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Hydrazyl, Nitronyl-, and Imino-Nitroxides: Synthesis, Properties and Reaction With Nitric Oxide and Nitrogen Dioxide

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Abstract: Ten novel and stable free radicals of nitronyl-, imino-nitroxide and hydrazyl type compounds were synthesized and their physico-chemical properties investigated. UV-Vis and ESR spectroscopic data, as well as the lipophilicities and specific hydrophobic areas of the compounds are compiled. The reaction of these radical compounds with nitric oxide and nitrogen dioxide was also investigated. The radicals synthesized, show selectivity in their reaction with these nitric oxides, depending on their structure (nitronyl-nitroxide radicals react with NO, while hydrazyl radicals react with NO_2). The processes are easily monitored by UV-Vis or ESR spectroscopy.

Keywords: hydrazyl, nitronyl, nitric oxide, eosine, TEMPO, lipophilicity, free radical

1 Introduction

The chemistry of nitric oxide has been intensely studied in recent years due to the discovery of its role played in the living cell. It is well known that NO is a novel transient cell messenger that augments intercellular communication and governs many intracellular events, as well as playing an essential role in the host immune response [1]. Nitric oxide is generated *in vivo* by the nitric synthase enzymes, using L-arginine, NADPH and oxygen as substrates. The simplest and most frequently used method for the assay of nitric oxide is the Griess reaction, which in fact determines nitrite as an oxidation product of nitric oxide [2]. It was found that nitric oxide and nitrogen dioxide can react with other stable radicals, namely nitronyl-nitroxide radicals (for NO), and hydrazyl radicals (for NO_2)

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[3, 4, 5]. These processes are easily monitored by ESR, which has been reported as an accurate technique for determination of nitric oxide in biological samples [1].

Among the molecular properties of radicals, lipophilicity is important since its biological activity can be correlated by quantitative structure-activity relationships (QSAR), eg. such as determining the capacity of a compound to cross the lipophilic cell membrane. In this regard, we studied the lipophilicity (hydrophobicity) of radicals using the method of reverse phase thin layer chromatography (RP-TLC). Besides the classical methods of lipophilicity determination by partition of the compound between an immiscible polar and a nonpolar solvent pair (usually *n*-octanol / water), RP-TLC is widely used owing to it's simplicity and rapidity [6, 7].

In this paper we investigated the synthesis and reactions of nitric oxide or nitrogen dioxide with some novel stable radicals and diradicals (hybrid molecules) of nitronyl-nitroxide, imino-nitroxide and those of the hydrazyl type, which can act as spin probes [8, 9]. Figure 1 shows the structure of these new radicals.

