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An antenna triplet sensitiser for 1-acyl-7-nitroindolines improves the efficiency of carboxylic acid photorelease †

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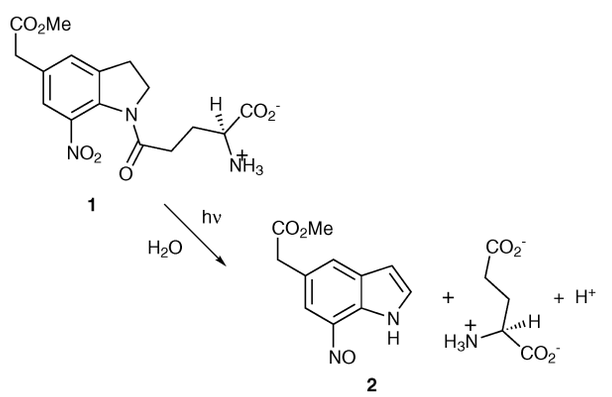
Conjugates of a triplet sensitiser (a 4,4′-dialkoxybenzophenone) with 1-acetyl-7-nitroindolines show up to 20-fold enhancement for photorelease of acetate (relative to the same indolines lacking the attached sensitiser) upon irradiation at 300 nm in neutral aqueous solution. The sensitised photolysis can be carried out in the presence of dissolved oxygen and will be applicable to photorelease of other carboxylates. The enhanced efficiency is mediated by an antenna function of the sensitiser, which transfers its triplet energy to populate the reactive, short-lived triplet state of the acylnitroindoline. This energy transfer is confirmed by a large reduction of the sensitiser's triplet lifetime in the conjugates compared with that of the sensitiser alone.

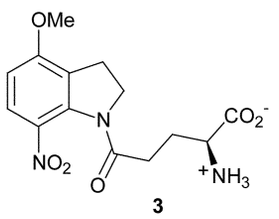
Introduction

Rapid release of biological effector species from photolabile precursors (colloquially termed caged compounds) upon flash photolysis is a valuable technique for probing sub-millisecond biological processes, such as muscular contraction and synaptic transmission.[1] Photocleavage of 2-nitrobenzyl derivatives has been the mainstay of this approach since it was first applied for photorelease of adenosine 5′-triphosphate[2] but has been less effective for rapid release of neuroactive amino acids, either because of slow release kinetics or hydrolytic instability of the photolabile precursors (see ref. 3 for a bibliography of much previous work). In an alternative chemical strategy, we have described[4] photorelease of L-glutamate from its 7-nitroindoline conjugate **1**. In aqueous solution the reaction by-product was the 7-nitrosoindole **2** (Scheme 1) whereas earlier work on related 1-acyl-7-nitroindolines irradiated in moist organic solvent had yielded a 7-nitroindoline by-product.[5]



Scheme 1 Overall reaction for photolysis of 1-acyl-7-nitroindolines in aqueous solution.

The caged glutamate **1** had various desirable properties: for example it was very resistant to hydrolysis and had clean photolytic stoichiometry, *i.e.* a 1 : 1 relationship between conversion of starting material and product formation. Furthermore, initial biological assessment indicated that **1** itself had no pharmacological activity and that it released L-glutamate with sub-millisecond kinetics upon flash photolysis.[4] In a later study of substituent effects, we found that the 4-methoxy compound **3** was approx. 2.5-fold more photosensitive than **1** (irradiation at 350 nm).[6] The enhanced photosensitivity of **3** arose from a combination of extinction coefficient and quantum yield effects. Our original synthesis gave **3** in very poor overall yield but we were later able to develop an efficient route[7] using a nitration step with the claycop reagent.[8] More recently, we have studied the solvent-dependent photocleavage mechanisms of 1-acyl-7-nitroindolines analogous to **1** and found a smooth transition from nitroindoline to nitrosoindole by-products as the water content of the solvent was increased.[9] Other significant findings of that work (for the photochemistry in 100% aqueous solution) included determination of the release rate of the carboxylate product (~5×10^6 s^{-1} at ambient temperature) and that photolysis probably proceeds exclusively *via* a short-lived triplet state ($\tau < 20$ ns). This short lifetime ensures that quenching by oxygen is minimised so photolysis proceeds efficiently in oxygenated solutions, as is required for a reagent intended for use in physiological experiments. Several biological applications of these compounds have already been described[10] and other groups have recently reported synthetic applications of nitroindoline photochemistry.[11]



To take advantage of the results of our mechanistic study, we were attracted to the possibility of further improving the photolytic susceptibility of 1-acyl-7-nitroindolines within biological preparations by triplet sensitisation. In considering possible strategies, it seemed impractical to use traditional intermolecular sensitisation, since the long-lived triplet states of most sensitisers are efficiently quenched[12] by the molecular oxygen that would necessarily be present to maintain the viability of living tissue preparations. However, intramolecular

† Electronic supplementary information (ESI) available: Synthetic details for starting materials and photolysis protocols for **4** plus the calculated absorption spectrum for **4**, spectra of its progressive photolysis and comparisons of calculated and experimental absorption spectra for **4** and **5**. See http://www.rsc.org/suppdata/pp/b3/b316251f/

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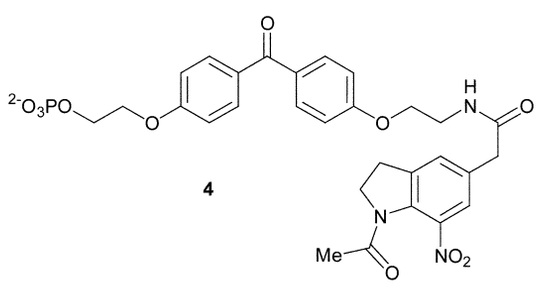
energy transfer, at least over a small number of bonds, takes place on a time scale that should compete effectively with diffusion-controlled quenching by dissolved oxygen.[12] We therefore aimed to construct a 1 : 1 conjugate of a triplet sensitiser with a 1-acyl-7-nitroindoline. For ease of synthesis in initial evaluation studies, the proposed conjugate bore a simple 1-acetyl substituent and should release acetate upon photolysis in neutral aqueous solution.

The choice of sensitiser was dictated by several considerations. Firstly, the sensitiser in a 1 : 1 conjugate needs to have a high absorption coefficient in order that the sensitiser and not the moiety to be sensitised is the principal light absorber. This contrasts with traditional intermolecular sensitisation, where a low absorption coefficient of the sensitiser can be compensated by a high concentration. Furthermore it is desirable that the sensitiser's absorption is as far to the red as possible, since light is generally less damaging to biological systems as its wavelength increases. These design criteria and the necessity to have a triplet energy at least 5 kcal mol^{-1} greater than the estimated triplet energy of the nitroindoline (~60 kcal mol^{-1})[9] led us to a sensitiser based on 4,4′-dimethoxybenzophenone (E_T ~70 kcal mol^{-1})[13] and thus to a proposed initial conjugate **4**. Significant features of **4** are the stable amide linkage between the two halves of the conjugate, and the phosphate group that provides aqueous solubility. For comparison, we also made a conjugate **5** related to the 4-methoxynitroindoline **3**.



