189 (25); NMR δ 0.88 (6 H, m, term Me), 1.00 (3 H, s, ring Me), 1.07 (3 H, s, ring Me), 1.68 (4 H, m, ring CH_2), 3.57 (3 H, m, OCH_3).

Fluorine-19 NMR spectra (proton decoupled) were determined in $CHCl_3$ with 3% CF_3CO_2H as an internal standard. Compound **20** gave two peaks, 538.8 and 530.3 Hz downfield from CF_3CO_2H. Compound **21** gave one peak, 524.3 Hz downfield from CF_3CO_2H.

***cis*-2,5-Dimethyl-5-nonyl-2-(6′-iodohexyl)tetrahydropyr-**

role-1-oxyl (11). To a stirred solution of 9.9 mg (0.029 mmol) of **9** and 6.1 mg (0.06 mmol) of Et_3N in 1 mL of dry CH_2Cl_2 at −20 °C (dry ice–CCl_4) was added 5.7 mg (0.05 mmol) of methanesulfonyl chloride. The mixture was allowed to warm to 21 °C, 8 mL of cyclohexane was added, the mixture was filtered, and the solvent was then evaporated to give crude **12** as an orange oil. This was taken up in 1 mL of methyl ethyl ketone along with 27 mg (0.18 mmol) of NaI and stirred at reflux for 30 min. The mixture was diluted with cyclohexane and filtered, and the solvent was evaporated. Preparative TLC ($CHCl_3$) of the resulting yellow oil gave 11.6 mg (88%) of cis iodide **11:** single spot by TLC ($CHCl_3$, R_f 0.3); MS *m/e* 450.221 (23) (calcd for $C_{21}H_{41}NOI$, 450.223), 324 (99), 308 (21), 294 (10), 254 (41), 240 (100), 223 (82), 198 (33), 196 (36), 182 (56), 127 (24), 96 (21), 85 (35), 71 (56), 69 (35), 55 (46), 43 (44), 41 (36). Anal. Calcd for $C_{21}H_{41}NOI$: C, 55.99; H, 9.17; N, 3.11. Found: C, 55.69; H, 9.06; N, 2.71.

***cis*-2,5-Dimethyl-2,5-dinonyltetrahydropyrrole-1-oxyl (17) via (11).** To a stirred solution of 11.6 mg (0.026 mmol) of **11** in 0.1 mL THF at 0 °C under N_2 was added 0.13 mL of a 1.0 M solution of propylmagnesium chloride in THF followed immediately with 0.01 mL of a 0.1 M THF solution of Li_2CuCl_4.[14] After 2 min, saturated aqueous NH_4Cl was added. The mixture was diluted with ether, and the ether was decanted and combined with an ether washing of the residue. The solvent was evaporated, and the residue was taken up in CH_3OH and stirred with 5 mg of $Cu(OAc)_2 \cdot H_2O$ for 30 min. Evaporation of the solvent and silica gel chromatography of the residue gave 3.7 mg (35%) of *O*-propyl derivative **22** as a colorless oil [NMR δ 0.8–1.0 (9 H, m, term Me), 1.09 (6 H, s, ring Me), 1.51 (4 H, m, ring CH_2), 3.66 (2 H, t, J = 7 Hz, CH_2O); MS *m/e* 409.430 (calcd for $C_{27}H_{55}NO$, 409.428)] and 4.3 mg (46%) of **17**, which by TLC was identical with that obtained from nonylmagnesium bromide addition to nitrone **2**. Catalytic reduction and acetylation gave **19**, identical by NMR with that obtained as described above.

