ARTICLE

OBC
www.rsc.org/obc

Synthesis of profluorescent isoindoline nitroxides *via* palladium-catalysed Heck alkenylation

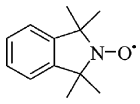
Daniel J. Keddie, Therese E. Johnson, Dennis P. Arnold and Steven E. Bottle*
School of Physical and Chemical Sciences, Queensland University of Technology, GPO Box 2434, Q4001, Australia. E-mail: s.bottle@qut.edu.au

Received 29th March 2005, Accepted 10th May 2005
First published as an Advance Article on the web 13th June 2005

The synthesis of a new structural class of isoindoline nitroxides (aminoxyls), accessible *via* the palladium-catalysed Heck reaction, is presented. Reaction of the aryl bromoamine, 5-bromo-1,1,3,3-tetramethylisoindoline (**4**) or dibromoamine, 5,6-dibromo-1,1,3,3-tetramethylisoindoline (**5**) or the analogous bromonitroxides **6** and **7** with methyl acrylate gives the acrylate substituted tetramethylisoindoline amines **8** and **10** and nitroxides **12** and **14**. Similarly, the reaction of the aryl bromides and dibromides **4**–**7** with methyl 4-vinylbenzoate gives the carboxystyryl substituted tetramethylisoindoline amines **9** and **11** and the analogous nitroxides **13** and **15**. The carboxystyryl tetramethylisoindoline nitroxides demonstrate strongly suppressed fluorescence, which is revealed upon removal of the free radical by reduction or radical coupling.

Introduction

The isoindoline class of nitroxides (aminoxyls), such as 1,1,3,3-tetramethylisoindolin-2-yloxyl[1] (**1**) (TMIO), possesses some advantages[2–5] over commercially available nitroxides. These advantages arise largely from the fused aromatic moiety, which imparts rigidity to the ring system. Isoindoline nitroxides can exhibit superior electron paramagnetic resonance (EPR) linewidths,[6–9] resulting in increased accuracy in EPR oximetry, as relative line broadening is more easily measured on narrower lines.[10] Isoindoline systems may also allow the synthesis of more complex structures, through substitution onto the ring.[11] This is particularly significant in the generation of structures where extended conjugation can give rise to useful spectroscopic properties.



1

In this context, nitroxide radicals have long been recognised as effective quenchers of excited states of fluorescent moieties.[12–18] Following the seminal work of Blough *et al.*[19–23] several nitroxide molecules containing a covalently linked fluorophore have been synthesised. The covalent linkage creates a permanent "collision complex" between the nitroxide free radical moiety and the fluorophore resulting in almost complete quenching of the expected fluorescence output.19 The conversion of such "profluorescent" nitroxides into diamagnetic species by radical scavenging or redox activity eliminates this process and consequently allows for their detection by fluorescence spectroscopy. These nitroxide–fluorophore species can therefore be used as sensitive probes for the investigation of processes involving reactive free radical/redox species. Few of the profluorescent nitroxides synthesised to date involve robust carbon frameworks linking the fluorophore and the nitroxide,[24–26] and usually contain more labile linkages such as esters,[19–21,27–31] amides[32–34] and sulfonamides[35–38] or utilise comparatively labile fluorescamine/amine adducts.[22,39] In this context we have recently demonstrated the use of a novel profluorescent nitroxide based on a stable fused phenanthrene structure to probe the thermo-oxidative degradation process in polypropylene.[26]

Here we describe the first application of palladium-catalysed alkenylation of isoindoline nitroxides to generate a range of novel profluorescent nitroxides and amines. The use of methyl acrylate (**2**) or methyl 4-vinylbenzoate (**3**) as the olefin starting materials allows for the production of isoindoline amines and nitroxides containing substituted styryl and substituted stilbene functionality respectively. These molecules combine the advantages of the isoindoline class of nitroxides with a robust carbon–carbon bonded fluorophore–nitroxide linkage. The free radical compounds display weak fluorescence which can be switched on by reduction to generate highly fluorescent hydroxylamines. Compounds which contain the stilbene functionality are often used as fluorescent probe molecules in the study of photophysical properties of various media, due to the characteristically high quantum yields and short excited state lifetimes of stilbene derivatives.[40] The incorporation of a stilbene moiety imparts these key fluorescent properties to the novel nitroxide probes, albeit suppressed by the nitroxide function. Upon reaction at the nitroxide moiety, the fluorescence potency of the stilbene is revealed.

Results and discussion

Due to the relative stability of bis(*tert*-alkyl) nitroxides, many reactions can be performed in the presence of the free radical moiety. Reactions performed on the isoindoline systems have included nitration,[6] Friedel–Crafts alkylation,[41] as well as many functional group interconversions. Little work to date has dealt with palladium-catalysed coupling reactions which are a powerful tool in current synthetic methodology. Whilst Kalai *et al.*[42] have successfully applied such coupling reactions to pyrroline nitroxides, here we report the first examples where palladium-catalysis has been performed on the isoindoline class of nitroxides.

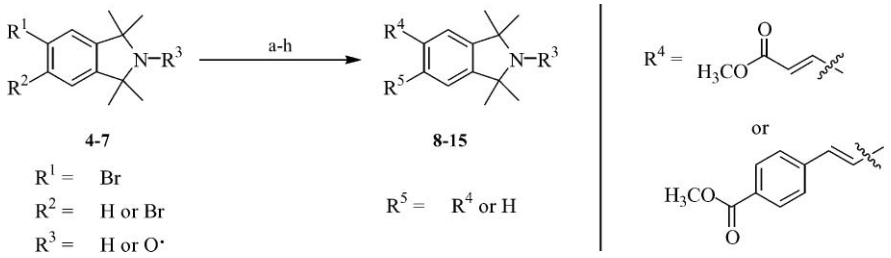
Palladium-catalysed Heck alkenylation of 5-bromo-1,1,3,3-tetramethylisoindoline (4) and 5-bromo-1,1,3,3-tetramethylisoindolin-2-yloxyl (6) with methyl acrylate (2) and methyl 4-vinylbenzoate (3)

The reaction of bromoisoindoline **4** and the nitroxide analogue **6** with methyl acrylate (**2**) in the presence of 2 mol% palladium catalyst in anhydrous *N*,*N*-dimethylformamide (DMF) at 100 °C, gave the acrylate substituted isoindoline (**8**) and isoindoline nitroxide (**12**) in modest yields of 29% and 13% respectively (Scheme 1, Table 1). Due to its low boiling point (80.7 °C),[43] a large excess of the olefin was used to maximise the available concentration in the reaction mixture.