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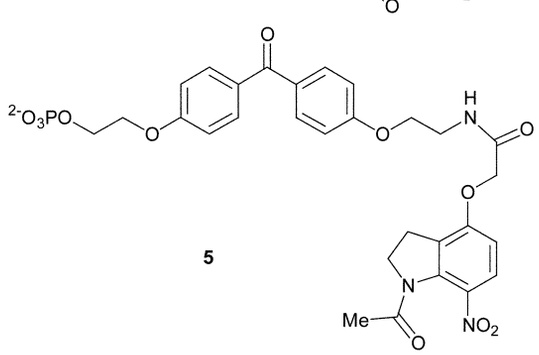

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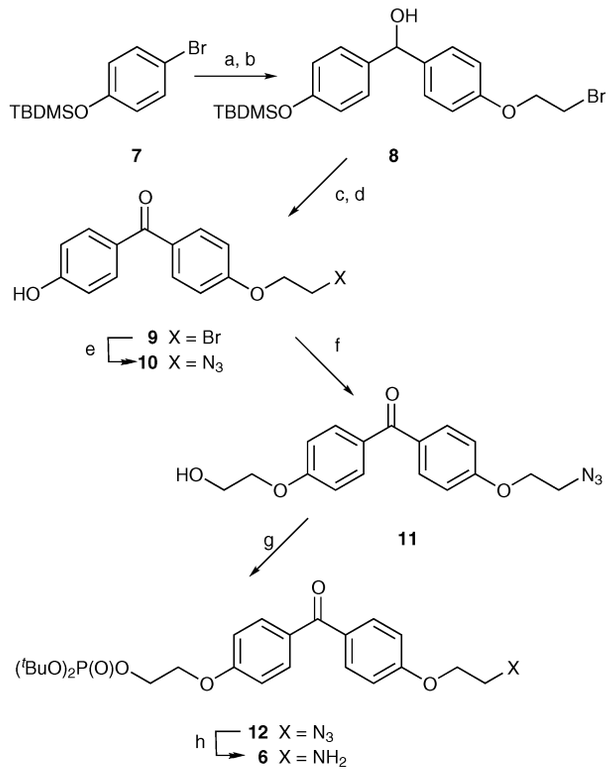
Results and discussion

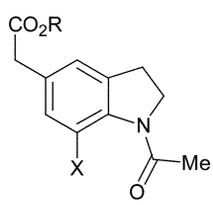
Synthesis of the functionalised sensitiser component **6**, shown in Scheme 2, was essentially straightforward and is described in the Experimental section. Ketone **9** was known from previous work[14] but we were unable to reproduce this: in particular, the reported[14] dichromate oxidation of an analogue of alcohol **8**, where a tetrahydropyranyl ether was used in place of the present TBDMS protecting group was unsatisfactory in our hands. However, oxidation of **8** with activated manganese(IV) oxide gave **9** in quantitative yield. Subsequent elaboration of **9** to the crystalline phosphotriester **12** proceeded with excellent yields and the oily amine **6** was prepared by reduction of the azido group of **12** immediately before use. In the present work, this reduction was achieved by catalytic hydrogenation, but was accompanied by variable reduction of the carbonyl group. Improved methods are currently under investigation.

For construction of conjugate **4**, the necessary nitroacid **15** was obtained by nitration of acid **14**, itself available from the previously-described ester **13**.[4] This was feasible in the present



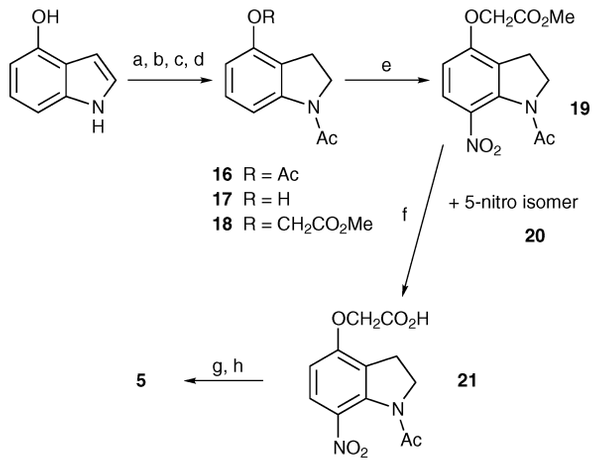
Scheme 2 Reagents: (a) *tert*-BuLi–THF, −78 °C; (b) 4-($BrCH_2CH_2$)OC_6H_4CHO; (c) MnO_2–CH_2Cl_2; (d) TBAF–THF; (e) NaN_3–DMF, 100 °C; (f) 2-bromoethanol–K_2CO_3–butanone–Δ; (g) (*tert*-BuO)$_2$PNEt_2–tetrazole–THF, then MCPBA; (h) H_2–Pd-on-C–EtOH.

case but would present problems with more complex 1-acyl substituents, as discussed below. Carbodiimide-mediated coupling of **6** and **15**, followed by TFA treatment to remove the protecting *tert*-butyl esters, liberated the desired conjugate **4** that was soluble in water to at least 40 mM concentration. The concentrations of aqueous solutions of **4** were determined using an absorbance coefficient of 25,500 M^{-1} cm^{-1} at 300 nm, calculated by summing spectra of the separate chromophores of acylnitroindoline **1** and benzophenone **11** (see Fig. 1 of the ESI†). The validity of this approach was confirmed by comparing the calculated and experimental spectra of **4**: the maximum absorbance was red-shifted by only 1.5 nm in the experimental spectrum and there was no major difference in the band shapes, suggesting that there was no perturbation of the combined chromophore by stacking or other interactions. The comparative spectra are shown in Fig. 2 of the ESI.†



13 R = Me, X = H
14 R = H, X = H
15 R = H, X = NO_2

Synthesis of **5** required the acid **21**, which was prepared from 4-hydroxyindole (Scheme 3). Synthetic procedures were generally straightforward (see Experimental). Claycop-mediated nitration of **18** gave the 7-nitro isomer **19** as the major product, together with the 5-nitro isomer **20**. The isomer ratio was less favourable (2 : 1 of **19** and **20**, respectively) than for the 4-methoxy series, where a 5 : 1 ratio in favour of the 7-nitro isomer was obtained.[7] These substituent effects on the regiochemistry may reflect differences in interactions of the substrate with the clay support. The isomers **19** and **20** were easily separated and hydrolysis of the ester group in **19** then proceeded readily (0.5 h at room temp with 1.5 equivalents of hydroxide) to give the acid **21**, without detectable hydrolysis of the *N*-acetyl



