Acyl-Protected Hydroxylamines as Spin Label Generators for EPR Brain Imaging

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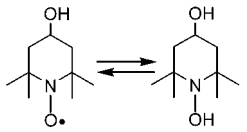
In a search for novel electron paramagnetic resonance (EPR) brain imaging agents, we have designed and synthesized the acyl-protected hydroxylamines 1-acetoxy-4-methoxycarbonyl-2,2,6,6-tetramethylpiperidine (AMCPe), 1-acetoxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine (AMCPy), and 1-acetoxy-3-(acetoxymethoxy)carbonyl-2,2,5,5-tetramethylpyrrolidine (DACPy), in which both the ring size and the number of ester functions were varied. In all of them, the nitroxide was first reduced and the resultant hydroxylamine was then protected with an acetyl group. These compounds are lipophilic, which is a major prerequisite for blood−brain barrier penetration. Once in the brain, esterases and oxidants quickly convert these derivatives into ionic, water-soluble radicals and thus EPR detectable species that then reside in the central nervous system for periods of time sufficient for detection and imaging. The biological relevancy of these new compounds in mice has been assessed, and their biodistribution patterns have been compared. The five-membered ring derivative AMCPy emerged as a potent EPR brain imaging agent while the other two derivatives, AMCPe and DACPy, were quite ineffective.

Introduction

Electron spin resonance (ESR) or electron paramagnetic resonance (EPR) imaging operating in lower frequency bands (less than 1 GHz) is an emerging technique, which allows the observation of distribution of exogenous free radicals, i.e., spin labels, in vivo.[1−12] Initially, water-soluble free radicals such as TEMPOL (4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl) were explored as spin labels for in vivo EPR imaging. However, these otherwise in vitro stable nitroxides are prone to bioreduction to the corresponding diamagnetic, EPR silent hydroxylamines such as TEMPOL-H (Scheme 1). The oxidized (nitroxide) and the reduced (hydroxylamine) species rapidly establish an equilibrium in vivo that strongly favors the hydroxylamine form.[13] Thus, in vivo, the short half-life of nitroxides ($t_{1/2} \approx 3$ min) has limited the efficacy of the latter and has prevented their development for a wide range of potential diagnostic and therapeutic applications. Recently, polynitroxyl-albumin (PNA) or polynitroxyl-dendrimers (PND), in which 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) moieties are attached to the macromolecule surface, have been reported to be capable of reoxidizing TEMPOL-H back to TEMPOL.[14−16]

Despite the fact that the half-lives of nitroxide radicals have been increased to clinically relevant periods of time and preliminary experiments with in vivo EPR imaging in the past have successfully lead to the imaging of rat tails, heads, kidneys, and hearts,[14,15,17−20] ordinary spin labels do not readily penetrate the blood−brain barrier (BBB).[21,22] This is why the development of nitroxides for EPR brain imaging, recently an area of increasing interest, has proven to be a difficult task. So far, all studies have focused only on five-membered ring carbamoyl-PROXYL derivatives, and in all of them, the lipophilicity has been increased by modifications of only either the carbonyl (PCAM) or the hydroxylamine (ACP) functional groups in the molecule (Figure 1), but simultaneous modification of both of these functional groups has hitherto been unexplored.[23−26]

Scheme 1



TEMPOL TEMPOL-H

Herein, we report the synthesis and the structure−activity relationship (SAR) evaluation of the biodistribution properties of three such doubly modified spin label generating agents. Our approach includes not only variation of the ring size of the label but also variation in the number of the ester functions present in the molecule. We have prepared 1-acetoxy-4-methoxycarbonyl-2,2,6,6-tetramethylpiperidine (**3**, AMCPe), a derivative of the six-membered ring TEMPO radical, and 1-acetoxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine (**7**, AMCPy) and 1-acetoxy-3-(acetoxymethoxy)-carbonyl-2,2,5,5-tetramethylpyrrolidine (**10**, DACPy); both compounds are derivatives of the five-membered ring PROXYL radical (Figure 1). In all three target compounds, the lipid solubility was further enhanced by the introduction of two (AMCPe and AMCPy) or three (DACPy) ester functions in the molecules. The ester functions were expected to be hydrolyzed by esterase enzymes in the intracellular environment. This finding suggests that **7** (AMCPy) is a novel compound possess-