2 Results and Discussion

2.1 Synthesis

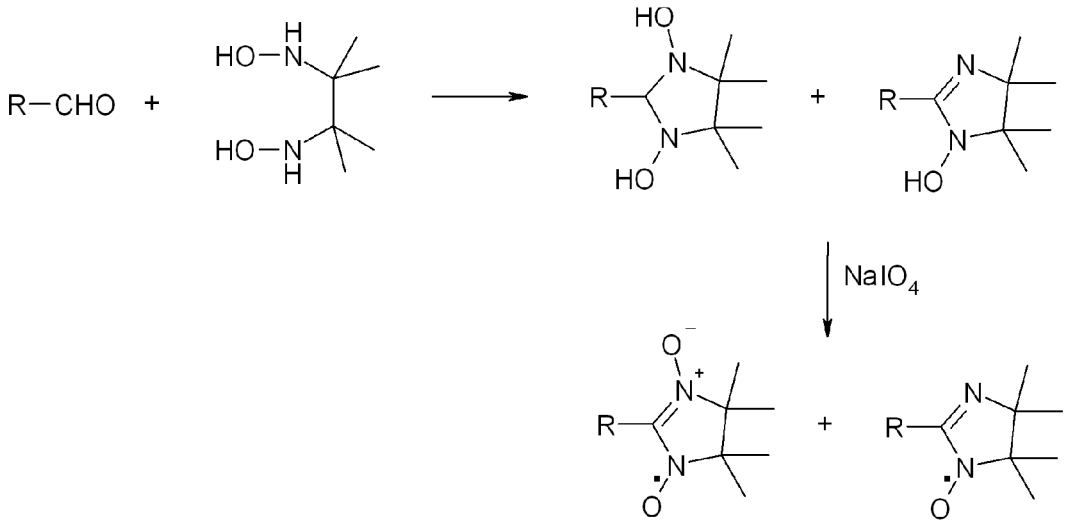
The literature has shown that 2,3-bis(hydroxyamino)-2,3-dimethylbutane is a volatile and unstable compound, which cannot be stored; thus, the sulphate derivative was synthesized, but unfortunately this method afforded many impurities , which were unsuitable for our purposes [10]. However, 2,3-bis(hydroxyamino)-2,3-dimethylbutane can easily be converted into the corresponding dihydrochloride, and stored in it's pure form without any decomposition. 2,3-Bis(hydroxyamino)-2,3-dimethylbutane dihydrochloride reacts readily in the presence of potassium carbonate with aldehydes, leading to the corresponding condensation products, which are quickly oxidized by sodium periodate to the corresponding radicals (Scheme 1) and giving overall yields of around 40%.

For the synthesis of compounds 5, 7, 9, and 10, the method used involves the coupling of an acid with an amine or alcohol using the DCC method (Scheme 2), with general yields of 80%.

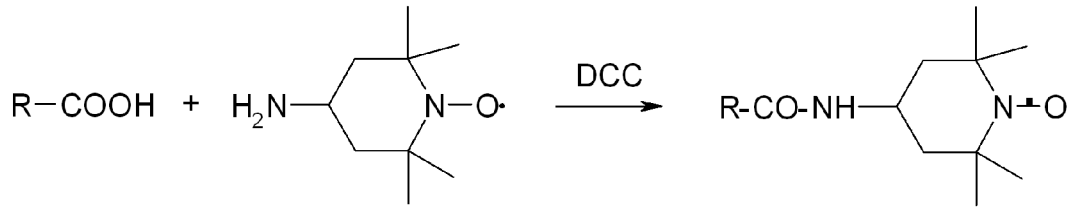
The diradicals 6 and 8 were obtained in almost quantitative yields by oxidation of 5 and 7 with solid potassium permanganate or lead dioxide. The hydrazine group is oxidized to the corresponding hydrazyl stable radical, Scheme 3.

2.2 Physico-chemical properties of radicals 1-10

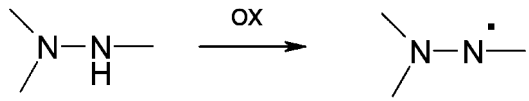
Table 1 contains all the data obtained for the radicals used in this work, including their UV-Vis and ESR characteristic data, as well as data obtained from lipophilicity determination and reaction with NO/NO_2.



Scheme 1 General method for compounds 1-4.



Scheme 2 General method for obtaining radicals 5, 7, 9, and 10.



Scheme 3 Oxidation of hydrazines to hydrazyl radicals.

2.3 Characteristic absorption spectra and chromatographic (partition) properties

All the radicals 1-10 have strong absorption in UV-Vis spectra. The nitronyl-nitroxides 1 and 3 have shown the greatest bathocromic shift (Table 1) due to the delocalisation of the unpaired electron of the respective molecule. The chromatographic behavior of the compounds has been studied using TLC and RP-TLC. TLC was performed on silica precoated plates using chloroform as mobile phase, or on impregnated silica (with paraffin oil), using a mixture of acetone/water as eluent. The method is simple, inexpensive, quick, sensitive and can be used successfully for the separation and purity control of these compounds. The R_F values of the compounds are given in Table 1.

