Eur. J. Med. Chem. **24** (1989) 335–340

Original paper

Influence of chemical structure on nitroxyl spin label magnetic relaxation characteristics

Robert C. BRASCH[1], Michael T. McNAMARA[1], Richard L. EHMAN[1], William R. COUET[2]**, Thomas N. TOZER[2], George SOSNOVSKY[3]*, Nuti Uma MAHESWARA RAO[3] and Indra PRAKASH[3]

[1]*Contrast Media Laboratory, Department of Radiology, University of California San Francisco, San Francisco, CA 94143,*
[2]*Department of Pharmaceutical Chemistry, University of California San Francisco, CA 94143, and*
[3]*Department of Chemistry, University of Wisconsin-Milwaukee, Milwaukee, WI 53201, USA*

(Received May 31, 1988, accepted November 10, 1988)

Summary — An attempt was made to develop guidelines for the design of new contrast agents using nitroxyl spin labels (NSL). The structural parameters of ring size and of substituents were correlated with the stability towards reduction and the relaxation effectiveness using 5 piperidine and 5 pyrrolidine nitroxyls containing the same substituents. The susceptibility of NSL to reduction was assessed by EPR spectroscopy. The relaxation effectiveness of NSL on protons in buffer and plasma solution was measured on a NMR spectrometer. The ring size and substituents has a decisive effect on the stability of NSL, whereby the ring size effect was dominant. In the case of spin–lattice relaxivities (R_1) and spin–spin relaxivities (R_2), the ring size and the substitution effect were marginal in buffer solution, while in plasma these effects were more pronounced. A number of guidelines were proposed for the design of suitable NSL contrast agents for MRI.

Résumé — Influence de la structure chimique sur les caractéristiques de relaxation magnétique de nitroxyles marqueurs de spin. *Une tentative d'élaboration de règles pour la conception de nouveaux agents de contraste utilisant des marqueurs de spin nitroxyles (NSL) a été réalisée. Les paramètres structuraux de la taille du cycle et des substituants ont été reliés à la stabilité vis-à-vis de la réduction et l'efficacité de relaxation en utilisant 5 pipéridines et 5 pyrrolidines nitroxylées contenant les mêmes substituants. La sensibilité du NSL à la réduction a été étudiée par spectroscopie RPE. L'efficacité de relaxation du NSL sur des protons dans un tampon ou dans le plasma a été mesurée par spectrométrie RMN. La taille du cycle et les substituants ont un effet décisif sur la stabilité du NSL, alors même que la taille du cycle avait un effet dominant. Dans le cas des relaxations spin–réseau (R_1) et spin–spin (R_2), l'effet de la taille du cycle et de la substitution était marginal en solution tamponnée, alors que dans le plasma les effets étaient plus prononcés. Des règles ont été proposées pour la mise au point d'agents de contraste NSL convenables pour la MRI.*

contrast agents / MR imaging / nitroxyl radical stability / spin–lattice relaxivity / spin–spin relaxivity

Introduction

In the search for safe and effective paramagnetic compounds to be used as contrast-enhancing agents in magnetic resonance imaging (MRI), considerable attention has been focused on nitroxyl spin labels (NSL) [1–7]. NSL are synthetic organic compounds containing one unpaired electron in the nitroxyl moiety >N·O that imparts the paramagnetic property to the molecule. The nitroxyl moiety is shielded to some extent from chemical interactions by 4 bulky methyl groups located on the adjacent α-carbon atoms. As a consequence, the NSL are often resistant to a variety of reagents. Nevertheless, the nitroxyl moiety can be readily reduced by various reducing agents including suitable enzymes, to the diamagnetic hydroxylamine derivative. NSL have been effectively employed for the past quarter-century as spin probes in a variety of chemical, biochemical, and medicinal projects using electron paramagnetic spectroscopy (EPR). The popularity of NSL can be attributed, in part, to the synthetic versatility of nitroxyl structures enabling them to be attached covalently to strategic parts of molecules under investigation and, in part, to the sensitive and informative EPR spectroscopy. This chemical versatility combined with their paramagnetic properties is the reason for their appeal as potential contrast agents for MRI.