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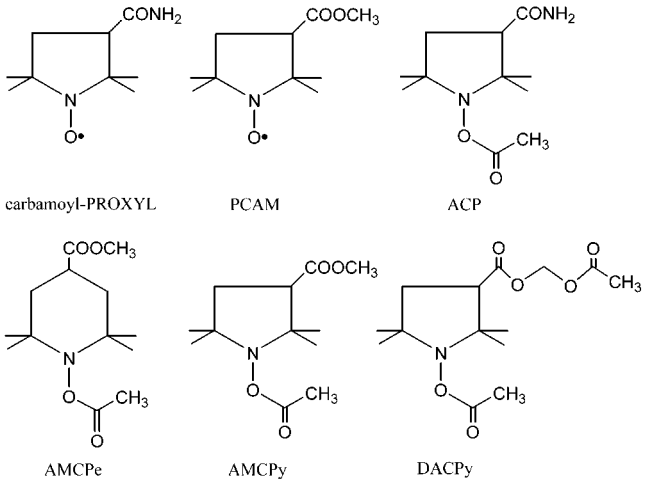
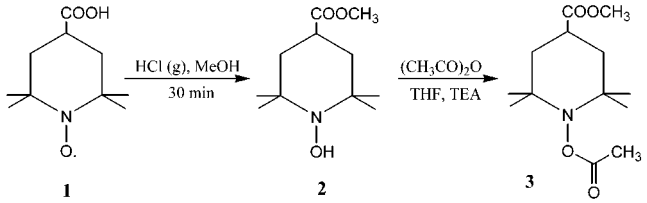


Figure 1.

Scheme 2



ing the optimal lipophilicity and thus has a promising role as a future spin label generator for EPR brain imaging.

Chemistry

The design strategy for our target compounds is shown in Schemes 2−4. All compounds were characterized by their exact fast atom bombardment (FAB) mass spectra, ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra (if no free radical functional group was present in the molecule), and elemental analysis, where applicable.

The six-membered ring nitroxide radical, 1-oxyl-4-carboxy-2,2,6,6-tetramethylpiperidine **1** (4-carboxy-TEMPO) was simultaneously reduced and esterified to its hydroxylamine methyl ester **2** in a one step procedure by bubbling dry HCl(g) through a methanol solution of the compound. This reaction was completed within 30 min (Scheme 2). Under identical conditions, however, the five-membered ring nitroxide radical, 1-oxyl-3-carboxy-2,2,5,5-tetramethylpyrrolidine **4** (3-carboxy-PROXYL) underwent esterification only, and no reduction to its hydroxylamine was observed within the short period of time employed for the synthesis of **2** (Scheme 3). However, if the HCl(g) saturated methanol solution of the nitroxide methyl ester **5** is left at ambient temperature for 24 h or longer, the reduction eventually proceeds, and the hydroxylamine methyl ester **6** could be isolated in quantitative yields. The reduction rate of six-membered ring nitroxides is well-known to be greater than that of five-membered rings both in vitro and in vivo.[27−29] Hydroxylamine methyl ester **6** could also be prepared by the reduction of **5** with an excess of hydrazine in methanol. Both hydroxylamines **2** and **6** were acetylated by using acetic anhydride in tetrahydrofuran (THF) in the presence of triethylamine (TEA) to yield **3** (AMCPe) and **7** (AMCPy), respectively. Each of these acyl-protected hydroxylamine methyl esters was purified by flash chromatography.

A somewhat different approach was used in the preparation of **10** (DACPy). In this case, 4-carboxy-PROXYL **4** was first reduced by hydrazine to 1-hydroxy-3-carboxy-2,2,5,5-tetramethylpyrrolidine **8** (Scheme 4), which was consequently acetylated to 1-acetoxy-3-carboxy-2,2,5,5-tetramethylpyrrolidine **9**. This acid was then reacted with bromomethyl acetate in the presence of TEA to yield DACPy, which was also purified by flash chromatography.

