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Effects of Aromatic Substituents on the Photocleavage of 1-Acyl-7-nitroindolines

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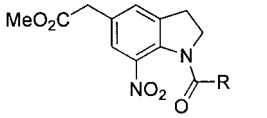
Abstract—Photolysis of 1-acyl-7-nitroindolines in aqueous solution gives a carboxylic acid and a 7-nitrosoindole. These compounds are useful as photolabile precursors of carboxylic acids, particularly neuroactive amino acids. The effect of electron-donating substituents at the 4-position has been explored for its effect on photolysis efficiency. 4-Methoxy substitution improved the photolysis efficiency >2-fold but the 4-dimethylamino analogue was essentially inert. A 5-alkyl substituent, that blocks unwanted nitration at this position, reduced the beneficial effect of the 4-methoxy group.

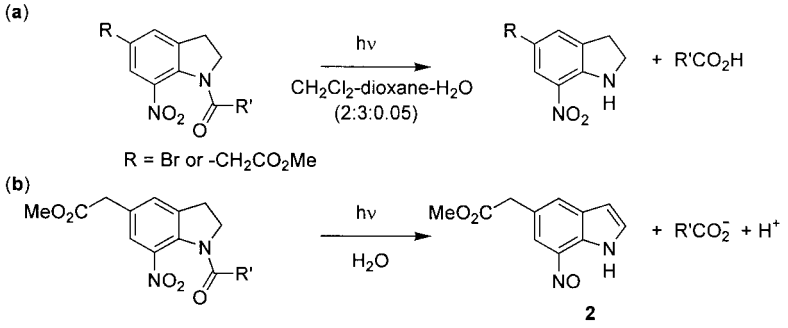
Introduction

Many compounds have been developed in the past two decades that, upon photolysis with a pulse of near-UV light, release biological effector species at or near their site of action within living preparations on a μs to ms time scale. These photolabile precursors are used particularly because they enable study of fast biological processes without interference by diffusion kinetics. The concept is covered in a number of reviews.[1] Despite these successes, there has been a lack of reagents capable of rapid and efficient release of amino acids, such as L-glutamate, that are key neurotransmitters in synapses of the central nervous system (see Ref. 2 for a survey of previous work). Recently we described the 7-nitroindolyl derivative **1a** as a particularly promising reagent for rapid photolytic generation of L-glutamate.[3] Initial neurophysiological studies in mammalian brain slices, triggered by photochemical release of L-glutamate from **1a**, have been reported in conference proceedings.[4]



1a R = $CH_2CH_2CH(NH_3^+)CO_2^-$
1b R = Me

Our exploration of this photochemistry originated from the known photohydrolysis of 1-acyl-5-bromo-7-nitroindolines, which yields 5-bromo-7-nitroindoline and a carboxylic acid when irradiated in an aprotic organic solvent containing ~1% water (Scheme 1a).[5] Under identical conditions, we observed the same photohydrolysis when the bromine substituent was replaced by a methoxycarbonylmethyl group as in **1**.[3] However, in fully aqueous solution the reaction took an entirely different course to yield a carboxylic



Scheme 1. Photolytic reactions of 1-acyl-7-nitroindolines: (a) in aprotic organic solvent containing a trace of water; and (b) in fully aqueous solution.

Keywords: amino acids and derivatives; indoles; indolines; photochemistry.
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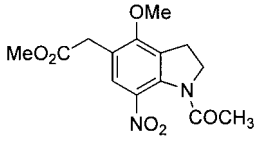
acid and the 5-substituted 7-nitrosoindole **2** (Scheme 1b), without incorporation of solvent water into the products.[3] Studies of this mechanistic dichotomy are in progress, but the present paper describes efforts to optimise the efficiency of the aqueous photoreaction by exploring different substituents on the aromatic ring. Good photolysis efficiency is important in biological experiments in order to maximise conversion by a single light flash. For simplicity, all the optimisation experiments were carried out with *N*-acetyl compounds.

Efficiency in single flash experiments is normally determined by a combination of the absorption coefficient at the wavelength of the irradiating light and the product quantum yield. Our strategy was focused on enhancing the absorption coefficient by introducing electron-donating substituents *para* to the nitro group. The results encompass a range of chemistry of 4-substituted indoles and indolines and in practice the effects on photolysis efficiency were mediated by changes in both product quantum yield and absorption coefficient.

Results and Discussion

Our first goal was to study the effect of a 4-methoxy substituent, i.e. compound **3**, and initially we followed an analogous route to that used for synthesis of **1**.[3] Thus 1-acetyl-4-methoxyindoline **4** was treated with acetyl chloride under Friedel–Crafts conditions and the product ketone was oxidised with thallium(III) nitrate[6] to give the corresponding methyl indolineacetate. Nitration ($NaNO_3$–TFA) gave a product that was initially assumed to be the required compound **3**, but this material was photostable and we were concerned that the first electrophilic substitution could have taken place at the 7-position rather than at C-5 as expected. Single crystal X-ray diffraction analysis of the nitrated product (Fig. 1) confirmed this[†]. The true sequence is therefore as shown in Scheme 2 to yield the 5-nitro compound **7**.



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Since Friedel–Crafts acylation of **4** was regiospecific at the 7-position, we examined direct nitration of **4** in the hope of obtaining only a compound with the nitro group at C-7. However, nitration of **4** gave the 5- and 7-nitro compounds **8** and **9** in equal proportion. Nevertheless, the two compounds were readily separated by chromatography and the regiochemistry of **9** was assigned by observation of a strong NOE enhancement between the methoxy protons and the adjacent aromatic proton. The lack of regioselectivity in this reaction compared to the Friedel–Crafts acylation can be explained by higher reactivity of the

[†] Crystallographic details will be deposited at the Cambridge Crystallographic Data Centre.

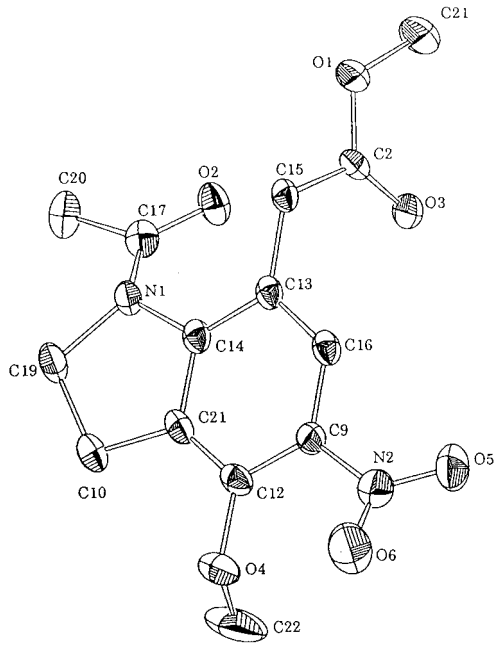
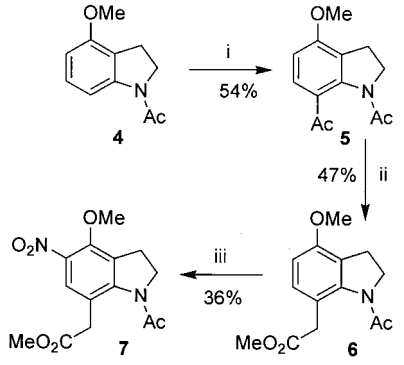
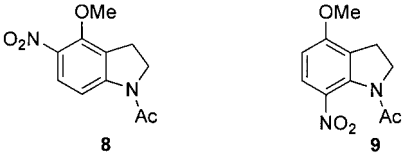


Figure 1. X-Ray crystal structure of compound **7**, showing 30% thermal ellipsoids.



