SYNTHESIS OF DINITROXIDES OF POTENTIAL USE AS CONTRAST AGENT IN MRI

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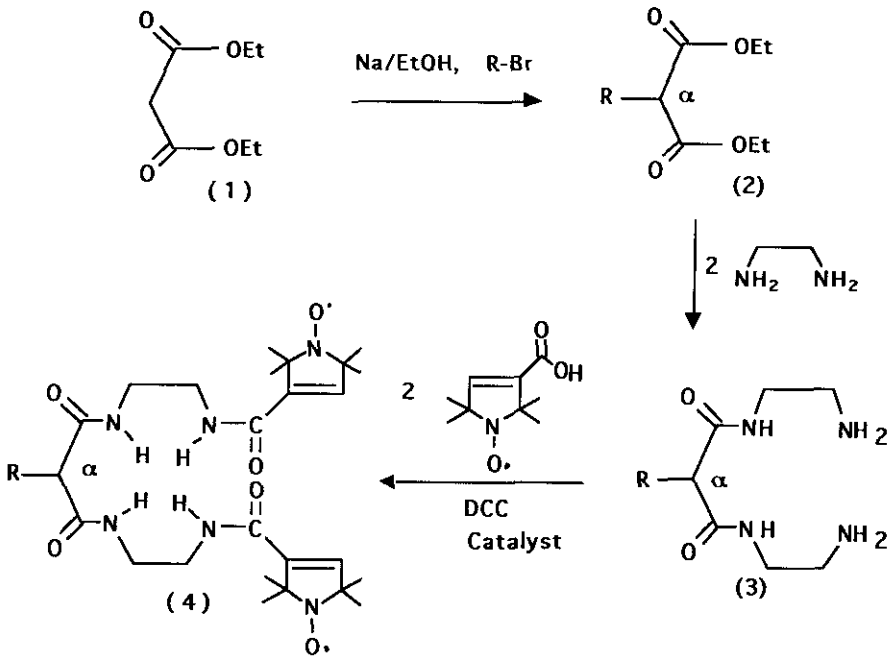
Abstract- Dinitroxides derived from 6-alkyl-1,4,8,11-tetraaza undecane-5,7-dione have been synthesized in three steps. These compounds, which contain two paramagnetic centers, are more effective proton relaxation agents than those containing only one nitroxyl moiety and could be used in smaller quantities as contrast agents in MRI.

Nuclear magnetic resonance has been widely used for medical imaging research.[1-4] The rapid development of this technique in the last ten years has stimulated the synthesis of a great number of new paramagnetic species as contrast agents.[5-8] Among these stable free radicals, such as nitroxyl groups, which though they are weaker relaxation accelerators than metal ions, are of interest as they can be covalently bound to other molecules or biomolecules. The nitroxide group is highly sensitive to its environment and its esr spectrum is greatly influenced by the polarity and mobility of the environment. A wide range of nitroxide spin labels, suitable for probing particular environments, have been synthesized and found to be relatively stable under most biological conditions.[9] The type of spin label chosen for a particular experiment depends upon the chemical structure of the system to be investigated. The interaction of the electron with the magnetic moment of the N atom gives rise to an isotropic spectrum with three lines of equal intensity. However the spectrum can be substantially different when the nitroxide is incorporated in a biological system, as it often results in a powder structure, where the radical mobility is low and the hyperfine structure disappears.[10] The esr spectra of dinitroxide depends not only on the average distance between the paramagnetic centers but also on their relative orientations. The intensity and the width of the lines also depends on temperature and on the distance between the nitroxides.[11,12] As the nitroxide moities are brought nearer to one another, their esr signal becomes increasingly different from the mononitroxide signal. However, in

order to improve the MRI contrast without injection of a high dose, a family of polynitroxides [13,14] having a strong proton relaxation effect have been recently developed. The increase in MRI intensity produced by biradicals is attributable to their strong electron paramagnetism resulting from the presence of the two paramagnetic centers, which hastens the relaxation of neighbouring hydrogen nuclei. Various substituted alkyl groups of ranging polarity were introduced in order to induce specific biodistribution and biospecificity to some target organs. Five-membered cyclic nitroxides (pyrroline) were preferred over six membered ones (piperidine derivatives) because of their higher resistance to *in vivo* reduction as demonstrated elsewhere.[15,16] Synthesis of dinitroxides involved three subsequent steps. (Scheme 1). The reaction of diethyl malonate (1) with bromoalkane in ethanol led to the formation of diethyl 2-alkylmalonate (2) which was coupled to ethylenediamine to give 6-alkyl-1,4,8,11-tetraazaundecane-5,7-dione (3) in yields ranging from 50 to 70%. Finally the biradicals (4) were obtained by coupling of 2,2,5,5-tetramethylcarboxypyrroline-1-oxyl with (3) at room temperature using *N,N'*-dicyclohexylcarbodiimide and 4-pyrrolidinopyridine as catalyst.