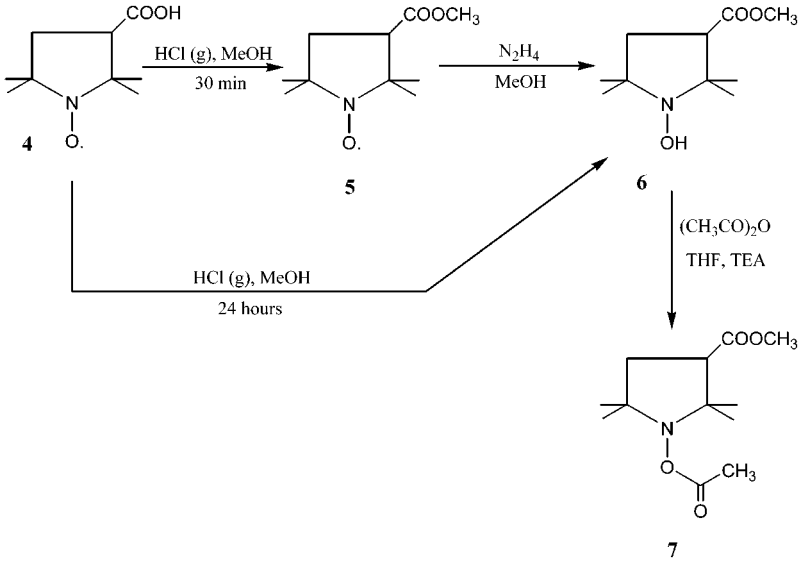
All three target spin label generators—AMCPe, AMCPy, and DACPy—are stable nonradical compounds that are not affected by oxidation. They show poor water solubility and could not be dissolved without an organic cosolvent such as dimethyl sulfoxide (DMSO) or ethanol, which did not prevent their proper biological evaluation.

Results and Discussion

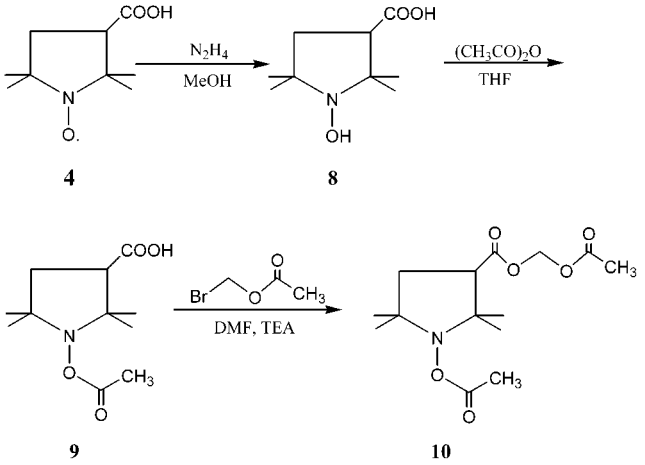
The originally used water-soluble spin labels in previous experiments did not penetrate the BBB.[14,15,17,18,21,22] Thus, in all EPR imaging experiments of rat heads achieved by use of carbamoyl-PROXYL, the brain was imaged as a nitroxide-deficient area while the blood vessels and/or the extracranium tissues were imaged as a nitroxide-rich area. During periods when high intensities of EPR signals were maintained, spots of nitroxide accumulation were imaged at the central part of the brain. However, those spots were assigned to the moderate-sized blood vessels in the brain.[22] Some brain EPR images utilizing carbamoyl-PROXYL have also been recently reported. In those studies, the label was perfused by microdialysis. However, it has been difficult to image cortex and striatum simultaneously. Furthermore, this administration method has taken a long time (6 h) to perfuse the spin label.[30] This inconvenience has prompted investigators to look for new spin labels that penetrate the BBB more efficiently or spin labels that can be generated within the intracellular compartment from stable, nonradical precursors.[23−26] This has led to some encouraging results in rat brain imaging.

We report, herein, the synthesis and the biodistribution SAR evaluation of three new in vivo spin label generators, AMCPe (**3**), AMCPy (**7**), and DACPy (**10**). AMCPe differs from AMCPy and DACPy by its six-membered ring size while DACPy differs from the rest by having three ester functions in its molecule. It was expected that these three compounds would possess higher lipophilicity for efficient BBB penetration than the previously reported carbamoyl-PROXYL, PCAM, and ACP (Figure 1). Compound **3**, AMCPe, is a derivative of TEMPO free radical and contains a six-membered ring and two ester functions. Both **7** (AMCPy) and **10** (DACPy) are derivatives of PROXYL free radical and contain a five-membered ring. AMCPy and DACPy have two and three ester functions, respectively. Upon entering the intracellular compartment, esterases should hydrolyze the ester functional groups in the compounds to produce ionic, water-soluble species, which cannot penetrate back across the BBB and should be retained in the brain intracellularly for longer periods of time (Figure 2). In addition to that, oxidants in the intracellular environment (among them, free radicals) should oxidize the formed hydroxylamines to nitroxides,[31,32] the