Scheme 2. *Reagents:* (i) MeCOCl, $AlCl_3$; (ii) Tl(III)NO_3, MeOH; (iii) $NaNO_3$, TFA.

electrophile in the nitration reaction.[7] Comparative photolysis of aqueous solutions of **1b** and **9** at equal concentrations indicated that the latter photolysed with ~3-fold greater efficiency, as assessed by spectroscopic changes upon progressive photolysis (Fig. 2). A more quantitative measurement is described below, but the effect was sufficiently marked to warrant further exploration of substituent effects, especially as the isosbestic point in the progressive photolysis spectra (Fig. 2b) suggested that the photochemical reaction took place cleanly, as previously demonstrated for **1a**.[3]



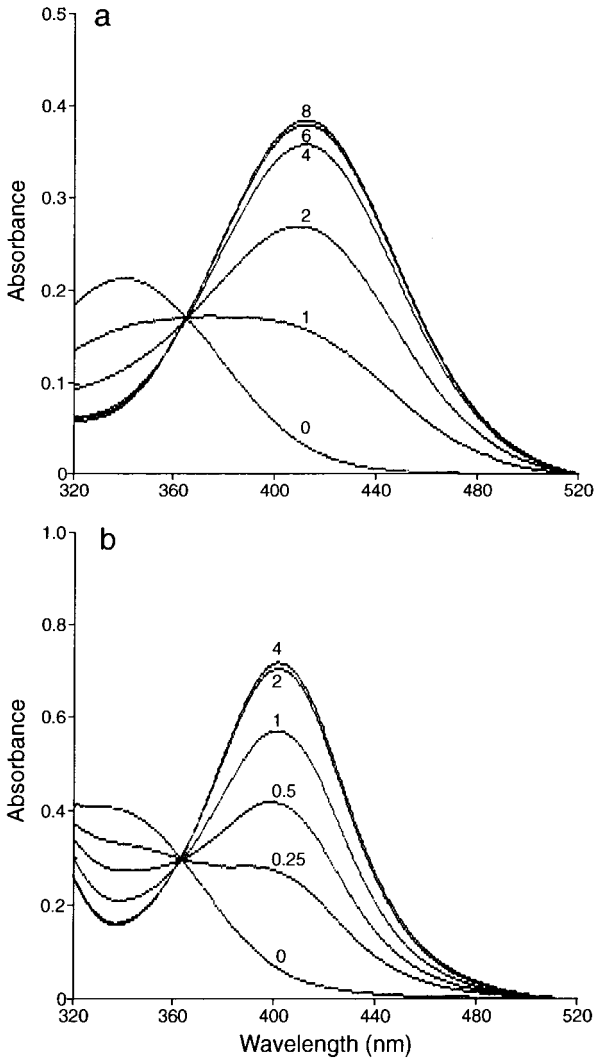
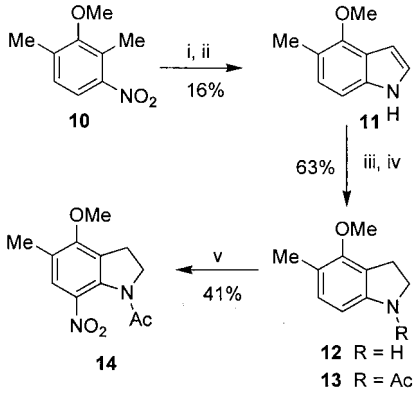


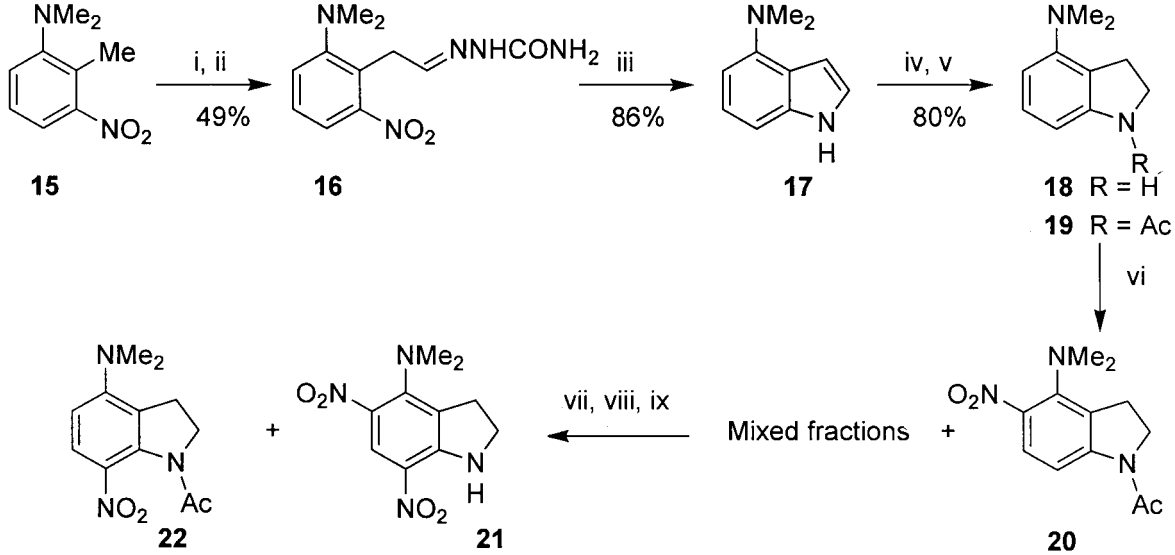
Figure 2. UV–Vis spectra showing the time course for photolysis of: (a) compound **1b**, and (b) compound **9**, both in neutral aqueous solution. The numbers on the traces show the cumulative irradiation time in minutes.

We first considered whether a compound similar to our original target **3** could be easily accessed, since a 5-substituent would eliminate the problem of regioselectivity during nitration. For simplicity in this phase of the work, we used a 5-methyl substituent (Scheme 3). The required indole **11** was prepared in poor yield from 2,6-dimethyl-3-nitroanisole **10** by a Leimgruber–Batcho synthesis,[8] reduced to the indoline **12** with $NaBH_3CN$–acetic acid[9] and acetylated to give **13**. Nitration with $NaNO_3$–TFA then gave the single isomer **14**. Photolysis of **14** in aqueous solution was ~2-fold less efficient than for **9**, probably because the 5-methyl group in **14** sterically inhibits interaction between the methoxy group and the aromatic ring. The implication of these combined results is that an electron-releasing group at C-4 has a beneficial effect on photolysis efficiency and that the effect is greater without a C-5 substituent. On these grounds we decided to examine the effect of a 4-dimethylamino group alone.



Scheme 3. *Reagents:* (i) $(MeO)_2CHNMe_2$, pyrrolidine; (ii) Fe, silica gel, toluene, HOAc; (iii) $NaBH_3CN$, HOAc; (iv) Ac_2O, HOAc; (v) $NaNO_3$, TFA.

Synthesis of the required 7-nitroindoline **22** (Scheme 4) required some changes to the route used for the compounds that had a 4-methoxy substituent. The Leimgruber–Batcho



Scheme 4. *Reagents:* (i) $(Me_2N)_3CH$, DMF; (ii) semicarbazide, aq. HCl; (iii) H_2, Pd–C; (iv) $BH_3{\cdot}Me_2S$, TFA; (v) Ac_2O, HOAc; (vi) $NaNO_3$, TFA; (vii) MeOH, aq. HCl; (viii) chromatography; (ix) MeCOCl, HOAc.