To determine the lipophilicity (R_{M0}) and the specific hydrophobic surface area (b),

the linear correlation between the R_M values of the compounds and the concentration of organic solvent (C) in the eluent was calculated. Where, R_{M0} is the R_M value of a compound extrapolated to zero organic phase concentration in the eluent. These values (R_{M0} and b) are the best indicators of the lipophilicity and the specific hydrophobic surface area of the compounds [6, 7]. Table 1 shows the R_M, b and r values obtained for the compounds employed (where r represent the correlation coefficient). A good linear correlation was found between R_M and C, characterized by high values of r (85-99%). The values for the specific hydrophobic surface area (b) are higher in the compounds which have a higher lipophilicity. The attempt to correlate the apparent lipophilicity R_{M0} determined by RP-TLC with the calculated partition coefficient (between n-octanol-water, using the Hanch-Leo method), $\log P$ [9], led to inconclusive data, suggesting that there are other factors which should be included in the calculation.

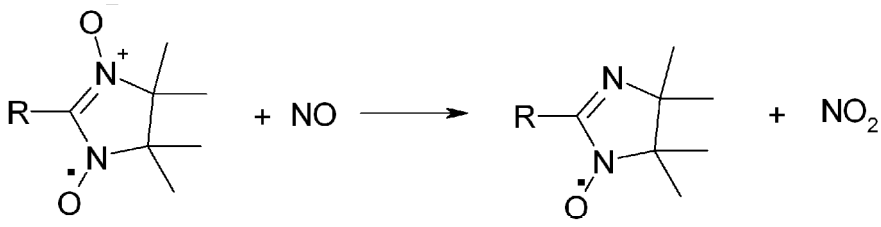
2.4 ESR spectroscopy

ESR spectroscopy is the best tool for the study of free radicals, due to the sensitivity and accuracy of the method [11, 12]. Different types of radicals show different patterns in the ESR spectra, thus our compounds could be divided into four types: Fig. 2a shows the ESR spectra of a nitronyl-nitroxide, Fig. 2b the spectra of an imino-nitroxide, Fig. 2c the classical spectra of a nitroxide, and Fig. 2d the spectra of a diradical of hydrazyl-nitroxide type. The spectra were simulated with a good fit using a Bruker software program (SimFonia).

Spin populations (Table 1) of the unpaired electron on the nitrogen atoms of the radicals 1-10 were obtained using molecular orbital calculations, following the procedure described earlier [5]. Essentially, the geometry of the molecules was first approximated using molecular mechanics MM+ force fields, and then optimized using the AM1 semi-empirical method. The values obtained are in relatively good agreement with the experimental data (see Table 1), as judged by comparison of the observed hyperfine splittings with those calculated using Q values (Q^N_{NO} = 33.1 G and Q^N_{NN} = 22.8 G for nitroxide and hydrazyl moiety) [5, 13-15].

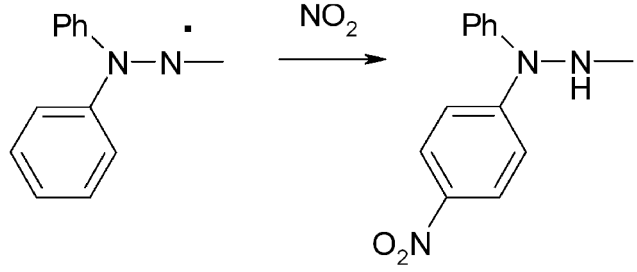
2.5 Reaction with NO and NO_2

It is well known that nitronyl-nitroxides react with NO, as described in the reaction shown in Scheme 4 [1]. The process is easily monitored by UV-Vis and ESR. The initial spectrum of nitronyl-nitroxides 1 and 3 has 5 lines (with an intensity ratio of 1/2/3/2/1 and the same a_N coupling constants),which on reaction changes to give spectras of the corresponding imino-nitroxides 2 and 4, with seven lines (intensity ratio 1/1/2/1/2/1/1 and different a_N coupling constants). See Fig. 2 for the shape of the spectra and Table 1 for a_N values. The imino-nitroxides do not react with NO or NO_2, as shown by our results and is confirmed by the literature [1, 5].