Scheme 3 Reagents: (a) $NaBH_3CN$–HOAc; (b) Ac_2O–AcOH; (c) NaOH-aq. MeOH; (d) $BrCH_2CO_2Me$–K_2CO_3–acetone; (e) claycop–Ac_2O–CCl_4; (f) 1.5 equiv. NaOH–aq. MeOH; (g) **6** with EDC; (h) TFA.

substituent. Coupling of **6** and **21** and subsequent TFA deprotection then gave the conjugate **5** which, like **4**, was readily soluble in water. As for compound **4**, an absorbance coefficient was calculated by summing the individual chromophores of **11** and **19** (Fig. 1), which gave an absorption maximum for **5** at 300 nm (ε 27,900 M^{-1} cm^{-1}). As was done for conjugate **4**, the experimental and calculated spectra of **5** were compared (see Fig. 4 of the ESI †) and again showed no evidence of perturbation by interactions between the sensitiser and nitroindoline chromophores.

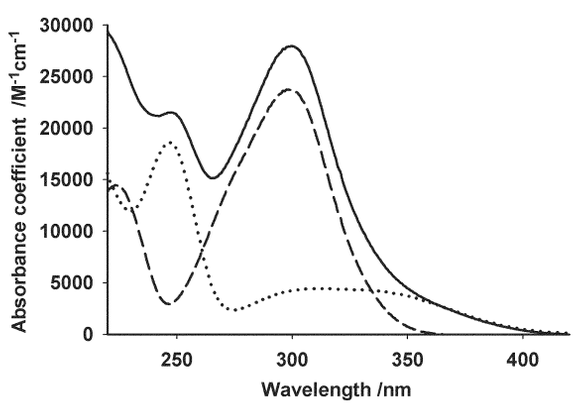
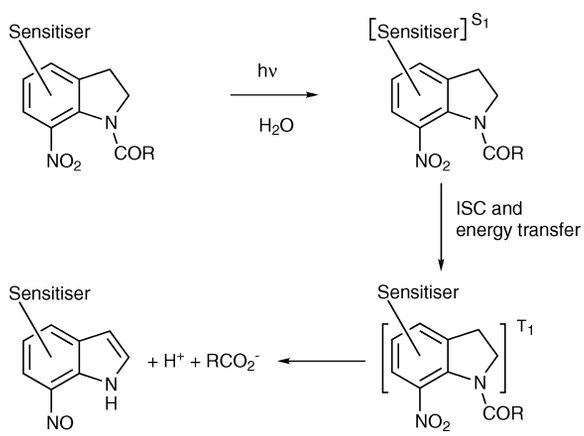


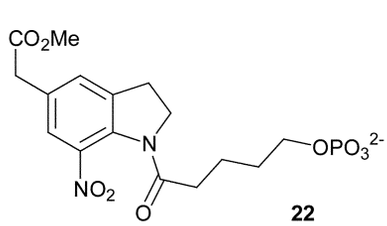
Fig. 1 Measured absorption spectra for aqueous solutions of **3** (· · ·) and **11** (------) and the sum of these two spectra, *i.e.* calculated spectrum of **5** (——).

A significant issue, relevant to the synthesis of future conjugates with more complex 1-acyl substituents, relates to preparation of nitroindoline acids analogous to **21** that are required for conjugation with the amine **6**. During assembly of either type of nitroindoline used here, it is necessary to protect the carboxylate group in the side chain that is ultimately used to link with **6**. It is convenient to use a methyl ester for this protection, but conditions for its removal differed greatly in the two series used for construction of **4** and **5**. Hydrolysis of the phenylacetate-like ester in **13** or its 7-nitro analogue required >24 h with 1.5 equivalents of sodium hydroxide in aqueous methanol while, as noted above, hydrolysis of the ester in **19** was complete under the same conditions in 30 min. Prolonged exposure to alkaline conditions risks partial cleavage of the amide bond between the nitroindoline and the acyl substituent (previous measurements[4] gave $t_{½}$ 29 h at pH 12, 30 °C for hydrolysis of a simple acyl substituent such as in **15**). Furthermore, protecting groups within a more complex acyl group, such as would be present in synthesis of a glutamate derivative, could also be at risk. The rapid hydrolysis of the ester in **19** should avoid either problem. Note that faster hydrolysis of this ester function compared to that in **13** and similar compounds was predictable on the basis of the pK_a values of relevant carboxylic acids (3-nitrophenylacetic acid, a model for **13**, has pK_a 4.0 and 4-nitrophenoxyacetic acid, a model for **19**, has pK_a 2.8).[15] These values reflect the relative susceptibility of the ester groups in **13** and **19** to electrophilic attack.

With the two planned conjugates in hand, we evaluated the effects of the internal sensitiser. Irradiation of a pH 7 aqueous solution of **4** (300 nm lamps, Rayonet reactor) showed rapid spectroscopic changes (see Fig. 3 of the ESI †), most marked by the progressive development of a broad peak at 418 nm that was similar in appearance to the spectrum of the nitrosoindole **2** (λ_{max} 412 nm).[4] This absorption band disappeared upon addition of a thiol, which was consistent with the presence of the expected nitrosoindole photoproduct (see Scheme 4). Upon comparative 300 nm irradiations of **4** and **22** (a water-soluble analogue of **1** available from previous work[4]) in separate solutions exposed to atmospheric oxygen and containing equal concentrations of one or other compound, the sensitised compound **4** was 41% converted after 8 s illumination. In contrast, **22** showed <3% conversion over this time and required illumination for 2 min to achieve 40% conversion, *i.e.* compound **4** is ~15-fold more photosensitive than **22**. The estimates of relative conversion were determined by HPLC analysis of the extent of conversion of the initial compounds.