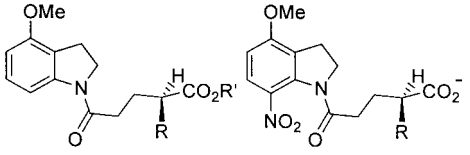
procedure with DMF dimethyl acetal was ineffective when applied to 2-dimethylamino-6-nitrotoluene **15**. However, a modified procedure[10] using tris(dimethylamino)methane and isolation of the semicarbazone **16** formed from the intermediate enamine was more satisfactory. Hydrogenation of **16** gave 4-(*N*,*N*-dimethylamino)indole **17** in excellent yield. Reduction of **17** to the corresponding indoline with $NaBH_3CN$–acetic acid, as used with the methoxy compounds, was unsatisfactory but was cleanly effected with $BH_3{\cdot}Me_2S$ in TFA.[11] The product indoline was immediately acetylated to give **19** in acceptable overall yield.

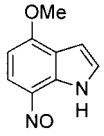
Nitration of **19** ($NaNO_3$–TFA) gave a complex mixture, from which the unwanted 5-nitro isomer **20** could be isolated by chromatography. We were unable to isolate other components in pure form and the remaining mixture was subjected to acid hydrolysis, after which two components could be separated. One was characterised as 5,7-dinitroindoline **21** while the second, after re-acetylation, gave the required 7-nitro compound **22**. Although the overall yield of **22** via this route was very poor, sufficient material was obtained to investigate its properties. First, NOE experiments in which the dimethylamino group of **20** or **22** was irradiated showed strong enhancement of one aromatic proton signal for **22** but had no effect on either aromatic proton for **20**, thereby confirming the assigned regiochemistry. Disappointingly, near-UV irradiation of an aqueous solution of **22** showed no change in the UV–Vis spectrum over time periods longer than those in which comparable solutions of **1b**, **9** or **14** were completely transformed. As our aim in the present work was specifically to seek substituents that would enhance photolysis efficiency, we have not further investigated this compound. However, in retrospect it seems likely that formation of a low-lying triplet state (as is known to occur with high efficiency for *N*,*N*-dialkyl-4-nitroanilines[12]) is responsible for quenching the photoreactivity.

The experiments described above define that the substitution pattern of **9** is optimal for efficiency of photolysis. At this point we had assumed the spectroscopic changes produced upon photolysis of **9** (Fig. 2b) indicated that its photochemistry in aqueous solution was the same as previously demonstrated for compounds of the general structure **1**.[3] With the substitution pattern optimised, we needed to establish that photolysis of **9** did indeed proceed with high stoichiometry for carboxylate release upon photolysis and that a nitrosoindole by-product analogous to **2** was formed.

To investigate these issues we required the glutamate and glutarate derivatives **25** and **26**, respectively. The glutamate derivative was needed to enable easy quantification of the released amino acid, as in our previous work,[3] while the glutarate derivative was a reasonably easily accessed compound that was water soluble at pH 7 and that enabled preparative scale photolysis for isolation of the anticipated nitrosoindole by-product. Each compound was prepared by appropriate acylations of 4-methoxyindoline to give the compounds **23** and **24** which were subsequently nitrated as for the preparation of **9**. As expected, nitration yielded both 5- and 7-nitro compounds in each case but the mixtures were separable by appropriate chromatography (see Experimental).

Photolysis of **25** was monitored by reverse-phase HPLC to quantify the extent of reaction and the photolysed solution was also analysed for free glutamate. The measured amount of glutamate was in the range 88–93% of that expected from the amount of starting material consumed. Given the 1:1 stoichiometry previously demonstrated for glutamate photorelease from **1a**, isolation of the comparable nitrosoindole by-product (see below) and the very clean conversion seen spectroscopically on progressive photolysis of the acetyl derivative **9** (Fig. 2b), we consider glutamate photo-liberation from **25** to be stoichiometric within the experimental errors of the analysis. The product quantum yield, measured using laser flash irradiation at 347 nm was 0.085, almost twice that of the previous compound **1a** under the same conditions. Comparative photolysis of **1a** and **25** using solutions of equal concentration showed that **25** was converted with 2.2-fold greater efficiency under the particular experimental conditions (continuous irradiation, 350 nm lamps, Rayonet photochemical reactor).





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To characterise the photo-product of the methoxynitroindolyl group, an aqueous solution of the glutarate **26** was irradiated until UV analysis indicated that the starting material was consumed. The isolated by-product had properties fully consistent with 4-methoxy-7-nitrosoindole **27**. This result suggests that the methoxy substituent does not alter the photolysis mechanism in aqueous solution from that followed by our earlier nitroindolines such as **1**.

Further work with these compounds will aim to optimise the nitration step to avoid or minimise formation of unwanted 5-nitro isomers such as **8**. This would facilitate access to these more efficient methoxy-substituted caged species. Biological testing of the new caged glutamate **25** will be reported in a future communication.

Experimental

General procedures

General experimental details were as previously described[2,3] except that high field ^1H NMR spectra were recorded on a Varian Unityplus 500 spectrometer. IR spectra were determined for Nujol mulls unless otherwise specified.

1,7-Diacetyl-4-methoxyindoline 5. 1-Acetyl-4-methoxyindoline **4** was prepared from 4-methoxyindole[8] by

reduction to the indoline[9] and acetylation, mp 115–116°C (lit. mp[13] 112°C). A stirred solution of **4** (4.02 g, 21 mmol) in dry 1,2-dichloroethane (30 mL) was cooled in ice and acetyl chloride (2.1 mL, 30 mmol) was added, followed by portionwise addition of powdered anhydrous $AlCl_3$ (8.4 g, 63 mmol). The mixture was stirred at room temperature overnight, then heated at 50°C for 2 h. The red/brown slurry was poured into acidified cold water (200 mL) and extracted with CH_2Cl_2. The combined organic phases were washed with 0.5 M aq. NaOH and brine, dried and evaporated to give a brown oil. Flash chromatography (EtOAc) followed by trituration with Et_2O afforded **5** as pale yellow needles (2.22 g, 54%), mp 88–89°C: IR: ν_{max}/cm^{-1} 1670, 1650, 1600, 1355, 1270, 1095; ^1H NMR (90 MHz): δ 7.33 (1H, d, J=8.3 Hz, H6), 6.61 (1H, d, J=8.3 Hz, H5), 4.17 (2H, t, J=8.1 Hz, H2), 3.85 (3H, s, OMe), 3.05 (2H, t, J=8.1 Hz, H3), 2.44 (3H, s, Ac), 2.21 (3H, s, Ac). Calcd for $C_{13}H_{15}NO_3$: C, 66.94; H, 6.48; N, 6.00. Found: C, 66.89; H, 6.50; N, 5.95.

