Synthesis, single crystal X-ray structure and W-band (95 GHz) EPR spectroscopy of a new anionic isoindoline aminoxyl: synthesis and characterisation of some derivatives

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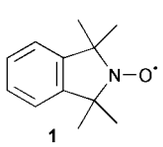
Here we describe the improved synthesis and full characterisation, including W-band EPR analysis and X-ray crystallography, of an anionic isoindoline aminoxyl, 5-carboxy-1,1,3,3-tetramethylisoindolin-2-yloxyl **6** (CTMIO), which is more water soluble than the parent 1,1,3,3-tetramethylisoindolin-2-yloxyl **1** (TMIO). We also report the synthesis and characterisation of three novel CTMIO derivatives: the *n*-hexyl ester **9**, anhydride **10**, and *N*-hydroxysuccinimide ester **11**.

Selective $AlCl_3$-catalysed bromination of 2-benzyl-1,1,3,3-tetramethylisoindoline **2** produces the aminoxyl precursor, 5-bromo-1,1,3,3-tetramethylisoindoline **3** in high yield. Lithiation and subsequent carboxylation of this species generates 5-carboxy-1,1,3,3-tetramethylisoindoline **5**, which undergoes tungstate–H_2O_2 oxidation to give the target aminoxyl, CTMIO **6**. The analogous synthesis of 5-formyl-1,1,3,3-tetramethylisoindolin-2-yloxyl **8** is also described.

W-band (95 GHz) EPR studies of toluene solutions of CTMIO, both liquid and frozen, indicate that aggregates are formed: the liquid state was used to measure the equilibrium dimer formation constant (30 ± 40 M^{-1} at 298 K); the solid state was used to estimate the distance (19.3 Å) between the two aminoxyl groups in the dimer. A single crystal X-ray examination reveals that there is short-range intermolecular hydrogen bonding between aminoxyl and carboxylic groups, and that the aminoxyl groups are separated by 10.2 Å. However, it is concluded from the EPR spectral evidence that, in toluene solution, the dimers are formed by hydrogen bonding between pairs of carboxylic groups. Using the molecular dimensions determined by X-ray analysis, the distance between the aminoxyl groups in the dimer in frozen solution was estimated to be 17 Å.

Introduction

Isoindoline-based aminoxyls such as 1,1,3,3-tetramethylisoindolin-2-yloxyl[1] **1** (TMIO) are known to have a variety of advantages[2–5] over commercially available aminoxyls, including the commonly used pyrrolidine and piperidine species. The fused aromatic moiety of the isoindoline skeleton provides resistance to the ring-opening reactions that are significant decomposition pathways for pyrrolidine and piperidine aminoxyls. In addition, isoindoline aminoxyls demonstrate superior intrinsic electron paramagnetic resonance (EPR) linewidths[6] and excellent thermal and chemical stability in a wide variety of chemical environments.



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Isoindoline aminoxyls, such as TMIO **1**, scavenge carbon-, sulfur- and phosphorus-centred radicals at near diffusion-controlled rates, and have subsequently been extensively utilised as radical traps in the investigation of radical-mediated polymerisation processes.[4,5,7,8] The UV chromophore of the aromatic ring facilitates HPLC separation and subsequent characterisation of the spin adducts.

More recently, following full NMR and EPR analyses[9,10] of **1** and its isotopically labelled ^{15}N and tetrakis(trideuteriomethyl)[11,12] analogues, isoindoline aminoxyls have been successfully applied as EPR spin probes in a variety of systems. The 5-*n*-octyltetrakis(trideuteriomethyl) analogue of TMIO[11] has been successfully used as a matched spin probe of molecular motion in a low traction lubricant fluid.[13] TMIO, its 5-*n*-pentyl[11] and water-soluble sulfonate (NaTMIOS) analogues,[14] have been utilised to study the effects of heating on the starch and lipid components of dough.[15]

The ease of synthesis of ^2H and ^{15}N labelled derivatives[11] of the isoindoline aminoxyls provides access to probes with specifically tailored spectroscopic characteristics, widening the scope of their application, as has been demonstrated by the development of a simple EPR technique for the measurement of translational diffusion constants using the tetrakis(trideuteriomethyl) analogue (TMIOD) of TMIO in conjunction with the deuterated 5-sulfonate derivative (NaTMIODS).[16–18]

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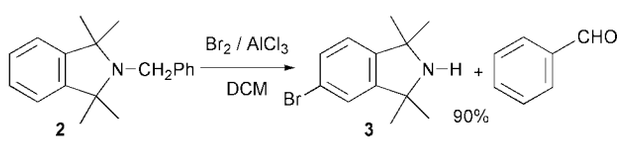
While the suitability of isoindoline aminoxyls for use as spin probes has been adequately demonstrated, their full potential has been somewhat limited by a lack of structural variation, especially with regards to water solubility. To this end, we have synthesised a range of new substituted isoindoline aminoxyls, including halogenated[19] and carboxy species (CTMIO, **6**)[20] suitable for further derivatisation. An EPR method has been used to measure the partitioning properties of CTMIO and a variety of other isoindoline and piperidine aminoxyls.[6] Such characterisation of these compounds paves the way for their application as spin probes/labels in a variety of systems, including biological environments.

Here we report the improved synthesis, EPR characterisation and X-ray crystallography of 5-carboxy-1,1,3,3-tetramethylisoindolin-2-yloxyl **6** (CTMIO). CTMIO appears to form aggregates in toluene solutions and this has been investigated by a high-field (W-band) EPR study and by X-ray examination of a single crystal. We also report the synthesis and characterisation of several CTMIO derivatives: the *n*-hexyl ester **9**, anhydride **10**, and *N*-hydroxysuccinimide ester **11**, as well as the synthesis of a related aminoxyl, 5-formyl-1,1,3,3-tetramethylisoindolin-2-yloxyl **8**.