Scheme 3



Scheme 4



latter being the EPR detectable species. Our study aimed not only to prove this hypothesis but also to investigate whether there were any profound differences in the biological behavior of the six- (**3**) and the five- (**7** and **10**) membered ring derivatives. A second aspect of this study was to assess whether varying the number of ester functions in the molecule would make any difference in the biological behavior of these compounds.

To achieve these goals, a biodistribution study was performed in mice. The concentration of the oxidized form of the compounds (nitroxides) and the total concentration of both oxidized and reduced forms (nitroxides + hydroxylamines) were monitored in brain, heart, liver, kidney, spleen, and blood. The animals were sacrificed 10 and 60 min after intraperitoneal injections of each compound in DMSO solution. The organs were immediately collected and homogenized in ice-cold phosphate-buffered saline (PBS), and the nitroxide concentration in them was measured by its EPR signal intensity. The total (nitroxides + hydroxylamines) concentration was measured afterward by EPR signal intensity of the same sample when any free hydroxylamine was oxidized to nitroxide by addition of excess potassium ferricyanide ($K_3[Fe(CN)_6]$). Figure 3 displays the biodistribution patterns of all three compounds in

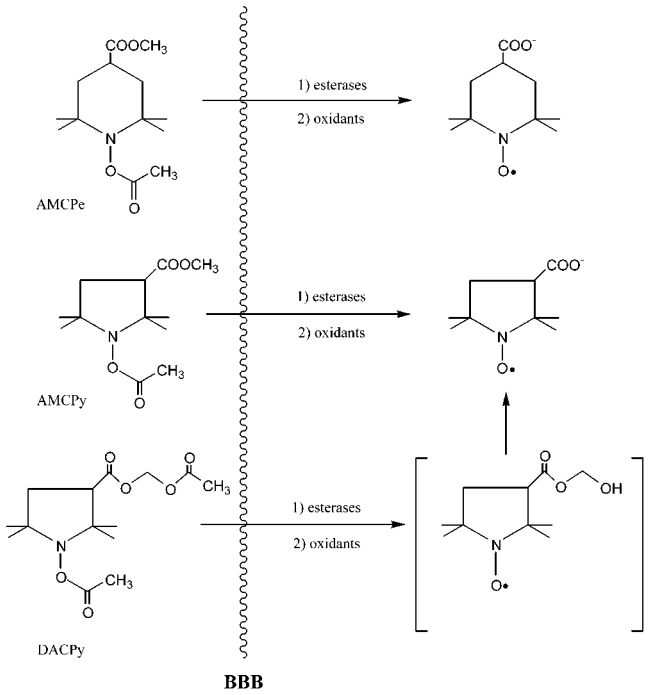


Figure 2.

their nitroxide and total form after injection in mice. It is clearly seen that AMCPy (**7**) penetrates the BBB easily, as soon as 10 min postinjection, where it undergoes hydrolysis by esterases to free hydroxylamine, part of which is then oxidized by intracellular oxidants to an EPR detectable nitroxide. After a clinically relevant period of time (60 min), the nitroxide species can still be observed in the brain in significant concentrations as well as the heart, liver, kidney, spleen, and blood. Thus, AMCPy is a useful spin label generator not only for the purposes of brain imaging but also for the imaging of other organs, especially kidney.