Scheme 4 Proposed reaction scheme for photolysis of sensitised nitroindolines. The detailed steps between the nitroindoline triplet and products are described in Morrison *et al.*[9]



Progressive photolysis of **5** under the same conditions as for **4** also showed a clean transition from the initial spectrum, with isosbestic points near 265 and 355 nm, and development of a band at 405 nm (Fig. 2). This band, like the similar one in the photolysis spectrum of **4**, disappeared upon addition of dithiothreitol and was assigned to the presence of a nitrosoindole. It is similar to a band at 404 nm in the spectrum of 4-methoxy-7-nitrosoindole formed on photolysis of **3** and related compounds.[6] Comparative 300 nm irradiation of neutral aqueous solutions of **5** and its non-sensitised analogue **3** showed that **5** was approx. 6-fold more photosensitive than **3**. A similar comparison of **5** with **22**, our original type of nitroindoline, showed an overall 20-fold gain in photoefficiency, measured as the extent of conversion of each compound upon exposure to an identical incident light dose. When **4** and **5** were directly

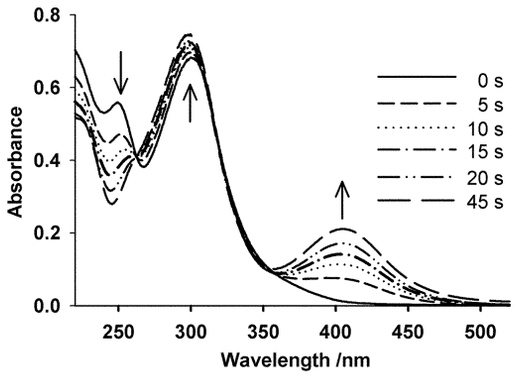


Fig. 2 UV-Vis absorption spectra for an aqueous solution of **5** after 300 nm irradiation for the cumulative time periods indicated. The arrows indicate the direction of absorbance changes with increasing irradiation time.

compared, the latter compound was found to be ~30% more photosensitive, consistent with the other data.

Once these large gains in photoefficiency had been observed, it was important also to establish that the expected carboxylate product, *i.e.* acetate ion from the present conjugates, was released upon photolysis. This was demonstrated for photolysis of **5** by the development of a singlet at δ 1.91 in the ^{1}H NMR spectrum of a partly-photolysed aqueous solution (pH 7). The chemical shift was consistent with that of acetate ion in aqueous solution and addition of sodium acetate to the solution specifically enhanced this signal. Scheme 4 shows the overall photoreaction, consistent with the present spectroscopic and product data and our previous mechanistic study.[9] Quantitative data on the stoichiometry of product release will be more readily available from future work with an aminoacyl side chain (such as from L-glutamate). However, our previous data from the nitroindolines themselves,[4,6] together with the clean nature of the UV-Vis spectra recorded upon progressive photolysis, strongly suggest that the sensitised compounds will also have good product stoichiometry.

As well as demonstrating release of the expected acetate photoproduct, we wished to investigate the photophysics of these conjugates. As discussed in the Introduction, the enhanced photoreactivity of **4** and **5** was expected to be a consequence of triplet energy transfer from the 4,4′-dialkoxybenzophenone moiety to the nitroindoline chromophore. To obtain evidence for such energy transfer between these chromophores, **4** and **5** were studied by nanosecond laser flash photolysis (LFP) with the expectation that the triplet states of the 4,4′-dialkoxybenzophenone moieties in these compounds would show shorter lifetimes than untethered 4,4′-dimethoxybenzophenone as a result of the additional energy transfer pathway available to the conjugates. The triplet state of 4,4′-dimethoxybenzophenone is known to show long wavelength absorption with maxima at 350, 440, 545, and 675 nm in aqueous acetonitrile[16] and the long wavelength absorption ($\lambda > 500$ nm) of these triplets allows their observation in a spectral region free of interference from nitroindoline-derived species, that had absorption maxima in the range 420–450 nm.[9]

To provide a reference for the 4,4′-dialkoxybenzophenone triplets of **4** and **5**, the parent 4,4′-dimethoxybenzophenone was studied by LFP in aqueous solution. Under deoxygenated conditions, the lifetime was measured to be 27 μs, which is in a similar range to the reported[17] 47 μs lifetime in benzene solution. Saturation of the solution with oxygen reduced the lifetime to 0.2 μs. Assuming a dissolved oxygen concentration[13] of 1.39×10^{-3} M, the bimolecular rate constant for the reaction of the 4,4′-dimethoxybenzophenone triplet with dissolved oxygen is 3.6×10^9 M^{-1} s^{-1}, which approaches the diffusion controlled limit.

LFP of **4** and **5** was carried out in water with λ_{ex} 308 nm, so the dialkoxybenzophenone moiety is primarily excited, as is evident in Fig. 1. LFP of **5** in deoxygenated water gave rise to a strong transient absorption above 600 nm, that qualitatively resembled that of 4,4′-dimethoxybenzophenone.[16] Decay of this transient was monitored at 675 nm and showed first order kinetics with a lifetime of 1.6 μs. The lifetime was reduced to 50 ns when the solution was saturated with oxygen, consistent with an assignment of the transient to the triplet state of the dialkoxybenzophenone moiety. The lifetime for the triplet of **5** in deaerated water is more than 10-fold shorter than for 4,4′-dimethoxybenzophenone under the same conditions, consistent with the presence of an energy transfer pathway to the nitroindoline moiety, that is available to **5** but not to the ketone alone. LFP of **4** in deoxygenated water gave a transient with an absorption profile similar to that obtained for **5**. Its decay (monitored at 675 nm) was first order and gave a lifetime of 0.6 μs, that shortened to 45 ns when the solution was saturated with oxygen.

The high rate constant measured for quenching of the 4,4′-dimethoxybenzophenone triplet by dissolved oxygen suggests that the reactivity of **4** and **5** might be significantly attenuated by the presence of dissolved oxygen. The extent of this attenuation can be quantified with the available data. From the previously determined bimolecular rate constant (see above), the rate of quenching of the triplet state of 4,4′-dimethoxybenzophenone under atmospheric conditions ($[O_2] = 0.21$ atm, solubility in water[13] $= 2.9 \times 10^{-4}$ M) is calculated to be 1.0×10^6 s^{-1}, and this value is expected to be similar for quenching of the triplet states of the dialkoxybenzophenone moieties in **4** and **5**. Under deoxygenated conditions, the lifetimes of the dialkoxybenzophenone moieties of **4** and **5** are reduced from 27 μs (for 4,4′-dimethoxybenzophenone) to 0.6 and 1.6 μs, respectively, giving energy transfer rate constants of 1.7×10^6 s^{-1} for **4** and 5.9×10^5 s^{-1} for **5**. Both of these rate constants are competitive with quenching by oxygen, with ~38% quenching expected for **4** and ~63% quenching expected for **5** in aerated solutions. This permits compounds such as **4** and **5** to be used under physiologically relevant conditions.

We did not measure absolute quantum yields in the present work, as it seems preferable to carry out such measurements when the reaction stoichiometry can more readily be measured, for example with a conjugate of L-glutamate. However, some indication that the enhanced efficiency is largely mediated by the high absorption coefficient of the sensitiser can be obtained by consideration of the relative absorption coefficients for the sensitiser and the nitroindolines. Thus, at 300 nm, the ratio of molar absorption coefficients for the benzophenone and for nitroindoline **22** is 12.8, while the same ratio for the benzophenone and compound **3** is 5.6. These ratios are similar to the photosensitivity enhancements of **4** and **5** of approximately 15- and 6-fold compared to the non-sensitised respective nitroindolines and suggest that enhanced light absorption is the major factor in the more efficient photolysis. Future measurements of absolute quantum yields, using a monochromatic light source to eliminate differences in spectral overlap between the light source and the various chromophores, should further substantiate this.

