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Synthesis of Isotopically-Labelled Nitroxyl Radicals for Use as Spin Probes

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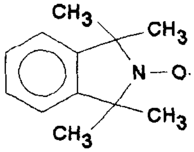
ABSTRACT

The synthesis of [^{15}N]-1,1,3,3-tetrakis(trideuteriomethyl)isoindolin-2-yloxyl and of some 5-alkyl derivatives have been achieved by standard synthetic routes.[1] The presence of deuterium improves the sharpness of the signals, and this, together with the incorporation of nitrogen-15, enhances the application of such compounds as spin probes; alkyl substituents at C-5 improve the lipophilicity.

Keywords: Nitroxyl radicals, spin probes, isoindolin-2-yloxyl.

INTRODUCTION

We have recently reported the synthesis of the stable nitroxide radical 1,1,3,3-tetramethylisoindolin-2-yloxyl,[2]



and the preparation of some 5-alkylated derivatives. Our detailed EPR studies of these radicals have revealed the considerable potential of this class of radicals as spin probes[2].

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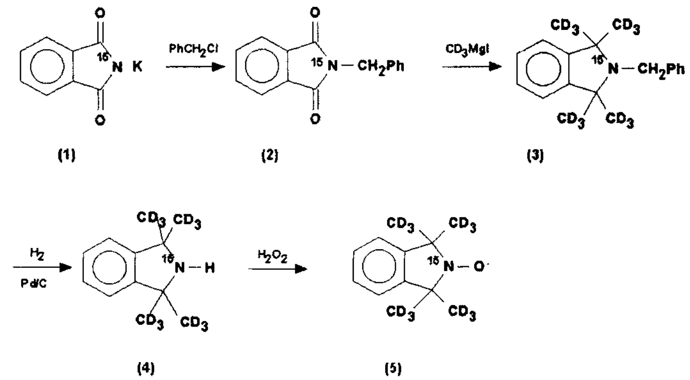
Deuterium and nitrogen-15 labelled radicals show increased sensitivity in EPR measurements by simplifying the EPR spectrum and by sharpening the lines; this has particular importance in *in vivo* EPR and for EPR imaging, and for dynamic nuclear polarisation experiments at low magnetic fields[3].

In this Paper we report the synthesis of deuterated and nitrogen-15 labelled 1,1,3,3-tetramethylisoindolin-2-yloxyl, and of some deuterated 5-alkyl analogues. These radicals are of especial interest when fat-soluble spin probes are required. The synthetic routes are shown in Schemes 1 and 2.

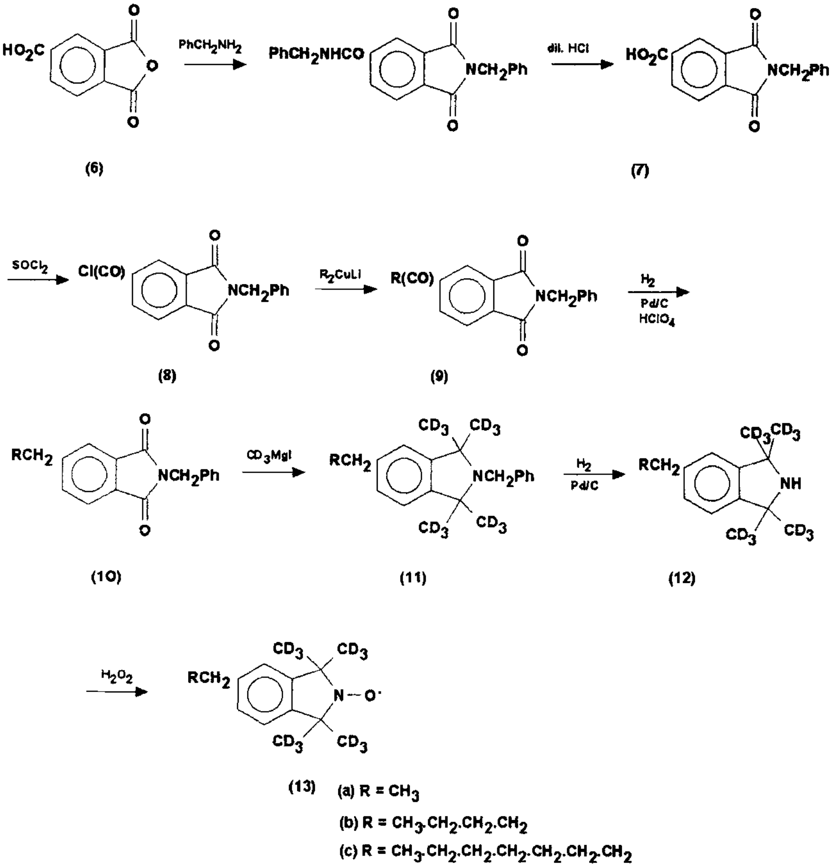


Scheme 1: Synthesis of ^{15}N-1,1,3,3-Tetrakis(trideuteriomethyl)isoindolin-2-yloxyl

EXPERIMENTAL

^{1}H-NMR spectra were measured at 300 MHz using a Bruker AC-300E spectrometer, with $CDCl_3$ as solvent and $SiMe_4$ as internal standard. EPR spectra were measured usng a Jeol RE1X spectrometer. We thank Mr J P Bloxsidge for help with the NMR spectra and Mrs. N Walker for elemental analyses. Melting points were obtained using a Koffler hot stage. Methyl-lithium, n-butyl-lithium, potassium $[^{15}N]$-phthalimide and $[^2H_3]$-methyl magnesium iodide were commercial materials (Aldrich).