Although to date the NSL have not been used as pharmaceuticals in humans, their effectiveness as contrast agents has been well documented by studies in animals

*Author to whom correspondence should be addressed.
**Present address:* CEMAF, 144 rue de la Gibauderie, 86000 Poitiers, France.

[1–5]. In these studies, primarily two compounds have been explored, namely, the succinic acid *N*-(2,2,6,6-tetramethyl-1-oxyl)-4-piperidinyl monoamide (**1c**, TES) and the 3-carboxyl-2,2,5,5-tetramethylpyrrolidine-1-oxyl (**2b**, PCA) (Fig. 1). TES(**1c**) and PCA(**2b**) have been shown to be effective in urographic enhancement of kidneys [1], in the definition of blood–brain barrier disruption [2], in the enhancement of experimentally induced neoplasms [3], in inflammatory lesions [2], and in acutely infarcted myocardium [5]. The minimum effective dose of PCA and TES for tissue enhancement is ≈0.1 mmol/kg [1–5]. The pharmacokinetics and metabolic properties of TES and PCA are uncomplicated [5] as compared to many pharmaceuticals in use. The principal route of elimination appears to be a partial reduction of the nitroxyl group to the hydroxylamine group [5]. Furthermore, both compounds are rapidly excreted by glomerular filtration after an intravenously administered dose ≈80–85 percent can be recovered in urine in ≈6 h [5].

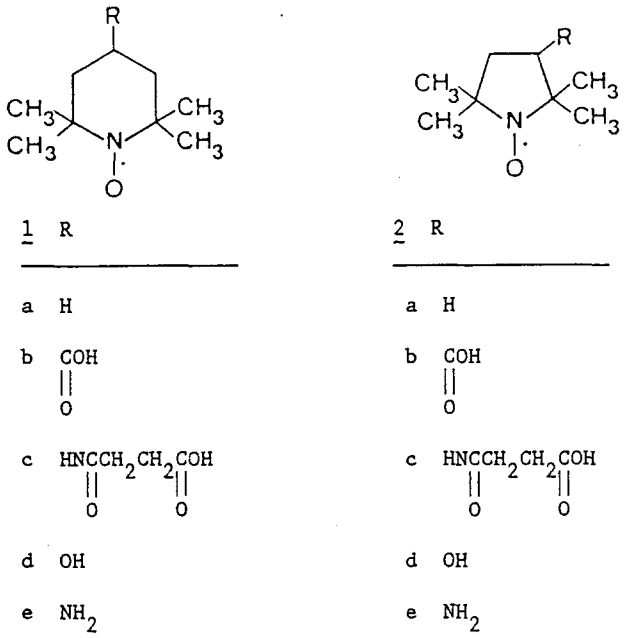


Fig. 1. Structures of piperidine and pyrrolidine nitroxyls.

The susceptibility of NSL to partial bioreduction *in vivo* can be viewed either as an advantage or a disadvantage for their application as contrast agents in MRI. On the positive side, the reduction of paramagnetic nitroxyls to the diamagnetic hydroxylamines may be expected to vary between tissues, depending on the total redox potentials and oxygen tensions [8]. These differences between tissues may then be potentially highlighted on MR images following NSL administration. On the negative side, the bioreduction of NSL diminishes the concentration of the paramagnetic nitroxyl species in a given tissue, and thereby reduces the degree of contrast enhancement. Consequently, one can envisage a parallel development of two kinds of NSL contrast agents, depending on the projected applications. For the assessment of local redox potentials and oxygen concentrations, a series of nitroxyl probes with a range of susceptibilities to reduction would be developed; whereas for a general purpose contrast enhancement a series of nitroxyl probes with the highest possible resistance to reduction would be the goal.

In the present study, an attempt was made to correlate structural elements of 10 NSL with their reducibilities and relaxation effectiveness, and ultimately, to define the structural parameters of NSL which determine their suitability as contrast agents in MRI.