DOI: 10.1039/b504354a

Table 1

Starting material	R^1	R^2	R^3	Reagents/ conditions	Alkene	Product	R^3	R^4	R^5	Reaction time/h	Purified yield (%)
4	Br	H	H	a	2	8	H	$CH{=}CHCO_2CH_3$	H	160	29
				b	3	9	H	$CH{=}CH(C_6H_4)CO_2CH_3$	H	96	33
5	Br	Br	H	c	2	10	H	$CH{=}CHCO_2CH_3$	R^4	66	15
				d	3	11	H	$CH{=}CH(C_6H_4)CO_2CH_3$	R^4	142	11
6	Br	H	O•	e	2	12	O•	$CH{=}CHCO_2CH_3$	H	72	50
				f	3	13	O•	$CH{=}CH(C_6H_4)CO_2CH_3$	H	72	85
7	Br	Br	O•	g	2	14	O•	$CH{=}CHCO_2CH_3$	R^4	72	62
				h	3	15	O•	$CH{=}CH(C_6H_4)CO_2CH_3$	R^4	72	53



Scheme 1 *Reagents and conditions*: (a) K_2CO_3 (1.8 eqv.), $Pd(OAc)_2$ (0.025 eqv.), PPh_3 (0.04 eqv.), **2** (9.6 eqv.), DMF, 100 °C, 160 h (b) K_2CO_3 (1.8 eqv.), $Pd(OAc)_2$ (0.025 eqv.), PPh_3 (0.04 eqv.), **3** (1.25 eqv.), DMF, 100 °C, 96 h (c) K_2CO_3 (3.0 eqv.), $Pd(OAc)_2$ (0.06 eqv.), PPh_3 (0.10 eqv.), **2** (19.1 eqv.), DMF, 100 °C, 66 h (d) K_2CO_3 (3.0 eqv.), $Pd(OAc)_2$ (0.06 eqv.), PPh_3 (0.1 eqv.), **3** (2.5 eqv.), DMF, 100 °C, 142 h (e) K_2CO_3 (1.95 eqv.), $Pd(OAc)_2$ (0.05 eqv.), PPh_3 (0.10 eqv.), **2** (9.6 eqv.), DMF, 120 °C, 72 h (f) K_2CO_3 (1.9 eqv.), $Pd(OAc)_2$ (0.05 eqv.), PPh_3 (0.10 eqv.), **3** (1.3 eqv.), DMF, 120 °C, 72 h (g) K_2CO_3 (3.26 eqv.), $Pd(OAc)_2$ (0.10 eqv.), PPh_3 (0.20 eqv.), **2** (19.1 eqv.), DMF, 120 °C, 72 h (h) K_2CO_3 (3.27 eqv.), $Pd(OAc)_2$ (0.10 eqv.), PPh_3 (0.20 eqv.), **3** (2.6 eqv.), DMF, 120 °C, 72 h.

In a similar reaction, this bromoisoindoline (**4**) and the nitroxide analogue (**6**) successfully reacted with methyl 4-vinylbenzoate (**3**) to give the carboxystyryl-substituted isoindoline (**9**) and isoindoline nitroxide (**13**) in yields of 33% and 34% respectively (Scheme 1). These initial reactions were not optimised, with the methyl acrylate being used to demonstrate the viability of Heck methodology to achieve this coupling. Palladium catalysed coupling with 1° and 2° amines in this context can be problematic, leading to amine cross coupling *via* Buchwald–Hartwig amination of the aryl halide. This may not be a major factor under the conditions used here due to the high steric demand of the amine in these tetamethylisoindoline systems.

This reaction was quite sensitive to the conditions used, as subtle alterations were shown to lead to substantial changes in the isolated yields. For instance, with the nitroxides, optimal reaction conditions (5 mol% palladium catalyst and temperature 120 °C) improved the syntheses of the acrylate substituted nitroxide (**12**) and carboxystyryl substituted nitroxide (**13**) from the 13% and 34% described above to 50% and 85% respectively.

Palladium-catalysed Heck alkenylation of 5,6-dibromo-1,1,3,3-tetramethylisoindoline (5) and 5,6-dibromo-1,1,3,3-tetramethylisoindolin-2-yloxyl (7) with methyl acrylate (2) and with methyl 4-vinylbenzoate (3)

The reaction of the aryl *ortho*-dibromides **5** and **7** with methyl acrylate (**2**) at 100 °C, in the presence of 5.5 mol% palladium catalyst, successfully gave the bis-acrylate substituted isoindoline (**10**) and isoindoline nitroxide (**14**) in yields of 15% and 32% respectively (Scheme 1).

Similarly, reaction of aryl *ortho*-dibromides **5** and **7** with methyl 4-vinylbenzoate (**3**) at 100 °C gave the bis-carboxystyryl-substituted isoindoline (**11**) and isoindoline nitroxide (**15**) in yields of 11% and 36% respectively (Scheme 1).

Again this reaction was quite sensitive to the conditions used, and optimal reaction conditions improved the syntheses of the extended aryl nitroxide products (**14**) and (**15**) from the 32% and 36% described above to 62% and 53% respectively.

Electron-rich aryl-halides, such as **4**, **5**, **6** and **7**, are somewhat deactivated with respect to the Heck reaction,[44] because oxidative addition of the halide to the palladium complex becomes more difficult in the presence of electron-donating substituents.

This is also evident from the yields of the disubstituted Heck products (**10**, **11**, **14** and **15**) which, under the same conditions, are obtained in comparable amounts to the monosubstituted products (**8**, **9**, **12** and **13**). Vinylation would be expected to lower electron density in the ring and therefore promote reactivity for the second substitution.