In comparison, the EPR signals after AMCPe (**3**) injections showed very little intensity in all organs collected. As a matter of fact, the signal from this compound barely registers on the scale employed in Figure 3. Because AMCPe is expected to be not any less lipophilic and penetrate the BBB not any less easily

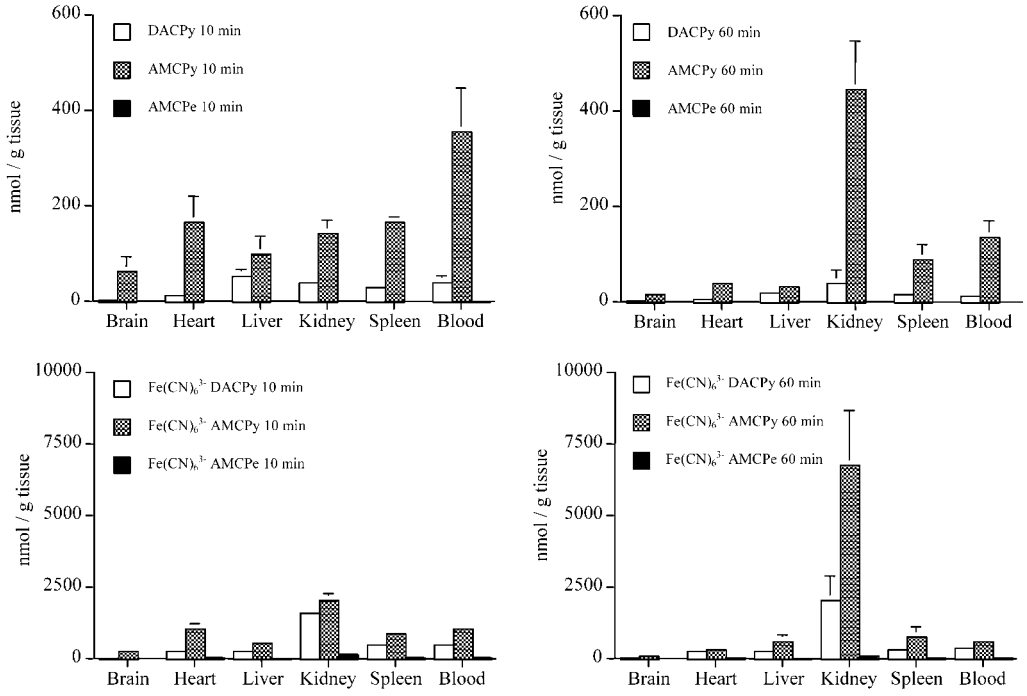


Figure 3.

than AMCPy, this difference is no doubt due to either a slower rate of deacetylation for AMCPe to a free hydroxylamine as compared to AMCPy or to a much faster clearance of the compound from the body. Also, previous reports have shown that the five-membered ring radicals are much more stable in biological systems than the six-membered ones.[33] Therefore, the observation that AMCPe does not behave entirely as expected is believed due to a combination of some or all of these properties. The addition of a third ester group in DACPy (**10**) has a negative effect on the BBB penetration ability and the whole body retention of this spin label generator.

In conclusion, the most important finding of the present investigation is that the five-membered ring PROXYL derivative AMCPy (**7**) possesses the optimal lipophilicity for BBB penetration where it is retained in the brain for clinically relevant periods of time. Once there, AMCPy is subsequently deacetylated and oxidized into its nitroxide form, the latter being an EPR detectable species. Clearly, AMCPy is a very promising candidate for a spin label generating agent in EPR imaging. Currently, murine models are under investigation to further evaluate and promote use of AMCPy in EPR imaging techniques.

Experimental Section

Chemistry. Reagents and anhydrous solvents were purchased from Aldrich Chemical Co. (Milwaukee, WI) unless otherwise stated, and all were used without further purification. All spin label generators were stored at −20° C before use. Anhydrous HCl(g) was supplied by Roberts Oxygen Co. (Gaithersburg, MD). Proton and ^{13}C NMR spectra were recorded on a Varian Gemini 300 instrument (Palo Alto, CA). NMR peak patterns were described by the following abbreviations: br = broad, d = doublet, t = triplet, q = quartet, m = multiplet, and arom = aromatic protons. Chemical shifts were expressed as parts per million as referenced to tetramethylsilane (TMS) ($CDCl_3$ as an internal standard for ^{13}C) unless otherwise noted. Low-resolution chemical ionization (CI) and electron ionization (EI) mass spectra were obtained on a JEOL JMS-SX102 instrument (Peabody, MA). Exact FAB mass spectra were obtained on an Extrel 4000 (Pittsburgh, PA) instrument in the positive ion detection mode. Elemental analyses were performed by Galbraith Laboratories, Inc. (Knoxville, TN). When the elemental analysis is not included, the crude compounds were used in the next step without further purification. Chromatographic separations were performed on silica gel columns by flash (Silica Gel 60, 0.035−0.070 mm; Fluka).