Conclusion

The high extinction coefficient of the sensitiser in these compounds allows it to act as an antenna, efficiently absorbing incident light and passing its energy to the nitroindoline, thereby populating the reactive triplet state of the latter. Similar approaches have been reported in related areas. For example, recent work describes enhancement of the luminescence of weakly-absorbing lanthanide chelates by attachment of an antenna species to harvest light and transfer energy to the metal ion.[18] In extensive studies directed to promoting photochemical reactions, Morrison and co-workers used a dimethylphenylsilyloxy antenna and observed photoreaction at remote sites in

steroid frameworks that involve both singlet and triplet intramolecular transfers.[19] All present evidence suggests that the photolysis mechanism does not differ significantly from the non-sensitised case that has been previously investigated.[9] To summarise, progressive photolysis indicates that a clean transformation takes place, just as for the non-sensitised nitroindolines, and the nitroindoline is converted to a nitrosoindole photoproduct (Scheme 4). The rate constants for energy transfer are in the region of 10^6 s^{-1} so may impose a slight limitation on the rate of product release (5×10^6 s^{-1} from the non-sensitised compounds[9]) but this would not be significant for photorelease of a neuroactive amino acid.

Overall, our results offer promise of substantially higher product release for a given light dose. The possibility of achieving this by means of triplet sensitisation was suggested for caged compounds several years ago[1a] but the present work appears to be the first practical example. The fact that the series with a 4-alkoxy substituent is slightly more photosensitive is particularly satisfactory in synthetic terms, as discussed above, since it makes practicable the synthesis of more complex species, such as a glutamate-releasing compound. Current efforts are directed to this goal and to further optimisation of the overall system in terms of both synthesis and photochemical efficiency.

Experimental

General

^{1}H NMR spectra were determined on Varian Unityplus 500 or JEOL FX90Q spectrometers in $CDCl_3$ solution with TMS as internal reference, unless otherwise specified. Infrared spectra were determined in Nujol mulls. Elemental analyses were carried out by MEDAC Ltd., Surrey, UK. Merck 9385 silica gel was used for flash chromatography. Analytical HPLC was performed on a 250 × 4 mm Merck Lichrospher RP8 column at 1.5 mL min^{-1} flow rate. Preparative HPLC was carried out on a 2 × 30 cm column (Waters C_{18} packing, Cat. No. 20594) at 2 mL min^{-1} flow rate. Detection for analytical and preparative work was at 254 nm. Photolysis experiments were performed in a Rayonet RPR-100 photochemical reactor fitted with 16 × 300 nm lamps. Petroleum ether (bp 40–60 °C) was redistilled before use.

Details of the preparation of 4-(2-bromoethoxy)benzaldehyde and compounds **7**–**9** are given in the ESI, as are photolysis protocols and spectroscopic data for **4**.†

4-(2-Azidoethoxy)-4′-hydroxybenzophenone 10. A mixture of the bromide **9** (5.78 g, 18 mmol) and NaN_3 (3.51 g, 54 mmol) in dry DMF (180 mL) was heated at 100 °C for 2.5 h. The solvent was removed under high vacuum and the residue was diluted with EtOAc, washed with water, dried and evaporated. Flash chromatography [EtOAc–petroleum ether (2 : 3)] gave **10** as white crystals (4.59 g, 90%), mp 105–106 °C (from EtOAc–petroleum ether); found: C, 63.56; H, 4.64; N, 14.73; calcd. for $C_{15}H_{13}N_3O_3$: C, 63.60; H, 4.65; N, 14.83%; IR (ν_{max}/cm^{-1}): 3320, 2100, 1625, 1600, 1565, 1320, 1255; ^{1}H NMR (δ, 500 MHz, $CDCl_3$ + DMSO-d_6): 9.80 (1H, br s), 7.75 (2H, AA′BB′, J = 8.8 Hz,), 7.67 (2H, AA′BB′, J = 8.8 Hz), 6.99 (2H, AA′BB′, J = 8.8 Hz), 6.90 (2H, AA′BB′, J = 8.8 Hz), 4.25 (2H, t, J = 4.8 Hz) and 3.66 (2H, t, J = 4.8 Hz).

4-(2-Azidoethoxy)-4′-(2-hydroxyethoxy)benzophenone 11. A solution of the phenol **10** (1.19 g, 4.2 mmol) in 2-butanone (80 mL) was mixed with anhydrous K_2CO_3 (1.16 g, 8.4 mmol), NaI (50 mg) and 2-bromoethanol (2.62 g, 21 mmol) and the mixture was heated under reflux. The progress of the reaction was followed by TLC [EtOAc–petroleum ether (3 : 2)]. More 2-bromoethanol (2.62 g) and K_2CO_3 (1.16 g) were added at 4 and 6 h and heating was continued for 7 h total. The solvent was evaporated and the residue was dissolved in water (100 mL) and washed with EtOAc (3 × 60 mL). The combined organic phases were washed with brine, dried and evaporated to give **11** (1.07 g, 78%), mp 117–118 °C (white flakes from EtOAc–petroleum ether); found: C, 62.41; H, 5.23; N, 12.81; calcd. for $C_{17}H_{17}N_3O_4$: C, 62.38; H, 5.23; N, 12.83%; UV [λ_{max} (EtOH)/nm (ε/M^{-1} cm^{-1})]: 221 (22 200), 293 (25 700); UV {λ_{max} [EtOH–25 mM Na phosphate, pH 7.0 (1 : 50)]/nm (ε/M^{-1} cm^{-1})}: 223 (15 500), 299 (23 700); IR (ν_{max}/cm^{-1}): 3280, 2110, 1635, 1605, 1260; ^{1}H NMR (δ, 500 MHz): 7.77–7.80 (4H, m), 6.97–7.00 (4H, m), 4.23 (2H, t, J = 4.8 Hz), 4.17 (2H, t, J = 4.8 Hz), 3.99–4.03 (2H, m), 3.65 (2H, t, J = 4.8 Hz) and 2.09–2.15 (1H, m).