Fluorescence

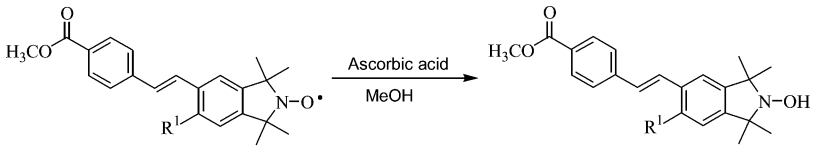
A measure of the fluorescence suppression of nitroxides **13** and **15** can be determined by *in situ* reduction with ascorbic acid measured directly in a spectrofluorimeter.

Complete reduction of the mono and disubstituted nitroxides **13** and **15** to give the hydroxylamines **16** and **17** is readily achieved, with maximum fluorescence being observed ~3 hours after addition of ascorbic acid (see Scheme 2). As expected, the reduced mono-carboxystyryl substituted nitroxide (**16**) has a maximum emission at a shorter wavelength (378 nm) than the corresponding reduced disubstituted nitroxide (**17**) (436 nm), due to the more extended conjugation present in the latter. Interestingly, the monosubstituted nitroxide displays a much stronger relative suppression (225 fold) compared to the disubstituted species (*cf.* 25 fold). Notably the disubstituted system generates the stronger emission intensity and displays greater fluorescence than the monosubstituted compound. Presumably incomplete excited state quenching by the nitroxide radical, when linked to a strongly fluorescent aromatic structure such as the one present in **15**, does not allow complete suppression of fluorescence emission.

The difference in the fluorescence intensity between the profluorescent nitroxides (**13** and **15**) and the diamagnetic hydroxylamines (**16** and **17**) indicates that these compounds may be useful fluorescent probes for the detection of reactive radical–redox species (Figs. 1 and 2).

Radical coupling

As further proof of the structure of the nitroxides generated using this Heck methodology, the carboxystyryl substituted



13 or 15 16 or 17

	R^1
13	H
15	$CH=CH(C_6H_4)CO_2CH_3$
16	H
17	$CH=CH(C_6H_4)CO_2CH_3$

Scheme 2 Reduction of nitroxides to hydroxylamines with ascorbic acid.

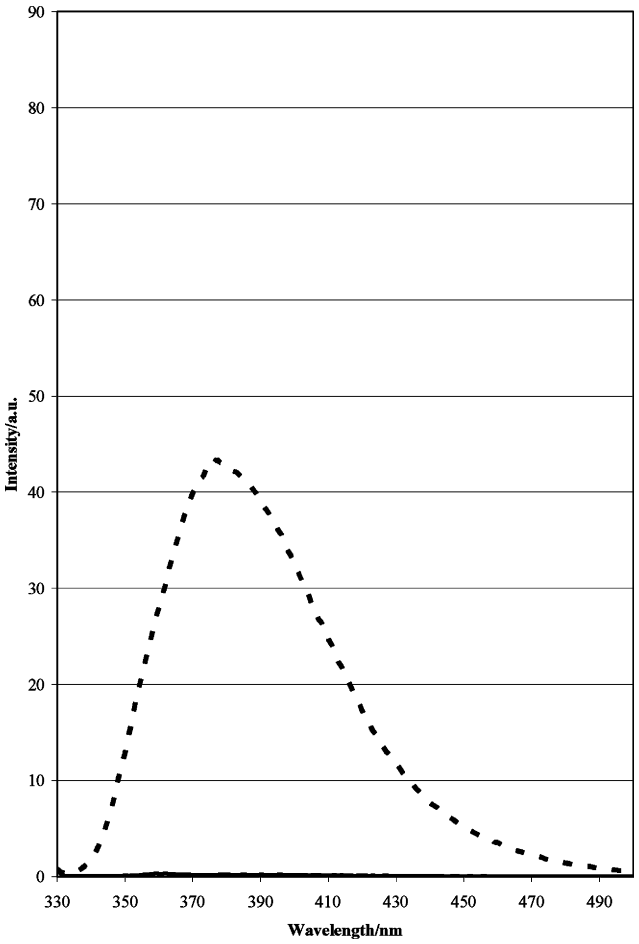


Fig. 1 Fluorescence spectra of **13** (—) and **16** (---) normalised to 1 μM.

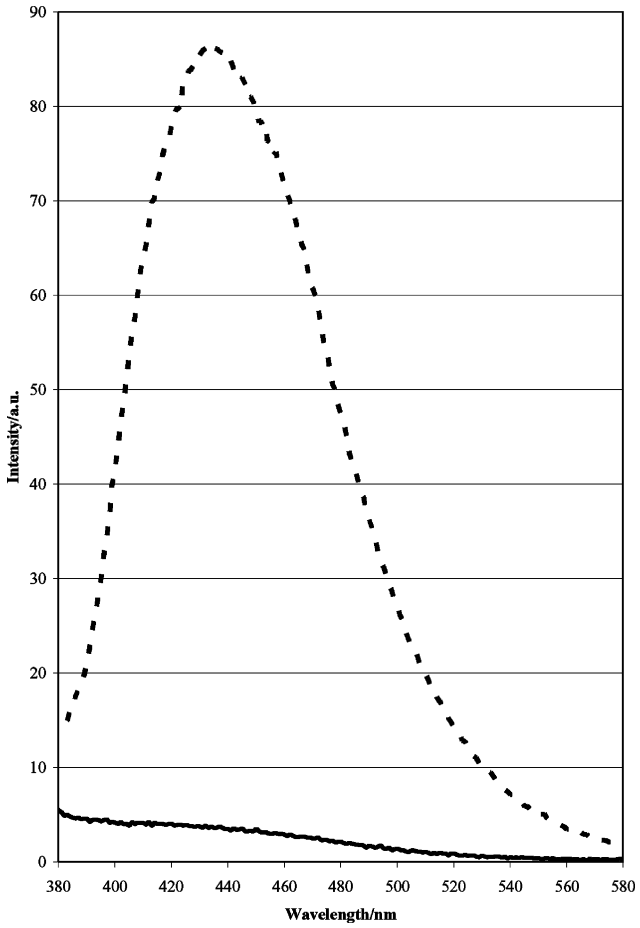
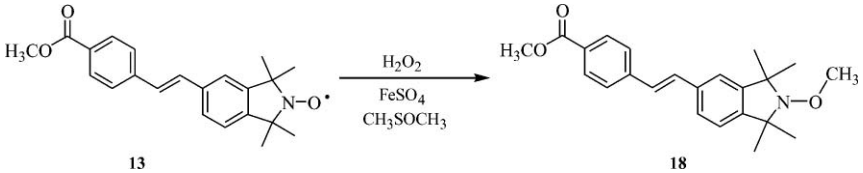


