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# Synthesis and EPR spin trapping properties of a new isoindole-based nitrone: 1,1,3-trimethylisoindole *N*-oxide (TMINO)

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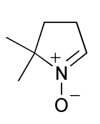
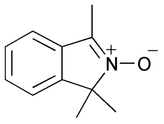
Here we describe the synthesis and characterisation of a new isoindole-based nitrone spin trap, 1,1,3-trimethylisoindole *N*-oxide (TMINO). This nitrone and its radical adducts (isoindoline nitroxides) exhibit enhanced stability with respect to other commonly used spin traps and their adducts. We also report EPR trapping studies of this new nitrone with some carbon- and oxygen-centred radicals including alkyl, aryl, hydroxyl and benzoyloxyl systems. The narrow EPR line-widths and stability of the resulting nitroxide spin adducts allowed the detection of the expected radicals as well as secondary and minor radical components in the reaction mixtures.

## Introduction

Spin trapping is a valuable technique for the investigation of short-lived free radicals present in steady-state concentrations too low to be detected by direct EPR spectroscopy. When nitrones are utilised as spin traps the capture of primary radicals results in the formation of persistent nitroxide species which may be readily analysed by EPR. While various cyclic and acyclic nitrones have been employed as spin traps, pyrroline-based compounds such as DMPO are those most commonly employed. Several issues however, complicate the use of these compounds. These include the formation of spurious EPR signals due to non-radical reactions such as hydrolysis[1–3] and metal-mediated oxidation processes.[1,2,4,5] The stability of the resulting spin adducts (nitroxides) is critical, with decomposition of these species complicating their detection, identification and quantification. Many spin traps, including DMPO, possess a hydrogen atom at the α-carbon, making their spin adducts susceptible to decomposition *via* disproportionation.



DMPO



TMINO

Here we report the development of a new spin trap, 1,1,3-trimethylisoindole *N*-oxide (TMINO), which avoids some of the limitations of pyrroline-based nitrones. The fused isoindole ring structure inhibits decomposition by ring-opening reactions and the absence of a hydrogen atom at the α-carbon minimises the formation of artifactual nitroxide species *via* non-radical pathways, as well as precluding decomposition of the radical adducts by disproportionation. The radical adducts of TMINO are persistent isoindoline nitroxides which exhibit enhanced thermal and chemical stability with respect to other nitroxides.[6–8] We also report preliminary spin-trapping studies using TMINO, with some carbon- and oxygen-centred radicals including alkyl, aryl, hydroxyl and benzoyloxyl systems.

## Results and discussion

### Synthesis of TMINO

The preparation of an aryl-fused, five-membered ring nitrone such as TMINO by a one step synthesis from a known nitroxide precursor was an attractive route compared to standard methodology. Accordingly, the synthetic strategy for TMINO was devised following our observations of the thermal decomposition of the isoindoline nitroxide 1,1,3,3-tetramethylisoindolin-2-yloxyl (TMIO),[9] during GC-MS studies using elevated injector temperatures (up to 290 °C). Fig. 1 shows the total ion count (TIC) trace obtained from one such experiment.

In these investigations TMIO was found to undergo homolytic cleavage to form TMINO and a methyl radical which could be trapped by further TMIO to give **1**, or undergo further reaction (Scheme 1). Deoxygenation of the nitrone gives rise to the corresponding imine (**2**) while a small amount of amine **4** is