4-(2-Azidoethoxy)-4′-{2-[di(*tert*-butoxy)phosphoryloxy]ethoxy}-benzophenone 12. A solution of **11** (0.72 g, 2.2 mmol) in dry THF (30 mL) was treated under nitrogen with 1*H*-tetrazole (0.93 g, 13.2 mmol) and di-*tert*-butyl-*N*,*N*-diethylphosphoramidite (93% purity; 1.18 g, 4.4 mmol) and the mixture was stirred at room temperature for 18 h. The solution was cooled to 0 °C and treated dropwise with a solution of *m*-chloroperbenzoic acid (55% peracid; 2.07 g, 6.6 mmol) in CH_2Cl_2 (30 mL). The solution was stirred at 4 °C for 1 h, then diluted with CH_2Cl_2 (100 mL) and washed with 10% aq. $Na_2S_2O_5$. The organic phase was washed with saturated aq. $NaHCO_3$ and brine, dried and evaporated. Flash chromatography [EtOAc–petroleum ether (7 : 3)] and trituration with ether gave **12** as white crystals (1.04 g, 91%), mp 53–55 °C (from Et_2O–petroleum ether); found: C, 57.79; H,6.78; N, 8.03; calcd. for $C_{25}H_{34}N_3O_7P$: C, 57.80; H, 6.60; N, 8.09%; IR (ν_{max}/cm^{-1}): 2100, 1640, 1600, 1375, 1275, 1250; ^{1}H NMR (δ, 500 MHz): 7.76–7.81 (4H, m), 6.95–7.01 (4H, m), 4.31–4.35 (2H, m), 4.25–4.29 (2H, m), 4.23 (2H, t, J = 4.8 Hz), 3.65 (2H, t, J = 4.8 Hz) and 1.50 (18H, s).

1-Acetylindolin-5-yl-acetic acid 14. A solution of methyl 1-acetylindoline-5-acetate **13**[4] (4.66 g, 20 mmol) in a mixture of MeOH (150 mL) and 2 M aq. NaOH (15 mL, 30 mmol) was stirred at room temperature for 17 h. The solvent was evaporated and the residue was dissolved in water and washed with ether, and the aqueous phase was acidified with conc. HCl. The precipitated solid was filtered, washed with cold water and dried to give **14** as light brown crystals (4.26 g, 97%), mp 216–218 °C (from aq. MeCN); found: C, 65.85; H, 6.01; N, 6.51; calcd. for $C_{12}H_{13}NO_3$: C, 65.74; H, 5.98; N, 6.39%; IR (ν_{max}/cm^{-1}): 1720, 1605, 1580, 1500, 1190; ^{1}H NMR (δ, 90 MHz, DMSO-d_6): 7.99 (1H, d, J = 8 Hz), 6.88–7.20 (2H, m), 4.25 (2H, t, J = 8 Hz), 3.47 (2H, s), 3.12 (2H, t, J = 8 Hz) and 2.16 (3H, s).

1-Acetyl-7-nitroindolin-5-yl-acetic acid 15. The acid **14** (389 mg, 1.77 mmol) was added to a well-stirred suspension of claycop[8] (1.92 g) and acetic anhydride (6 mL) in CCl_4 (12 mL) and the mixture was stirred at room temperature for 18 h. The solid was filtered off, washed with EtOAc (50 mL) and the filtrate was washed with brine, dried and evaporated. Flash chromatography [$CHCl_3$–MeOH (3 : 2)] afforded **15** as a yellow viscous oil (304 mg, 65%) which was used in the next step without further purification. ^{1}H NMR (δ, 90 MHz): 7.52 (1H, s), 7.36 (1H, s), 4.20 (2H, t, J = 8 Hz), 3.60 (2H, s), 3.16 (2H, t, J = 8 Hz) and 2.22 (3H, s).

4-{[2-(1-Acetyl-7-nitroindolin-5-yl)acetamido]ethoxy}-4′-[2-(dihydroxyphosphoryloxy)ethoxy]benzophenone 4. A solution of the azide **12** (260 mg, 0.5 mmol) in EtOH (20 mL) was treated with 10% Pd–C (200 mg) and hydrogenated at atmospheric pressure for 30 min. The catalyst was filtered off and washed with EtOH, and the filtrate was evaporated to give the amine **6** (210 mg, 0.42 mmol, 85%) as a viscous oil. ^{1}H NMR (δ, 90 MHz): 7.60–7.86 (4H, m), 6.76–7.04 (4H, m), 3.98–4.40 (6H, m), 3.12 (2H, t, J = 8 Hz), 2.66 (2H, br s) and 1.48 (18H, s). The crude amine was dissolved in dry MeCN (10 mL) and

treated with the acid **15** (200 mg, 0.75 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide.HCl (115 mg, 0.6 mmol). The mixture was stirred at room temperature for 18 h, then evaporated and the residue was dissolved in EtOAc and washed successively with 0.5 M aq. HCl, saturated aq. $NaHCO_3$ and brine, dried and evaporated. Flash chromatography [$CHCl_3$–MeOH (95 : 5)] gave the crude product as a yellow gum (184 mg), which was dissolved in TFA (10 mL), stirred at room temperature for 1 h and concentrated *in vacuo*. The residue was dissolved in water (100 mL) and adjusted to pH 7.0 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–MeOH (5 : 4)], t_R 5.4 min. The solution was lyophilised, dissolved in 25 mM Na phosphate, pH 6.0 (80 mL) and pumped onto the preparative HPLC column. After loading, the column was washed with 25 mM Na phosphate, pH 6.0 for 2 h, then with water for 2 h and finally the product was eluted with water–MeOH (4 : 1). Fractions containing the product were analysed as above, combined and concentrated *in vacuo*. The residue was dissolved in water, passed through a 0.2 μm membrane filter and lyophilised. The dried product was dissolved in water (5.4 mL) and quantified by UV spectroscopy to give **4** (Na^+ salt) (32.4 mM, 175 μmol, 35% from **12**); LRMS (ESI) (*m*/*z*): $(M + H)^-$ found: 626.3; calcd. for $(C_{29}H_{28}N_3O_{11}P + H)^-$: 626.2; ^{1}H NMR ($\delta$, 500 MHz, D_2O, acetone ref.): 7.69 (2H, d, J = 8.7 Hz), 7.57 (2H, d, J = 8.7 Hz), 7.48 (1H, s), 7.35 (1H, s), 7.11, (2H, d, J = 8.7 Hz), 6.77 (2H, d, J = 8.7 Hz), 4.30 (2H, t, J = 4.9 Hz), 4.11–4.15 (4H, m), 4.03 (2H, t, J = 8 Hz), 3.61 (2H, t, J = 4.9 Hz), 3.53 (2H, s), 2.92 (2H, t, J = 8 Hz) and 2.13 (3H, s).