Fig. 2 Fluorescence spectra of **15** (—) and **17** (---) normalised to 1 μM.

nitroxide (**13**) was reacted with methyl radicals generated from dimethylsulfoxide and hydrogen peroxide to give the methoxyamine **18** (Scheme 3). The fluorescence intensity of **18** mirrored the hydroxylamine **16** with an excitation at 342 nm producing a strong fluorescence at 392 nm.

Experimental

^{1}H NMR spectra were recorded on a Bruker Avance 400 spectrometer in $CDCl_3$ and ^{13}C NMR spectra on a Varian Unity 300 spectrometer in $CDCl_3$. Coupling constants are given in Hz. IR spectra were recorded on a Nicolet 870 Nexus Fourier



13 18

Scheme 3 Coupling of carboxystyryl substituted nitroxide (**13**) to give the methyl radical adduct.

Transform infrared spectrometer equipped with a DTGS TEC detector and an ATR objective. High resolution mass 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, at a resolution of 6000 ppm, with perfluorokerosene used as an internal mass reference. Spectrofluorimetry was performed on a Varian Cary Eclipse fluorescence spectrophotometer equipped with a standard multicell Peltier thermostatted sample holder. 5-Bromo-1,1,3,3-tetramethylisoindoline[11] (**4**), 5,6-dibromo-1,1,3,3-tetramethylisoindoline[11] (**5**) 5-bromo-1,1,3,3-tetramethylisoindolin-2-yloxyl[11] (**6**), 5,6-dibromo-1,1,3,3-tetramethylisoindolin-2-yloxyl[11] (**7**) and methyl 4-vinylbenzoate[45] (**3**) were synthesised by methods previously described in the literature.

Heck reactions with methyl acrylate (2)

The procedure for the synthesis of acrylate substituted isoindoline amines **8** (monosubst.) and **10** (disubst.) and nitroxides **12** (monosubst.) and **14** (disubst.) from methyl acrylate (**2**) and the brominated amines **4** and **5** or nitroxides **6** and **7** is shown below, using the synthesis of the monosubstituted acrylate isoindoline amine **8** as an example. Alterations of the procedure for the synthesis of disubstituted amine **10** and the mono and disubstituted nitroxides, **12** and **14** respectively, are also shown below. Notably, standard oxidations of the substituted amines to generate the nitroxides were not successful, possibly due to oxidation of the alkene moiety.

5-(2-Methylcarboxyethenyl)-1,1,3,3-tetramethylisoindoline (8)

To 5-bromo-1,1,3,3-tetramethylisoindoline (**4**) (300 mg, 1.18 mmol), K_2CO_3 (300 mg, 2.17 mmol), $Pd(OAc)_2$ (6 mg, 0.027 mmol) and PPh_3 (12 mg, 0.046 mmol) in a Schlenk vessel, under argon were added methyl acrylate (**2**) (0.96 cm^3, 10.66 mmol) and anhydrous DMF (15 cm^3) and the resulting solution was degassed *via* several freeze–evacuate–thaw cycles then sealed under argon and stirred at 100 °C for 160 hours. Water was then added to the resultant brown solution and the reaction mixture was extracted with DCM. The combined organic phases were washed with brine, dried (Na_2SO_4) and the solvent was removed under reduced pressure. 5-(2-Methylcarboxyethenyl)-1,1,3,3-tetramethylisoindoline (**8**) was purified by column chromatography (30% EtOAc, 70% *n*-hexane) to give an orange oil (86 mg, 0.34 mmol, 29%) which did not crystallise; δ_H (400.162 MHz; $CDCl_3$) 1.45 (6H, s, CH_3), 1.46 (6H, s, CH_3), 1.81 (1H, br, NH), 3.80 (3H, s, OCH_3), 6.46 (1H, d, *J* 15.9 Hz, =CH), 7.14 (1H, d, *J* 7.8 Hz, 7-H), 7.27 (1H, d, *J* 1.5 Hz, 4-H), 7.42 (1H, dd, *J* 7.8 Hz and 1.5 Hz, 6-H), 7.75 (1H, d, *J* 15.9 Hz, =CH); δ_C (75.430 MHz; $CDCl_3$) 29.9 (CH_3), 32.0 (CH_3), 51.9 (OCH_3), 63.1 (C-1), 117.4 (=CH), 121.3 (C-5), 122.3 (C-6), 127.9 (C-7), 133.9 (C-4), 141.9 (=CH), 149.8 (C-7a), 151.6 (C-3a), 167.1 (C=O); ν_{max} (ATR) 3369 (NH), 2960 (alkyl CH), 1716 (C=O), 1636 (C=C), 1463 and 1436 (aryl C–C), 1164 (OCH_3) cm^{-1}; EI MS found $(M–H)^+$ 258.14939 (0.04 ppm from calc. mass of $C_{16}H_{20}NO_2$ (M–H)); *m/z* 258 ($(M–H)^+$, ~1%), 244 (100), 229 (33).