Results

Reductions

The differences in reducibility of NSL on exposure to a 5-fold molar excess of ascorbic acid are shown in Table I expressed as the percentage of the initial concentration remaining 1 min after the addition of ascorbic acid [7]. Although the rank order of resistance to reduction by ascorbic acid is the same in the piperidine (**1a–e**) and the pyrrolidine (**2a–e**) series, namely, R = H>CO_2H>OH>$NHC(O)CH_2CH_2CO_2H$>NH_2, all pyrrolidine derivatives were markedly more resistant than the correspond-

Table I. Stability of NSL solution to ascorbic acid. Percentage remaining after 1-min exposure.

Piperidines 1	R	Percent of nitroxyls remaining	Pyrrolidines 2	R	Percent of nitroxyls remaining
a	H	51±4.0	a	H	98±0.2
b	COH ‖ O	33±3.2	b	COH ‖ O	96±1.4
c	HNCCH$_2$CH$_2$COH ‖ O ‖ O	13±2.5	c	HNCCH$_2$CH$_2$COH ‖ O ‖ O	91±0.3
d	OH	15±0.8	d	OH	94±0.7
e	NH_2	1±0.3	e	NH_2	72±4.0

ing analogs of the piperidine series. The most resistant compounds (**2a** and **2b**) were only marginally different. The least resistant compound (**2e**, R=NH_2) of the pyrrolidine series was, nevetheless, superior to the most resistant compound (**1a**) of the piperidine series. Thus, the rank order of both series was as follows: **2a>2b>2d>2c>2e>1a >1b>1d>1c>1e**.

Proton relaxivities in buffer and plasma solutions

The spectrometer-derived T_1 and T_2 relaxivities, R_1 and R_2, of the piperidine (**1a–e**) and pyrrolidine (**2a–e**) series in plasma and buffer solutions are shown in Tables II and III. The R_1 and R_2 values of the piperidine (**1a–e**) and pyrrolidine (**2a–e**) derivatives were found to be, in general, higher in plasma than in buffer solutions. Thus, the values for R_1 and R_2 for compound **2a, 2b** and **2d** were higher in plasma than in buffer solutions, whereas the values for **2c** and **2e** were only marginally different. All R_1 values in the piperidine series (**1a–e**) in plasma were higher than the R_1 values of the corresponding pyrrolidine analogs (**2a–e**). However, the R_2 values in plasma of the piperidines **1a, 1b, 1d**, and **1e** were lower, and **1c** was higher than those of the corresponding pyrrolidine analogs (**2a–e**). The R_1 values of piperidines **1a, 1b, 1c,** and **1e** in buffer solutions were somewhat higher than the R_1 values of the corresponding pyrrolidine analogs **2a, 2b, 2c,** and **2e**, whereas the values **1d** and **2d** were the same. The R_2 values of both series in buffer solutions were only marginally different. On the basis of this evaluation of data in Tables II and III, the following sequences of R_1 and R_2 relaxivities in plasma can be established: R_1 = **1a>1b>1d, 2b>1c>2a> 1e,2d>2e>2c**; R_2 = **2a>2b>1b>1a>2d>1c>1d>2e>1e >2c**.

Discussion

The analysis of relaxivity and stability data of NSL in the preceding section (Tables I–III) indicates certain trends in the influence of structural parameters on the suitability of NSL for contrast enhancement in MRI.

Thus, while the spin–lattice relaxivities (R_1) of the piperidine derivatives (**1a–e**) were only marginally higher

Table II. T_1 relaxivities (R_1) and T_2 relaxivities (R_2) of plasma NSL solutions at 10.7 MHz.

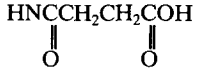
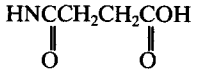
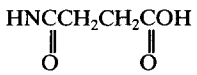
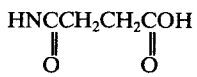
Piperidines		Relaxivities ($sec^{-1}\,mM^{-1}$)		Pyrrolidines		Relaxivities ($sec^{-1}\,mM^{-1}$)	
1	R	R_1	R_2	**2**	R	R_1	R_2
a	H	0.66	0.67	a	H	0.49	1.02
b		0.59	0.71	b		0.52	0.83
c		0.50	0.48	c		0.29	0.33
d	OH	0.52	0.48	d	OH	0.45	0.55
e	NH_2	0.46	0.38	e	NH_2	0.33	0.42

Table III. T_1 relaxivities (R_1) and T_2 relaxivities (R_2) of buffered NSL solutions at 10.7 MHz.