Methyl 1-acetyl-4-methoxyindoline-7-acetate 6. Thallium-(III) nitrate trihydrate (3.21 g, 7.1 mmol) was added to a solution of **5** (1.65 g, 7.1 mmol) in MeOH (70 mL) that contained perchloric acid (60% w/w, 1.5 mL) and the mixture was stirred at room temperature for 4 h. The precipitated solid was filtered off and the filtrate concentrated to ~10 mL, diluted with EtOAc (50 mL) and washed with water. The aqueous phase was extracted with EtOAc and the combined organic phases were washed with saturated aq. $NaHCO_3$ and brine, dried and evaporated to give a viscous oil. Flash chromatography (EtOAc–light petroleum (4:1)) gave **6** as white crystals (0.88 g, 47%), mp 80–81°C (EtOAc–light petroleum): IR: ν_{max}/cm^{-1} 1730, 1660, 1615, 1500, 1390, 1345, 1270, 1150; ^1H NMR (90 MHz): δ 7.04 (1H, d, J=8.3 Hz, H6), 6.63 (1H, d, J=8.3 Hz, H5), 4.06 (2H, t, J=8.1 Hz, H2), 3.81 (3H, s, OMe), 3.76 (2H, s, $ArCH_2$), 3.71 (3H, s, CO_2Me), 2.96 (2H, t, J=8.1 Hz, H3), 2.24 (3H, s, Ac). Calcd for $C_{14}H_{17}NO_4$: C, 63.86; H, 6.51; N, 5.32. Found: C, 63.60; H, 6.50; N, 5.28.

Methyl 1-acetyl-4-methoxy-5-nitroindoline-7-acetate 7. To a stirred solution of $NaNO_3$ (75 mg, 0.9 mmol) in TFA (4 mL) was added **6** (211 mg, 0.8 mmol). The mixture was stirred at room temperature for 4 h and the red/brown solution was poured into ice-cold water and extracted with EtOAc. The combined organic phases were washed with saturated aq. $NaHCO_3$ and brine, dried and evaporated. The residue was flash chromatographed (EtOAc) and crystallised (EtOAc–light petroleum) to give **7** as pale yellow needles (90 mg, 36%), mp 181–182°C: UV: λ_{max} (EtOH)/nm 314 ($\epsilon/M^{-1}\,cm^{-1}$ 7250); λ_{max} (EtOH–25 mM Na phosphate, pH 7.0 (5:95))/nm 325 ($\epsilon/M^{-1}\,cm^{-1}$ 7370); IR: ν_{max}/cm^{-1} 1735, 1680, 1615, 1575, 1515, 1380, 1320, 1200, 1165; ^1H NMR (90 MHz): δ 7.74 (1H, s, H6), 4.16 (2H, t, J=8.1 Hz, H2), 3.90 (3H, s, OMe), 3.78 (2H, s, $ArCH_2$), 3.69 (3H, s, CO_2Me), 3.15 (2H, t, J=8.1 Hz, H3), 2.27 (3H, s, Ac). Calcd for $C_{14}H_{16}N_2O_6$: C, 54.54; H, 5.23; N, 9.09. Found: C, 54.59; H, 5.24; N, 9.03.

1-Acetyl-4-methoxy-5-nitroindoline 8 and 1-acetyl-4-methoxy-7-nitroindoline 9. 1-Acetyl-4-methoxyindoline **4** (96 mg, 0.5 mmol) was added to a stirred solution of $NaNO_3$ (43 mg, 0.5 mmol) in TFA (2 mL) at −15°C and the mixture was stirred for 2 h, allowing the temperature to rise to −10°C. The solution was poured into ice-cold water and extracted with EtOAc. The combined organic phases were washed with saturated aq. $NaHCO_3$ and brine, dried and evaporated to give a brown viscous oil. Flash chromatography (EtOAc–light petroleum (1:1)) afforded two products. The less polar product was the 7-nitro isomer **9** as yellow crystals (43 mg, 36%) mp 180–182°C (EtOAc–light petroleum) and the more polar was the 5-nitro isomer **8** as yellow crystals (44 mg, 37%) mp 101–102°C (EtOAc–light petroleum).

The compound **8** had UV: λ_{max} (EtOH)/nm 238 ($\epsilon/M^{-1}\,cm^{-1}$ 9100), 327 (8600); λ_{max} (EtOH–25 mM Na phosphate, pH 7.0 (5:95))/nm 238 ($\epsilon/M^{-1}\,cm^{-1}$ 8550), 341 (9200); IR: ν_{max}/cm^{-1} 1665, 1600, 1520, 1385, 1335; ^1H NMR (500 MHz): δ 8.01 (1H, d, J=8.5 Hz, H7), 7.86 (1H, d, J=8.5 Hz, H6), 4.18 (2H, t, J=8.5 Hz, H2), 3.93 (3H, s, OMe), 3.28 (2H, t, J=8.5 Hz, H3), 2.26 (3H, s, Ac). Calcd for $C_{11}H_{12}N_2O_4$: C, 55.93; H, 5.12; N, 11.85. Found: C, 55.89; H, 5.10; N, 11.82.

The compound **9** had UV: λ_{max} (EtOH)/nm 248 ($\epsilon/M^{-1}\,cm^{-1}$ 18300), 298 (4700), 335sh (3600); λ_{max} (EtOH–25 mM Na phosphate, pH 7.0 (5:95))/nm 246 ($\epsilon/M^{-1}\,cm^{-1}$ 17500), 322 (4800); IR: ν_{max}/cm^{-1} 1670, 1605, 1520, 1340, 1275; ^1H NMR (500 MHz): δ 7.75 (1H, d, J=9.0 Hz, H6), 6.63 (1H, d, J=9.0 Hz, H5), 4.23 (2H, t, J=8.1 Hz, H2), 3.91 (3H, s, OMe), 3.08 (2H, t, J=8.1 Hz, H3), 2.24 (3H, s, Ac). Calcd for $C_{11}H_{12}N_2O_4$: C: 55.93; H, 5.12; N, 11.85. Found: C, 56.05; H, 5.12; N, 11.80.

2,6-Dimethyl-3-nitroanisole 10. A mixture of 2,6-methyl-3-nitrophenol (4.18 g, 25 mmol; prepared from 2,6-dimethyl-3-nitroaniline according to the general method described[14]) and anhydrous K_2CO_3 (4.15 g, 30 mmol) in acetone (100 mL) was treated with dimethyl sulfate (2.84 mL, 30 mmol) and the mixture was heated under reflux. The progress of the reaction was followed by TLC (EtOAc–light petroleum (1:3)). Further aliquots of dimethyl sulfate (2×2 mL) were added after 4 and 6 h and the mixture was refluxed overnight. After cooling to room temperature the solid was filtered and washed with acetone and the combined filtrate was evaporated. The residue was dissolved in conc. aq. ammonia (100 mL) to destroy excess dimethyl sulfate, stirred at room temperature for 30 min and extracted with ether. The combined organic phases were washed with 2 M aq. HCl, 1 M aq. NaOH and brine, dried and evaporated to give a brown oil (4.30 g). TLC indicated the presence of starting phenol. The product was dissolved in light petroleum (100 mL), washed with Claisen's alkali[15] (2×100 mL) and the petroleum phase was dried and evaporated to give a brown oil (4.08 g). Flash chromatography (EtOAc–light petroleum (1:9)) afforded **10** as an oil (3.43 g, 76%) that was used without further purification; ^1H NMR (90 MHz): δ 7.60 (1H, d, J=8.3 Hz, H4), 7.11 (1H, d, J=8.3 Hz, H5), 3.74 (3H, s, OMe), 2.48 (3H, s, Me), 2.35 (3H, s, Me).