5,6-Bis-(2-methylcarboxyethenyl)-1,1,3,3-tetramethylisoindoline (10)

,6-Dibromo-1,1,3,3-tetramethylisoindoline (**5**) (300 mg, 0.90 mmol), K_2CO_3 (378 mg, 2.73 mmol), $Pd(OAc)_2$ (12 mg, 0.054 mmol), PPh_3 (24 mg, 0.092 mmol), methyl acrylate (**2**) (1.44 cm^3, 16 mmol), were treated as above and heated in anhydrous DMF (15 cm^3) for 66 hours at 100 °C. Column chromatography (95% $CHCl_3$, 5% MeOH) gave the disubstituted acrylate isoindoline amine **10** as a yellow oil (45 mg, 0.132 mmol, 15%), which did not crystallise; δ_H (400.162 MHz; $CDCl_3$) 1.46 (12H, s, CH_3), 1.92 (1H, br, NH), 3.82 (6H, s, OCH_3), 6.35 (2H, d, *J* 15.8 Hz, =CH), 7.29 (2H, s, Ar–H), 8.04 (2H, d, *J* 15.8 Hz, =CH); δ_C (75.430 MHz; $CDCl_3$) 31.9 (CH_3), 52.1 (OCH_3), 63.0 (C-1 and C-3), 120.8 (=CH), 121.2 (C-5 and C-6), 134.1 (C-4 and C-7), 141.9 (=CH), 151.7 (C-3a and C-7a), 167.1 (C=O); ν_{max} (ATR) 3365 (NH), 2961 (alkyl CH), 1716 (C=O), 1637 (C=C), 1463 and 1436 (aryl C–C), 1167 (OCH_3) cm^{-1}; EI MS found $(M–H)^+$ 342.17020 (0.96 ppm from calc. mass of $C_{20}H_{24}NO_4$ (M–H)); *m/z* 342 ($(M–H)^+$, <1%), 328 (100), 268 (9).

5-(2-Methylcarboxyethenyl)-1,1,3,3-tetramethylisoindolin-2-yloxyl (12)

5-Bromo-1,1,3,3-tetramethylisoindolin-2-yloxyl (**6**) (100 mg, 0.38 mmol), K_2CO_3 (100 mg, 0.72 mmol), $Pd(OAc)_2$ (4 mg, 0.008 mmol), PPh_3 (8 mg, 0.031 mmol) and methyl acrylate (**2**) (0.32 cm^3, 3.55 mmol) were treated as above and heated in anhydrous DMF (5 cm^3) for 72 hours at 120 °C. Column chromatography (30% EtOAc, 70% *n*-hexane) and recrystallisation from MeCN gave yellow irregular platelets of the monosubstituted acrylate nitroxide **12** (52 mg, 0.189 mmol, 50%) mp 162–164 °C; ν_{max} (ATR) 3023 (aryl CH), 2973 (alkyl CH), 1704 (C=O), 1641 (C=C), 1492 and 1432 (aryl C–C), 1359 and 1330 (NO), 1164 (OCH_3) cm^{-1}; EI MS found M^+ 274.14427 (0.18 ppm from calc. mass of $C_{16}H_{20}NO_3$); *m/z* 274 (M^+, 66%), 259 (100), 244 (83), 229 (60).

5,6-Bis-(2-methylcarboxyethenyl)-1,1,3,3-tetramethylisoindolin-2-yloxyl (14)

5,6-Dibromo-1,1,3,3-tetramethylisoindolin-2-yloxyl (**7**) (100 mg, 0.28 mmol), K_2CO_3 (154 mg, 1.11 mmol), $Pd(OAc)_2$ (8 mg, 0.016 mmol), PPh_3 (16 mg, 0.062 mmol) and methyl acrylate (**2**) (0.5 cm^3, 5.55 mmol) were treated as previously indicated above and heated in anhydrous DMF (5 cm^3) for 72 hours at 120 °C. Column chromatography (30% EtOAc, 70% *n*-hexane) and recrystallisation from MeCN gave yellow needles of the disubstituted acrylate nitroxide **14** (62 mg, 0.173 mmol, 62%) mp 216–218 °C (decomp.); ν_{max} (ATR) 3066 (=CH), 3033 (aryl CH), 2967 (alkyl CH), 1702 (C=O), 1627 (C=C), 1488 and 1436 (aryl C–C), 1367 and 1328 (NO), 1166 (OCH_3) cm^{-1}; EI MS found M^+ 358.16511 (0.95 ppm from calc. mass of $C_{20}H_{24}NO_5$); *m/z* 358 (M^+, 90%), 343 (14), 328 (100), 283 (53), 268 (75), 253 (44).

Heck reactions with methyl 4-vinylbenzoate (3)

The procedure for the synthesis of the carboxystyryl isoindoline amines **9** and **11** and nitroxides **13** and **15** from methyl 4-vinylbenzoate (**3**) and the brominated amines **4** and **5** or nitroxides **6** and **7** is shown below using the synthesis of the monosubstituted carboxystyryl isoindoline amine **9** as an example. Alterations of the procedure for the synthesis of the disubstituted carboxystyryl isoindoline amine **11** or the mono and disubstituted carboxystyryl nitroxides, **13** and **15** respectively, are also shown below.

5-[2-(4-Methylcarboxyphenyl)ethenyl]-1,1,3,3-tetramethylisoindoline (9)

To 5-bromo-1,1,3,3-tetramethylisoindoline (**4**) (300 mg, 1.18 mmol), K_2CO_3 (300 mg, 2.17 mmol), $Pd(OAc)_2$ (6 mg, 0.027 mmol) and PPh_3 (12 mg, 0.046 mmol) in a Schlenk vessel under argon were added methyl 4-vinylbenzoate (**3**) (240 mg, 1.47 mmol) and anhydrous DMF (15 cm^3) and the resulting solution was degassed *via* several freeze–evacuate–thaw cycles, sealed under argon and stirred at 100 °C for 96 hours. Water was added to the resultant dark brown solution and this reaction mixture was extracted with DCM. The combined organic phases were washed with brine, dried (Na_2SO_4) and the solvent removed under reduced pressure. The product, 5-[2-(4-methylcarboxyphenyl)ethenyl]-1,1,3,3-tetramethylisoindoline (**9**), was purified by column chromatography (98% $CHCl_3$, 2% MeOH) and recrystallised from EtOH, forming colourless crystals (132 mg, 0.394 mmol, 33%) mp 144–146 °C; δ_H