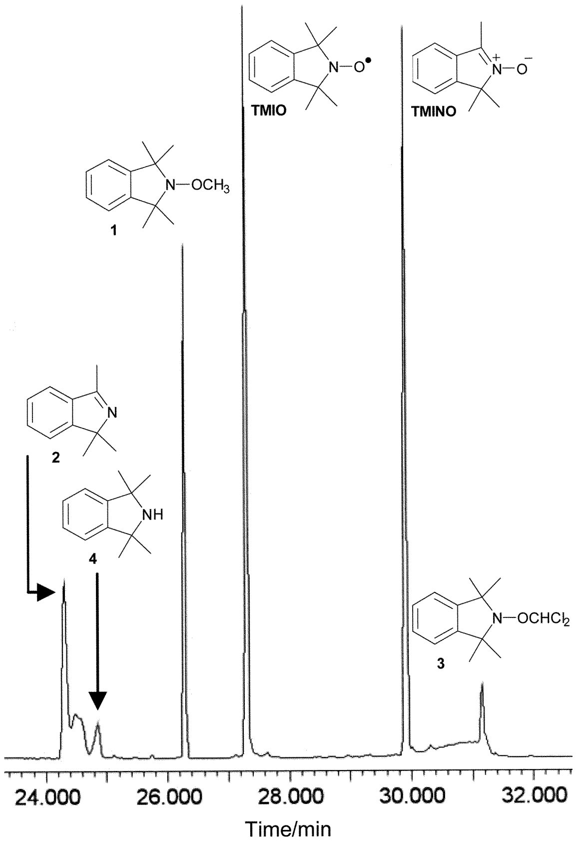


**Fig. 1** GC-MS TIC trace obtained from thermal cracking of TMIO using an injector temperature of 290 °C. Thermolysis products were resolved using a 60 m, 0.25 mm id, DB1 capillary column and a temperature program of 10 min at 50 °C, 10 °min$^{-1}$ to 260 °C, 30 min at 260 °C.

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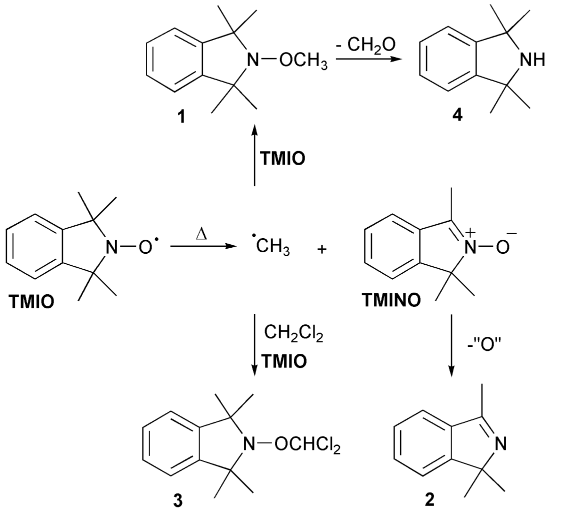


**Scheme 1** Thermolysis of and radical trapping by TMIO in the GC-MS injector.

generated presumably *via* the loss of $CH_2O$ from alkoxyamine **1**. Solvent-derived dichloromethyl radicals trapped by TMIO formed alkoxyamine **3**, which was observed as a minor component in the chromatogram, as was a trace of 1,1,2,2-tetrachloroethane. Analogous thermolysis products were noted for the piperidine nitroxide TEMPO, although a greater number of unidentified minor products were generated.

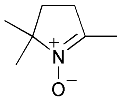
While solution-based thermolysis of TMIO proved unsuitable for the preparative-scale production of TMINO due the generation of excessive quantities of by-products such as methyl adduct **1**, flash vacuum pyrolysis (FVP) proved more successful. Thus FVP of TMIO (420 °C; <0.5 mm Hg) gave good yields of TMINO (up to 73%), even for gram scale preparations. It proved crucial to maintain the pressure below 0.5 mm Hg to prevent gas-phase recombination and methyl radical trapping by TMIO (to give **1**). Equally, higher temperatures increased the yields of the imine by-product (**2**). Temperatures close to 420 °C provided the best balance between starting material conversion and product yields. To the best of our knowledge, this is the first described preparative synthesis of a nitrone from a stable nitroxide precursor *via* flash vacuum pyrolysis.

The target nitrone was isolated by flash column chromatography to give a clear oil infrequently contaminated with traces of a pinkish paramagnetic impurity. TMINO proved very stable and could be stored at −18 °C under argon for several months without significant decomposition. Under the same conditions DMPO was found to develop significant paramagnetic contamination, most probably due to dimerisation.[5,10–13] Traces of paramagnetic impurities in TMINO were readily removed by further flash chromatography on silica.