4-Methoxy-5-methylindole 11. To a stirred solution of **10** (3.26 g, 18 mmol) in dry DMF (36 mL) were added DMF dimethyl acetal (94%; 2.51 g, 19.8 mmol) and pyrrolidine

(1.8 mL). The mixture was stirred under nitrogen at 125°C for 3 h, then cooled to room temperature and concentrated in vacuo. The oily residue was dissolved in toluene–acetic acid (5:3, 36 mL) and added to a stirred mixture of iron powder (18 g) and silica gel (70–230 mesh, 45 g) in toluene–acetic acid (5:3, 235 mL) under nitrogen. The mixture was heated under reflux for 1 h, cooled to rt, diluted with CH_2Cl_2 and filtered. The filter cake was washed thoroughly with CH_2Cl_2 and the combined filtrates were washed successively with aq. $Na_2S_2O_5$, saturated aq. $NaHCO_3$ and brine, dried and evaporated. Flash chromatography (CH_2Cl_2–light petroleum (3:2)) gave **11** as a colourless viscous oil (0.48 g, 16%) that was used without further purification; ^{1}H NMR (90 MHz): δ 8.10 (1H, br s, NH), 7.08–6.88 (3H, m, ArH), 6.64–6.50 (1H, m, H3), 3.98 (3H, s, OMe), 2.32 (3H, s, Me).

4-Methoxy-5-methylindoline 12. $NaBH_3CN$ (0.75 g, 9 mmol) was added portionwise over 10 min to a solution of **11** (0.48 g, 2.98 mmol) in acetic acid (20 mL) (exothermic reaction) and the mixture was stirred at room temperature for 0.5 h. Water (3 mL) was added and the solvent was removed under reduced pressure. The residue was dissolved in EtOAc and washed with saturated aq. $NaHCO_3$ and brine, dried and evaporated to give **12** as a viscous oil (449 mg, 92%) that was used immediately without purification; ^{1}H NMR (90 MHz): δ 6.80 (1H, d, J=9 Hz, H6), 6.31 (1H, d, J=9 Hz, H7), 3.76 (3H, s, OMe), 3.56 (2H, t, J=8 Hz, H2), 3.12 (2H, t, J=8 Hz, H3), 2.14 (3H, s, Me).

1-Acetyl-4-methoxy-5-methylindoline 13. Crude 4-methoxy-5-methylindoline **12** (449 mg, 2.75 mmol) was dissolved in a mixture of acetic anhydride (10 mL) and glacial acetic acid (10 mL) and the mixture was heated under reflux for 3 h, then cooled to room temperature and concentrated. The residue was dissolved in EtOAc and washed with water, saturated aq. $NaHCO_3$ and brine, dried and evaporated to give **13** as white crystals (388 mg, 69%), mp 139–140°C (EtOAc–light petroleum); IR: ν_{max}/cm^{-1} 1650, 1610, 1335, 1220, 1080; ^{1}H NMR (90 MHz): δ 7.84 (1H, d, J=8.3 Hz, H7), 6.99 (1H, d, J=8.3 Hz, H6), 4.05 (2H, t, J=8.3 Hz, H2), 3.77 (3H, s, OMe), 3.19 (2H, t, J=8.3 Hz, H3), 2.23 (3H, s, Me), 2.19 (3H, s, Ac). Calcd for $C_{12}H_{15}NO_2$: C, 70.22; H, 7.37; N, 6.82. Found: C, 70.17; H, 7.37; N, 6.85.

1-Acetyl-4-methoxy-5-methyl-7-nitroindoline 14. To a solution of $NaNO_3$ (140 mg, 1.65 mmol) in TFA (5 mL) was added **13** (308 mg, 1.5 mmol) and the mixture was stirred at room temperature for 3 h. The solution was poured into ice-cold water and extracted with EtOAc. The combined organic phases were washed with saturated aq. $NaHCO_3$ and brine, dried and evaporated to give a red viscous oil which after trituration with Et_2O–light petroleum gave **14** as red microcrystals (155 mg, 41%), mp 153–154°C (EtOAc–light petroleum); UV (EtOH): λ_{max}/nm 247 (ϵ/M^{-1} cm^{-1} 22600), 329 (3100); UV (EtOH–25 mM Na phosphate, pH 7.0 (1:9)): λ_{max}/nm 246 (ϵ/M^{-1} cm^{-1} 18900), 338 (3300); IR: ν_{max}/cm^{-1} 1670, 1600, 1515, 1375, 1335; ^{1}H NMR (90 MHz): δ 7.52 (1H, s, H6), 4.22 (2H, t, J=8.1 Hz, H2), 3.86 (3H, s, OMe), 3.23 (2H, t, J=8.1 Hz, H3), 2.25 (3H, s, Ac), 2.22 (3H, s, Me). Calcd for $C_{12}H_{14}N_2O_4$: C, 57.59; H, 5.64; N, 11.19. Found: C, 57.68; H, 5.62; N, 11.20.

Comparative photolysis of 1-acetyl-4-methoxy-7-nitroindoline 9 and methyl 1-acetyl-7-nitroindoline-5-acetate 1b

Separate solutions (1 mL) of **9** and **1b** (each 89.6 μM in EtOH–25 mM Na phosphate, pH 7 (5:95)) in 1 cm path length cells were irradiated simultaneously for intervals of up to 8 min in a Rayonet Photochemical Reactor (16×350 nm lamps) and UV–Vis spectra were recorded after each time interval (see Fig. 2).

Comparative photolysis of 1-acetyl-4-methoxy-7-nitroindoline 9 and 1-acetyl-4-methoxy-5-methyl-7-nitroindoline 14

Separate solutions (1 mL) of **9** and **14** (each 74.8 μM in EtOH–25 mM Na phosphate, pH 7 (5:95)) in 1 cm path length cells were irradiated simultaneously for intervals of up to 6 min as described above and UV–Vis spectra were recorded after each time interval (data not shown).

***N,N*-Dimethyl-2-methyl-3-nitroaniline 15.** This compound has been prepared previously[16] but the amount of dimethyl sulfate specified was insufficient to achieve full alkylation. The procedure given here is satisfactory. 2-Methyl-3-nitroaniline (20.9 g, 137 mmol) was added to a solution of K_2CO_3 (47.5 g, 343 mmol) in water (130 mL) and the mixture was heated to 100°C. Dimethyl sulfate (36.4 g, 27.3 mL, 288 mmol) was added slowly over 1 h the mixture was heated overnight. The progress of the reaction was monitored by TLC (EtOAc–light petroleum (1:3)). More K_2CO_3 (47.5 g) and dimethyl sulfate (27 mL) were added and heating was continued until all the starting material was consumed. The solution was then cooled to rt, diluted with water and extracted with Et_2O. The combined organic phases were evaporated, treated with conc. aq. ammonia (100 mL) and stirred at room temperature for 20 min. The solution was concentrated, diluted with water and extracted with Et_2O. The combined organic phases were dried evaporated and the residual oil was dissolved in Ac_2O (50 mL) and stirred at room temperature overnight (to acetylate the monomethyl product). The solution was concentrated under reduced pressure and the residue was dissolved in Et_2O and washed with 1 M aq. HCl. The aqueous phase was basified to pH 13 with 2 M aq. NaOH and extracted with Et_2O. The organic extract was washed with brine, dried and evaporated and the residue was distilled under reduced pressure to give **15** as a yellow oil (17.04 g, 69%), bp 110°C/0.7 mmHg (lit.[17] bp: 191–192°C/100 mmHg); IR: ν_{max}/cm^{-1} (film) 2940, 2830, 2780, 1600, 1520, 1350, 1285, 1145, 1045, 955, 815; ^{1}H NMR (90 MHz): δ 7.19–7.49 (3H, m, ArH), 2.72 (6H, s, NMe_2), 2.42 (3H, s, Me).