(400.162 MHz; $CDCl_3$) 1.47 (6H, s, CH_3), 1.50 (6H, s, CH_3), 1.61 (1H, br, NH), 3.92 (3H, s, OCH_3), 7.11 (1H, d, *J* 16.1 Hz, =CH), 7.12 (1H, d, *J* 7.8 Hz, 7-H), 7.25 (1H, d, *J* 16.1 Hz, =CH), 7.28 (1H, d, *J* 1.2 Hz, 4-H), 7.42 (1H, dd, *J* 1.2 Hz and 7.8 Hz, 6-H), 7.57 (2H, d, *J* 8.3 Hz, ArH), 8.02 (2H, d, *J* 8.3 Hz, ArH); δ_C (75.430 MHz; $CDCl_3$) 31.8 (CH_3), 31.9 (CH_3), 52.0 (OCH_3), 62.7 (C-1 and C-3), 119.6 (=CH), 121.8 (=CH), 126.2 (C-6), 126.3 (C-4), 127.0 (C-7), 128.8 (ArC), 130.0 (ArC), 131.3 (ArC), 136.1 (C-5), 142.0 (ArC), 149.3 (C-7a), 149.5 (C-3a), 166.9 (C=O); ν_{max} (ATR) 3351 (NH), 2962 (alkyl CH_3), 1719 (C=O), 1604 (C=C), 1477 and 1435 (aryl C–C), 1178 (OCH_3) cm^{-1}; EI MS found $(M–H)^+$ 334.18164 (2.81 ppm from calc. mass of $C_{22}H_{24}NO_2$ (M–H)); *m*/*z* 334 ($(M–H)^+$, 14%), 320 (100), 305 (23), 304 (18), 144 (10).

5,6-Bis-[2-(4-methylcarboxyphenyl)ethenyl]-1,1,3,3-tetramethylisoindoline (11)

5,6-Dibromo-1,1,3,3-tetramethylisoindoline (**5**) (300 mg, 0.90 mmol), K_2CO_3 (378 mg, 2.73 mmol), $Pd(OAc)_2$ (12 mg, 0.054 mmol), PPh_3 (24 mg, 0.092 mmol) and methyl 4-vinylbenzoate (**3**) (360 mg, 2.21 mmol) were treated as described above and heated in anhydrous DMF (15 cm^3) for 142 hours at 100 °C. Column chromatography (70% EtOAc, 30% *n*-hexane; then 80% EtOAc, 20% EtOH) and recrystallisation by mixed solvent layer recrystallisation from DCM–*n*-pentane gave off-white crystals of the disubstituted carboxystyryl isoindoline amine **11** (50 mg, 0.101 mmol, 11%) mp 176–178 °C; δ_H (400.162 MHz; $CDCl_3$) 1.53 (12H, s, CH_3), 1.74 (1H, br, NH), 3.93 (6H, s, OCH_3), 7.04 (2H, d, *J* 15.8 Hz, =C–H), 7.34 (2H, s, 4-H and 7-H), 7.57 (2H, d, *J* 15.8 Hz, =C–H), 7.59 (4H, d, *J* 8.2 Hz, ArH), 8.04 (4H, d, *J* 8.2 Hz, ArH); δ_C (75.430 MHz; $CDCl_3$) 31.7 (CH_3), 52.1 (OCH_3), 62.9 (C-1 and C-3), 119.8 (=CH), 119.9 (=CH), 126.4 (C-4 and C-7), 129.1 (ArC), 130.1 (ArC), 130.3 (ArC), 135.4 (C-5 and C-6), 141.8 (ArC), 149.3 (C-3a and C-7a), 166.8 (C=O); ν_{max} (ATR) 3345 (NH), 3018 (aryl CH), 2955 (alkyl CH), 1708 (C=O), 1603 (C=C), 1479 and 1433 (aryl C–C), 1178 (OCH_3) cm^{-1}; EI MS found $(M–H)^+$ 494.23332 (0.38 ppm from calc. mass of $C_{32}H_{32}NO_4$ (M–H)): *m*/*z* 494 ($(M–H)^+$, ~1%), 480 (100), 465 (8), 320 (24).

5-[2-(4-Methylcarboxyphenyl)ethenyl]-1,1,3,3-tetramethylisoindolin-2-yloxyl (13)

5-Bromo-1,1,3,3-tetramethylisoindolin-2-yloxyl (**6**) (100 mg, 0.38 mmol), K_2CO_3 (100 mg, 0.72 mmol), $Pd(OAc)_2$ (4 mg, 0.018 mmol), PPh_3 (8 mg, 0.031 mmol) and methyl 4-vinyl benzoate (**3**) (80 mg, 0.491 mmol) were treated as above and heated in anhydrous DMF (5 cm^3) for 72 hours at 120 °C. Column chromatography (70% EtOAc, 30% *n*-hexane) and recrystallisation from EtOH gave light orange needles of the monosubstituted carboxystyryl isoindoline nitroxide **13** (113 mg, 0.323 mmol, 85%) mp 164–165 °C (found: C, 75.2; H, 6.9; N, 3.9. $C_{22}H_{24}NO_3$ requires C, 75.4; H, 6.9; N, 4.0%); ν_{max} (ATR) 2976 (alkyl CH), 1715 (C=O), 1604 (C=C), 1492 and 1434 (aryl C–C), 1373 and 1358 (NO), 1177 (OCH_3) cm^{-1}; EI MS found M^+ 350.17546 (0.46 ppm from calc. mass of $C_{22}H_{24}NO_3$); *m*/*z* 350 (M^+, 30%), 335 (25), 320(100), 305 (42).

5,6-Bis-[2-(4-methylcarboxyphenyl)ethenyl]-1,1,3,3-tetramethylisoindolin-2-yloxyl (15)

5,6-Dibromo-1,1,3,3-tetramethylisoindolin-2-yloxyl (**7**) (100 mg, 0.28 mmol), K_2CO_3 (154 mg, 1.11 mmol), $Pd(OAc)_2$ (8 mg, 0.036 mmol), PPh_3 (16 mg, 0.062 mmol) and methyl 4-vinylbenzoate (**3**) (120 mg, 0.737 mmol) were treated as above and heated in anhydrous DMF (5 cm^3) at 120 °C for 72 hours. Column chromatography (30% EtOAc, 70% *n*-hexane) and subsequent recrystallisation from MeCN gave fine light yellow needles of the disubstituted carboxystyryl isoindoline nitroxide **15** (78 mg, 0.152 mmol, 54%) mp 212–214 °C (decomp.); ν_{max} (ATR) 2973 (alkyl CH), 1708 (C=O), 1602 (C=C), 1484 and 1403 (aryl C–C), 1373 and 1359 (NO) cm^{-1}; EI MS found M^+ 510.22812 (0.14 ppm from calc. mass of $C_{32}H_{32}NO_5$); *m*/*z* 510 (M^+, 15%), 495 (9), 480 (100), 465 (10), 330 (40).