Results and discussion

Bromination of 2-benzyl-1,1,3,3-tetramethylisoindoline (2)

The treatment of 2-benzyl-1,1,3,3-tetramethylisoindoline **2** with bromine in the presence of anhydrous $AlCl_3$ results in the oxidative cleavage of the benzyl group and bromination of the isoindoline ring to give 5-bromo-1,1,3,3-tetramethylisoindoline **3** in high yield (Scheme 1). The oxidative cleavage of the benzyl



Scheme 1 Bromination of 2-benzyl-1,1,3,3-tetramethylisoindoline **2**.

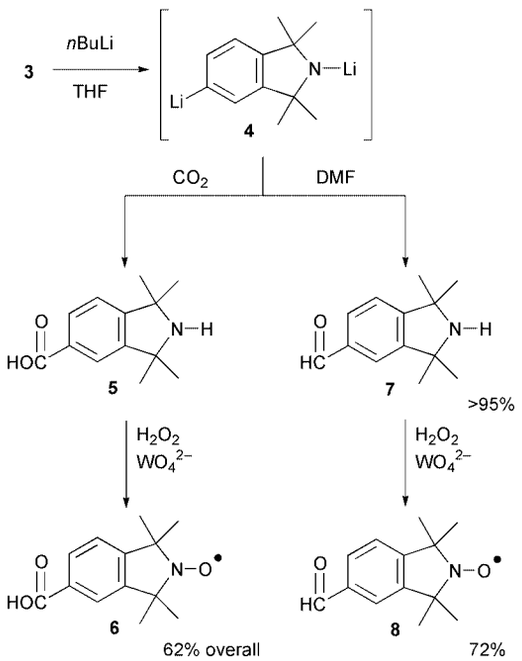
group by Br_2 to give benzaldehyde and the isoindoline species has been discussed previously.[19]

Selective monobromination of the isoindoline aromatic ring is highly dependent upon the use of an excess (3.5 equiv.) of anhydrous powdered $AlCl_3$ and an approximately stoichiometric volume of Br_2 (2.2 equiv.). The presence of a small excess of Br_2 upon quenching results in formation of the bromoamine, although this is not problematic as the free amine is regenerated by H_2O_2 during workup. A greater excess of Br_2 is avoided, however, as it results in dibromination of the aromatic ring to give the 5,6-dibromo analogue of **3**. The reaction is also sensitive to solvent effects with dichloromethane giving the most consistent results and highest yields.

Synthesis of 5-carboxy-1,1,3,3-tetramethylisoindolin-2-yloxyl (6)

Standard lithiation and subsequent carboxylation of **3** produces zwitterionic 5-carboxy-1,1,3,3-tetramethylisoindoline **5**, as shown by NMR. Isolation of this compound from inorganic salts produced during workup is problematic. Subsequently, the water-soluble mixture of product and salts is treated using standard H_2O_2–tungstate oxidation to generate the target species, 5-carboxy-1,1,3,3-tetramethylisoindolin-2-yloxyl **6** (CTMIO) in good yield (Scheme 2). This compound is easily isolated and purified *via* column chromatography and recrystallisation.

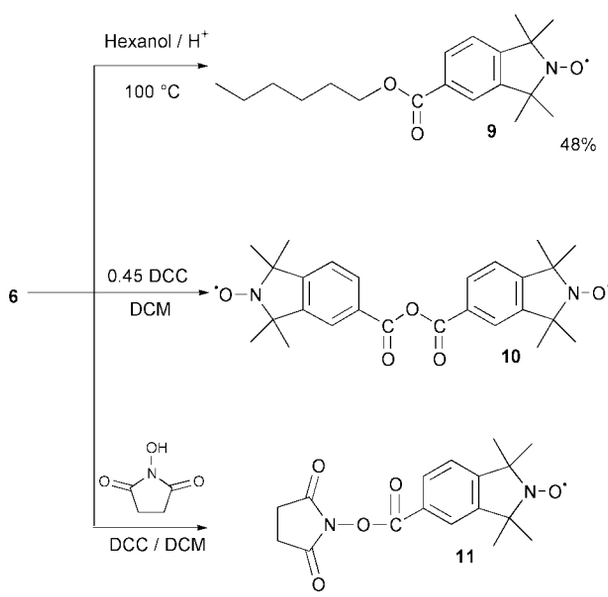
CTMIO (**6**) is water soluble at concentrations up to ~2 mM, although dissolution from well-formed crystals is difficult. The pK_a of the compound (pK_a 3.3 ± 0.1) was determined by titration of a 1 mM aqueous solution with 1 mM NaOH. CTMIO has been found to partition between water and *n*-octanol in a ratio of 17 : 83 (log K_{ow} = 0.69).[6]



Scheme 2 Synthesis of 5-carboxy- and 5-formyl-1,1,3,3-tetramethylisoindolin-2-yloxyl (**6** and **8**).

Derivatives of 5-carboxy-1,1,3,3-tetramethylisoindolin-2-yloxyl (6)

Several simple derivatives of CTMIO **6** were synthesised in order to demonstrate the possibilities for spin labelling with this compound (Scheme 3). The *n*-hexyl ester **9** was produced



Scheme 3 Synthesis of CTMIO derivatives.

in reasonable yield (48%) using standard methodology. This compound offers a lipophilic spin label with extended alkyl functionality as an alternative to the 5-alkylisoindoline aminoxyls,[11] which require more demanding syntheses. In addition, under the appropriate conditions, **9** may be hydrolysed to the parent CTMIO, which possesses significantly different partitioning characteristics.