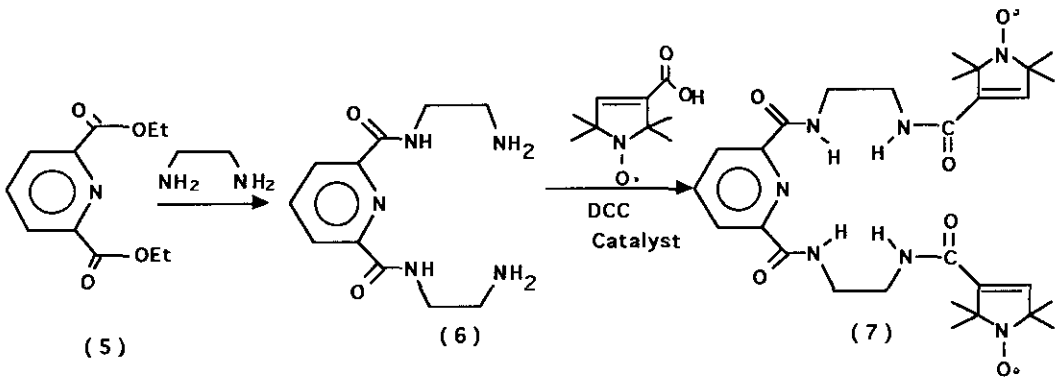


a) R= $C_{16}H_{33}$ b) R= C_9H_{19} c) R= C_4H_9

Scheme 1

Using the same sequence, we have also synthesized a biradical in which the alkyl group in the α position is replaced by pyridine group (Scheme 2). This increases its solubility in

water by converting the secondary amine group into the hydrochloride and consequently *in vitro* esr biodistribution together with the spin clearance of these biradicals and their imaging in the whole body of animals could be easily undertaken.



Scheme 2

At room temperature, the esr spectra of a dilute solution (10^{-4} M) of mononitroxide exhibit a three lines spectrum. In general the hyperfine interaction value $\mathbf{a_n}$ ranges from 14.5 to 16.2 G and depends on temperature and the solvent used.[17] This interaction is due to correlation and the delocalization of the unpaired electrons resulting from the spin individually induced spin density at the site of each spin. When there is an electron-nuclear interaction producing a hyperfine structure in one of the spin-systems, the following sequence of changes will be observed in the esr spectrum as the exchange is enhanced. In the case of a biradical, the spin exchange in solution causes the appearance of two lines (marked 2 and 4 in Figure 1) in addition to the three expected for interaction between an unpaired electron and one nitrogen nucleus. This exchange occurs only through space. The physical meaning of the phenomena arising from exchange interaction may be visualized with the aid of a model that describes the behavior of spins in a constant magnetic field. The theoretical treatment took into acount the energy of the exchange interaction **J** which characterizes the magnitude of the overlapping between the orbitals of the unpaired electron in an encounter.[18]

This exchange interaction occurs in the case of a dilute solutions of biradicals (**4a**, **4b**, **4c** and **7**) when the nitroxide fragments approach each other as a result of intramolecular motion. The spectrum displays the esr features of a long chain dinitroxide biradical that are typical both for the spectrum of the elongated conformation (weak exchange interaction 3 lines) and for all possible folded conformations in which the radical fragments are drawn together (strong exchange interaction $\mathbf{J > a_n}$: 5 lines spectrum Figure 1).