Scheme 4 The reaction of a nitronyl-nitroxide radical with NO.

According to the literature, hydrazyl radicals react with NO_2 and not with NO [5]. Indeed, our expected results were confirmed by ESR data, where only the hydrazine or hydrazyl compounds 5-8 show a change in their ESR spectra. Radical 5 lead's to the diradical 6 when it was treated with small amounts of NO_2, however, an excess of NO_2 reverse's the ESR spectra to that of the original. The process is explained as follows: NO_2 can oxidize the hydrazine to the hydrazyl radical (Scheme 3), leading to a change in the ESR spectra from the shape shown in Fig. 2c (monoradical) into the shape shown in Fig. 2d (diradical); the hydrazyl-nitroxide diradical then subsequently reacts with the excess NO_2 leading to the corresponding hydrazine-nitroxide (Scheme 5), with an ESR spectrum similar to that shown in Fig. 2c.



Scheme 5 Reaction of a hydrazyl radical with NO_2.

All these changes can be monitored by UV-Vis spectroscopy, the colour of the samples changing according to the data shown in Table 1. The compounds described can also be useful in magnetic materials studies or as sensors [16-19].

3 Conclusions

The radicals synthesized show selectivity in reaction with nitric oxide and nitrogen dioxide, depending on their structure. The processes are easily monitored by UV-Vis or ESR spectroscopy.

4 Experimental

4.1 Chemicals

3,4-(Methylendioxy)benzaldehyde, *p*-(dimethylamino)benzaldehyde, 4-amino-TEMPO, 4-hydroxy-TEMPO, eosine (tetrabromo-fluorescein), dicyclohexyl-carbodiimide (DCC), sodium nitrite, sodium periodate, potassium permanganate, lead dioxide, hydrochloric acid, as well as all the solvents employed, were used as received from Aldrich or Chimopar. TLC plates (silica gel GF 254) were from Merck. 2,3-Bis(hydroxyamino)-2,3-dimethylbutane was synthesized according to literature data [5, 10]. The precursors of the compounds 5 and 7 were synthesized as previously described [5]. The purity of the synthesized compounds was checked by TLC (single spot), low resolution mass spectra, and nitrogen elemental analysis (see Table 1). Compound 3 is already reported, see also ref [8].

4.2 Instruments

UV-Vis: Specord M40, Carl Zeiss Jena; spectra were recorded at room temperature in methylene chloride (DCM), using 1 cm quartz cell and scanning the samples from 250 nm to 800 nm. ESR: Jeol Jes-FA, the following settings were used: center field 3352 G, field width 50 G, time constant 0.3, power 0.2 mW, modulation amplitude 0.2 G, sweep time 2 min. Low resolution electrospray impact mass spectra ESI-MS (solutions in DCM) were also recorded in order to certificate the identity of the compounds 1-10.

4.3 Synthesis

2,3-Bis(hydroxyamino)-2,3-dimethylbutane dihydrochloride. Bubbling dry hydrochloric acid gas into a dichloromethane (DCM) solution which contains 2,3-bis(hydroxyamino)-2,3-dimethylbutane, the corresponding dihydrochloride is obtained as a precipitate, which was washed with DCM and dried. The compounds are pure and stable.

General procedure for obtaining compounds 1-4. To a solution in methanol of the corresponding aldehyde (1 mM) was added an excess of potassium carbonate (2 mM) and 2,3-bis(hydroxyamino)-2,3-dimethylbutane dihydrochloride (1.2 mM) and the mixture stirred overnight. Water was added and the solution was extracted with DCM. The organic solution was dried over anhydrous sodium sulphate and the solvent was partially removed. After stirring for 1 h the biphasic system obtained from the DCM solution and an aqueous solution of sodium periodate (amount?), a colour change from very pale yellow to red-purple was observed. The organic layer was separated, dried over anhydrous sodium sulphate and the solvent was removed. Chromatography on a silica gel column with chloroform as eluent afforded the desired compounds (as coloured spots).