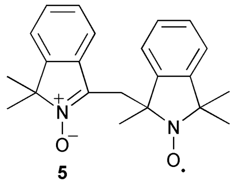
### Loss of paramagnetic purity of TMINO samples with time

Unless purified immediately prior to use, solutions of TMINO exhibited trace levels of a paramagnetic impurity which exhibited a typical nitroxide three-line EPR spectrum with a nitrogen hyperfine interaction characteristic of tetra-alkyl substitution (average $A/g\beta$ = 14.26 G in benzene and 16.07 G in water). The EPR signal of this impurity ranged from being absent in freshly purified samples, to being relatively strong in material which had been either stored under benchtop conditions, or at −18 °C in the presence of oxygen for extended periods (weeks).

The identity of the impurity remains unconfirmed, not least due to its low levels, however it is possible to draw analogies with the chemistry of related trimethyl substituted pyrroline nitrones[5,11–14] (*e.g.* $M_3PO$) which have been found to contain similar paramagnetic contamination attributed to dimeric nitrone–nitroxide species. At equilibrium, a small proportion of TMINO is expected to be present as its enamine tautomer and this may add to further TMINO to form a dimer which, under aerobic conditions, should oxidise to give nitrone–nitroxide **5**.



**$M_3PO$**



**5**

In most cases the TMINO contaminant was present in such low levels that its detection required the maximum gain setting on the spectrometer (with other parameters as per normal). In such cases the impurity did not interfere with the spin trapping experiments and the stock solution was considered suitable for use. If required, contaminated TMINO could be rendered essentially EPR-silent immediately prior to use by flash column chromatography as described above. In contrast, paramagnetic contamination of $M_3PO$ proves difficult to remove by simple chromatography or distillation.

### Simple spin-trapping experiments with TMINO

TMINO was heated in the presence of the standard radical initiators di-*tert*-butylperoxyoxalate (DtBPO), benzoyl peroxide and azo-bis-isobutyronitrile (AIBN), to assess its applicability as a spin trap. The reactions were conducted in benzene, in sealed EPR tubes under anaerobic conditions and the trapping products were monitored by X-band EPR. All of the adducts proved to be persistent with essentially no degradation of the EPR signal detected over the timeframe of the measurements (several hours).

**Di-*tert*-butylperoxyoxalate (DtBPO).** The thermal decomposition of DtBPO results in the formation of *tert*-butoxyl radicals. In the absence of further reaction, these decompose to form methyl radicals and acetone. When a solution of TMINO and DtBPO (half-life: 1 minute at 60 °C[15]) was heated at 70–80 °C for 10 minutes, a strong EPR signal was observed. Deconvolution of the experimental spectrum using EWVoigtN[16] indicated the presence of two nitroxide species. These were observed in a 2 : 3 ratio and exhibited nitrogen hyperfine interactions ($A/g\beta$) of 12.97 G and 14.19 G respectively (Fig. 2). The spectra were attributed to the *tert*-butoxyl radical adduct (**6**) and the methyl radical adduct (TMIO) respectively, on the basis of their relative nitrogen hyperfine interactions (Scheme 2). These hyperfine couplings are comparable to those of the *tert*-butoxyl[17] and methyl adducts[18] of DMPO in benzene (13.1 G and 14.3 G respectively). The nitrogen hyperfine interaction of the *tert*-butoxyl adduct of the related trimethyl pyrroline *N*-oxide ($M_3PO$) in benzene has been reported at 13.44 G and 13.49 G.[13]

**Benzoyl peroxide.** Thermal decomposition of benzoyl peroxide generates benzoyloxyl radicals which lose $CO_2$ to form phenyl radicals in solvents that are poor hydrogen donors, such as benzene.[19] When benzoyl peroxide (half-life: 7 h at 70 °C[15]) was heated for 90 minutes at 70–80 °C in the presence of TMINO, a strong composite EPR signal was obtained. This was deconvoluted to give two constituent spectra in a ratio of 1 : 4, exhibiting nitrogen hyperfine interactions ($A/g\beta$) of 13.09 G and 14.24 G respectively (Fig. 2). These were assigned to the benzoyloxyl (**7**) and phenyl (**8**) radical adducts of TMINO respectively, on the basis of the nitrogen hyperfine interactions (Scheme 2). The scaling data suggests that addition of the benzoyloxyl radical to TMINO is relatively inefficient with respect to addition of the phenyl radical.