Piperidines		Relaxivities ($sec^{-1}\,mM^{-1}$)		Pyrrolidines		Relaxivities ($sec^{-1}\,mM^{-1}$)	
1	R	R_1	R_2	**2**	R	R_1	R_2
a	H	0.44	0.32	a	H	0.34	0.24
b		0.40	0.35	b		0.37	0.39
c		0.45	0.30	c		0.35	0.31
d	OH	0.43	0.42	d	OH	0.43	0.40
e	NH_2	0.39	0.36	e	NH_2	0.36	0.32

than those of the corresponding pyrrolidine derivatives (**2a–e**) in buffer solutions, more importantly, in plasma, the R_1 values of piperidine and the R_2 values of pyrrolidine NSL were more enhanced. In buffer solutions no pronounced ring size or substituent effect could be detected. In plasma solutions, a ring size effect was observed whereby the R_1 relaxivities were higher for the piperidine rings and the R_2 relaxivities were, in most cases, higher for the pyrrolidines. Furthermore, in general, in piperidine series the R_1 values were similar to those of the corresponding R_2 values, while in the pyrrolidine series the R_2 values were higher than those of the corresponding R_1 values. The ranking of the substituent effect in plasma was similar to that observed in the stability series, *i.e.*, the substituent R = H, CO_2H>OH>$HNC(O)CH_2CH_2CO_2H$,NH_2. It should be noted that the effectiveness of contrast enhancement on spin echo MRI tends to increase with higher values of R_1 (lower T_1) and lower values of R_2 (higher T_2) [6] in accordance with the following equation:

$$I \propto N(H)\,(1-e^{-T_R/T_1})e^{-T_E/T_2}$$

wherein I = signal intensity, H = proton density, T_R = pulse repetition time, and T_E = echo delay.

The observation that the relaxivities R_1 and R_2 of protons in the presence of all NSL compounds in plasma were higher than those in buffer solutions is of importance, and can be explained [9–16]. It is hypothesized that in plasma the NSL compounds interact with plasma macromolecular components, presumably forming either ionic or hydrogen bonds, or both, depending on the functional groups. These intermolecular interactions are assumed to form larger paramagnetic species than the original NSL, resulting in diminished rotational [9, 10, 12, 13, 15, 16] and translational [11, 14] motions, and hence, in lengthening of correlation times. As a result, the relaxation times of protons will become shorter, and the relaxivity values higher. In support of this contention, it was shown [17] that the nitroxyl labeled albumin derivatives using piperidine and pyrroline NSL possess much higher R_1 relaxivity values (≈ 11-fold per nitroxyl group) as compared to the values of low molecular weight nitroxyls.

Alternatively, differences in viscosity of plasma and buffer solutions could contribute either additionally or exclusively to higher relaxivity values in plasma. The dependence of relaxation time on viscosity is not linear; instead, the relaxation times are longer at low and high viscosities, and shorter at intermediate viscosities. This result can be explained as follows. In fluids of low viscosities the fluctuating magnetic fields are composed of a wide range of frequencies, so that the individual frequencies, including the frequency that matches the precessional frequency of the proton nuclei, make only small contributions. In fluids of high viscosity the molecular motions are slower and hence, the fluctuating magnetic fields are composed of frequencies lower than the precessional frequency of protons. Thus, the intensity of the frequency that matches the Larmor frequency is low. As a consequence, at either low or high viscosities the relaxation time T_1 will be higher, while in the intermediate range of viscosities it will be lower. Although at high viscosities the T_1 values are long, the T_2 values may become short. Consequently, the T_1 relaxivity values R_1 will be lower than the T_2 relaxivity values R_2. This assumption could explain the higher R_1 and R_2 values in plasma than those obtained in buffer solutions, and the higher R_2 values than R_1 in plasma solution (Table III). Analogous results were obtained in an earlier investigation with various mono- and di-nitroxyl derivatives using piperidine and pyrrolidine NSL [18], and with glucose derivatives using piperidine and pyrroline NSL [19].