Of somewhat more interest are the activated forms of **6**, the *N*-hydroxysuccinimidyl ester **11** and anhydride **10**. These species allow the possibility of labelling molecular species with CTMIO under relatively mild conditions. The *N*-hydroxysuccinimidyl esters of carboxylic acids are well-established and

Table 1 Comparison of observed and predicted chemical shifts for 5- and 5,6-substituted 1,1,3,3-tetramethylisoindoline species

		Chemical shifts observed (predicted[a])/ppm			
Nucleus	Position	5-Bromo[19]	5,6-Dibromo[19]	5-Carboxy	5-Formyl
^{1}H	CH_3	1.43, 1.45	1.42	1.70	1.46, 1.47
	4	7.23 (7.30)	7.34 (7.22)	7.88 (7.97)	7.64 (7.68)
	6	7.34 (7.42)	—	7.94 (8.09)	7.75 (7.80)
	7	6.98 (7.04)	7.34 (7.22)	7.45 (7.30)	7.25 (7.34)
^{13}C	CH_3	31.7	31.5	28.5	31.8, 31.9
	1	62.5	62.4	68.0	62.4
	3	62.6	62.4	68.0	62.8
	3a	151.2 (150.7)	150.2 (149.7)	143.0 (148.5)	149.9 (149.7)
	4	124.7 (124.8)	126.8 (127.0)	123.3 (122.7)	122.4[b] (122.7)
	5	121.3 (121.6)	122.8 (124.9)	130.7 (129.1)	136.2 (136.0)
	6	130.1 (130.3)	122.8 (124.9)	123.7 (128.2)	130.0 (128.2)
	7	123.0 (123.7)	126.8 (127.0)	122.3 (121.5)	122.0[b] (122.7)
	7a	147.8 (147.5)	150.2 (149.7)	146.8 (152.9)	155.8 (154.5)

[a] Calculated using reassigned aromatic chemical shifts for 1,1,3,3-tetramethylisoindoline; δ_H (299.95 MHz; $CDCl_3$,) 7.12 (2H, dd, *J* 3.2 and 5.4, 4-H and 7-H), 7.24 (2H, dd, *J* 3.2 and 5.4, 5-H and 6-H); δ_C (75.43 MHz; $CDCl_3$) 121.5 (C-4 and C-7), 127.0 (C-5 and C-6), 148.5 (C-3a and C-7a). [b] Assignment of these signals is arbitrary due to a lack of distinguishing characteristics and identical predicted shifts.

effective acylating agents, and are commonly utilised in the spin labelling of biological species.

Synthesis of 5-formyl-1,1,3,3-tetramethylisoindolin-2-yloxyl (8)

A dimethylformamide quench of the lithiated isoindoline **4** gives 5-formyl-1,1,3,3-tetramethylisoindoline **7** in almost quantitative yield (Scheme 2). This compound was identified by NMR and mass spectroscopy, and oxidised without further characterisation, *via* standard H_2O_2–tungstate oxidation to give the corresponding aminoxyl 5-formyl-1,1,3,3-tetramethylisoindolin-2-yloxyl **8**, which was fully characterised. Oxidation of the secondary amine was achieved without oxidising the aldehyde moiety.

NMR Spectroscopy

The unequivocal assignment of the aromatic NMR signals of 1,1,3,3-tetramethylisoindoline has proven difficult previously.[10] The synthesis and NMR spectroscopy, however, of a variety of substituted analogues of this compound, such as **3**, **5** and **7**, have permitted unambiguous allocation of most of the ^{1}H and ^{13}C NMR signals. Prediction of the aromatic chemical shifts of 5- or 5,6- substituted tetramethylisoindoline analogues, using published substituent constants in conjunction with previously reported[10,19] base values for the unsubstituted compound, has been found to give shifts which are in poor agreement with experimental results. Reassignment of the aromatic chemical shifts of 1,1,3,3-tetramethylisoindoline and recalculation, however, have been found to give very good agreement with observed values (Table 1).

W-Band EPR spectroscopy of 5-carboxy-1,1,3,3-tetramethylisoindolin-2-yloxyl (6) (CTMIO)

In a previous paper,[6] we presented the X-band (9 GHz) EPR spectrum of CTMIO in frozen perdeuteriotoluene. The broad spectrum obtained is indicative of dimer formation and a simulation assuming the presence of a biradical indicated that the paramagnetic NO centres are separated by about 20 Å. In order to obtain a more accurate estimate of this distance, we have recorded the W-band spectrum of CTMIO in perdeuteriotoluene at low temperatures. The higher frequency provides a spectrum more sensitive to biradical formation as may be seen from Fig. 1 (the experimental spectrum is shown as dots). The presence of a biradical species is evident from the splitting of the nitrogen hyperfine components of the *z*-manifold. The computer program SIMPIP (based upon the powder

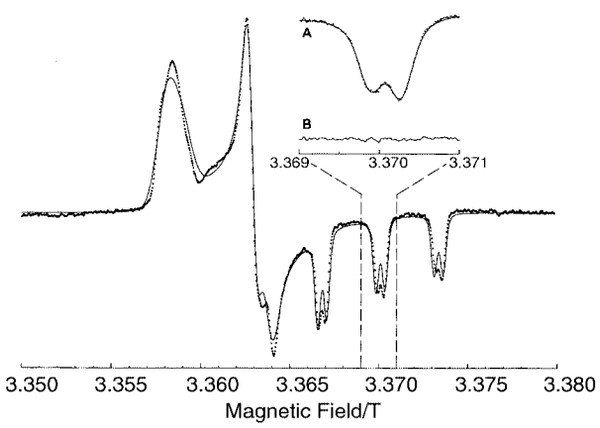


Fig. 1 W-Band EPR spectrum (dots) of 5-carboxy-1,1,3,3-tetramethylisoindolin-2-yloxyl (**6**) (CTMIO) in perdeuteriotoluene at 130 K. The full line shows the simulated spectrum obtained using the parameters: $D = 0.38$ mT, $g_{xx} = 2.00909$, $g_{yy} = 2.00616$, $g_{zz} = 2.00220$, $A_{xx} = 0.550$ mT, $A_{yy} = 0.414$ mT, $A_{zz} = 3.271$ mT, and Gaussian line widths of 0.90, 0.43 and 0.26 mT for the *x*, *y* and *z* directions respectively; the isotropic Lorentzian linewidth is 0.71 mT. The more precise fitting of the central component of the *z*-manifold is shown in the expansion: **A** shows the experimental spectrum (dots) and the fitting (full line); **B** is the residual.