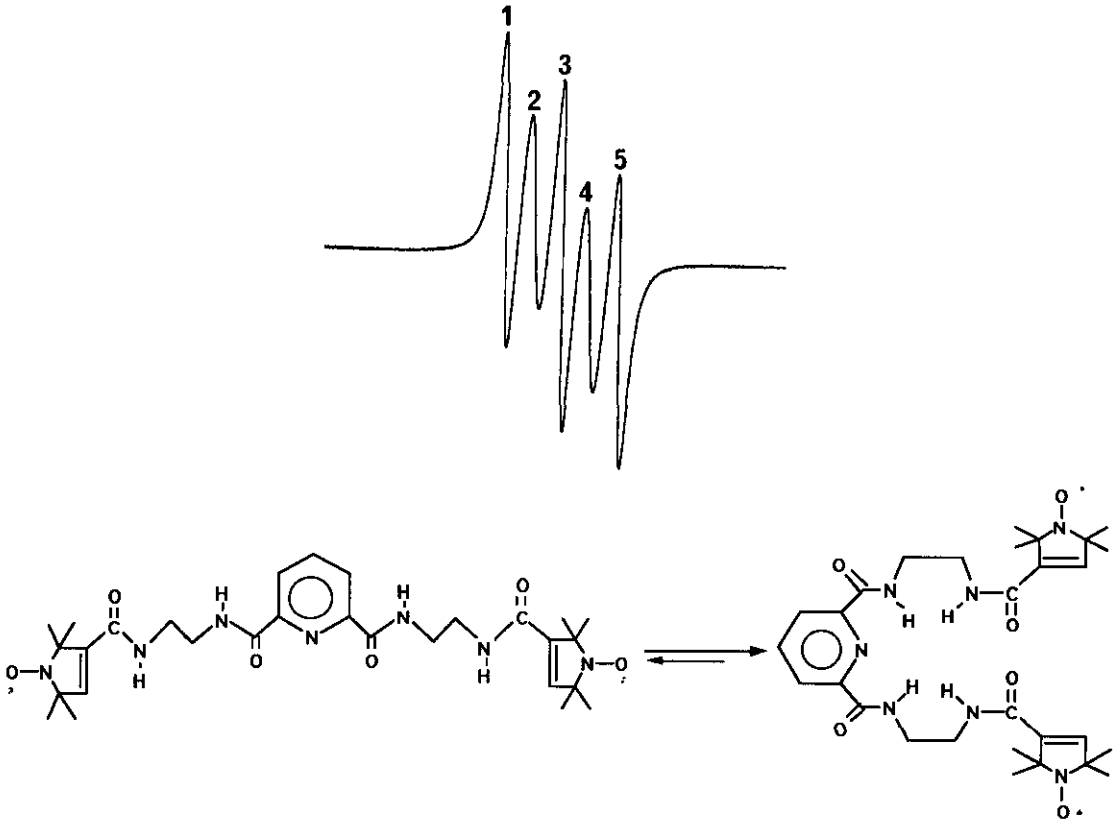


Figure 1: Esr spectra of biradical (7) in CH_2Cl_2 at room temperature.

However, the esr spectra of biradical can be transformed experimentally from a quintet to a high triplet by varying the specific conditions, for instance by slowing the exchange when the temperature is decreased (frozen solution) or when the centers are rigidly bound to very large macromolecules (contrast agent for MRI [13] or spin label for biological system. [19,20]) Under these conditions, the molecular motion of the spins and the solvent (water) virtually ceased and the relaxivity of the medium is enhanced.[13] These results show that these lipophilic biradicals synthesized in three steps are of interest as potential contrast agents in MRI.

EXPERIMENTAL

All reagents were of the finest commercially available quality. All solvents were distilled prior to use. Ether was distilled and stored over sodium. Ethylenediamine, 1-bromohexadecane, 1-bromobutane, 1-bromononane, *N,N'*-dicyclohexylcarbodiimide, 2,6-

pyridinedicarboxylic acid, diethyl malonate and 4-pyrrolidinopyridine were obtained from Janssen Chimica Company. 2,2,5,5-Tetramethyl-3-carboxypyrroline-1-oxyl was obtained in a three steps as described elsewhere.[17]

The ^{1}H-Nmr spectra were recorded on a Bruker 250 AM high resolution spectrometer. Chemicals shifts are reported in ppm (δ) downfield from internal Me_4Si and coupling constants (J) are given in Hz. CI-ms spectra were obtained on a Nermag equipment using direct insertion probe with a source pressure of 10^{-1} torr and ammonia as the reactant gas. The electron paramagnetic resonance spectra of 10^{-4} M of nitroxides in CH_2Cl_2 as solvent were obtained on a Bruker ER 200E spectrometer.