1-Hydroxy-4-carbamoyl-2,2,6,6-tetramethylpiperidine methyl ester (2). A sample of the commercially available 4-carboxy-TEMPO **1** (0.50 g, 2.5 mmol) was stirred in methanol (50 mL) and placed in an ice bath. Dry HCl(g) was then bubbled through the solution for 30 min. The solvent was removed by evaporation, and the resulting oil was neutralized with 10% $NaHCO_3$. The product was then extracted with ethyl acetate (3 × 100 mL). The organic layers were combined and dried over Na_2SO_4, and the solvent was evaporated to give the product as an oil; yield 0.51 g (94%). Exact FAB mass spectrum: calcd for $C_{11}H_{21}NO_3 + H^+$, 216.1600; found, 216.1606. ^1H NMR ($CDCl_3$): δ 1.15 (s, 6H, CH_3), 1.22 (s, 6H, CH_3), 1.66 (m, 2H, CH_2), 1.80 (m, 2H, CH_2), 2.68 (t of t, 1H, CH), 3.68 (s, 3H, OCH_3). ^{13}C NMR ($CDCl_3$): δ 7.18, 20.0, 22.07, 28.90, 40.37, 46.63, 163.00.

1-Acetoxy-4-methoxycarbonyl-2,2,6,6-tetramethylpiperidine, AMCPe (3). Hydroxylamine **2** (0.51 g, 2.4 mmol) was dissolved in a mixture of THF (40 mL) and TEA (5 mL). The solution was placed in an ice bath, and then, acetic anhydride (5 mL, 53 mmol) was added dropwise over 10 min. After 8 h of stirring at ambient temperature, the excess of acetic anhydride was carefully destroyed with water and the resulting solution was neutralized with aqueous 10% $NaHCO_3$. The THF was evaporated, and the product was then extracted with ethyl acetate (3 × 100 mL) and dried over Na_2SO_4, and the solvent was evaporated almost to dryness. Subsequent flash chromatography (hexane:ethyl acetate = 9:1) gave **3** as a yellowish crystalline solid; yield 0.41 g (67%). Exact FAB mass spectrum: calcd for $C_{13}H_{23}NO_4 + H^+$, 258.1705; found, 258.1711. Elemental analysis calcd: C, 60.7%; H, 9.01%; N, 5.44%. Found: C, 58.8%; H, 9.28%; N, 6.65%. ^1H NMR ($CDCl_3$): δ 1.02 (s, 6H, CH_3), 1.11 (s, 6H, CH_3), 1.76 (m, 4H, CH_2), 2.02 (s, 3H, CH_3), 2.64 (m, 1H, CH), 3.60 (s, 3H, OCH_3). ^{13}C NMR ($CDCl_3$): δ 16.63, 18.08, 29.13, 32.0, 38.44, 49.43, 56.88, 168.00.

1-Oxyl-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine (5). A sample of commercially available 3-carboxy-PROXYL **4** (1.50 g, 8.05 mmol) was stirred in methanol (100 mL) and placed in an ice bath. Dry HCl(g) was then bubbled through the solution for 30 min. The solvent was evaporated quickly at ambient temperature, the resulting oil was neutralized with 10% $NaHCO_3$, and the product was extracted with ethyl acetate (3 × 100 mL). The organic layers were combined and dried over Na_2SO_4, and the solvent was evaporated to complete dryness to produce **5** as an orange oil; yield 1.40 g (87%). Exact FAB mass spectrum: calcd for $C_{10}H_{18}NO_3$, 200.1287; found, 200.1283.

1-Hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine (6). Method I. Nitroxide **5** (1.40 g, 7.0 mmol) was dissolved in methanol (100 mL). Anhydrous hydrazine (10 mL, 0.32 mol) was added dropwise to that solution. After 18 h of stirring, the solvent and the unreacted hydrazine were evaporated at room temperature almost to dryness. Toluene (15 mL) was added, and the solvent was vacuum-evaporated to dryness again (no heat!). This procedure, which removes any residual hydrazine, was repeated as many times as necessary to give **6** as an oil; yield 1.30 g (92%).