***trans*-2,5-Dimethyl-5-nonyl-2-(6′-iodohexyl)tetrahydropyrrole-1-oxyl (10) and *cis*-2,5-Dimethyl-5-nonyl-2-(6′-iodohexyl)tetrahydropyrrole-1-oxyl (11).** Using the procedure for the preparation of **11** as described above, the mixture (394 mg, 0.94 mmol) of cis and trans nitroxide alcohols **4** was converted to a mixture of the cis and trans mesylates **5:** IR (CCl_4) 1370 (S=O), 1180 (S–O) cm^{-1}; MS *m/e* 418.300 (6) (calcd for $C_{22}H_{44}NO_4S$, 418.299), 292 (50), 276 (23), 240 (100), 234 (42), 224 (47), 126 (25), 113 (29), 108 (25), 96 (62), 69 (22), 55 (31), 41 (21).

The mixture of **5** was then converted to the mixture of nitroxide iodides **10** and **11** (381 mg, 90% from **4**). The isomers were separated by dry column silica gel chromatography eluting with $CHCl_3$ to give **10** (82%) as a yellow waxy solid: single spot by TLC ($CHCl_3$, R_f 0.4); MS *m/e* 450.221 (9) (calcd for $C_{21}H_{41}NOI$, 450.223), 324 (100), 308 (12), 240 (100), 224 (26), 196 (27), 96 (20), 69 (43), 57 (47), 55 (61), 43 (33), 41 (56). Anal. Calcd for $C_{21}H_{41}NOI \cdot H_2O$: N, 2.99. Found: N, 2.64. Isomer **11**, identical with that obtained previously from **9**, was also obtained (18% yield).

Trans Oxazoline Nitroxide 23 and Cis Oxazoline Nitroxide 24. The procedure used was that of Meyers.[15] To a stirred solution of 340 mg (3.00 mmol) of 2,4,4-trimethyloxazoline in 2 mL of THF at −78 °C (dry ice–acetone) under N_2 was added 1.6 mL of a 1.6 M solution of butyllithium in hexane. After 3 min, the iodide **10** (290.9 mg, 0.646 mmol) in 1.0 mL of THF was added. A white precipitate formed after 1 min, and the mixture was stirred for an additional 20 min at −78 °C before the bath was removed and the mixture allowed to warm to 0 °C, during which time the precipitate dissolved. The solution was treated with saturated aqueous NH_4Cl, diluted with ether, washed with H_2O and brine, dried over K_2CO_3, and evaporated to give a yellow oil which was chromatographed on silica gel to give 202.2 mg (72%) of **23** as a yellow waxy solid: single spot by TLC (ether, R_f 0.4); MS *m/e* 436 (72), 435 (68), 422 (9), 421 (8), 405 (17), 309 (75), 293 (66), 240 (100), 224 (68), 196 (34), 126 (40), 113 (68), 96 (18), 69 (16), 55 (25), 43 (12), 41 (16).

Similar treatment of **11** gave **24** (59%) as a yellow oil: single spot by TLC (ether, R_f 0.4); MS *m/e* 436 (32), 435.396 (33) (calcd for $C_{27}H_{51}N_2O_2$, 435.395), 422 (5), 421 (6), 405 (7), 309 (64), 293 (21), 240 (100), 224 (27), 196 (30), 126 (44), 113 (56), 96 (29), 69 (38), 55 (77), 43 (39), 41 (53).

***trans*-2,5-Dimethyl-5-nonyl-2-(8′-carboxyoctyl)tetrahydropyrrole-1-oxyl (25) and *cis*-2,5-Dimethyl-5-nonyl-2-(8′-carboxyoctyl)tetrahydropyrrole-1-oxyl (26).** Using the procedure of Meyers,[15] a solution of 196.8 mg (0.45 mmol) of **23** in 3 mL of methyl iodide was allowed to stand in the dark at 21 °C for 14 h. The methyl iodide was evaporated and the residue stirred with 8 mL of CH_3OH and 2 mL of 4 N NaOH for 20 h. The solution was acidified with cold 1 N aqueous HCl and washed with ether. The ether solution was washed with H_2O and brine, dried over $MgSO_4$, and evaporated to give a yellow oil. Silica gel chromatography gave 145.7 mg (84%) of **25** as a yellow waxy solid: single spot by TLC (ether/0.5% HOAc, R_f 0.7); IR (CCl_4) 2800–3400 (OH), 1710 (acid carbonyl) cm^{-1}; MS *m/e* 382.332 (12) (calcd for $C_{23}H_{44}NO_3$, 382.332), 368 (2), 352 (2), 256 (71), 240 (100), 224 (10), 113 (10), 81 (10), 69 (10), 55 (19), 43 (11), 41 (13). Anal. Calcd for $C_{23}H_{44}NO_3$: C, 72.20; H, 11.59; N, 3.66. Found: C, 71.77; H, 11.76; N, 3.47.