General procedure for obtaining compounds 5, 7, 9, and 10. To the corresponding acid precursor dissolved in DCM (1mM) was added DCC (1.2 mM) and 1 mM of 4-

amino- or 4-hydroxy-TEMPO, and the reaction mixture was left for three days at room temperature. The organic solution was washed with dilute aqueous hydrochoric acid, dried over anhydrous sodium sulphate and the solvent was removed under vacuum. The residue was chromatographed on silica gel column with chloroform/methanol 9/1 v/v, affording the desired compounds.

General procedure for obtaining compounds 6 and 8. Compound 5 or 7 dissolved in DCM afforded 6 or 8 by oxidation with solid potassium permanganate or lead dioxide (stirring the reaction mixture for 2 h). Filtration and removal of the solvent afforded the diradicals as stable dark solids.

4.4 ESR measurements

ESR spectra were recorded in DCM at room temperature. The spectra were simulated with a good fit using a Bruker software program (SimFonia). Molecular modeling of the radicals was also performed using the same technique, described elsewhere (AM1 semi empirical method) [5].

4.5 Lipophilicity Determination

RP-TLC was performed on silica precoated plates impregnated by overnight predevelopment in n-hexane-paraffin oil (95/5 v/v). Chromatography was performed on 20 × 10 cm plates developed in a tank chamber at room temperature using different mixtures of acetone/water (7/3, 6/4, 5/5, 4/6, 3/7 v/v). The data were omitted from the calculation when the spot of solute remained close to the start or was very close to the front. The lipophilicities and specific hydrophobic surface area were determined using the equations $R_M = \log(1/R_F - 1)$ (1) and $R_M = R_{M0} + bC$ (2), in which R_M is the apparent lipophilicity, R_{M0} is the R_M value extrapolated to zero organic phase concentration and C the organic phase concentration of the eluent. The $\log P$ values were calculated according to the fragment methods using the fragmental constants and the relationship $\log P = \sum_{1}^{n} a_n f_n$, where a stands for the occurrences of type n structure for fragment f [9].

4.6 Reaction of compounds 1-10 with nitric oxide and nitrogen dioxide

These processes were performed in two ways: by stirring a DCM solution of the corresponding radical with an acidic aqueous solution of sodium nitrite, then separating and drying the organic layer, or by bubbling into the DCM solution the gas resulting from the reaction of sodium nitrite with hydrochloric acid. The resulting solutions were monitored by UV-Vis and ESR spectroscopy.

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Compound	1	2	3	4	5	6	7	8	9	10
λ^a_{max}	694	464	683	338	395	534	402	539	537	542
R^b_f	0.45	0.49	0.66	0.69	0.80	0.82	0.14	0.15	0.77	0.77
$\log P$ [c]	5.11	3.73	5.90	4.52	6.32	6.22	6.66	6.56	2.21	2.86
R^d_{M0}	1.79	1.65	0.93	0.49	0.10	0.10	2.16	2.15	1.20	1.28
b [d]	-2.99	-2.73	-2.08	-1.43	-0.92	-0.95	-2.05	-2.05	-3.14	-3.16
r [d]	99.8	99.3	98.9	99.4	85.1	99.3	98.9	99.7	99.5	99.1
a^e_N	7.68	8.81	7.69	8.82	15.62	15.62	15.77	15.54	15.78	15.77
	7.68	4.51	7.69	4.51		9.00		9.00		
						9.00		9.00		
Spin	0.242	0.334	0.251	0.352	0.389	0.389	0.387	0.388	0.393	0.393
population [f]	0.221	0.198	0.252	0.185		0.301		0.322		
						0.285		0.179		
Reaction	Yes	No	Yes	No	No	No	No	No	No	No
with NO [g]										
Reaction	No	No	No	No	Yes	Yes	Yes	Yes	No	No
with NO_2 [g]										
Nitrogen	10.17	10.80	15.49	16.46	12.91	12.93	15.40	15.42	3.50	1.75
elemental	10.10	10.65	15.44	16.37	12.88	12.86	15.42	15.45	3.40	1.66
analysis [h]										
ESI-Mass	275	259	276	260	548	547	727	726	801	802
Spectra (M^+)										