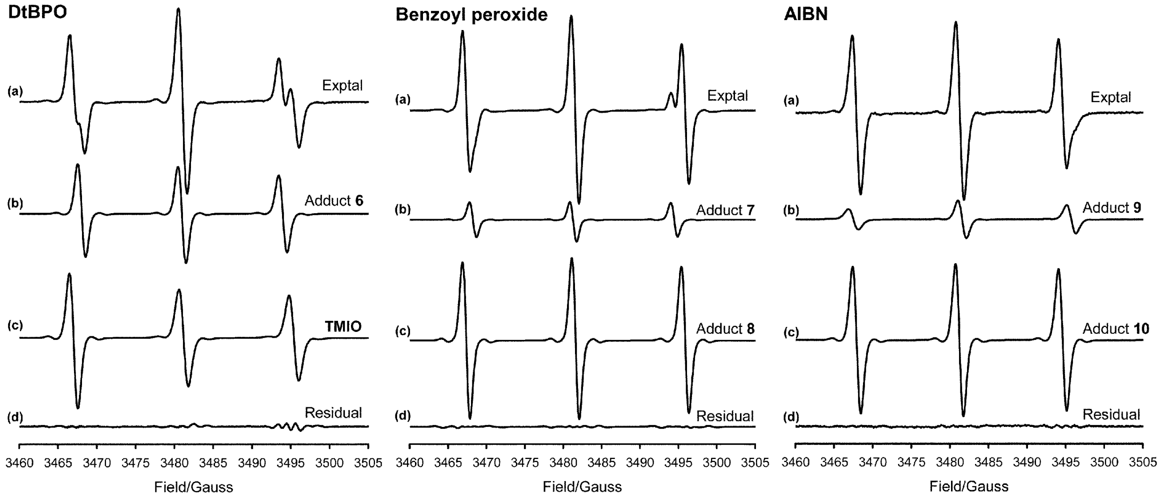
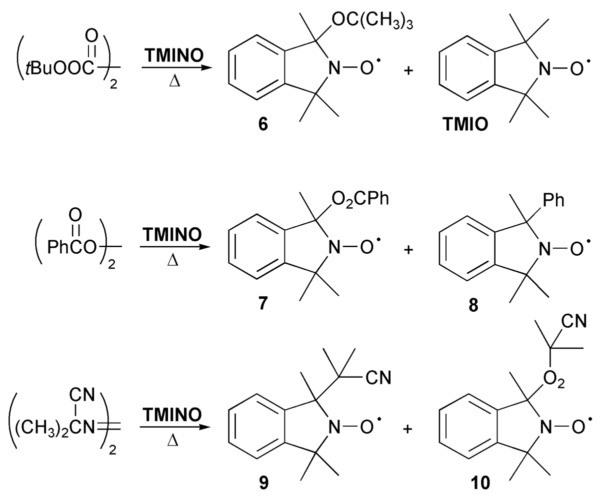


**Fig. 2** EPR spectra of the radical adducts of TMINO. The radical source for each set of spectra is listed at the top left. For each set of spectra: (a) experimental spectrum; (b, c) computer simulations of the constituent radical adducts. Relative weightings are listed in Table 1; (d) the residual obtained by subtracting spectrum a − (b + c).



**Scheme 2** Formation of spin adducts of TMINO from radical initiators in benzene.

**Azo-bis-isobutyronitrile (AIBN).** Thermolysis of a benzene solution of TMINO and AIBN at 70–80 °C for 105 minutes gave a strong EPR signal. Spectral deconvolution using EWVoigtN gave two constituent nitroxide spectra (Fig. 2), which was unexpected as 2-cyano-2-propyl radicals were anticipated to be the only radical species produced and trapped in this experiment. According to scaling data, the major radical adduct was present in 3.4 times the amount of the minor product. The minor product displayed a typical tetraalkyl nitroxide nitrogen hyperfine interaction ($A/g\beta$) of 14.09 G, and this was assigned to the expected 2-cyano-2-propyl radical adduct (**9**). The major component exhibited a nitrogen hyperfine interaction ($A/g\beta$) somewhat smaller than usual for a tetraalkyl nitroxide (13.32 G).