1-Heptyl-lithium was obtained from 1-chloroheptane (0.10 mole) and lithium wire (0.20 mole) in light petroleum (b.p. 40°-60°) by boiling for 7 hours. Alkyl-lithium solutions were standardised by titration with diphenylacetic acid.[4]



Scheme 2: Synthesis of 5-alkyl-^{15}N-1,1,3,3-Tetrakis(trideuteriomethyl)isoindolin-2-yloxyl

[^{15}N]-Benzylphthalimide (**2**) was prepared by boiling a mixture of potassium -[^{15}N]-phthalimide (1.0 g., 5.4 mmol) with benzyl chloride (2.5 ml., 20 mmol) for 8 hours, removing the excess of reagent under reduced pressure, and extracting (Et_2O) the product (1.28 g., 100%. M.p. 115° *ex* EtOH. ^{1}H-NMR δ 7.84 (q, 2H), 7.70 (q, 2H). 7.44 (d, 2H), 7.34-7.26 (m, 6H), 4.85 (s, 2H)).

[^{15}N]-Benzyl-1,1,3,3-tetrakis(trideuteriomethyl)isoindoline (**3**) d_3-Methyl magnesium iodide in diethyl ether (1M; 40 ml, 40 mmol) was treated under N_2 with a solution of **2** (1.3 g., 5.4 mol) in dry toluene (100 ml.) dropwise, with stirring. The mixture was then distilled until the flask temperature reached 110° and held at this temperature for 5 hours. The mixture was then concentrated to 20 ml. by distillation and the cooled residue was treated with light petroleum (20 ml.; b.p. 60°-80°) and filtered through Celite. The flask residue was extracted with light petroleum (2 x 10 ml.; b.p. 60°-80°) and also passed through the Celite bed, and the combined solutions were then passed through a short column of alumina (alkaline; I). Evaporation of the colourless eluate gave **3** (0.3 g., 1.1 mmol, 21%. M.p. 63° *ex* EtOH. ^{1}H-NMR δ 7.45 (d, 2H), 7.33-7.16 (m, 5H), 7.14 (q, 2H), 3.98 (s, 2H)).

Analogously, ***2-benzyl-5-pentyl-1,1,3,3-tetrakis(trideuteriomethyl)isoindoline*** (**11**) was obtained from **10** (^{1}H-NMR δ 7.44 (d, 2H), 7.28 (m, 3H), 7.06 (q, 2H), 6.95 (s, 1H), 3.96 (s, 2H), 2.60 (t, 2H), 1.59 (quintet, 2H), 1.27 (m, 4H), 0.88 (t, 3H)).

[^{15}N]-1,1,3,3-Tetrakis(trideuteriomethyl)isoindoline (4) A solution of **3** (0.3 g., 1.1 mmol) in acetic acid (50 ml.) and Pd/C (10%; 1 g) was hydrogenated (60 p.s.i.) for 3 hours at room temperature. The filtered solution was treated with water (5 ml.), made alkaline with sodium hydroxide (10%) and extracted (Et_2O; 3 x 20 ml.). The combined organic extract gave **4** in quantitative yield (m.p. 36°. ^{1}H-NMR δ 7.25 (q, 2H), 7.12 (q, 2H)).

Similarly, ***5-pentyl-1,1,3,3-tetrakis(trideuteriomethyl)isoindoline*** (**12**) was obtained from **11** (^{1}H-NMR δ 7.07 (d, 1H), 7.03 (d, 1H), 2.61 (t, 2H), 1.62 (quintet, 2H), 1.34 (m, 4H), 0.90 (t, 3H)).

[^{15}N]-1,1,3,3-Tetrakis(trideuteriomethylisoindolin-2-yloxyl (5) A solution of **4** (0.2 g., 1.1 mmol) in methanol (20 ml.) - acetonitrile (2 ml.) was treated successively with sodium hydrogen carbonate (0.26 g., 3 mmol), sodium tungstate dihydrate (0.04 g., 0.1 mmol) and hydrogen peroxide (30 % v/v; 2 ml, 18 mmol). The suspension was stirred at room temperature for 48 hours when the resulting bright-yellow solution was diluted with distilled water and extracted with light petroleum (b.p. 60°-80°; 2 x 20 ml.). The combined organic layers were washed twice with sulphuric acid (2M) and brine; on evaporation 1,1,3,3-tetrakis(trideuteriomethyl)isoindolin-2-yloxyl was obtained as bright yellow crystals, m.p. 125° *ex* light petroleum and showing two broad peaks in the ^{1}H-NMR spectrum at δ 9.5-7.5 and 6.5-4.5. Found: C, 71.05; H, 8.22; N, 6.92. $C_{12}H_4D_{12}NO$ requires C, 70.90; H, 7.90; N, 6.89%.