(**2a**) After dissolving 2.3 g (0.1 mol) of Na in 250 ml of anhydrous EtOH, 16 g (0.1 mol) of diethyl malonate are added dropwise under argon. After 1 h at 50°C, 30.6 g (0.1 mol) of 1-bromohexadecane are added and the mixture is stirred for 15 h. The solution is concentrated to dryness and washed with hot $CHCl_3$. The precipitate of NaBr is filtered and the solution is dried over Na_2SO_4. After evaporation, a yellow oil is obtained and distilled to give 24 g (62%) of **2a**, bp: 195°C/0.5 torr. Ir (KBr): υ_{CO}=1730 cm^{-1}. ^{1}H-Nmr ($CDCl_3$) δ_H: 0.85 (t, 3H, $C\underline{H}_3$, J=7 Hz); 1.20 (t, 6H, $(C\underline{H}_3CH_2O)_2$, J=7 Hz); 1.15 (m, 28H, $(C\underline{H}_2)_{14}$); 1.73 (q, 2H, $C\underline{H}_{2\beta}$, J=7 Hz); 3.18 (t, 1H, $\underline{H}_\alpha$, J=7 Hz); 4.10 (q, 4H, $(C\underline{H}_2O)_2$, J=7 Hz).

(**2b**) yield 47%, bp: 180°C/2 torr. Ir (KBr): υ_{CO}=1730 cm^{-1}. ^{1}H-Nmr ($CDCl_3$) δ_H: 0.90 (t, 3H, $C\underline{H}_3$, J=7 Hz); 1.25 (m, 20H, $(C\underline{H}_2)_7$ and $(C\underline{H}_3CH_2O)_2$, J=7 Hz); 1.85 (m, 2H, $C\underline{H}_{2\beta}$); 3.30 (t, 1H, $C\underline{H}_\alpha$, J=7 Hz); 4.20 (q, 4H, $(C\underline{H}_2O)_2$, J=7 Hz).

(**2c**) yield 53%, bp: 122°C/4 torr. Ir (KBr): υ_{CO}=1730 cm^{-1}. ^{1}H-Nmr ($CDCl_3$) δ_H: 0.82 (t, 3H, $C\underline{H}_3$, J=7 Hz); 1.20 (t, 6H, $(C\underline{H}_3CH_2O)_2$, J=7 Hz); 1.25 (m, 4H, $(C\underline{H}_2)_2$); 1.81 (dt, 2H, $C\underline{H}_{2\beta}$, J and J'=7.2 Hz); 3.24 (t, 1H, $C\underline{H}_\alpha$, J=7.2 Hz); 4.13 (q, 4H, $(C\underline{H}_2O)_2$, J=7 Hz).

(**3a**) 4.25 g (11 mmol) of **2a** are added dropwise under argon to 1.32 g (22 mmol) of ethylenediamine. The mixture is stirred during 3 days at room temperature. After concentration, a white precipitate is obtained and washed by water and ethyl acetate to remove traces of unreacted ethylenediamine and ester. 3.50 g (77%) of **3a** are obtained, mp: 120 °C. Ir (KBr): υ_{CO}=1660 cm^{-1}. ^{1}H-Nmr ($CDCl_3$) δ_H: 0.85 (t, 3H, $C\underline{H}_3$, J=6 Hz); 1.22 (s, 30H, $(C\underline{H}_2)_{15}$); 1.81 (m, 4H, $(N\underline{H}_2)_2$); 2.95 (t, 1H, $\underline{H}_\alpha$, J=6 Hz); 2.79 (t, 4H, $(C\underline{H}_2NH_2)_2$, J=6 Hz); 3.26 (t, 4H, $CONHC\underline{H}_2$, J=6 Hz); 7.23 (m, 2H, $(N\underline{H})_2$).

(**3b**) yield 78%, mp: 110°C. Ir (KBr): υ_{CO}=1660 cm^{-1}. ^{1}H-Nmr (DMSO-d_6) δ_H: 0.90 (t, 3H, $C\underline{H}_3$, J=6 Hz); 1.30 (s, 14H, $(C\underline{H}_2)_7$); 1.70 (m, 2H, $C\underline{H}_{2\beta}$); 2.50 (m, 4H, $(N\underline{H}_2)_2$); 2.60 (m, 4H, $(C\underline{H}_2NH_2)_2$); 3.10 (m, 5H, $(C\underline{H}_2NH)_2$ and $C\underline{H}_\alpha$); 7.95 (t, 2H, $(N\underline{H})_2$, J=6 Hz).