Similarly prepared from 23 mg of **24** was *cis*-azethoxyleicosanoic acid **26** (73%) as a yellow waxy solid: single spot by TLC (ether/0.5% HOAc, R_f 0.7); IR (CCl_4) 2800–3400 (OH), 1710 (acid carbonyl) cm^{-1}; MS *m/e* 382.330 (21) (calcd for $C_{23}H_{44}NO_3$, 382.332), 368 (4), 352 (3), 256 (81), 240 (100), 224 (17), 113 (5), 96 (8), 69 (10), 55 (13), 43 (10), 41 (14). Anal. Calcd for $C_{23}H_{44}NO_3 \cdot 0.25H_2O$: C, 71.36; H, 11.59; N, 3.62. Found: C, 71.55, H, 11.07; N, 3.21.

Acetate Esters 29 and 30. Treatment of 7.0 mg (0.18 mmol) of **25** in 0.5 mL of ether with 0.2 mL of a 0.5 M ether solution of diazomethane followed by silica gel TLC (ether, R_f 0.8) gave the corresponding methyl ester **27:** IR (CCl_4) 1745 cm^{-1} (ester carbonyl). Using the procedure described for acetate **6**, **27** was converted to acetate ester **29:** IR (CCl_4) 1770 (N—O—C=O), 1745 (CH_3OC=O) cm^{-1}; NMR δ 0.89 (3 H, m, term Me), 1.14 (6 H, s, ring Me), 2.08 (3 H, s, acetate), 2.33 (2 H, t, J = 7 Hz, CH_2C=O), 3.69 (3 H, s, CH_3O).

Similarly prepared from **26** was acetate ester **30:** IR (CCl_4) 1770 (N—O—C=O), 1745 (CH_3OC=O) cm^{-1}; NMR δ 0.88 (3 H, m, term Me), 1.12 (6 H, s, ring Me), 1.66 (4 H, m, ring CH_2), 2.06 (3 H, s, acetate), 2.31 (2 H, t, J = 7 Hz, CH_2C=O), 3.68 (3 H, s, OCH_3).

1-Palmitoyl-2-(10′-aza-9′,11′-dimethyl-9′,11′-ethano-*N*-oxyleicosanoyl)-*sn*-glycerol-3-phosphatidylcholine (31). The procedure followed was patterned after that of Boss.[17] To a solution of 47.7 mg (0.122 mmol) of **25** in 0.4 mL of dry $CHCl_3$ was added 22.3 mg (0.137 mmol) of carbonyldiimidazole. After stirring for 20 min at 21 °C, 35.2 mg (0.071 mmol) of lysopalmitoylglycerolphosphatidylcholine (Sigma) in 0.2 mL of $CHCl_3$ was added and the mixture was heated under N_2 to 50–55 °C for 5 days, during which time most of the solvent had evaporated. The reaction was quenched with H_2O and taken up in $CHCl_3$. Azeotropic removal of the H_2O by several evaporations from $CHCl_3$ gave a yellow foam which was put on a silica gel column prewashed with a 50:50 mixture of $CH_3OH/CHCl_3$ and then $CHCl_3$ only. The starting nitroxide acid and imidazole were eluted with $CHCl_3/CH_3OH$ (85:15). Crude **31** was eluted with $CHCl_3/CH_3OH$ (50:50). Final purification on an 18 × 1650 cm Sephadex LH-20 column eluting with 95% EtOH gave 37.3 mg (61%) of **31** as a yellow solid. Anal. Calcd for $C_{47}H_{92}N_2O_9P \cdot 3H_2O$: C, 62.84; H, 10.10; N, 3.12. Found: C, 63.07; H, 10.44; N, 3.47.