spectrum simulation program QPOW[21]) was used to simulate the spectrum (Fig. 1). In order to simplify the simulations, we have assumed that the axes of the aminoxyl groups are parallel. In this approximation, the zero-field splitting, *D*, is axial but its axis could be tilted with respect to the aminoxyl groups. The best least-squares fit is shown in Fig. 1 (solid line). If the rings carrying the aminoxyl groups are in the plane of the inter-spin vector, then the splitting on the *z*-manifold is *D*. The best value of *D* was obtained by fitting the three hyperfine components of the *z*-manifold, giving a value of 0.38 ± 0.01 mT (the fitting of the central component of the *z*-manifold is shown in Fig. 1A). This leads to an estimate[22] of 19.3 Å for the separation of the two NO groups, which we assume to be equivalent in the dimer. It is worthwhile noting that recently,[23] more sophisticated simulation methods were developed for the analysis of the EPR spectra from aminoxyl pairs in which the NO groups adopt an arbitrary mutual orientation. Model simulations showed that the splitting on the *z*-manifold is particularly sensitive to the angle between the aminoxyl *z*-axis and the inter-spin vector. For example, if the *z*-axes of the NO groups of a biradical are parallel to each other but if the angle

Table 2 Concentration of CTMIO **6** monomer and dimer in toluene solution

	Concentration/mM	
Temperature/K	Monomer	Dimer
270	0.828 ± 0.153	0.0861 ± 0.0108
250	0.663 ± 0.102	0.169 ± 0.016
240	0.422 ± 0.050	0.289 ± 0.022
230	0.267 ± 0.036	0.366 ± 0.033
220	0.129 ± 0.021	0.436 ± 0.041

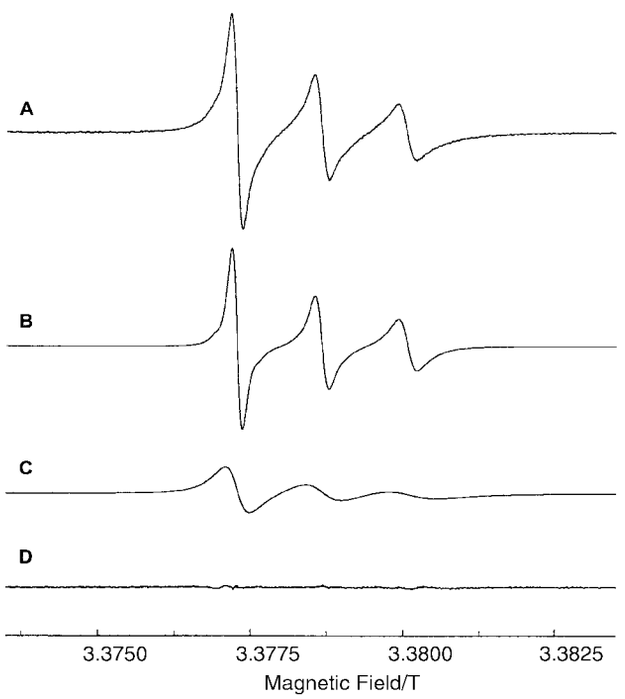


Fig. 2 The W-band solution spectrum of 5-carboxy-1,1,3,3-tetramethylisoindolin-2-yloxyl (**6**) (CTMIO) in perdeuteriotoluene at 240 K, showing the experimental and simulated spectra: **A** is the experimental spectrum; **B** is the deconvoluted spectrum of the monomer; **C** is the deconvoluted spectrum of the dimer; **D** is the residual.

between these axes and the inter-spin vector is approximately 54.7° then there is no splitting on the *z*-manifold. If in the dimer there is some tilt between the aminoxyl's *xy*-plane and the inter-spin vector, then the distance we estimated of 19.3 Å is just an upper limit for the NO group separation. Clearly, a multi-frequency EPR study and global simulation analysis are necessary to obtain an unambiguous determination of the dimer geometry from the EPR data.

Toluene solution W-band spectra of CTMIO obtained in the temperature range 220 to 270 K were used to get an estimate of the equilibrium constant for dimer formation. The spectra of the monomer and dimer are different due to the dipolar broadening effect and differences in the rotation correlation times, that is, the spectra have different linewidths. The dimer population can be seen more clearly at high microwave frequency (W-band) because of the enhanced sensitivity of the EPR line shape to the aminoxyl tumbling rate. The spectra were deconvoluted (see Fig. 2) using the program EWVOIGTN (Scientific Software Services, 305 E. Locust, Bloomington, IL 61701). Integration of the separate spectra provided a measure of the concentrations of the monomer and dimer, the total concentration of CTMIO being 1 mM (Table 2).

From these data, the enthalpy of dimer formation was found to be -43 ± 8 kJ mol^{-1} and the formation constant and entropy of formation at 298 K were estimated to be 30 ± 40 M^{-1} and -120 ± 40 J mol K^{-1} respectively.

Table 3 Selected molecular dimensions of CTMIO **6**. Bond lengths are in Ångstroms; angles are in degrees. E.s.d's are in parentheses

C(1)–C(6)	1.389(3)	C(7)–N(2)	1.480(3)
C(1)–C(2)	1.391(3)	C(7)–C(8)	1.520(3)
C(1)–C(13)	1.486(3)	C(7)–C(9)	1.523(3)
C(2)–C(3)	1.379(3)	C(10)–N(2)	1.486(3)
C(3)–C(4)	1.382(3)	C(10)–C(11)	1.517(3)
C(4)–C(5)	1.383(3)	C(10)–C(12)	1.521(3)
C(4)–C(7)	1.512(3)	C(13)–O(2)	1.196(3)
C(5)–C(6)	1.387(3)	C(13)–O(1)	1.312(3)
C(5)–C(10)	1.515(3)	N(2)–O(3)	1.282(2)
C(6)–C(1)–C(2)	120.3(2)	N(2)–C(7)–C(9)	109.8(2)
C(6)–C(1)–C(13)	122.2(2)	C(4)–C(7)–C(9)	112.2(2)
C(2)–C(1)–C(13)	117.5(2)	C(8)–C(7)–C(9)	112.1(2)
C(3)–C(2)–C(1)	120.9(2)	N(2)–C(10)–C(5)	99.4(2)
C(2)–C(3)–C(4)	118.5(2)	N(2)–C(10)–C(11)	110.0(2)
C(3)–C(4)–C(5)	121.2(2)	C(5)–C(10)–C(11)	113.2(2)
C(3)–C(4)–C(7)	126.6(2)	N(2)–C(10)–C(12)	110.2(2)
C(5)–C(4)–C(7)	112.2(2)	C(5)–C(10)–C(12)	112.2(2)
C(4)–C(5)–C(6)	120.4(2)	C(11)–C(10)–C(12)	111.2(2)
C(4)–C(5)–C(10)	112.1(2)	O(2)–C(13)–O(1)	122.6(2)
C(6)–C(5)–C(10)	127.5(2)	O(2)–C(13)–C(1)	123.6(2)
C(5)–C(6)–C(1)	118.7(2)	O(1)–C(13)–C(1)	113.8(2)
N(2)–C(7)–C(4)	99.6(2)	O(3)–N(2)–C(7)	122.8(2)
N(2)–C(7)–C(8)	110.2(2)	O(3)–N(2)–C(10)	120.6(2)
C(4)–C(7)–C(8)	112.2(2)	C(7)–N(2)–C(10)	116.6(2)