4-Acetoxy-1-acetylindoline 16. A solution of 4-hydroxyindole (6.66 g, 50 mmol) in acetic acid (250 mL) was treated with $NaBH_3CN$ (9.42 g, 150 mmol) over 0.5 h, keeping the temperature at ~15 °C. The mixture was then stirred at room temperature for 1 h and water (5 mL) was added and the solvent evaporated. The residue was dissolved in EtOAc (150 mL) and washed with saturated aq. $NaHCO_3$ and brine, dried and evaporated to give 4-hydroxyindoline as pale crystals (6.76 g. 100%); ^{1}H NMR (δ, 90 MHz, $CDCl_3$ + DMSO-d_6): 6.82 (1H, t, J = 8 Hz), 6.20 (1H, d, J = 8 Hz), 6.16 (1H, d, J = 8 Hz), 3.52 (2H, t, J = 8 Hz) and 2.90 (2H, d, J = 8 Hz). The crude indoline was dissolved in a mixture of acetic acid (50 mL) and acetic anhydride (50 mL) and heated under reflux for 1 h. The solution was diluted with water (10 mL) and the solvents evaporated. The residue was dissolved in EtOAc (150 mL) and washed with saturated aq. $NaHCO_3$ and brine, dried and evaporated to give **16** as pale crystals (9.01 g, 82%), mp 98–99 °C (EtOAc–petroleum ether); found: C, 65.56; H, 6.07; N, 6.39; calcd. for $C_{12}H_{13}NO_3$: C, 65.74; H, 5.98; N, 6.39%; IR (ν_{max}/cm^{-1}): 1755, 1635, 1610, 1215; ^{1}H NMR (δ, 90 MHz): 8.07 (1H, d, J = 8 Hz), 7.19 (1H, t, J = 8 Hz), 6.72 (1H, d, J = 8 Hz), 4.05 (2H, t, J = 8 Hz), 3.03 (2H, t, J = 8 Hz) 2.28 (3H, s) and 2.19 (3H, s).

1-Acetyl-4-hydroxyindoline 17. A solution of **16** (8.77g, 40 mmol) in MeOH (250 mL) was treated with 2 M aq. NaOH (22 mL, 44 mmol), stirred at room temperature for 0.75 h, diluted with water (100 mL) and concentrated. The residue was acidified to pH 3 with 2 M aq. HCl and the precipitate was filtered, washed with water and dried under vacuum. The filtrate was extracted with EtOAc and the organic phase was washed with saturated aq. $NaHCO_3$ and brine, dried and evaporated to give more solid. The combined solids were recrystallised (EtOAc) to give **17** as white crystals (5.75 g, 82%), mp 230–231 °C; found: C, 67.80; H, 6.26; N, 7.86; calcd. for $C_{10}H_{11}NO_2$: C, 67.78; H, 6.26; N, 7.90%; IR (ν_{max}/cm^{-1}): 3150, 1630, 1610, 1295; ^{1}H NMR (δ, 90 MHz, $CDCl_3$ + DMSO-d_6): 9.10 (1H, br s), 7.57 (1H, d, J = 8 Hz), 6.93 (1H, t, J = 8 Hz), 6.48 (1H, d, J = 8 Hz), 4.05 (2H, t, J = 8 Hz), 3.04 (2H, t, J = 8 Hz) and 2.16 (3H, s).

Methyl (1-acetylindolin-4-yloxy)acetate 18. A suspension of anhydrous K_2CO_3 (6.64 g, 48 mmol) in acetone (250 mL) was mixed with **17** (5.67 g, 32 mmol). After 15 min, methyl bromoacetate (7.34 g, 48 mmol) was added and the mixture was heated under reflux for 4 h. The solid was filtered, washed with acetone and the filtrate was evaporated, then re-evaporated from toluene to give **18** as white crystals (7.19 g, 90%), mp 129–131 °C (from EtOAc–petroleum ether); found: C, 62.33; H, 6.06; N, 5.54; calcd. for $C_{13}H_{15}NO_4$: C, 62.64; H, 6.07; N, 5.62%; IR (ν_{max}/cm^{-1}): 1770, 1660, 1605, 1440, 1230, 1125; ^{1}H NMR (δ, 500 MHz): 7.88 (1H, d, J = 8 Hz), 7.14 (1H, t, J = 8 Hz), 6.44 (1H, d, J = 8 Hz), 4.66 (2H, s), 4.08 (2H, t, J = 8.5 Hz), 3.79 (3H, s), 3.20 (2H, t, J = 8.5 Hz) and 2.21 (s, 3H).

Methyl (1-acetyl-7-nitroindolin-4-yloxy)acetate 19 and methyl (1-acetyl-5-nitroindolin-4-yloxy)acetate 20. A solution of **18** (2.49 g, 10 mmol) in a mixture of CCl_4 (80 mL) and acetic anhydride (40 mL) was treated with claycop[8] (6.4 g) and the mixture was stirred at room temperature for 4 h. The solid was filtered and washed with CCl_4 and the filtrate was evaporated. The residue was dissolved in EtOAc and washed with saturated aq. $NaHCO_3$ and brine, dried and evaporated to give a brown viscous oil. Flash chromatography [EtOAc–petroleum ether (4 : 1)] afforded two products. The less polar was the 7-nitro isomer **19** as pale yellow crystals (1.76 g, 60%) mp 136–137 °C (EtOAc–petroleum ether); found: C, 53.13; H, 4.82; N, 9.49; calcd. for $C_{13}H_{14}N_2O_6$: C, 53.06; H, 4.80; N, 9.52%; UV [λ_{max} (EtOH)/nm (ε/M^{-1} cm^{-1})]: 249 (19 200), 295 (4000), 321(sh) (3600); UV [λ_{max} [EtOH–25 mM Na phosphate, pH 7.0 (1 : 25)/nm (ε/M^{-1} cm^{-1})]: 247 (18 600), 322 (4400); IR (ν_{max}/cm^{-1}): 1750, 1685, 1615, 1595, 1510, 1460, 1300, 1205, 1110; ^{1}H NMR (δ, 90 MHz): 7.68 (1H, d, J = 9 Hz), 6.45 (1H, d, J = 9 Hz), 4.73 (2H, s), 4.25 (2H, t, J = 8 Hz), 3.80 (3H, s), 3.17 (2H, t, J = 8 Hz) and 2.24 (3H, s).

The more polar product was the 5-nitro isomer **20** as pale yellow needles (0.87 g, 30%) mp 134–135 °C (EtOAc–petroleum ether); found: C, 53.20; H, 4.83; N, 9.48; calcd. for $C_{13}H_{14}N_2O_6$: C, 53.06; H, 4.80; N, 9.52%; UV [λ_{max} (EtOH)/nm (ε/M^{-1} cm^{-1})]: 240 (11 900), 329 (10 200); UV {λ_{max} [EtOH–25 mM Na phosphate, pH 7.0 (1 : 25)]/nm (ε/M^{-1} cm^{-1})}: 238 (9500), 342 (10 000); IR (ν_{max}/cm^{-1}): 1765, 1690, 1605, 1585, 1510, 1460, 1315, 1205, 1095; ^{1}H NMR (δ, 90 MHz): 7.80–8.20 (2H, m), 4.65 (2H, s), 4.18 (2H, t, J = 8 Hz), 3.79 (3H, s), 3.33 (2H, t, J = 8 Hz) and 2.26 (3H, s).