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Registry No.—**1**, 67408-72-4; **2**, 67408-73-5; *cis*-**3**, 67462-27-5; *trans*-**3**, 67462-28-6; *cis*-**4**, 67408-74-6; *trans*-**4**, 67408-75-7; *cis*-**5**, 67408-76-8; *trans*-**5**, 67408-77-9; **6**, 67408-78-0; **7**, 67408-79-1; **8**, 67408-75-7; **9**, 67408-74-6; **10**, 67408-80-4; **11**, 67408-81-5; **12**, 67408-76-8; **13**, 6931-10-8; **14**, 67408-82-6; **15**, 67408-83-7; **16**, 67408-84-8; **17**, 67408-85-9; **18**, 67408-86-0; **19**, 67408-87-1; **20** (isomer 1), 67408-88-2; **20** (isomer 2), 67408-89-3; **21**, 67462-29-1; **22**, 67425-69-8; **23**, 67408-90-6; **24**, 67408-91-7; **25**, 67408-92-8; **26**, 67408-93-9; **27**, 67408-94-0; **28**, 67408-95-1; **29**, 67408-96-2; **30**, 67408-97-3; **31**, 67408-98-4; nonyl bromide, 693-58-3; tetrahydropyranyl 6-chlorohexyl ether, 2009-84-9; (+)-methoxy(trifluoromethyl)phenylacetyl chloride, 20445-33-4; methanesulfonyl chloride, 124-63-0; propyl chloride, 540-54-5; 2,4,4-trimethyloxazoline, 1772-43-6; diazomethane, 334-88-3; lysopalmitoylglycerolphosphatidylcholine, 17364-16-8.

References and Notes

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(18) Acyl migration in lysophosphatidylcholine is known to occur; see B. Serdarevich, *J. Am. Oil Chem. Soc.*, **44**, 381 (1967). The extent to which acyl migration might have occurred in our experiment has not been determined.
(19) Infrared spectra were recorded with either a Beckman IR-5 or IR-7 spectrophotometer. NMR spectra were recorded on a Varian XL-100 high-resolution spectrometer in $CDCl_3$, and only the characteristic peaks are reported. Chemical shifts are reported in parts per million (δ) downfield from internal Me_4Si. Mass spectra (70 eV) (*m/e*) are given followed by the relative peak height in parentheses and were determined on a CEC 110-2B double-focusing mass spectrometer equipped with a direct inlet. Elemental analyses were performed at the University of Oregon by Dr. R. Wielesek. Ultraviolet spectra were determined on a Cary 15 UV spectrometer. Unless otherwise noted, all silica gel column chromatography was done with Baker 60–200 mesh silica gel packed dry and eluted in the usual fashion. Preparative thin-layer chromatography (TLC) was done on Analtech 1000 silica gel plates with the fluorescent indicator. Analytical TLC used EM silica gel F-254 plates. Solvents were routinely distilled. Commercial reagents were used as received.
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Notes