On the basis of data in Table I, it appears that the ring size has a more dominant effect than the substituent effect on the stability of NSL. Thus, all 5 membered pyrrolidine NSL (**2a–e**) were more resistant in the ascorbic acid test than the 6-membered piperidine NSL analogs (**1a–e**). The influence of substituents on the reducibility was parallel in both series (Table I). However, the most resistant compound (**1a**) in the piperidine series was less stable than the least resistant compound (**2e**) in the pyrrolidine series. This ranking of *in vitro* stabilities **1a>1b>1d>1c>1e>2a>2b>2d>2c>2e** was also reflected in experiments using rat kidney and liver homogenate [5, 7, 20], with the exception that the unsubstituted compounds **1a** and **2a** ranked together with the least resistant compounds **1e** and **2e** [7]. Similar results were also obtained in erythrocyte suspension and lysate [21]. Furthermore, the stability of all NSL, independent of structure, was significantly increased in homogenates *in vitro* and erythrocyte suspension and lysate [21], and in animal models *in vivo* [1–5]. For example, the comparatively readily reducible TES (**1c**) in the ascorbic acid test was reduced to about 13% in 1 min [22] while in rat kidney homogenate it was reduced to ≈ 27.3±3.7% in 15 min [7]. This result clearly indicates that while the severe ascorbic acid test is useful in a preliminary screening, in order to rank newly developed contrast agents for MRI, a subsequent stability test will be required using homogenates to confirm the rankings, before proceeding to imaging experiments on animals *in vivo* where even more favorable conditions may exist for extending the longevity of NSL agents, since the biostability *in vivo* of NSL will depend on the relative concentrations and access of reducing substances to the contrast agent.

The high resistance of carboxylic acid derivatives **1b** and **2b**, and the low resistance of the amino derivatives **1e** and **2e** in the ascorbic acid test can be plausibly explained by the electrostatic repulsion effect between the carboxylate anion of **1b** and **2b** and the ascorbate, and by the electrostatic attraction effect of the protonated amino groups in **1e** and **2e**, and the ascorbate anion. Incidentally, the pyrroline carboxylate analog was shown also to have a high stability comparable to that of **2c** [22].

In recapitulating, for the design of new NSL contrast-enhancing agents, the following guidelines can be proposed based on the present study, and on previous studies in our laboratories [1–7, 17–23].

1. The ascorbic acid test [7, 21, 22] can be advantageously used for a preliminary rapid estimate of the relative stability ranking of a new potential NSL contrast agent for MRI.

2. In general, the pyrrolidine and pyrroline NSL containing a carboxyl moiety are more resistant to reduction *in vitro* and to bioreduction *in vitro* and *in vivo* than the piperidine NSL analogs [1–7, 20–22]. In cases where highest resistance to bioreduction is of paramount importance, pyrrolidine and pyrroline NSL should be used. However, in cases where highest biostability is not essential [8], *e.g.*, in studies of redox potentials or in assessment of oxygen concentrations, the piperidine NSL could be a viable and even a preferred alternative.

3. In general, in plasma, the piperidine NSL exhibit similar R_1 and R_2 relaxivity characteristics, while the pyrrolidine derivatives have higher R_2 values than the corresponding R_1 values. The R_1 values of the piperidine series are slightly higher than the corresponding values in the pyrrolidine series, while the R_2 values in the pyrrolidine series are, in general, higher than the corresponding R_2 values of the piperidine series. These properties favor [6] the piperidines as contrast-enhancing agents for MRI.

4. Considering the osmolality, at present, there are insufficient data available [6] to substantiate the importance of this parameter in MRI. Nevertheless, in developing new contrast agents, it would be advisable to replace the ionizable carboxyl groups with non-ionizable hydroxyl moieties [6], in analogy to the more recently developed [24] agents in CT tomography.

5. It would be advantageous to attach NSL covalently to larger molecular units, in order to improve the correlation time [9–17], at the same time, without eliciting antigenic responses.