2-(2-Dimethylamino-6-nitrophenyl)acetaldehyde semicarbazone 16. To a stirred solution of **15** (5.40 g, 30 mmol) in dry DMF (30 mL) was added tris(dimethylamino)methane (6.54 g, 45 mmol) and the mixture was heated under nitrogen to 115°C (internal temp.) for 4 h. The progress of the reaction was followed by the

consumption of the starting nitroaniline, monitored by TLC (EtOAc–light petroleum (1:3)). The dark red solution was cooled in an ice bath, diluted with DMF (20 mL) and a solution of semicarbazide hydrochloride (3.51 g, 31.5 mmol) and conc. HCl (5.4 mL) in water (50 mL) was added. Stirring was continued for 30 min and the pH was raised to 5.0. After a further 30 min at 0°C the pH was raised to 7.5. The precipitated yellow solid was filtered, washed with ice-cold water and ether and dried in vacuo. Recrystallisation from aq. EtOH afforded **16** as yellow microcrystals (3.93 g, 49%), mp 156–157°C; IR: ν_{max}/cm^{-1} 3450, 3160, 1720, 1600, 1525, 1350; ^1H NMR (500 MHz): δ (~1:3 mixture of *syn* and *anti* stereoisomers) 9.91 and 9.69 (1H, 2×s, NH), 7.71 and 7.53 (1H, 2×dd, J=1.2, 7.9 Hz, H5) 7.45 and 7.37 (1H, 2×t, J=7.9 Hz, H4), 7.42 (1H, dd, J=1.2, 7.9 Hz, H3), 7.32 and 6.64 (1H, 2×t, J=4.9 Hz, CHN), 5.91 and 5.53 (2H, 2×br s, NH_2), 4.02 and 3.84 (2H, 2×d, J=4.9 Hz, $ArCH_2$), 2.79 and 2.69 (6H, 2×s, NMe_2). Calcd for $C_{11}H_{15}N_5O_3$: C, 49.80; H, 5.70; N, 26.40. Found: C, 49.65; H, 5.70; N, 26.14.

4-*N*,*N*-Dimethylaminoindole 17. A suspension of **16** (6.37 g, 24 mmol) in EtOH (200 mL) was hydrogenated for 6 h at 60 psi over 10% Pd–C (1.35 g). The reaction mixture was filtered and the filtrate evaporated. The residue was dissolved in EtOAc and washed with saturated aq. $NaHCO_3$ and brine, dried and evaporated to give a pink solid. Recrystallisation (EtOAc–light petroleum) followed by flash chromatography of the residue from the mother liquor (EtOAc–light petroleum (1:3)) gave **17** as white crystals (3.30 g, 86%), mp 104–106°C; UV: λ_{max} (EtOH)/nm 222 ($\epsilon/M^{-1}\,cm^{-1}$ 35900), 277 (10700); IR: ν_{max}/cm^{-1} 3070, 1605, 1575, 1365; ^1H NMR (90 MHz): δ 8.12 (1H, br s, NH), 7.24–6.84 (3H, m, ArH), 6.70–6.46 (2H, m, ArH), 2.99 (6H, s, NMe_2). Calcd for $C_{10}H_{12}N_2$: C, 74.97; H, 7.55; N, 17.48. Found: C, 74.74; H, 7.61; N, 17.48.

1-Acetyl-4-(*N*,*N*-dimethylamino)indoline 19. To a solution of **17** (0.80 g, 5 mmol) in dry THF (15 mL) cooled to 0°C under nitrogen was slowly added borane-dimethyl sulfide (10 M in THF; 1.90 g, 2.37 mL, 25 mmol). The solution was then treated with TFA (10 mL, 130 mmol) and the mixture was stirred at 0°C for 1 h, then at room temperature for 4 h. The progress of the reaction was followed by TLC (EtOAc–light petroleum (4:1)). After 5 h the solution was cooled to 0°C and more $BH_3{\cdot}Me_2S$ (1 mL) was added. After a further 1 h at room temperature the solution was quenched with water (5 mL), basified to pH 11 with 2 M aq. NaOH and extracted with CH_2Cl_2. The combined organic phases were washed with brine, dried and evaporated. The residue was dissolved in a mixture of acetic anhydride (15 mL) and glacial acetic acid (15 mL), stirred at room temperature overnight and concentrated in vacuo. The residue was dissolved in EtOAc and washed with 0.5 M aq. NaOH and brine, dried and evaporated to give a white solid. Flash chromatography (EtOAc–light petroleum (4:1)) afforded **19** as white needles (0.77 g, 80%), mp 101–102°C (EtOAc–light petroleum); IR: ν_{max}/cm^{-1} 1655, 1595, 1575, 1340, 1310, 790; ^1H NMR (90 MHz): δ 7.87 (1H, d, J=8.3 Hz, H7), 7.13 (1H, t, J=8.3 Hz, H6), 6.61 (1H, d, J=8.3 Hz, H5) 4.03 (2H, t, J=8 Hz, H2), 3.10 (2H, t, H3), 2.76 (6H, s, NMe_2), 2.20 (3H, s, Ac). Calcd for $C_{12}H_{16}N_2O$: C, 70.56; H, 7.89; N, 13.71. Found: C, 70.39; H, 7.91; N, 13.64.

Nitration of 1-acetyl-4-(*N*,*N*-dimethylamino)indoline. To a stirred solution of $NaNO_3$ (204 mg, 2.4 mmol) in TFA (20 mL), cooled to −10°C, was added 1-acetyl-4-*N*,*N*-dimethylaminoindoline **19** (408 mg, 2 mmol) and the mixture was stirred for 5 h, keeping the temperature below 0°C. The solution was poured into ice-cold water and extracted with EtOAc. The combined organic phases were washed with saturated aq. $NaHCO_3$ and brine, dried and evaporated to give a red viscous oil (640 mg). Flash chromatography (EtOAc–light petroleum (9:1)) gave two fractions. The first was 1-acetyl-4-*N*,*N*-dimethylamino-5-nitroindoline **20** as fine orange needles (74 mg, 15%), mp 163–164°C (EtOAc–light petroleum); UV (EtOH): λ_{max}/nm 245 ($\epsilon/M^{-1}\,cm^{-1}$ 25300), 266 (24250), 332 (11550); (EtOH–25 mM Na phosphate, pH 7.0 (2.5:97.5)): λ_{max}/nm 248 ($\epsilon/M^{-1}\,cm^{-1}$ 24800), 356 (11300); IR: ν_{max}/cm^{-1} 1665, 1595, 1385, 1305; ^1H NMR (500 MHz): δ 7.93 (1H, d, J=8.5 Hz, H7), 7.63 (1H, d, J=8.5 Hz, H6), 4.14 (2H, t, J=8.5 Hz, H2), 3.19 (2H, t, J=8.5 Hz, H3), 2.80 (6H, s, NMe_2), 2.24 (3H, s, Ac). Calcd for $C_{12}H_{15}N_3O_3$: C, 57.82; H, 6.07; N, 16.85. Found: C, 57.76; H, 6.13; N, 16.64.