In the hydrogen bond (the prime indicates the molecule at $x, y-1, z$):

O(1)–H(1)	0.86(3)	C(13)–O(1)–H(1)	113(2)
H(1)–O(3′)	1.86(3)	O(1)–H(1)···O(3′)	163(3)
O(1)···O(3′)	2.687(2)		

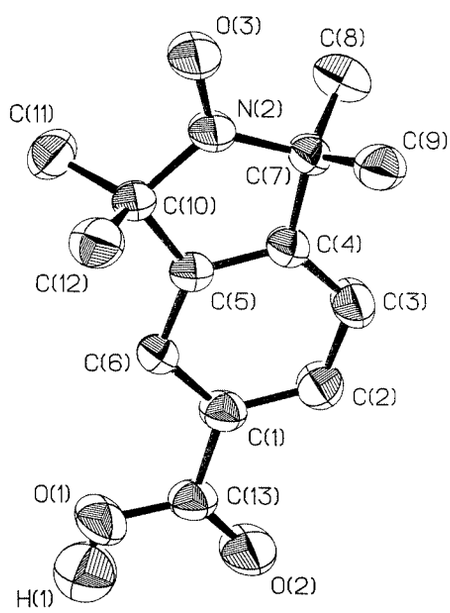


Fig. 3 ORTEP diagram of 5-carboxy-1,1,3,3-tetramethylisoindolin-2-yloxyl (**6**) (CTMIO), showing 50% probability ellipsoids and the atom numbering scheme.

X-Ray crystallography of 5-carboxy-1,1,3,3-tetramethylisoindolin-2-yloxyl (6) (CTMIO)

Selected molecular dimensions are listed in Table 3. Fig. 3 is a diagram of the molecule and Fig. 4 shows the packing of the unit cell. It may be seen from Fig. 4 that in the crystal of CTMIO, the radicals form linear chains with hydrogen bonding linking the NO group of one radical to the OH group ("head-to-tail") of an adjacent radical, with the H···O hydrogen bond having a length of 1.86 Å. Within a chain, the separation of the

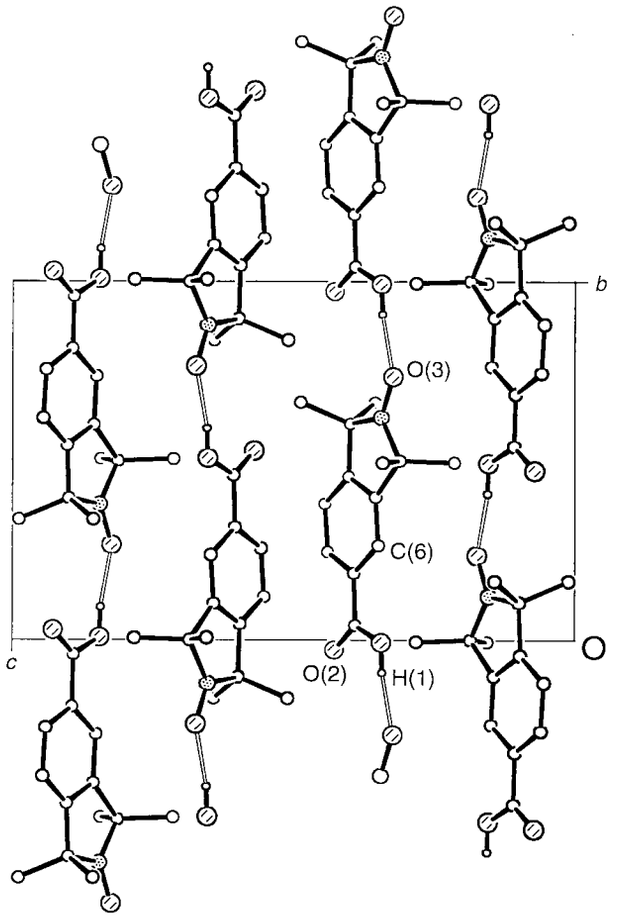


Fig. 4 Unit cell packing structure of 5-carboxy-1,1,3,3-tetramethylisoindolin-2-yloxyl (**6**) (CTMIO), showing the hydrogen-bonded linking of molecules in chains.