Synthesis of 3-Carboethoxyoxindoles

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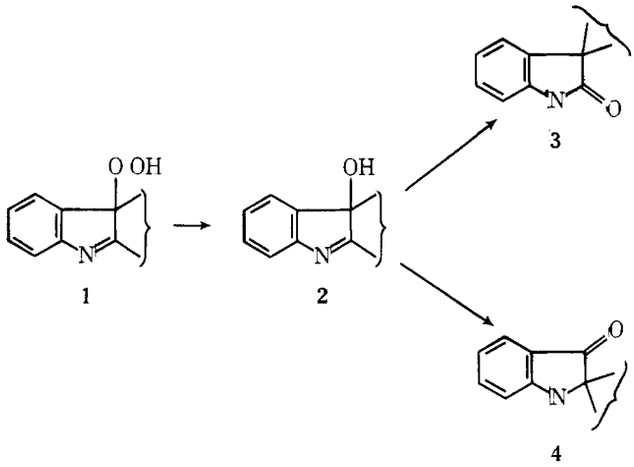
Indoles have been converted to 3-hydroperoxyindolenines **1** on autoxidation or peracid oxidation. Selective reduction of the hydroperoxy group in **1** gives 3-hydroxyindolenines **2**, which can rearrange to either oxindole **3**[1] or indoxyl **4**[2] de-

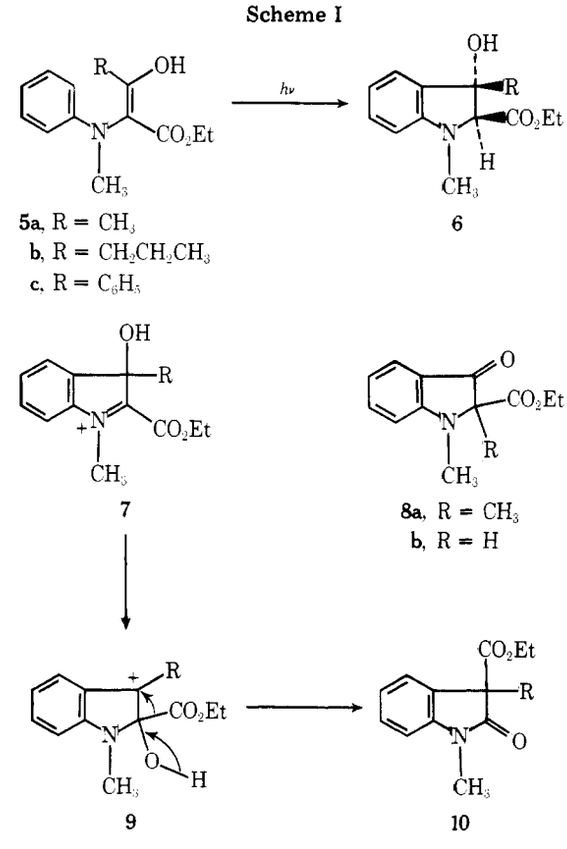


rivatives. Unfortunately, peracid oxidation can often be troublesome as a result of competitive N-oxide formation.[3] Other methods that circumvent this problem involve reaction of indoles with *tert*-butyl hypochlorite[4] or *N*-bromosuccinimide[5] to give 3-haloindolenines, and these can be converted to oxindoles. However, halogenation of the indole benzene ring[5] can sometimes be competitive with 3-haloindolenine formation (vide infra). In this paper, we present a new, high-yield method for oxindole preparation, which is based on the photosynthesis of 3-hydroxyindolines **6** and their oxidative rearrangement to oxindoles **9**.

We have reported that *N*-methyl-3-hydroxyindolines can be prepared in excellent yield by photocyclization–rearrangement of 2-(*N*-methylanilino)acetoacetates.[6] For example, irradiation of **5a** in *n*-pentane in the presence of suspended sodium carbonate gives 3-hydroxyindoline **6a** in quantitative yield. In similar fashion, indolines **6b** and **6c** also are prepared. Treatment of these 3-hydroxyindolines **6a–c** with lead tetraacetate (1.1 equiv) and pyridine (1.1 equiv) in benzene solution at room temperature results in a high-yield conversion to oxindoles **10a–c** (Table I).

In order to unambiguously establish the structure of the lead tetraacetate oxidation product, we attempted to prepare **10a** by treatment of *N*-methyl-2-carboethoxy-3-methylindole with *tert*-butyl hypochlorite using literature procedures.[4] Under these conditions, products resulting from chlorination of the benzene ring as well as the C(3) methyl substituent in the indole were obtained. Oxindole **10a** was eventually pre-

Scheme I



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