Busfield *et al.* have reported the competitive formation of a peroxyl radical from the 2-cyano-2-propyl radical due to trapping by adventitious $O_2$, even in thoroughly deoxygenated systems.[20] In this case, we suggest that it is possible that the unidentified nitroxide signal arises from peroxyl radical trapping by TMINO to give the corresponding nitroxide (**10**) (Scheme 2).

## Conclusion

TMINO is a new isoindole-based nitrone spin trap, readily synthesised in one step from a known stable nitroxide. It is apparent that TMINO is a potentially useful radical trap, capable of trapping both carbon- and oxygen-centred radicals. From the amounts of carbon-centred radicals detected with DtBPO and benzoyl peroxide, the rate of trapping may be governed by factors such as steric bulk and ionic character, which warrants further kinetic studies. The prime advantage of this new nitrone spin trap is the stability of the isoindoline nitroxide adducts formed by the spin-trapping. Isoindoline nitroxides possess some advantages over other classes of nitroxides and this is reflected by the stability and longevity of the paramagnetic compounds generated here. The EPR characteristics obtained from spectral fitting for the observed radical adducts of TMINO are listed in Table 1.

# Experimental

## General

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 operat-

**Table 1** X-band EPR characteristics of the radical adducts of TMINO obtained from radical initiators in benzene

| Radical source | Radical adduct | % | $g_{av}$ | $A_{av}(N)/g\beta$[a] |
|---|---|---|---|---|
| **DtBPO** | *t*BuO-TMINO (**6**) | 40 | 2.00588 | 12.97 |
| | $H_3C$-TMINO (TMIO) | 60 | 2.00586 | 14.19 |
| **$(PhCO_2)_2$** | $PhCO_2$-TMINO (**7**) | 20 | 2.00598 | 13.09 |
| | Ph-TMINO (**8**) | 80 | 2.00582 | 14.24 |
| **AIBN** | $CN(CH_3)_2C$-TMINO (**9**) | 23 | 2.00589 | 14.09 |
| | $CN(CH_3)_2COO$-TMINO (**10**) | 77 | 2.00610 | 13.32 |

[a] Units Gauss.

ing 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 (9 GHz) EPR spectra were obtained using a Bruker Elexsys E500 multifrequency continuous wave EPR spectrometer equipped with an EIP 548B microwave frequency counter and a Bruker ER035M gaussmeter for microwave frequency and magnetic field calibration. A conventional X-band rectangular $TE_{102}$ microwave cavity was utilised for all spectra measured in this study. $^1H$ and $^{13}C$ NMR spectra ($CDCl_3$) were recorded on a Varian Unity 300 spectrometer at 299.949 and 75.430 MHz respectively. Analytical grade dichloromethane was sourced from MBH and distilled immediately prior to use. The nitroxide 1,1,3,3-tetramethylisoindolin-2-yloxyl (TMIO) was synthesised from *N*-benzylphthalimide in three steps using established procedures.[9] Di-*tert*-butylperoxyoxalate (DtBPO) was synthesised as described elsewhere.[21] Samples of DtBPO were stored in the freezer in non-ground glass vessels until immediately before use and transferred using plastic spatulas. (**Caution**: explosive!) Benzoyl peroxide and azo-bis-isobutyronitrile (AIBN) were obtained from Sigma/Aldrich and were used without further purification.