(**3c**) yield 85%, mp: 110°C. Ir (KBr): υ_{CO}=1660 cm^{-1}. ^{1}H-Nmr (DMSO-d_6) δ_H: 0.90 (t, 3H, $C\underline{H}_3$, J=6 Hz); 1.25 (m, 4H, $(C\underline{H}_2)_2$); 1.70 (m, 2H, $C\underline{H}_{2\beta}$); 2.07 (m, 4H, $(N\underline{H}_2)_2$); 2.60 (t, 4H, $(C\underline{H}_2NH_2)_2$), J=6 Hz); 3.10 (m, 5H, $(CONHC\underline{H}_2)_2$ and $C\underline{H}_\alpha$); 7.95 (m, 2H, $(N\underline{H})_2$).

(**6**) yield 80%. Ir (KBr): υ_{CO}=1660 cm^{-1}. ^{1}H-Nmr (D_2O) δ_H: 3.00 (t, 4H, $(C\underline{H}_2NH_2)_2$, J=6 Hz); 3.42 (m, 4H, $(CONHC\underline{H}_2)_2$); 7.15 (m, 1H, H_{para}); 7.28 (m, 2H, H_{meta}).

(**4c**) A solution of 2,2,5,5-tetramethyl-3-carboxypyrroline-1-oxyl 1.84 g (0.01 mol), *N,N'*-dicyclohexylcarbodiimide 2.26 g (0.011 mol), the 6-butyl-1,4,8,11-tetraazaundecane-5,7-dione (**3b**) 1.22 g (0.005 mol) and 4-pyrrolidinopyridine 0.14 g (0.001 mol) in CH_2Cl_2 (30 ml) is stirred and allowed to stand at room temperature until the reaction is complete. The *N,N'*-dicyclohexylurea is filtered off and the filtrate is washed thoroughly with water (10x50 ml) to remove *N,N'*-dicyclohexylurea. The filtrate is then dried ($MgSO_4$) and the solvent is evaporated in vacuo to give dinitroxide (**4c**) yield 60%. CI-ms/NH_4^+: 579 ($(M+3)^+$, 32); 578 ($(M+2)^+$, 96); 577 ($(M+1)^+$, 100); 562 ($((M+1)^+-15)^+$, 3); 547 ($((M+1)^+-30)^+$, 3); 576 ($(M-30)^+$, 2).
Esr: 5 lines a_n= 15 gauss (biradical with an exchange interaction).

(**4b**) yield 55%. CI-ms/NH_4^+: 649 ($(M+3)^+$, 52); 648 ($(M+2)^+$, 100); 647 ($(M+1)^+$, 93); 616 ($(M-30)^+$, 2); 617 ($((M+1)^+-30)^+$, 4); 618 ($(M-30)^+$, 3).
Esr: 5 lines a_n= 15 (biradical with an exchange interaction).

(**4a**) yield 60%. CI-ms/NH_4^+: 749 ($(M+5)^+$, 34); 748 ($(M+4)^+$, 76); 746 ($(M+2)^+$, 50); 733 ($((M+4)^+-15)^+$, 26); 732 ($((M+3)^+-15)^+$, 42); 731 ($((M+2)^+-15)^+$, 24); 718 ($((M+4)^+-30)^+$, 6); 717 ($((M+3)^+-30)^+$, 12); 616 ($((M+2)^+-30)^+$, 13); 615 ($((M+1)^+-30)^+$, 6); 762 ($(M+18)^+$, 4)
Esr: 5 lines a_n= 15 gauss (biradical with a high exchange interaction).

(**7**) yield 53%. CI-ms/NH_4^+: 586 ($(M+3)^+$, 9); 585 ($(M+2)^+$, 52); 584 ($(M+1)^+$, 100); 570 ($((M+2)-15)^+$, 3); 569 ($((M+1)^+-15)^+$, 6); 555 ($((M+2)^+-30)^+$, 2); 554 ($((M+1)^+-30)^+$, 6); 553 ($(M-30)^+$, 5).
Esr: 5 lines a_n= 15 gauss (biradical with an exchange interaction).

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