6. Finally, the ease of compound procurement [17, 19–22] is an important factor. In this respect, at present, the piperidine and pyrroline NSL [7, 17–19, 23, 25] are more readily accessible, requiring less synthetic manipulations, and thus are less labor intensive, and more economical particularly, on a large scale than the pyrrolidine NSL derivatives [7, 22, 23, 25].

Experimental protocols

Materials

All reagents were of the finest quality available commercially. All solvents were distilled prior to use and stored over type 4A molecular sieves. Pyridine was stored over solid potassium hydroxide. Nitroxyl radicals **1a–e, 2a–d**, (Fig. 1) and **2f** and **2g** were prepared as described in the literature [22, 25, 26]. Compound **2e** was synthesized from **2f** by a modification of Rosen's method [26]. Compound **2b** was converted into the acid chloride **2g** which, in turn, was converted into the corresponding amide **2h**. Thus **2h** was prepared from **2b** by a different method than that reported in the literature [25]. The compound **2h** was found to be identical in all respects with the compound reported in literature [25].

Analytical procedures

Melting points were determined on the Thomas Hoover apparatus, model 6406-K with a calibrated thermometer. Mass spectra were recorded on a Hewlett–Packard mass spectrometer, model 5985 GS, using a direct insertion probe, a source pressure of 2×10^{-7} torr and methane as a reactant gas for chemical ionization. Therefore, for the molecular weight the $M^+ + 1$ values are reported. The EPR spectra of 10^{-4} solutions of the nitroxyl derivatives were recorded on a Varian E-115 EPR spectrometer. Microanalyses were performed on a Perkin–Elmer 240C elemental analyzer. Column chromatography was performed by conventional column chromatography on alumina (MC/B, type F-20, 80–200 mesh). The progress of reactions and purity of products was monitored by TLC analyses using silica gel 60 gel F_{254} precoated sheets (Merck), layer thickness 0.2 mm with visualization using UV light and/or iodine chamber.

Spectrometer analysis

Each of the 10 NSL compounds (Table I) was dissolved separately in 0.067 M phosphate buffer (PBS) at pH 7.4 and fresh human plasma in 10, 3.3, and 1.1. mmol concentrations. T_1 and T_2 relaxation times were measured for each aqueous and plasma NSL solution at 37°C using a 0.251 Tesla pulsed NMR analyzer (Praxis Model II, San Antonio, TX) with a resonant proton frequency of 10.7 MHz. A train of Hahn spin echoes with a TE range of 2.5–110.0 msec was utilized to calculate T_2. No correction was made for possible diffusion effects. T_1 relaxation times were determined by a saturation recovery method with a 90°–t–90° pulse sequence.

Data analysis

The paramagnetic contribution to the observed T_1 and T_2 values were calculated for each solution as follows:

$$1/T_{observed} = 1/T_{diamagnetic} + 1/T_{paramagnetic},$$

where $T_{observed}$ is the measured relaxation time, and $T_{diamagnetic}$ is the measured relaxation time of the solvent without NSL. Subsequently expressed data represent the paramagnetic contribution to relaxation as calculated by means of the equation.

To make a meaningful comparison of relaxation characteristics of various NSL, both T_1 and T_2 effects are expressed in terms of "relaxivity", defined respectively as either the slope of $1/T_1$ or $1/T_2$ *versus* concentration [17, 18]. An increase in relaxivity, or slope of the curve, thus represents an improvement in relaxation effectiveness.

Monitoring of reduction by EPR

Freshly prepared solutions of each NSL (2 mM) and of ascorbic acid (10 mM) in 0.067 M phosphate buffer at pH 7.4 were used [22]. Equal volumes (5 ml) of each solution were mixed and the nitroxyl concentration was measured continuously for 1 min at room temperature (20°C–25°C) by recording the height of the low field peak of the first derivative of the nitroxyl triplet spectrum using an electron paramagnetic resonance (EPR) spectrometer Varian model E 104A.