5-[2-(4-Methylcarboxyphenyl)ethenyl]-2-methoxy-1,1,3,3-tetramethylisoindoline (18)

To a solution of the carboxystyryl substituted nitroxide **13** (40 mg, 0.114 mmol) and $FeSO_4·7H_2O$ (64 mg, 0.230 mmol) in DMSO (4 cm^3) was added H_2O_2 (30%, 26 μL) dropwise and the mixture was stirred at room temperature for 30 minutes. The resultant solution was poured onto NaOH (1 M, 30 cm^3) and subsequently extracted with Et_2O (3 × 50 cm^3) and dried (Na_2SO_4). Removal of solvent under reduced pressure gave the carboxystyryl substituted *N*-methoxyisoindoline **18** (36.8 mg, 0.101 mmol, 89%). Recrystallisation from EtOH gave colourless crystals of **18** (32.2 mg, 0.088 mmol, 77%); δ_H (400.162 MHz; $CDCl_3$) 1.45 (12H, br s, CH_3), 1.61 (1H, br, NH), 3.80 (3H, s, $NOCH_3$), 3.92 (3H, s, ester OCH_3), 7.10 (1H, d, *J* 16.2 Hz, =CH), 7.11 (1H, d, *J* 8.1 Hz, 7-H), 7.23 (1H, d, *J* 16.2 Hz, =CH), 7.27 (1H, d, *J* 1.3 Hz, 4-H), 7.40 (1H, dd, *J* 1.3 Hz and 8.1 Hz, 6-H), 7.56 (2H, d, *J* 8.3 Hz, ArH), 8.02 (2H, d, *J* 8.3 Hz, ArH); δ_C (75.430 MHz; $CDCl_3$) 25.2 and 29.1 (CH_3 groups, broadened through ring inversion), 52.5 (ester OCH_3), 65.9 ($NOCH_3$), 67.8 (C-1 and C-3, broadened through ring inversion), 120.0 (=CH), 122.3 (=CH), 126.7 (C-6), 126.8 (C-4), 127.5 (C-7), 129.2 (ArC), 130.4 (ArC), 131.7 (ArC), 136.6 (C-5), 142.3 (ArC), 146.2 (C-7a and C-3a), 167.3 (C=O); ν_{max} (ATR) 2978 (alkyl CH_3), 1714 (C=O), 1603 (C=C), 1493 and 1435 (aryl C–C), 1178 (OCH_3) cm^{-1}; EI MS found M^+ 365.19948 (1.05 ppm from calc. mass of $C_{23}H_{27}NO_3$); *m*/*z* 365 (M^+, 5%), 350 (100), 319 (26), 304 (23).

Fluorescence measurements

5-[2-(4-Methylcarboxyphenyl)ethenyl]-1,1,3,3-tetramethylisoindolin-2-yloxyl (13). The carboxystyryl nitroxide **13** (0.4 mg) was dissolved in HPLC grade MeOH (100 cm^3) in a volumetric flask. The resulting solution (4.0 mg dm^{-3}, 1.14 × 10^{-5} M) was deoxygenated by bubbling with argon for 15 min. Any subsequent solvent loss was replaced to maintain a known concentration. Reduction of the carboxystyryl nitroxide **13** to the hydroxylamine **16** was achieved by addition of a saturated solution of ascorbic acid in HPLC grade MeOH (50 μL) to a solution of **13** in MeOH (3 cm^3) with stirring.

5,6-Bis-[2-(4-methylcarboxyphenyl)ethenyl]-1,1,3,3-tetramethylisoindolin-2-yloxyl (15). In a similar procedure to the one described above, disubstituted carboxystyryl nitroxide **15** (0.4 mg) was dissolved in HPLC grade MeOH (100 cm^3) in a volumetric flask. This solution was further diluted by a factor of 5. The resulting solution of **15** in MeOH (0.8 mg dm^{-3}, 1.57 × 10^{-6} M) was deoxygenated by bubbling with argon for 15 min. Any subsequent solvent loss was replaced to maintain the concentration. Similar to above, the reduction of disubstituted carboxystyryl nitroxide **15** to the hydroxylamine **17** was achieved by addition of a saturated solution of ascorbic acid in HPLC grade MeOH (50 μL) to the solution of **15** in MeOH (3 cm^3) with stirring.

Acknowledgements

The authors acknowledge the financial support of the Australian Research Council (DP0211669) and the National Cancer Institute (PO1 CA91597).