centres of the nearest NO groups is the length of the *b*-axis of the unit cell, that is, 10.2 Å. If, however, CTMIO dimerises in toluene by bringing together two carboxylic groups then, based on the crystallographically-determined intramolecular bond lengths, the separation of two aminoxyl groups can be estimated to be the much larger value of about 17 Å, assuming a centrosymmetric arrangement of the "tail-to-tail" carboxylic groups and an O(1)···O(2′) distance of 2.6 Å. The X-ray derived value is in good agreement with that of 19.3 Å estimated from the W-band spectrum. The difference could be due to several factors. One possibility is that the conformation of the dimer in the frozen solid is different from that assumed in the crystallographic model: the *D* parameter measured in the EPR experiment is very sensitive to the distance between the NO groups, as well as the angles between the aminoxyl axes and the interspin vector.[24] The intramolecular bond angles and bond distances of **6** are essentially the same as those we reported[25] for the X-ray structure of 5-nitro-1,1,3,3-tetramethylisoindolin-2-yloxyl, the only significant difference being that the NO bond length of the latter is 1.263(3) Å compared with 1.282(2) Å for CTMIO. This increase in bond length is consistent with the presence of hydrogen bonding. The nine-membered bicyclic unit of the CTMIO molecule is essentially planar and the plane of the carboxylic group is rotated just 0.17(4)° from that mean plane. The ring planes of the linked molecules, including the hydrogen-bonded groups, are also coincident with that plane, so that the crystal may be described as being composed of planar ribbons, with protruding methyl groups, running parallel to the *b*-axis; the angles between the normals to adjacent ribbons are 0° and (parallel ribbons) 71.94(6)°.

Experimental

Spectroscopy

NMR spectra were recorded on samples dissolved in deuteriochloroform (unless otherwise indicated) using a Varian Unity 300 spectrometer. *J* values are given in Hz.

Gas chromatography–mass spectrometry was performed in EI mode on a Fisons Instruments MD800 gas chromatograph–mass spectrometer (GC-MS) equipped with a 60 m, 0.25 mm id, DB1 capillary column.

Other EI mass spectra, including high resolution spectra, were recorded on a Kratos Concept ISQ mass spectrometer utilising a direct insertion probe and operating at 70 eV, 5.3 kV accelerating voltage and a source temperature of 200 °C. High resolution data were acquired by peak matching at a resolution of 10 000, using perfluorokerosene as an internal mass reference.

X-Band EPR spectra were obtained for samples dissolved in chloroform, using a Bruker ESP 300E EPR spectrometer fitted with an EIP 548B microwave frequency counter and an ER O35M gaussmeter.

W-Band (94.45 GHz) EPR spectra of CTMIO were obtained over a wide temperature range in perdeuteriotoluene at a concentration of about 1 mM using a spectrometer described elsewhere,[26–28] designed and built at the University of Illinois. For temperatures below 200 K, the incident power was set to about 3 μW in order to avoid saturation and/or rapid passage conditions. Spectra were recorded with the help of a low-noise microwave Millitech amplifier.[29] The best resolution for the powder spectrum was obtained at 130 K.

Materials

Dry THF was prepared by reflux over potassium–benzophenone immediately prior to use. Powdered anhydrous $AlCl_3$ and *n*-butyllithium (1.6 M in hexanes) were purchased from Sigma-Aldrich. 2-Benzyl-1,1,3,3-tetramethylisoindoline (**2**) was synthesised as previously described.[1]

Bromination of 2-benzyl-1,1,3,3-tetramethylisoindoline (2)

2-Benzyl-1,1,3,3-tetramethylisoindoline (**2**) (5.0 g, 18.9 mmol) was dissolved in dichloromethane (60 cm^3) under argon and cooled to 0 °C. A solution of Br_2 (2.15 cm^3, 42.0 mmol, 2.2 equiv.) in dichloromethane (40 cm^3) was added, with stirring, followed immediately by the addition of powdered anhydrous $AlCl_3$ (9.0 g, 3.5 equiv.). The reaction mixture was stirred at 0 °C for 1 h and then poured onto ice (~150 cm^3). After 15 min, the solution was basified with 10 M NaOH and extracted with dichloromethane (3 × 70 cm^3). The organic phase was washed with brine and the solvent removed under reduced pressure. The resulting golden oil was taken up in MeOH (~30 cm^3) and $NaHCO_3$ (~200 mg) added. H_2O_2 (30%) was then applied dropwise until the cessation of effervescence occurred, ensuring that excess $NaHCO_3$ remained. The mixture was treated with H_2SO_4 (2.0 M; 75 cm^3) (**CAUTION:** effervescence) and washed with dichloromethane (2 × 50 cm^3) to remove benzaldehyde. The combined organic phases were further extracted with H_2SO_4 (2 × 40 cm^3) and the combined acidic phases washed with dichloromethane. The acidic phase was cooled in an ice–water bath, basified with 10 M NaOH and extracted with dichloromethane (3 × 70 cm^3). The combined organics were washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure to give a golden oil which rapidly crystallised. The resulting 5-bromo-1,1,3,3-tetramethylisoindoline was of sufficient purity, according to TLC and ^1H NMR, for subsequent use without further purification (4.295 g, 90%); δ_H (299.95 MHz; $CDCl_3$) 1.43 (6H, s, CH_3), 1.45 (6H, s, CH_3), 1.87 (1H, br, NH), 6.98 (1H, d, *J* 7.8, 7-H), 7.23 (1H, d, *J* 2.0, 4-H), 7.34 (1H, dd, *J* 2.0 and 7.8, 6-H); δ_C (75.43 MHz; $CDCl_3$) 31.7 (CH_3), 62.5 (C-1), 62.6 (C-3), 121.3 (C-5), 123.0 (C-6), 124.7 (C-7), 130.1

(C-4), 147.8 (C-7a), 151.2 (C-3a); *m*/*z* 253/255 (M^+, 1%), 238/240 (100), 223/225 (29), 159 (13), 115 (20).