Table 1 Physico-chemical data of the radicals 1-10.

[a] wavelength (nm), in MeOH;
[b] retention factor; stationary phase: silica gel plates impregnated with paraffin oil, eluent acetone/water 6/4 v/v;
[c] partition coefficient, calculated (see text);
[d] R_{M0} is the lipophilicity, b is hydrophobic surface area, while r is the correlation factor, experimentally determined (see text);
[e] hyperfine splitting constants (G), in DCM at room temperature;
[f] spin population on the nitrogen atoms of the free radicals;
[g] as seen by UV-Vis and ESR, for details see text;
[h] calculated (top) *vs.* found (bottom).

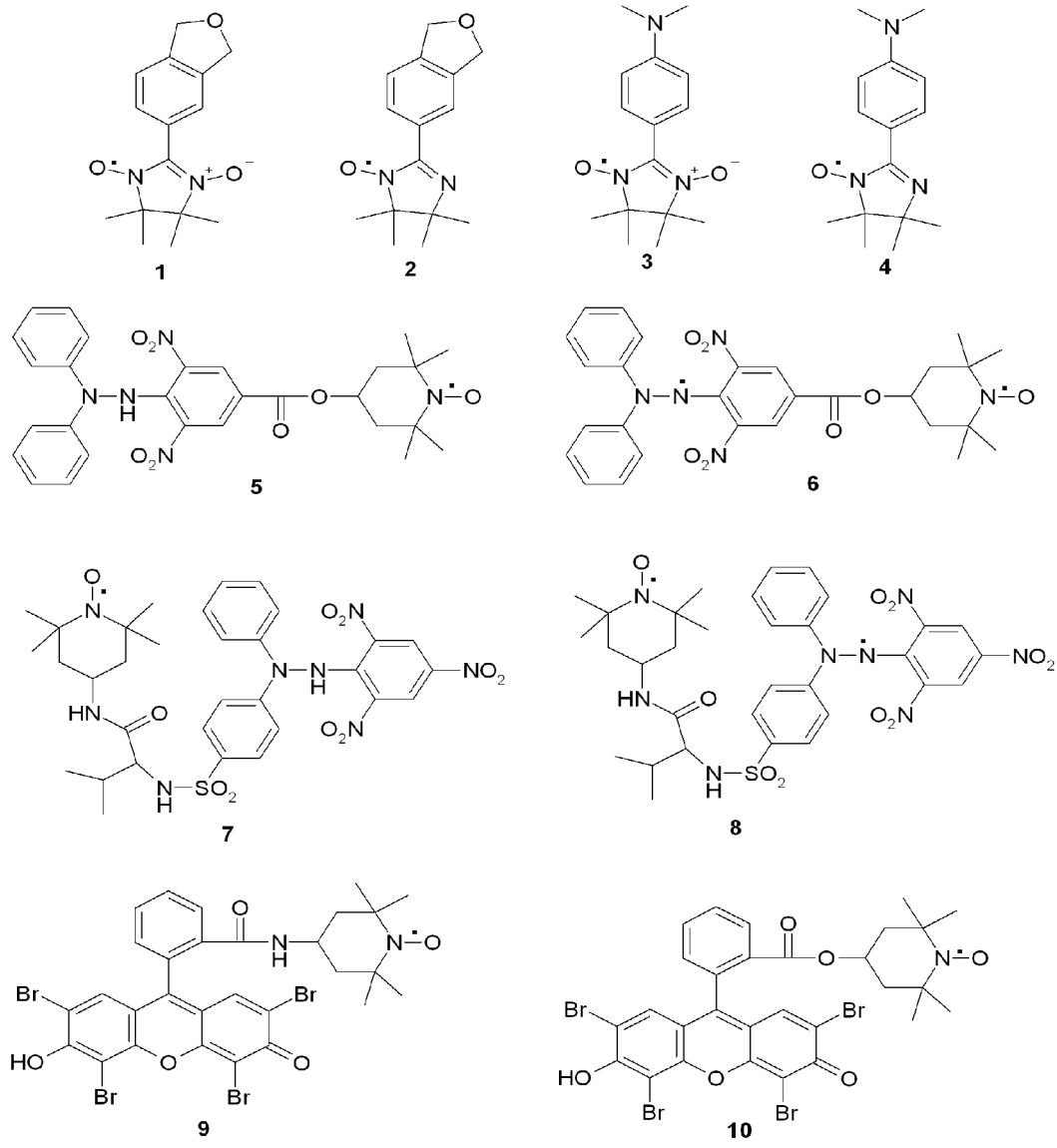
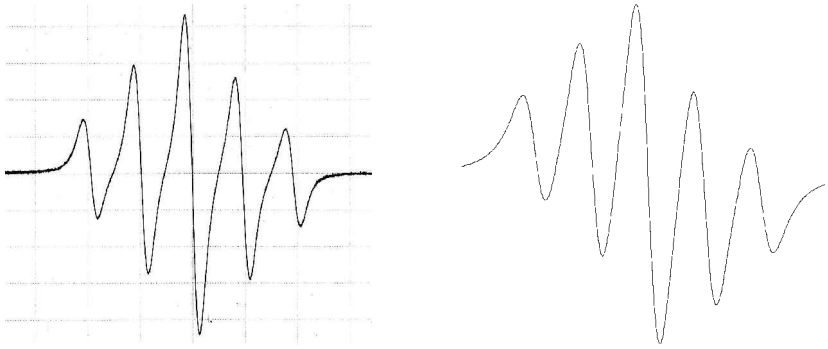
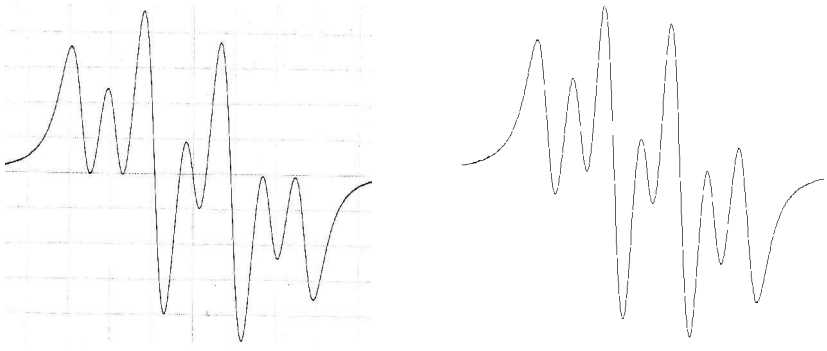