Method II. A sample of commercially available 3-carboxy-PROXYL **4** (1.50 g, 8.05 mmol) was stirred in methanol (100 mL) and placed in an ice bath. Dry HCl(g) was then bubbled through the solution for 30 min. The HCl-saturated solution was then stirred for 24 h at ambient temperature. The solvent was evaporated, the residue was neutralized with 10% $NaHCO_3$, and the product was extracted with ethyl acetate (3 × 100 mL). The organic layers were combined and dried over Na_2SO_4, and the solvent was evaporated to complete dryness (no heat!) to give **6** as an oil; yield 1.40 g (86%).

Exact mass spectrum: calcd for $C_{10}H_{19}NO_3-H$, 200.1287; found, 200.1280. ^{1}H NMR ($CDCl_3$): δ 1.02 (s, 3H, CH_3), 1.15 (s, 3H, CH_3), 1.23 (s, 3H, CH_3), 1.32 (s, 3H, CH_3), 1.77 (m, 1H, CH_2), 2.13 (m, 1H, CH_2), 2.74 (m, 1H, CH_2), 3.70 (s, 3H, OCH_3). ^{13}C NMR ($CDCl_3$): δ 14.17, 21.14, 21.78, 22.29, 32.25, 43.45, 46.52, 56.81, 60.77, 167.44.

1-Acetoxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine, AMCPy (7). Hydroxylamine **6** (9.4 g, 46.8 mmol) was dissolved in a mixture of THF (250 mL) and TEA (80 mL). The solution was placed in an ice bath, and then, acetic anhydride (30 mL, 0.32 mol) was added dropwise over 10 min. After 8 h of stirring at ambient temperature, the excess of acidic anhydride was carefully destroyed with water and the resulting solution was neutralized with aqueous 10% $NaHCO_3$. The THF was evaporated, and the product was then extracted with ethyl acetate (3 × 100 mL) and dried over Na_2SO_4, and the solvent was evaporated almost to dryness. Subsequent flash chromatography (hexane:ethyl acetate = 9:1) gave the product as a colorless oil; yield 10.5 g (92%). Exact FAB mass spectrum: calcd for $C_{12}H_{21}NO_4 + H^+$, 244.1549; found, 244.1547. Elemental analysis calcd: C, 59.3%; H, 8.70%; N, 5.76%. Found: C, 58.8%; H, 8.92%; N, 5.80%. ^{1}H NMR ($CDCl_3$): δ 1.06 (s, 3H, CH_3), 1.20 (s, 6H, CH_3), 1.28 (s, 3H, CH_3), 1.81 (m, 1H, CH_2), 2.09 (s, 3H, CH_3), 2.25 (m, 1H, CH_2), 2.91 (m, 1H, CH), 3.69 (s, 3H, OCH_3). ^{13}C NMR ($CDCl_3$): δ 11.00 (br), 13.27, 19.96 (br), 21.43 (br), 25.06 (br), 31.97 (br), 41.79 (br), 46.03, 56.01 (br), 60.29 (br), 164.53, 166.12.

1-Hydroxy-3-carboxy-2,2,5,5-tetramethylpyrrolidine (8). A sample of commercially available 3-carboxy-PROXYL **4** (1.0 g, 5.4 mmol) was stirred in methanol (100 mL). Anhydrous hydrazine (10 mL, 0.32 mol) was added dropwise to that solution. After 18 h of stirring at ambient temperature, the solvent and the unreacted hydrazine were removed by evaporation. Toluene (15 mL) was added, and the solvent was vacuum-evaporated to dryness again (no heat!). This procedure, which removes any residual hydrazine, was repeated as many times as necessary. The final residue was then dried over 48 h in vacuo to give **8** as a white solid; yield 0.93 g (93%). Exact FAB mass spectrum: calcd for $C_9H_{17}NO_3 + H^+$, 188.1287; found, 188.1294. ^{1}H NMR (DMSO-d_6): δ 0.92 (s, 3H, CH_3), 1.04 (s, 3H, CH_3), 1.09 (s, 3H, CH_3), 1.19 (s, 3H, CH_3), 1.62 (m, 1H, CH_2), 1.97 (m, 1H, CH_2), 2.52 (m, 1H, CH), 7.58 (br s, OH). ^{13}C NMR (DMSO-d_6): δ 18.94, 26.47, 27.65, 28.16, 38.27, 49.38, 60.71, 64.84, 175.05.