Synthetic methodologies

Preparation of 3-amino-2,2,5,5-tetramethylpyrrolidine-1-oxyl ***2e***

To a solution of 3-*oxo*-2,2,5,5-tetramethylpyrrolidine-1-oxyl (**2f**, 1.56 g, 10 mmol) and ammonium acetate (7.73 g, 100 mmol) in methanol (50 ml), was added sodium cyanoborohydride (0.86 g, 13.66 mmol) and the reaction mixture stirred at 25°C for 24 h. The solvent was removed on a rotating evaporator at 25°C/20 Torr and the oily residue dissolved in water (20 ml). The pH of this solution was adjusted to 12 with a 15% aqueous sodium hydroxide solution. The solution was extracted with chloroform (3 × 20 ml) and the combined chloroform extracts were dried over magnesium sulfate and filtered. Concentration of the filtrate on a rotating evaporator at 25°C/20 Torr gave an oil which was purified by column chromatography on alumina using first *t*-butyl methyl ether followed by a mixture of *t*-butyl methyl ether and methanol (9:1, v/v) as eluant. Concentration of appropriate fractions on a rotating evaporator at 25°C/20 Torr gave a yellow thick oil which was further purified by Kugelrohr distillation at 80–85°C/0.05–0.02 torr. Crystallization of the oil at 25°C produced 0.66 g (42%) of compound **2e**, mp 34–35°C (dec.), in the literature [25] described as oil. Purity control by the TLC (silica gel, chloroform and methanol, 7:1, v/v) indicated one product.

M.S. (C.I.) *m/e:* 158 ($M^+ + 1$, 100%), 140 ($M^+ - 17$, 23%); EPR (MeOH); 3 lines, a_N = 15.0 G. $C_8H_{17}N_2O$ (157.225). Anal. C, H, N.

Preparation of 3-chloroformyl-2,2,5,5-tetramethylpyrrolidine-1-oxyl ***2g***

To a stirred solution of **2b** (1.12 g, 6 mmol) in toluene (20 ml) was added pyridine (0.6 ml) and the solution cooled to 0°C. To the solution was added dropwise thionyl chloride (0.82 g, 0.50 ml, 6.9 mmol), keeping the temperature at 0°C. The reaction mixture was then stirred for 1 h at 25°C, and ethyl ether (10 ml) was added to precipitate pyridinium hydro

chloride which was collected by filtration. Concentration of the filtrate to dryness at 40°C/20 torr resulted in 0.77 g (62%) of a yellow crystalline solid (**2g**), mp 105–106°C (dec.).

M.S. (C.I.) *m/e:* 205 ($M^+ + 1$, 78%), 207 ($M^+ + 3$, 26%), 197 ($M^+ - 17$, 31%); EPR (C_6H_6): 3 lines, a_N = 15.0 G. $C_9H_{15}NO_2Cl$ (204.677). Anal. C, H, N.

Preparation of 3-aminocarbonyl-2,2,5,5-tetramethylpyrrolidine-1-oxyl **2h**
A solution of **2g** (0.612 g, 3 mmol) in methanol (20 ml) was saturated with ammonia gas at 0°C for 4 h and kept in a refrigerator overnight at 4°C. Concentration of the reaction mixture to dryness at 40°C/20 torr on a rotating evaporator gave a solid material. Recrystallization of this material from a mixture of chloroform and hexane (v/v, 4:1) gave 0.40 g (70%) of pure product **2h** mp 174–175°C (dec.), lit. [25] mp 174–175.5°C. Purity control by TLC (silica gel, chloroform and methanol, 9:1 v/v) indicated one single product. Compound **2h** was used in the preparation of **2e** by the literature method [25].

Acknowledgments

Supported in part by grant DK 31937 from the National Institute of Diabetes, Digestive and Kidney Diseases, and in part by the Graduate School of The University of Wisconsin–Milwaukee, J.D. and D. Shaw awards to G.S. in 1983, 1984, 1985 and 1986 for NMR imaging.