(1-Acetyl-7-nitroindolin-4-yloxy)acetic acid 21. A solution of the acetate **19** (0.47 g, 1.6 mmol) in a mixture MeOH (32 mL) and 1 M aq. NaOH (2.4 mL, 2.4 mmol) was stirred at room temperature for 0.5 h and diluted with water. The solvent was evaporated and the residue was acidified to pH 3 with 1 M aq. HCl and extracted with EtOAc. The combined organic phases were washed with brine, dried and evaporated to give **21** as orange crystals (0.39 g, 87%), mp 206–208 °C (from MeOH); found: C, 51.28; H, 4.30; N, 10.10; calcd. for $C_{12}H_{12}N_2O_6$: C, 51.43; H, 4.32; N, 9.99%; IR (ν_{max}/cm^{-1}): 1755, 1645, 1605, 1525, 1455, 1390, 1190, 1105; ^{1}H NMR (δ, 90 MHz, $CDCl_3$ + DMSO-d_6): 7.64 (1H, d, J = 9 Hz), 6.67 (1H, br s), 6.62 (1H, d, J = 9 Hz), 4.73 (2H, s), 4.26 (2H, t, J = 8 Hz), 3.16 (2H, t, J = 8 Hz) and 2.23 (3H, s).

4-{2-[(1-Acetyl-7-nitroindolin-4-oxy)acetamido]ethoxy}-4′-[2-(dihydroxyphosphoryloxy)ethoxy]benzophenone 5. The azide **12** (324 mg, 0.62 mmol) was hydrogenated and the crude amine (249 mg, 0.5 mmol, 81%) coupled with **21** (207 mg, 0.74 mmol) as described for **4** above and flash chromatographed [$CHCl_3$–MeOH (96 : 4)]. The recovered product was dissolved in TFA (10 mL), stirred at room temperature for 1 h and concentrated *in vacuo*. The residue was dissolved in water (95 mL) and adjusted to pH 7.0 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–MeOH (5 : 4 v/v)], t_R 5.6 min. The solution was lyophilised, redissolved in 25 mM Na phosphate, pH 6.0 (120 mL) and pumped onto the preparative HPLC column. The column was washed with 25 mM Na phosphate, pH 6.0 for 2 h, then with water for 2 h and finally eluted with water–MeOH (4 : 1). Fractions containing the product were analysed as above, combined and concentrated *in vacuo*. The residue was dissolved in water, passed through a 0.2 μm membrane filter, and lyophilised. The recovered material was dissolved in water (2.5 mL) and quantified by UV spectroscopy to give **5** (Na^+ salt) (30.5 mM, 76 μmol, 12% from **12**); LRMS (ESI) (*m*/*z*): $(M + H)^-$ found: 642.4. calcd. for $(C_{29}H_{28}N_3O_{12}P + H)^-$: 642.1; ^{1}H NMR ($\delta$, 500 MHz, D_2O, acetone ref.): 7.70 (2H, d, J = 8.8 Hz), 7.66 (2H, d, J = 8.8 Hz), 7.39 (1H, d, J = 9.2 Hz), 7.11 (2H, d, J = 8.8 Hz), 6.89 (2H, d, J = 8.8 Hz), 6.58 (2H, d, J = 9.2 Hz), 4.67 (2H, s), 4.31 (2H, t, J = 4.9 Hz), 4.12–4.20 (6H, m), 3.70 (2H, t, J = 4.9 Hz), 3.05 (2H, t, J = 8 Hz) and 2.20 (3H, s).

Progressive photolysis of 5. A solution of **5** (0.24 mM in 25 mM Na phosphate, pH 7.0) was irradiated in a 1 mm path length cell for increasing times in the range 0–45 s. The extent of photolysis was monitored by UV spectroscopy. Conversion was ~50% after 10 s and no further change was observed after 40 s (Fig. 2). As a control experiment, a solution of 1-[4*S*-(4-amino-4-carboxybutanoyl)]-4-methoxy-7-nitroindoline **3** (0.20 mM) was irradiated for increasing times up to 180 s under the same conditions. Conversion was ~50% after 45 s and photolysis was complete after 3 min.

Relative photolysis efficiencies of 5 and 3. Separate solutions of **5** and **3** (each 0.29 mM in 25 mM Na phosphate, pH 7.0 with 5 mM dithiothreitol) were simultaneously irradiated in 1 mm path length cells. The solutions were analysed by reverse-phase HPLC with mobile phases 25 mM Na phosphate, pH 6.0–MeCN (100 : 40 v/v) for **5**, t_R 4.6 min and 25 mM Na phosphate, pH 6.0–MeCN (100 : 25 v/v) for **3**, t_R 4.4 min. The extent of photolysis of each solution was determined by comparison of peak areas with those of non-irradiated controls and quantification was by measurement of peak heights. After 5 s irradiation, conversions for **5** and **3** were 35 and 8%, respectively. A 36% conversion of **3** was reached after 30 s irradiation, indicating that **5** photolysed ~6-fold more efficiently than **3**.

Relative photolysis efficiencies of 4 and 5. Separate solutions of **4** and **5** (each 0.29 mM in 25 mM Na phosphate, pH 7.0 with 5 mM dithiothreitol) were simultaneously irradiated for 5 s in 1 mm path length cells. The solutions were analysed by reverse-phase HPLC [mobile phase 25 mM Na phosphate, pH 6.0–MeCN (100 : 25 v/v)], (t_R 4.2 and 4.6 min for **4** and **5**, respectively). The extent of photolysis of each solution was determined by comparison of peak heights with those of non-irradiated controls. Conversions for **4** and **5** were 33 and 43%, respectively, indicating that **5** photolysed ~1.3-fold more efficiently than **4**.

Product identification on photolysis of 5. A solution of **5** (3.0 mM in 25 mM Na phosphate, pH 7.0) was lyophilised, re-dissolved in D_2O and irradiated in an NMR tube for 20 s. The NMR spectrum was recorded and compared with the spectrum of the non-irradiated solution. A new signal observed at 1.91 ppm was identified by an increase in its intensity when a solution of sodium acetate in D_2O was added.

Laser flash photolysis

LFP experiments were carried out at the University of Victoria LFP facility. Excitation was achieved using a Spectra-Physics excimer laser (308 nm, ~10 ns, < 30 mJ pulse^{-1}) and signals were digitised with a Tektronix TDS 520 recorder. Samples of OD ~ 0.3 cm^{-1} at 308 nm were prepared and excited in quartz cells of 7 mm path length. Samples were purged with N_2 or O_2 and sealed prior to photolysis. 8–10 shots were averaged to generate the decay traces to which monoexponential decay curves were fitted.

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