References

1 P. G. Griffiths, G. Moad, E. Rizzardo and D. H. Solomon, *Aust. J. Chem.*, 1983, **36**, 397–401.

2 G. Moad, E. Rizzardo and D. H. Solomon, *Macromolecules*, 1982, **15**, 909–914.
3 P. G. Griffiths, E. Rizzardo and D. H. Solomon, *Tetrahedron Lett.*, 1982, **23**, 1309–1312.
4 W. Adam, S. E. Bottle, R. Finzel, T. Kammel, E.-M. Peters, K. Peters, H. G. v. Schnering and L. Walz, *J. Org. Chem.*, 1992, **57**, 982–988.
5 S. E. Bottle, U. Chand and A. S. Micallef, *Chem. Lett.*, 1997, 857–858.
6 R. Bolton, D. G. Gillies, L. H. Sutcliffe and X. Wu, *J. Chem. Soc., Perkin Trans. 2*, 1993, 2049–2052.
7 D. G. Gillies, L. H. Sutcliffe and X. Wu, *J. Chem. Soc., Faraday Trans.*, 1994, **90**, 2345–2349.
8 S. E. Bottle, D. G. Gillies, A. S. Micallef, D. A. Reid and L. H. Sutcliffe, *Magn. Reson. Chem.*, 1999, **37**, 730–734.
9 S. E. Bottle, D. G. Gillies, D. L. Hughes, A. S. Micallef, A. I. Smirnov and L. H. Sutcliffe, *J. Chem. Soc., Perkin Trans. 2*, 2000, 1285–1291.
10 J. Shen, S. Bottle, N. Khan, O. Grinberg, D. Reid, A. Micallef and H. Swartz, *Appl. Magn. Reson.*, 2002, **22**, 357–368.
11 A. S. Micallef, R. C. Bott, S. E. Bottle, G. Smith, J. M. White, K. Matsuda and H. Iwamura, *J. Chem. Soc., Perkin Trans. 2*, 1999, **2**, 65–71.
12 J. A. Green, L. A. Singer and J. H. Parks, *J. Chem. Phys.*, 1973, **58**, 2690–2695.
13 J. A. Green and L. A. Singer, *J. Am. Chem. Soc.*, 1974, **96**, 2730–2733.
14 A. R. Watkins, *Chem. Phys. Lett.*, 1974, **29**, 526–528.
15 S. Atik and L. A. Singer, *J. Am. Chem. Soc.*, 1978, **100**, 3234–3235.
16 S. S. Atik, C. L. Kwan and L. A. Singer, *J. Am. Chem. Soc.*, 1979, **101**, 5696–5702.
17 S. K. Chattopadhyay, P. K. Das and G. L. Hug, *J. Am. Chem. Soc.*, 1983, **105**, 6205–6210.
18 J. C. Scaiano and C. I. Paraskevopoulos, *Can. J. Chem.*, 1984, **62**, 2351–2354.
19 N. V. Blough and D. J. Simpson, *J. Am. Chem. Soc.*, 1988, **110**, 1915–1917.
20 G. L. Gerlock, P. J. Zacmanidis, D. R. Bauer, D. J. Simpson, N. V. Blough and I. T. Salmeen, *Free Radical Res. Commun.*, 1990, **10**, 119–121.
21 S. A. Green, D. J. Simpson, G. Zhou, P. S. Ho and N. V. Blough, *J. Am. Chem. Soc.*, 1990, **112**, 7337–7346.
22 D. J. Kieber and N. V. Blough, *Free Radical Res. Commun.*, 1990, **10**, 109–117.
23 D. J. Kieber and N. V. Blough, *Anal. Chem.*, 1990, **62**, 2275–2283.
24 T. Kalai, E. Hideg, J. Jeko and K. Hideg, *Tetrahedron Lett.*, 2003, **44**, 8497–8499 .
25 T. Kalai, J. Jeko and K. Hideg, *Tetrahedron Lett.*, 2004, **45**, 8395–8398.
26 A. S. Micallef, J. P. Blinco, G. A. George, D. A. Reid, E. Rizzardo, S. H. Thang and S. E. Bottle, *Polym. Degrad. Stab.*, 2005, DOI: 10.1016/j.polymdegradstab.2005.01.030.
27 X.-F. Yang and X.-Q. Guo, *Analyst*, 2001, **126**, 1800–1804.
28 X.-F. Yang and X.-Q. Guo, *Anal. Chim. Acta*, 2001, **434**, 169–177.
29 C. Coenjarts, O. Garcia, L. Llauger, J. Palfreyman, A. L. Vinette and J. C. Scaiano, *J. Am. Chem. Soc.*, 2003, **125**, 620–621.
30 G. Moad, D. A. Shipp, T. A. Smith and D. H. Solomons, *J. Phys. Chem. A*, 1999, **103**, 6580–6586.
31 C. Aliaga, A. Aspee and J. C. Scaiano, *Org. Lett.*, 2003, **5**, 4145–4148.
32 C. Toniolo, M. Crisma and F. Formaggio, *Biopolymers*, 1998, **47**, 153–158.
33 B. Pispisa, A. Palleschi, L. Stella, M. Venanzi and C. Toniolo, *J. Phys. Chem. B*, 1998, **102**, 7890–7898.
34 B. Pispisa, C. Mazzuca, A. Palleschi, L. Stella, M. Venanzi, M. Wakselman, J.-P. Mazaleyrat, M. Rainaldi, F. Formaggio and C. Toniolo, *Chem.–Eur. J.*, 2003, **9**, 4084–4093.
35 E. Hideg, T. Kalai, K. Hideg and I. Vass, *Biochemistry*, 1998, **37**, 11405–11411.
36 T. Kalai, E. Hideg, I. Vass and K. Hideg, *Free Radical Biol. Med.*, 1998, **24**, 649–652.
37 E. Lozinsky, V. V. Martin, T. A. Berezina, A. I. Shames, A. L. Weis and G. I. Likhtenshtein, *J. Biochem. Biophys. Methods*, 1999, **38**, 29–42.
38 P. Bilski, K. Hideg, T. Kalai, M. A. Bilska and C. F. Chignell, *Free Radical Biol. Med.*, 2003, **34**, 489–495.
39 S. Pou, Y.-I. Huang, A. Bhan, V. S. Bhadti, R. S. Hosmane, S. Y. Wu, G.-L. Cao and G. M. Rosen, *Anal. Biochem.*, 1993, **212**, 85–90.
40 A. A. Kubicki, *Chem. Phys. Lett.*, 2003, **373**, 471–474.
41 C. D. Smith, R. C. Bott, S. E. Bottle, A. S. Micallef and G. Smith, *J. Chem. Soc., Perkin Trans. 2*, 2002, 533–537.
42 T. Kalai, M. Balog, J. Jeko, W. I. Hubbell and K. Hideg, *Synthesis*, 2002, **16**, 2365–2372.
43 *CRC Handbook of Chemistry and Physics*, ed. D. R. Lide, CRC Press, USA, 84th edn., 2003.
44 N. J. Whitcombe, K. K. Hii and S. E. Gibson, *Tetrahedron*, 2001, **57**, 7449–7476.
45 S. J. Webb and J. K. M. Sanders, *Inorg. Chem.*, 2000, **39**, 5920–5929.