References

1 Brasch R.C., London D.A., Wesbey G.E., Tozer T.N., Nitecki D.E., Williams R.D., Doemeny J., Tuck L.D. & Lallemand D.P. (1983) *Radiology* 147, 773–779
2 Brasch R.C., Nitecki D.E., Brant-Zawadzki M., Enzmann D.R., Wesbey G.E., Tozer T.N., Tuck L.D., Cann C.E., Fike J.R. & Sheldon P. (1983) *Am. J. Neuroradiology* 4, 1035–1039
3 Ehman R.L., Wesbey G.E., Moon K.L., Williams R.D., Couet W.R., Tozer T.N. & Brasch R.C. (1985) *Magn. Reson. Imaging* 3, 89–97
4 McNamara M.T., Wesbey G.E., Brasch R.C., Sievers R., Lipton M.J. & Higgins C.B. (1985) *Invest. Radiol.* 20, 591–595
5 Couet W.R., Eriksson U.G., Brasch R.C. & Tozer T.N. (1985) *Pharm. Res.* 69–72
6 Grodd W. & Brasch R.C. (1986) *Fortschr. Rontgenstr.* 145, 130–139
7 Couet W.R., Brasch R.C., Sosnovsky G. & Tozer T.N. (1985) *Magn. Reson. Imaging* 3, 83–88
8 Swartz H.M. (1987) *J. Chem. Soc. Faraday Trans. 1* 83, 191–202
9 Solomon I. (1955) *Phys. Rev.* 99, 559–565
10 Bloembergen N. (1957) *J. Chem. Phys.* 27, 572–573
11 Wehrli F.W. (1980) *in: ESR and NMR of Paramagnetic Species in Biological and Related Systems* (Bertini I. & Drago R.S., eds.), D. Reidel Publishing Company, Boston, pp. 1–28
12 Burton D.R., Forsen S., Karlstrom G. & Dwek R.A. (1979) *Prog. NMR Spectr.* 13, 1–45
13 Vaughn Jr. J.B., Stephens R.L., Lenkinski R.E., Heavner G.A., Goldstein G. & Krishna N.R. (1982) *Archiv. Biochem. Biophys.* 217, 468–472 references therein
14 Mansfield P. & Morris P.G. (1984) *in: NMR Imaging in Biomedicine.* Academic Press, New York
15 Koutcher J.A., Burt C.T., Lauffer R.B. & Brady T.J. (1984) *J. Nucl. Med.* 25, 506–513
16 Gadian D.G., Payne J.A., Bryant D.J., Young I.R., Carr D.H. & Bydder G.M. (1985) *J. Comp. Assist. Tomogr.* 9, 242–251
17 Sosnovsky G., Rao N.U.M., Lukszo J. & Brasch R.C. (1986) *Z. Naturforsch.* 41b, 1170–1177
18 Ehman R.L., Brasch R.C., McNamara M.T., Eriksson U., Sosnovsky G., Lukszo J. & Li S.W. (1986) *Invest. Radiol.* 21, 125–131
19 Sosnovsky G., Rao N.U.M., Lukszo J. & Brasch R.C. (1986) *Z. Naturforsch.* 41b, 1293–1305
20 Couet W.R., Eriksson U.G., Tozer T.N., Tuck L.D., Wesbey G.E., Nitecki D. & Brasch R.C. (1984) *Pharm. Res.* 203–209
21 Eriksson U.G., Tozer T.N., Sosnovsky G., Lukszo J. & Brasch R.C. (1986) *J. Pharm. Sci.* 75, 334–337
22 Couet W.R., Brasch R.C., Sosnovsky G., Lukszo J., Prakash I., Gnewuch G.T. & Tozer T.N. (1985) *Tetrahedon* 41, 1165–1172
23 Lovin J.D., Wesbey G.E., Engelstadt B.L., Sosnovsky G., Li S.W., Lukszo J., Mosley M., Tuck D.C. & Brasch R.C. (1985) *Magn. Reson. Imaging* 3, 73–81
24 Olsson B., Aulie A., Sveen K. & Andrew E. (1983) *Invest. Radiol.* 18, 177–182
25 Rozantsev E.G. (1974) *Free Nitroxyl Radicals.* Plenum Press, New York
26 Rosen G.M. (1974) *J. Med. Chem.* 17, 358–360