In analysing compounds containing hydrogen isotopes, we found that our combustion method allowed nearly complete exchange so that all hydrogen was assessed as H_2O.

Thus, naphthalene ($C_{10}H_8$ requires C, 93.71; H, 6.29%) gave combustion analyses C, 92.62; H, 6.13% and $C_{10}D_8$ gave C, 87.18 (required, 88.17%) and H, 6.00% (required, 11.83%). These results are consistent with nearly complete exchange between D and H so that the water of combustion was weighed as H_2O regardless of the isotope originally oxidised.

In the same way, **13** was obtained from **12**.

2-Benzyl-isoindolin-1,3-dione-5-carboxylic acid (7) 1,2,4-Benzenetricarboxylic acid anhydride (10 g., 50 mmol) and benzylamine (11 ml., 100 mmol) were boiled in acetic acid (250 ml.) for 2 hours. The solvent was then removed (rotary evaporator) and the solid residue was dissolved in hot water-acetic acid mixture (1:4, v/v). Upon treatment with hydrochloric acid (3M) a precipitate formed immediately; after boiling the mixture for 10 minutes, the cooled suspension was filtered to give 2-benzylisoindoline-1,3-dione-5-carboxylic acid (10 g., 36 mmol, 72%. M.p. 200°. Found, C, 68.56; H, 3.80; N, 4.80. $C_{16}H_{11}NO_4$ requires C, 68.32; H, 3.94; N, 4.98%. ^{1}H-NMR δ 8.56 (s, 1H), 8.46 (d, 1H), 7.96 (d, 1H), 7.44 (d, 2H), 7.30 (m, 3H), 4.88 (s, 2H)).

2-Benzylisoindolin-1,3-dione-5-carboxylic acid chloride (**8**) The acid 7 (10 g., 36 mmol) and $SOCl_2$ (5 ml., 95 mmol) was boiled for 2 h by which time HCl evolution had ceased. The residue was extracted exhaustively with boiling light petroleum (b.p. 60-80°); the acid chloride (70-85%; m.p. 79°-81°) precipitated upon cooling. This halide may be kept in light petroleum solution, or in a vacuum desiccator, for some while without decomposition but was usually used within 24 hours. (Found, C, 63.98; H, 3.21; N, 4.59. $C_{16}H_{10}ClNO_3$ requires C, 64.11; H, 3.34; N, 4.67%. ^{1}H-NMR δ 8.57 (s, 1H), 8.46 (d, 1H), 7.99 (d, 1H), 7.43 (d, 2H), 7.30 (m, 3H), 4.89 (s, 1H)).

2-Benzyl-5-pentanoylisoindolin-1,3-dione (9) Copper(I) iodide (1.52 g., 8.0 mmol) in anhydrous diethyl ether (30 ml.) was treated with butyl-lithium (1M; 16 ml., 16 mmol) at -40°. After 5 minutes at this temperature, the dark brown solution was cooled to -78° and a pre-cooled solution of **8** (0.80 g., 2.67 mmol) was added at this temperature. After 15 minutes at -78° the reaction mixture was quenched (methanol, 500 mg.) and the reaction mixture was allowed to reach room temperature.

It was worked up by pouring into an equal volume of saturated aqueous NH_4Cl, and the organic layer was washed, dried ($MgSO_4$) and concentrated to give **9** (1.20 g., 50 %. M.p. 115-117°. Found: C, 78.20; H, 5.76; N, 4.32. $C_{20}H_{21}NO_2$ requires C, 78.15; H, 5.96; N, 4.36%. ^{1}H-NMR δ 8.37 (s, 1H), 8.30 (d, 1H), 7.93 (d, 1H), 7.43 (d, 2H), 7.29 (m, 3H), 4.87 (s, 2H), 3.02 (t, 2H), 1.74 (quintet, 2H), 1.43 (sextet, 2H), 0.96, (t, 3H)).

Analogously, methyl-lithium at 0° gave ***2-benzyl-5-acetylisoindolin-1,3-dione.***

2-Benzyl-5-pentyl-isoindolin-1,3-dione (10) A solution of **9** (1.2 g., 3.7 mmol) in acetic acid (100 ml.) was treated with perchloric acid (70%; 1.0 ml.) and Pd/C (10%; 0.5 g.). Upon hydrogenation (r.t.; 35 psi; 2 hours) the filtered (Celite) reaction mixture was evaporated, treated with water (10 ml.) and extracted with ether to give the solid **10** in quantitative yield (m.p. 72° from EtOH. Found: C, 77.90; H, 6.82; N, 4.51. $C_{20}H_{21}NO_2$ requires C, 78.14; H, 6.89; N, 4.56%)

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