5-Carboxy-1,1,3,3-tetramethylisoindolin-2-yloxyl (6) (CTMIO)

n-Butyllithium (1.6 M in hexanes; 23.4 cm^3, 2.2 equiv.) was added *via* a syringe, over a period of approximately two minutes, to a stirred solution of 5-bromo-1,1,3,3-tetramethylisoindoline (4.295 g, 16.9 mmol) in dry THF (48 cm^3), at −78 °C under argon. After 10 min the reaction was poured onto a slurry of powdered dry ice in dry THF (~ 200 cm^3 total volume). This was allowed to stir until it had reached room temperature. The solvent and other volatiles were removed under reduced pressure. The residue was taken up in HCl (2 M; ~50 cm^3) and extracted with diethyl ether (3 × 50 cm^3) to remove pentanoic acid and neutral organics. The aqueous phase was then neutralised with Na_2CO_3 and extracted again with diethyl ether to remove basic species. Evaporation of the aqueous phase under reduced pressure gave a solid residue containing inorganic salts and 5-carboxy-1,1,3,3-tetramethylisoindoline **5**, which was not purified further; δ_H (299.95 MHz; [$^2H_6$]DMSO) 1.70 (12H, s, CH_3), 7.45 (1H, d, *J* 8.2, 7-H), 7.88 (1H, d, *J* 1.9, 4-H), 7.94 (1H, dd, *J* 1.9 and 8.2, 6-H), 9.55 (2H, br, NH_2^+); δ_C (75.43 MHz; [$^2H_6$]DMSO) 28.5 (CH_3), 68.0 (C-1, C-3), 122.3 (C-7), 123.3 (C-4), 123.7 (C-6), 130.7 (C-5), 143.0 (C-3a), 146.8 (C-7a), 166.8 (C=O).

The solid residue was taken up in H_2O–MeOH (40 cm^3:35 cm^3), and $NaHCO_3$ (1.14 g, 13.6 mmol, 0.8 equiv.), $Na_2WO_4{\cdot}2H_2O$ (500 mg, 1.5 mmol, 0.09 equiv.) and H_2O_2 (30%; 10.0 cm^3, 5.2 equiv.) added. After stirring at room temperature for 24 h, extra H_2O_2 (2.0 cm^3, 1.04 equiv.) was added. 48 h after the commencement of stirring, the reaction mixture was basified with 2 M NaOH and extracted with diethyl ether (3 × 40 cm^3) to remove basic species. The aqueous phase was then acidified with HCl and extracted with diethyl ether (3 × 70 cm^3). The organic phase was washed with brine, dried over Na_2SO_4, and the solvent removed under reduced pressure. 5-Carboxy-1,1,3,3-tetramethylisoindolin-2-yloxyl **6** was purified by column chromatography (SiO_2; 230–400 mesh; $CHCl_3$) (2.4 g, 62%) and recrystallised from MeCN, forming yellow prisms (1.69 g, 43%), mp 214–218 °C (decomp.) (Found: C, 66.57; H, 6.92; N, 6.28. $C_{13}H_{16}NO_3$ requires C, 66.67; H, 6.84; N, 5.98%); ν_{max}(KBr)/cm^{-1} 3050 (OH), 2982 (aryl CH), 1715 (CO), 1617 and 1585 (aryl C–C), 1392 and 1367 (NO), 1219 (C–O); *m*/*z* 234 (M^+, 75%), 220 (80), 219 (50), 204 (100), 189 (88); *g* = 2.0059, a^N = 1.445 mT ($CHCl_3$); pK_a 3.3 ± 0.1 (25 °C).

5-Carboxy-1,1,3,3-tetramethylisoindolin-2-yloxyl hexyl ester (9)

5-Carboxy-1,1,3,3-tetramethylisoindolin-2-yloxyl **6** (100 mg, 427 μmol) was added to stirring *n*-hexanol (2 cm^3) and the mixture heated to 100 °C to dissolve the aminoxyl. One drop of conc. H_2SO_4 was added, and the temperature maintained at 100 °C for a further 12 h. Water (~50 cm^3) was added and the reaction mixture basified with sat. $NaHCO_3$. Extraction with dichloromethane (3 × 30 cm^3) gave a yellow oil. The majority of the remaining *n*-hexanol was removed by crude vacuum distillation and the product resolved by flash chromatography (SiO_2; 230–400 mesh; 10% EtOAc–hexane), giving a pale yellow oil, which crystallised with difficulty (66 mg, 48%). Recrystallisation from *n*-pentane gave pale yellow platelets of 5-carboxy-1,1,3,3-tetramethylisoindolin-2-yloxyl hexyl ester **9**, mp 57 °C; EI MS found M^+ 318.20710 (0.57 ppm from calc. mass for $C_{12}H_{28}NO_3$); *m*/*z* 318 (M^+, 66%), 303 (100), 288 (38), 273 (29); *g* 2.0058, a^N 1.470 mT ($CHCl_3$).

5-Carboxy-1,1,3,3-tetramethylisoindolin-2-yloxyl anhydride (10)

5-Carboxy-1,1,3,3-tetramethylisoindolin-2-yloxyl **6** (100 mg, 427 μmol) was dissolved in a minimal volume of dichloromethane (~4 cm^3) and the solution cooled to 0 °C. Dicyclohexylcarbodiimide (DCC) (40 mg, 194 μmol, 0.45 equiv.) was added and the solution stirred at 0 °C for 3 h. Dicyclohexylurea was removed by filtration through Celite and the product, 5-carboxy-1,1,3,3-tetramethylisoindolin-2-yloxyl anhydride **10**, isolated as a yellow oil in almost quantitative yield. Flash column chromatography (SiO_2; 230–400 mesh; $CHCl_3$) was used to purify the product, giving **10** as a glassy solid (58 mg, 60%); EI MS found M^+ 450.21606 (1.31 ppm from calc. mass for $C_{26}H_{30}N_2O_5$); *g* 2.0059, a^N 1.453 mT ($CHCl_3$).

5-Carboxy-1,1,3,3-tetramethylisoindolin-2-yloxyl *N*-hydroxysuccinimidyl ester (11)

5-Carboxy-1,1,3,3-tetramethylisoindolin-2-yloxyl **6** (100 mg, 427 μmol) and *N*-hydroxysuccinimide (54 mg; 470 mmol, 1.1 equiv.) were dissolved in a minimal volume of dichloromethane (~4 cm^3) and the solution cooled to 0 °C. Dicyclohexylcarbodiimide (92 mg, 446 μmol, 1.05 equiv.) was added and the solution stirred at 0 °C for 3 h. Dicyclohexylurea was removed by filtration through Celite. Following flash column chromatography (SiO_2; 230–400 mesh; $CHCl_3$), 5-carboxy-1,1,3,3-tetramethylisoindolin-2-yloxyl *N*-hydroxysuccinimidyl ester **11** was isolated as an oily yellow solid (95 mg, 67%); EI MS found M^+ 331.12956 (0.50 ppm from calc. mass for $C_{17}H_{19}N_2O_5$); *g* 2.0059, a^N 1.456 mT ($CHCl_3$).