Fig. 1 Chemical structures of the synthesized free radicals **1**-**10:**

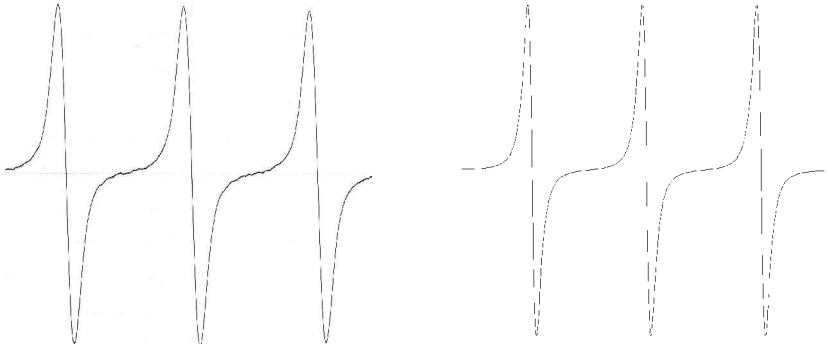
1 2-(1,3-Dihydro-isobenzofuran-4-yl)-4,4,5,5-tetramethyl-3-oxy-4,5-dihydro-imidazol-1-oxyl;
2 2-(1,3-Dihydro-isobenzofuran-4-yl)-4,4,5,5-tetramethyl-4,5-dihydro-imidazol-1-oxyl;
3 2-(4-Dimethylamino-phenyl)-4,4,5,5-tetramethyl-3-oxy-4,5-dihydro-imidazol-1-oxyl;
4 2-(4-Dimethylamino-phenyl)-4,4,5,5-tetramethyl-4,5-dihydro-imidazol-1-oxyl;
5 4-(N,N-Diphenyl-hydrazino)-3,5-dinitro-benzoic acid 1-oxyl-2,2,6,6-tetramethyl-piperidin-4-yl ester;
6 4-(N,N-Diphenyl-hydrazyl)-3,5-dinitro-benzoic acid 1-oxyl-2,2,6,6-tetramethyl-piperidin-4-yl ester;
7 N-(1-Oxyl-2,2,6,6-tetramethyl-piperidin-4-yl)-3-methyl-2-{4-[N-phenyl-N-(2,4,6-trinitro-phenyl)-hydrazino]-phenylsulfonylamino}-butyramide;
8 N-(1-Oxyl-2,2,6,6-tetramethyl-piperidin-4-yl)-3-methyl-2-{4-[N-phenyl-N-(2,4,6-trinitro-phenyl)-hydrazyl]-phenylsulfonylamino}-butyramide;
9 N-(1-Oxyl-2,2,6,6-tetramethyl-piperidin-4-yl)-2-(2,4,5,7-tetrabromo-6-hydroxy-3-oxo-3H-xanthen-9-yl)-benzamide;
10 N-(1-Oxyl-2,2,6,6-tetramethyl-piperidin-4-yl)-2-(2,4,5,7-tetrabromo-6-hydroxy-3-oxo-3H-xanthen-9-yl)-benzoic acid ester.



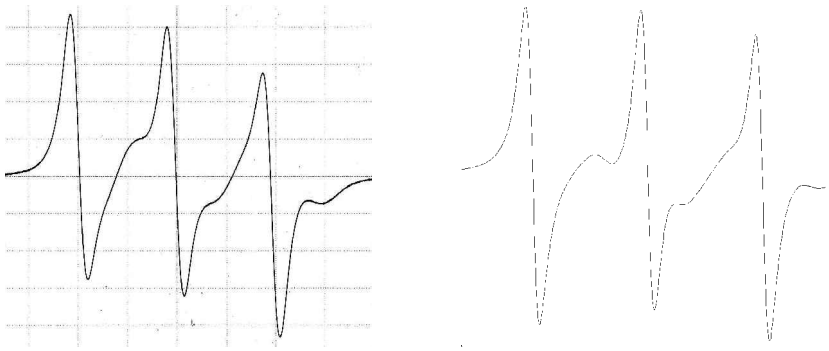
a) Typical ESR spectra of 1 and 3 (left) and simulation (right)



b) Typical ESR spectra of 2 and 4(left) and simulation (right)



c) Typical ESR spectra of 5, 7, 9 and 10 (left) and simulation (right)



d) Typical ESR spectra of 6 and 8 (left) and simulation (right)

Fig. 2 ESR spectra of the new radicals synthesized (left) and their simulation (right); for ESR hyperfine splitting constants see Table 1