1-Acetoxy-3-carboxy-2,2,5,5-tetramethylpyrrolidine (9). Hydroxylamine **8** (0.38 g, 2.0 mmol) was suspended in THF (150 mL). The suspension was placed in an ice bath, and then, acetic anhydride (8 mL, 84.8 mmol) was added dropwise over 10 min. The solution gradually cleared up, and after 18 h of stirring at ambient temperature, the excess of acidic anhydride was carefully destroyed with water. The THF was evaporated, and the product was then extracted with ethyl acetate (3 × 100 mL). The combined organic layers were extracted multiple times with water (until neutral pH) and dried over Na_2SO_4, and the solvent was evaporated almost to dryness. Subsequent drying in vacuo gave the product as an oil, which solidified upon standing; yield 0.40 g (80%). Exact FAB mass spectrum: calcd for $C_{11}H_{19}NO_4 + H^+$, 230.1392; found, 230.1391. ^{1}H NMR ($CDCl_3$): δ 1.10−1.28 (m, 9H, CH_3), 1.32 (m, 3H, CH_3), 1.91 (m, 1H, CH_2), 2.12 (s, 3H, CH_3), 2.25 (m, 1H, CH_2), 2.95 (m, 1H, CH). ^{13}C NMR ($CDCl_3$): δ 17.44 (br), 19.26, 26.21 (br), 27.76 (br), 31.54 (br), 38.09 (br), 48.17 (br), 66.86 (br), 171.25, 177.64.

1-Acetoxy-3-(acetoxymethoxy)carbonyl-2,2,5,5-tetramethylpyrrolidine, DACPy (10). Acid **9** (1.4 g, 6.1 mmol) was dissolved in dimethylformamide (DMF, 150 mL), and to this solution bromomethyl acetate (4.0 g, 26.1 mmol) was added, followed by TEA (15 mL). After 18 h of stirring at ambient temperature, the solvent was evaporated under reduced pressure and the residue was distributed between $CHCl_3$ and water. The organic phase was washed multiple times with water and dried over Na_2SO_4, and the solvent was evaporated almost to dryness. Subsequent flash chromatography (hexane:ethyl acetate = 7:1) gave the product as a colorless oil; yield 1.2 g (65%). Exact FAB mass spectrum: calcd for $C_{14}H_{23}NO_6 + H^+$, 302.1604; found, 302.1595. ^{1}H NMR ($CDCl_3$): δ 1.09−1.30 (m, 12H, CH_3), 1.85 (m, 1H, CH_2), 2.11 (s, 6H, CH_3), 2.27 (m, 1H, CH_2), 2.94 (m, 1H, CH), 5.72 (d, 1H, OCH_2O), 5.80 (d, 1H, OCH_2O). ^{13}C NMR ($CDCl_3$): δ 17.26 (br), 19.18, 20.70, 26.30 (br), 27.81 (br), 31.42 (br), 37.89 (br), 47.98 (br), 63.0 (br), 66.80 (br), 79.38, 169.55, 170.98.

In Vivo Biodistribution Experiment. C3H HenMTV$^-$ female mice were supplied by the National Cancer Institute Animal Production Area. The animals were 6−8 weeks old and weighed between 22 and 25 g. EPR experiments were performed in accordance with the Guide for Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources, National Research Council. The mice (three per time point) received intraperitoneal injections of each compound (500 μL of 40 mM in 20% ethanol in PBS). Ten and sixty minutes after injection, the mice were euthanized, and the brain, heart, liver, kidney, spleen, $(CN)_6$], and blood were immediately excised and homogenized separately in ice-cold PBS. Each sample was split in halves. Potassium ferricyanide ($K_3[Fe(CN)_6]$, final concentration 1 mM) was added to the second half of each sample in order to oxidize any residual hydroxylamine to a nitroxide and thus measure the total concentration of the oxidized and reduced form of the particular compound. The samples were stored on ice, and then, 50 μL of the solution was drawn into a gas permeable Teflon capillary tube and placed into the EPR cavity. EPR spectra were recorded on a Varian E9 X-band spectrometer with field of 3370 G, modulation frequency of 100 kHz, and modulation amplitude of 1 G.

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