The second material eluted (413 mg) was dissolved in a mixture of MeOH (75 mL), water (15 mL) and conc. HCl (7.5 mL) and heated under reflux for 3 h. The solution was diluted with water, concentrated in vacuo, basified to pH 12 and extracted with EtOAc. The combined organic phases were washed with brine, dried and evaporated to give a yellow solid (399 mg). Flash chromatography (EtOAc–light petroleum (2:3)) gave two fractions. The first was processed as described below, while the second was 4-*N*,*N*-dimethylamino-5,7-dinitroindoline **21** as bright red microcrystals (145 mg, 29%), mp 190–191°C (EtOAc–light petroleum); UV (EtOH): λ_{max}/nm 222 ($\epsilon/M^{-1}\,cm^{-1}$ 34100), 355 (46000); (EtOH–25 mM Na phosphate, pH 7.0 (5:95)): λ_{max}/nm 225 ($\epsilon/M^{-1}\,cm^{-1}$ 31700), 374 (40600); IR: ν_{max}/cm^{-1} 3380, 1590, 1400, 1370, 1320, 1295; ^1H NMR (90 MHz): δ 8.48 (1H, s, H6), 6.96 (1H, br s, NH), 3.92 (2H, t, J=9 Hz, H2), 3.20 (2H, t, J=9 Hz, H3), 2.91 (6H, s, NMe_2). Calcd for $C_{10}H_{12}N_4O_4$: C, 47.62; H, 4.80; N, 22.20. Found: C, 47.79; H, 4.81; N, 22.24.

The first fraction (48 mg) from the above column was dissolved in a mixture of acetyl chloride (5 mL) and acetic acid (5 mL) and heated under reflux for 6 h. The solution was poured into ice-water, basified to pH 12 with 2 M aq. NaOH and washed with CH_2Cl_2. The combined organic phases were washed with brine, dried and evaporated to give a brown viscous oil (66 mg). Flash chromatography (EtOAc–light petroleum (9:1)) gave 1-acetyl-4-*N*,*N*-dimethylamino-7-nitroindoline **22** as yellow microcrystals (23 mg, 5%), mp 177–178°C (EtOAc–light petroleum); UV (EtOH): λ_{max}/nm 227 ($\epsilon/M^{-1}\,cm^{-1}$ 35200), 244 (34300) 280 (12600) 373 (16700); UV: (EtOH–25 mM Na phosphate, pH 7.0 (5:95)) λ_{max}/nm 235 ($\epsilon/M^{-1}\,cm^{-1}$ 30800), 401 (12000); IR: ν_{max}/cm^{-1} 1670, 1590, 1375; ^1H NMR (500 MHz): δ 7.72 (1H, d, J=9 Hz, H6), 6.52 (1H, d, J=9 Hz, H5), 4.20 (2H, t, J=8 Hz, H2), 3.15 (2H, t, J=8 Hz, H3), 2.96 (6H, s, NMe_2), 2.22 (3H, s, Ac). Calcd for

$C_{12}H_{15}N_3O_3$: C, 57.82; H, 6.07; N, 16.85. Found: C, 57.66; H, 6.04; N, 16.80.

Irradiation of 1-acetyl-4-*N*,*N*-dimethylamino-7-nitroindoline 22

A solution (1 mL) of **22** (33.6 μM in EtOH–25 mM Na phosphate, pH 7) in a 1-cm path length cell was irradiated for up to 6 min in a Rayonet Photochemical Reactor (16×350 nm lamps) and monitored by uv spectroscopy, which indicated that no photolysis took place.

1-[*S*-(4-*t*-Butoxycarbonyl)-4-(*t*-butoxycarbonylamino)]-butanoyl-4-methoxyindoline 23. Crude 4-methoxyindoline (149 mg, 1 mmol) was prepared as described above, dissolved in dry MeCN (8 mL) and treated with DMAP (366 mg, 3 mmol) and *N*-BOC-L-glutamic acid α-*t*-butyl ester (364 mg, 1.2 mmol), followed by 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (268 mg, 1.4 mmol). The mixture was stirred at room temperature for 16 h, then evaporated and the residue, dissolved in EtOAc, was washed with 0.5 M aq. HCl, saturated aq. $NaHCO_3$ and brine, dried and evaporated to give a viscous oil. Flash chromatography (EtOAc–light petroleum (3:7)) and trituration with ether gave **23** as white crystals (315 mg, 72%), mp 131–132°C (EtOAc–light petroleum); IR: ν_{max}/cm^{-1} 3350, 1725, 1680, 1660, 1605, 1250, 1180; ^1H NMR (500 MHz): δ 7.84 (1H, d, *J*=8.1 Hz, H7), 7.17 (1H, t, *J*=8.1 Hz, H6), 6.58 (1H, d, *J*=8.1 Hz, H5), 5.22 (1H, d, *J*=7.7 Hz, NH), 4.18–4.26 and 4.10–4.18 (1H, 2×m, CH, rotamers), 4.043 and 4.039 (2H, 2×t, *J*=8.5 Hz, H2), 3.83 (3H, s, OMe), 3.10 and 2.96 (2H, 2×t, *J*=8.5 Hz, H3), 2.57–2.43 (2H, m, $COCH_2$), 2.30–2.23 (1H, m, CH), 2.07–1.99 (1H, m, CH), 1.47 (9H, s, CMe_3), 1.42 (9H, s, CMe_3). Calcd for $C_{23}H_{34}N_2O_6$: C, 63.57; H, 7.89; N, 6.45. Found: C, 63.42; H, 7.90; N, 6.41.

1-(4-Carboxybutanoyl)-4-methoxyindoline 24. A solution of crude 4-methoxyindoline (447 mg, 3 mmol), prepared as described above, in chloroform (30 mL) was treated with glutaric anhydride (376 mg, 3.3 mmol) and the solution was stirred at room temperature for 18 h. The solvent was removed in vacuo and the residue, dissolved in EtOAc, was washed with 1 M aq. HCl. The organic phase was washed with brine, dried and evaporated and the residue was triturated with ether to give **24** as white crystals (338 mg, 43%), mp 146–148°C (EtOAc–light petroleum); IR: ν_{max}/cm^{-1} 1700, 1660, 1605, 1250, 1205; ^1H NMR (500 MHz): δ 7.82 (1H, d, *J*=8.1 Hz, H7), 7.15 (1H, t, *J*=8.1 Hz, H6), 6.58 (2H, d, *J*=8.1 Hz, H5), 4.07 (2H, t, *J*=8.5 Hz, H2), 3.83 (3H, s, OMe), 3.10 (2H, t, *J*=8.5 Hz, H3), 2.51 (2H, t, *J*=7.2 Hz, $NCOCH_2$), 2.44 (2H, t, *J*=7.2 Hz, CH_2CO_2H) and 2.02 (2H, quintet, CH_2). Calcd for $C_{14}H_{17}NO_4$: C, 63.87; H, 6.51; N, 5.32. Found: C, 64.01; H, 6.57; N, 5.34.