5-Formyl-1,1,3,3-tetramethylisoindoline (7)

5-Bromo-1,1,3,3-tetramethylisoindoline **3** (1.0 g, 3.94 mmol) was lithiated according to the procedure for **6** given above, and was quenched by the addition of dimethylformamide (0.915 cm^3, 11.8 mmol, 3 equiv.). The reaction mixture was allowed to warm to room temperature over 30 min, followed by the addition of H_2O (~50 cm^3), to form two phases. The aqueous phase was acidified with HCl and the mixture washed with Et_2O (3 × 50 cm^3) to remove pentanoic acid and other non-basic species. The aqueous phase was then basified with 2 M NaOH and extracted with Et_2O (3 × 50 cm^3). The organic phases were washed with brine, dried over Na_2SO_4 and the solvent removed under reduced pressure. Purification by column chromatography (SiO_2; 230–400 mesh; $CHCl_3$) gave an almost quantitative yield of the product, 5-formyl-1,1,3,3-tetramethylisoindoline **7** (795 mg); δ_H (299.95 MHz; $CDCl_3$) 1.46 (6H, s, CH_3), 1.47 (6H, s, CH_3), 2.00 (1H, br, NH), 7.25 (1H, d, *J* 7.7, 7-H), 7.64 (1H, d, *J* 1.3, 4-H), 7.75 (1H, dd, *J* 1.3 and 7.7, 6-H), 10.02 (1H, s, HC=O); δ_C (75.43 MHz; $CDCl_3$) 31.8 (CH_3), 31.9 (CH_3), 62.4 (C-1), 62.8 (C-3), 122.0 (C-6), 122.4 (C-4), 130.0 (C-7), 136.2 (C-5), 149.9 (C-3a), 155.8 (C-7a), 191.8 (C=O); *m*/*z* 203 (M^+, <1%), 188 (100), 173 (46), 172 (26).

5-Formyl-1,1,3,3-tetramethylisoindolin-2-yloxyl (8)

5-Formyl-1,1,3,3-tetramethylisoindoline **7** (800 mg, 3.94 mmol) was dissolved in a mixture of MeOH (7.5 cm^3) and MeCN (0.5 cm^3) at room temperature. $NaHCO_3$ (266 mg, 3.17 mmol, 0.8 equiv.) and $Na_2WO_4{\cdot}2H_2O$ (38 mg, 115 μmol, 0.03 equiv.) were added, and the mixture treated with H_2O_2 (30%; 1.50 cm^3, 13.2 mmol, 3.36 equiv.). After stirring at room temperature for 40 h the reaction mixture was diluted with H_2O (~30 cm^3) and extracted with Et_2O. The combined organic phases were washed with 2 M H_2SO_4, followed by brine. After drying over Na_2SO_4, the solvent was removed under reduced pressure, giving a golden oil which was purified by column chromatography (SiO_2; 230–400 mesh, 0.2% MeOH–$CHCl_3$) (617 mg, 72%) and recrystallised from hexanes, giving yellow–orange crystals of 5-formyl-1,1,3,3-tetramethylisoindolin-2-yloxyl **8**, mp 139–141 °C (Found: C, 71.80; H, 7.45; N, 5.91. $C_{13}H_{16}NO_2$ requires C, 71.53; H, 7.39; N, 6.42%); ν_{max}(KBr)/cm^{-1} 2977 (aryl CH), 2788 and 2725 (aldehyde CH), 1698 (CO), 1616 and 1581 (aryl C–C), 1376 and 1358 (NO); *m*/*z* 218 (M^+, 100%), 203 (52), 188 (82), 173 (89), 145 (88); *g* 2.0058, a^N 1.456 mT ($CHCl_3$).

Crystal structure analysis of CTMIO 6†

Crystal data. $C_{13}H_{16}NO_3$, $M = 234.3$. Orthorhombic, space group $P2_12_12_1$ (no. 19), $a = 7.9732(7)$, $b = 10.1799(12)$, $c = 15.570(2)$ Å, $V = 1263.8(2)$ Å^3. $Z = 4$, $T = 293(2)$ K, μ(Mo-Kα) = 0.088 mm^{-1}, λ(Mo-Kα) = 0.71069 Å, crystal size 0.24 × 0.30 × 0.70 mm.

A crystal fragment was mounted on a glass fibre and transferred to an Enraf-Nonius CAD4 diffractometer (with monochromated radiation) for determination of accurate cell parameters (from the settings of 25 reflections, $\theta = 10$–$11°$, each centred in four orientations) and for measurement of diffraction intensities (1297 unique reflections to $\theta_{max} = 25°$; 1147 were 'observed' with $I > 2\sigma_I$). The structure was determined by the direct methods routines in the SHELX program[30] and refined by full-matrix least-squares methods, on F^2's, in SHELXL.[31] The non-hydrogen atoms were refined with anisotropic thermal parameters. All the hydrogen atoms were located in difference Fourier maps and were refined freely. At the conclusion of the refinement, $wR_2 = 0.083$ and $R_1 = 0.037$[31] for all 1297 reflections weighted $w = [\sigma^2(F_0^2) + (0.039P)^2 + 0.121P]^{-1}$ with $P = (F_0^2 + 2F_c^2)/3$; for the 'observed' data only, $R_1 = 0.033$. In the final difference map, the highest peak (*ca.* 0.1 e Å^{-3}) was close to a methyl group. Scattering factors for neutral atoms were taken from ref. 32. Computer programs used in this analysis have been noted above or in Table 4 of ref. 33, and were run on a DEC-AlphaStation 200 4/100 in the Biological Chemistry Department of the John Innes Centre.

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† CCDC reference number 188/248. See http://www.rsc.org/suppdata/p2/b0/b002497j/ for crystallographic files in .cif format.

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