### Synthesis of 1,1,3-trimethylisoindole *N*-oxide (TMINO)

The flash vacuum pyrolysis apparatus was constructed from standard laboratory equipment, including a Quickfit test-tube, 60 cm Pyrex column and cold trap, assembled linearly in that order. The apparatus was attached to a two stage electric high vacuum oil pump capable of generating a vacuum of <0.1 mm Hg. The test-tube (containing TMIO) was placed into a Kugelrohr oven which was heated gradually, in order to slowly sublime the nitroxide into the pyrolysis column. The Pyrex column passed through a standard tube oven which was thermostatically-controlled and maintained at 420 °C (determined by a digital thermometer and thermocouple placed directly alongside the Pyrex tube). The pyrolysis products were recovered in the cold trap, which was connected to the high vacuum pump *via* a three way tap that was attached to a manometer and permitted the introduction of argon if desired.

Typically, small scale flash vacuum pyrolysis of TMIO (100 mg, 526 μmol) at pressures <0.5 mm Hg and at a temperature of 420 °C, over one hour gave a pink–purple oil, which was recovered by rinsing the pyrex column and cold trap with $CH_2Cl_2$. Removal of the solvent under reduced pressure and resolution by flash chromatography ($SiO_2$; 230–400 mesh; $CHCl_3$) gave two components. The less polar component was recovered as a colourless or slightly yellow oil and was identified as 1,1,3-trimethylisoindole (**2**) (10 mg, 12%); $\delta_H$ (299.949 MHz, $CDCl_3$) 1.44 (6H, s, $CH_3$), 2.44 (3H, s, $CH_3$), 7.34–7.48 (4H, m, ArH); $\delta_C$ (75.430 MHz, $CDCl_3$) 16.5 ($CH_3$), 25.5 (2 × $CH_3$), 74.0 (C-1), 120.7 (C-6), 121.0 (C-5), 127.0 (C-7), 128.4 (C-4), 138.0 (C-3a), 159.0 (C-7a), 167.4 (C-3); *m*/*z* 159 ($M^+$, 77%), 158 (75), 144 (100), 103 (51).

The more polar compound, a colourless or slightly yellow oil, was identified as 1,1,3-trimethylisoindole *N*-oxide (TMINO) (68 mg, 73%); $\delta_H$ (299.949 MHz, $CDCl_3$) 1.55 (6H, s, $CH_3$), 2.35 (3H, s, $CH_3$), 7.26 (1H, m, ArH), 7.35 (3H, m, ArH); $\delta_C$ (75.430 MHz, $CDCl_3$) 9.5 ($CH_3$), 24.0 (2 × $CH_3$), 76.0 (C-1), 119.2 (C-6), 120.3 (C-5), 127.8 (C-7), 128.2 (C-4), 133.8 (C-7a), 140.8 (C-3), 144.8 (C-3a); *m*/*z* 175 ($M^+$, 88%), 160 (100), 158 (55), 126 (46), 115 (45); EI MS found $M^+$ 175.09967 (−0.24 ppm from calc. mass for $C_{11}H_{13}NO$). Scale up of this preparation produced similar yields, with the main determination for success being control of the vacuum, oven temperature and rate of sublimation from the Kugelrohr oven.

Further purification of TMINO was sometimes required to produce EPR-silent material for spin-trapping purposes. Material recovered from initial flash chromatography was occasionally contaminated with a pink-coloured impurity. Treatment of aqueous or $CHCl_3$ solutions of TMINO with activated charcoal and filtration though Celite successfully removed the coloured impurity. The resulting nitrone was again purified *via* flash chromatography at this point, if necessary, to give EPR-silent material. The purified nitrone was successfully stored under argon at −18 °C, with little or no decomposition, for a period of at least three months.

### TMINO spin-trapping experiments

The paramagnetic purity of the TMINO stock solution was assessed by EPR at the commencement of each set of experiments. The general procedure for reactions between radical initiators and TMINO involved the addition of solid initiator (approximately 0.45 equiv.) to a solution of the nitrone in benzene (1 $cm^3$; 10 mg $cm^{-3}$, 57 mM), which was then deoxygenated by bubbling with argon for 5–10 minutes. Reaction mixtures were sealed in 4 mm od EPR tubes and heated in a water bath at 70–80 °C. X-band EPR spectra were recorded upon removal from the water bath.

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