1-[*S*-(4-Amino-4-carboxybutanoyl)]-4-methoxy-7-nitroindoline 25. The protected glutamate derivative **23** (250 mg, 0.575 mmol) was added to a stirred solution of sodium nitrate (49 mg, 0.575 mmol) in TFA (5 mL) and the mixture was stirred at −15°C for 40 min. The dark solution was concentrated in vacuo and the residue was dissolved in water (50 mL) and adjusted to pH 6.7 with 1 M aq. NaOH. The solution was washed with ether and analysed by reverse-phase HPLC (mobile phase 25 mM Na phosphate, pH 6.0+30% MeCN v/v at 1.5 mL/min) to show two peaks with t_R 3.8 and 5.0 min. The solution was lyophilised and the components were separated by preparative HPLC. The column was eluted first with 25 mM Na phosphate, pH 6.0 for 1 h (all flow rates 2.5 mL/min), then with 25 mM Na phosphate, pH 6.0+12.5% MeCN. Fractions containing the first peak were combined, analysed and quantified (UV spectroscopy) to give **25** (38 μmol). The solution was lyophilised and desalted by re-application to the preparative HPLC column. The column was first eluted with water for 1 h, then with water+20% MeCN. Fractions containing the product were combined, analysed and quantified (UV spectroscopy) to give a solution of **25** (22 μmol) that was evaporated and redissolved in a small volume of water for storage at −20°C; ^1H NMR (500 MHz, D_2O, acetone ref.): δ 7.82 (1H, d, *J*=9.1 Hz, H6), 6.93 (1H, d, *J*=9.1 Hz, H5), 4.32 (2H, t, *J*=7.9 Hz, H2), 3.96 (3H, s, OMe), 3.75 (1H, t, *J*=5.8 Hz, CH), 3.11 (2H, t, *J*=7.9 Hz, H3), 2.78 (2H, t, *J*=7.5 Hz, $COCH_2$), 2.15–2.19 (2H, m, CH_2CH); HRMS (FAB) *m/z* 346.1020 $(M+Na)^+$. $C_{14}H_{17}N_3O_6$+Na requires 346.1015.

Fractions of the second peak from the original preparative HPLC, that contained the 5-nitro isomer, were processed similarly and had ^1H NMR (500 MHz, D_2O, acetone ref.): δ 7.94 (1H, d, *J*=9.0 Hz, H7), 7.87 (1H, d, *J*=9.0 Hz, H6), 4.24 (2H, t, *J*=8.4 Hz, H2), 3.94 (3H, s, OMe), 3.84 (1H, t, *J*=6.2 Hz, CH), 3.31 (2H, t, *J*=8.4 Hz, H3), 2.72–2.80 (2H, m, $COCH_2$), 2.14–2.22 (2H, m, CH_2CH).

1-(4-Carboxybutanoyl)-4-methoxy-7-nitroindoline 26. The acid **24** (210 mg, 0.8 mmol) was added to a stirred solution of $NaNO_3$ (68 mg, 0.8 mmol) in TFA (4 mL), cooled to −15°C, and the mixture was stirred for 1 h while allowing the temperature to rise to −10°C. The dark solution was poured into ice-cold water and extracted with EtOAc. The combined organic phases were washed with brine, dried and evaporated to give a dark viscous oil. Flash chromatography (EtOAc–light petroleum–AcOH (2.5:7:0.5)) gave two products. The first compound eluted was mostly the required 7-nitro isomer **26** as a brown viscous oil (76 mg, 31%) which was photolysed without further purification (see below); ^1H NMR (90 MHz): δ 9.98 (1H, br s, CO_2H), 7.64 (1H, d, *J*=8 Hz, H6), 6.58 (1H, d, *J*=8 Hz, H5), 4.20 (2H, t, *J*=8.5 Hz, H2), 3.92 (3H, s, OMe), 3.04 (2H, t, *J*=8.5 Hz, H3), 2.64–2.32 (4H, m, 2×CH_2), 2.00 (2H, quintet, CH_2).

The material that eluted second was principally the 5-nitro isomer but was not fully characterised.

4-Methoxy-7-nitrosoindole 27. A solution of crude **26** (193 mg, 0.62 mmol) in EtOH (4 mL) was diluted with 50 mM ammonium phosphate, pH 7.0 (76 mL) and irradiated for 2 h under nitrogen in a Pyrex flask, using a 100 W mercury arc lamp. The solution was diluted with water and extracted with EtOAc. The combined organic phases were washed with saturated aq. $NaHCO_3$ and brine, dried and evaporated. The residue was flash chromatographed (EtOAc–light petroleum (1:4)) to give

27 as small green needles (7 mg, 6%), mp 120–121°C (Et_2O–light petroleum); UV: λ_{max} (EtOH)/nm 232 ($\epsilon/M^{-1}\,cm^{-1}$ 4700), 256 (9300), 289 (5500), 392 (13200); λ_{max} (EtOH–25 mM Na phosphate, pH 7.0 (1:9))/nm 257 ($\epsilon/M^{-1}\,cm^{-1}$ 7500), 300 (6300), 404 (14500); ν_{max}/cm^{-1} ($CHCl_3$) 3440, 1600, 1585, 1500, 1490, 1330, 1170, 955, 895; ^1H NMR (500 MHz): δ 10.59 (1H, br s, H1), 8.97 (1H, d, J=8.5 Hz, H6), 7.13 (1H, t, $J_{1,2}=J_{2,3}$=2.7 Hz, H2), 6.91 (1H, d, J=8.5 Hz, H5), 6.66 (1H, t, $J_{1,3}$=2.7 Hz, H3), 4.16 (3H, s, OMe); HRMS (FAB) m/z 177.0659 $(M+H)^+$. $C_9H_8N_2O_2$+H requires 177.0664.

Photolysis and product analysis for 25

Solutions of **25** (0.45–0.54 mM) in 25 mM ammonium phosphate, pH 7.0 containing 1 or 5 mM dithiothreitol were irradiated for varying times (30 or 45 s) in 1 mm path length cells in a Rayonet Photochemical Reactor (16×350 nm lamps). Solutions were analysed by reverse-phase HPLC (Merck Lichrosphere RP8 column; mobile phase 25 mM Na phosphate, pH 6.0+30% MeCN v/v, flow rate 1.5 mL/min) and the extent of photolysis was determined by comparison of peak areas with those of unphotolysed controls. Aliquots of the photolysed solutions were also subjected to quantitative amino acid analysis as previously described.[3] Measured glutamate concentrations were 88–93% of the values expected from the extent of photolysis and were not affected by the concentration of dithiothreitol.

Comparative photolysis of 1a and 25

Separate solutions of **1a** and **25** (each 0.50 mM in 25 mM ammonium phosphate, pH 7.0 containing 5 mM dithiothreitol) were irradiated for 30 s (Rayonet, see above) in 1 mm path length cells. The extent of photolysis was determined by reverse-phase HPLC (column as above, mobile phase 25 mM Na phosphate, pH 6.0+20% MeCN v/v, flow rate 1.5 mL/min). Retention times were 6.7 and 6.4 min, respectively and quantification was by measurement of peak heights. Extents of conversion were 23.3 and 50.5% for **1a** and **25** respectively.

Quantum yield estimation for 25

A solution of 1-(2-nitrophenyl)ethyl phosphate[18] and **25** (each 0.5 mM) and dithiothreitol (5 mM) in 25 mM ammonium phosphate, pH 7.0 was irradiated in aliquots (7×30 μl) in a 1 mm path length cell, using 347 nm light from a frequency-doubled pulsed ruby laser (average energy 122 mJ, one pulse per aliquot). The irradiated aliquots were combined and the extent of photolysis for each compound was determined by reverse-phase HPLC (column as above, mobile phase 25 mM Na phosphate, pH 6.0+20% MeCN v/v, flow rate 1.5 mL/min). Retention times were 2.7 and 6.4 min for the phosphate and **25** respectively and the corresponding absorption coefficients at 347 nm were 510 and 4330 $M^{-1}\,cm^{-1}$. Extents of conversion were 28.4 and 38.1% for the phosphate and **25**, respectively.

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