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Optimised synthesis and photochemistry of antenna-sensitised 1-acyl-7-nitroindolines☆

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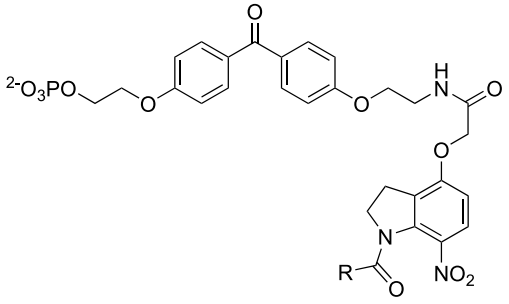
Abstract—Benzophenone antenna-sensitised 1-acyl-7-nitroindolines show a significantly enhanced extent of photochemical cleavage in aqueous solution over their non-sensitised analogues and release the carboxylate derived from their 1-acyl group. The present work investigates length and functional group effects in the linker between the benzophenone sensitiser and the nitroindoline and concurrently establishes a more efficient synthetic route to an effective conjugate than previously described. An incidental finding is that a TBDMS ether is stable during claycop-mediated nitration.

1. Introduction

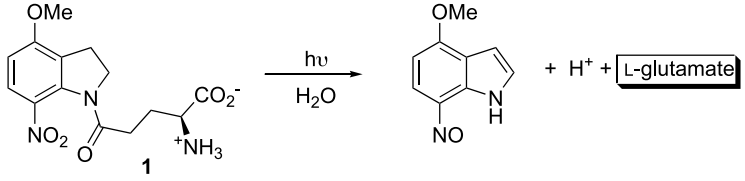
We have previously reported[1] the efficient photochemistry of 1-acyl-7-nitroindolines such as **1**, that release a carboxylate derived from the 1-acyl group upon irradiation by near-UV light in neutral aqueous solution, as shown in Scheme 1. Our interest in this photochemistry arose from a requirement for reagents that could rapidly release neuroactive amino acids by flash photolysis within biological preparations, particularly mammalian brain slices: the L-glutamate conjugate **1** exemplifies a means to achieve one aspect of this goal and has been used in a number of published studies.[2]

Based on mechanistic studies of this photochemistry,[3] we have described triplet-sensitised antenna conjugates of nitroindolines with substantially enhanced photosensitivity,[4] which is desirable as it should enable the photorelease of higher concentrations of the amino acid than from simple, non-sensitised nitroindolines such as **1**. The L-glutamate conjugate **2a** has recently been reported and shown to fulfil the latter requirement.[5]



2a R = $CH_2CH_2CH(NH_3^+)CO_2^-$ (*S*-isomer)
2b R = CH_3

With the general approach and efficacy of the antenna-sensitised methodology established, we wished to optimise



Scheme 1. Overall photocleavage reaction of **1**.

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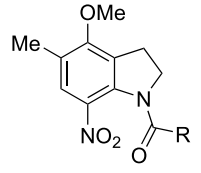
the synthetic chemistry required to assemble conjugates such as **2** and to examine the effects on photolysis efficiency of changes in the link between the benzophenone antenna and the nitroindoline. In particular, two broad aspects relevant to synthesis of the conjugates needed to be addressed. The first was the formation of some 5-nitro isomer during introduction of the nitro group, which required separation either before or after linkage to the benzophenone. The second was a convolution of the relatively cumbersome previous synthesis[4] of the sensitiser moiety of **2** and the more fundamental matter of whether the length of the linker had a significant effect on the photochemistry. Here we report our studies to explore these two issues.

2. Results and discussion

The obvious means to address the first of these issues was to block the 5-position with a suitable substituent. A previous attempt to achieve this in the unsensitised compounds such as **1** used a 5-methyl group, as in **3**, but this caused a significant reduction in photoefficiency that we ascribed to steric inhibition of the resonance interaction between the methoxy group and the aromatic ring.[1b] However we speculated for the sensitised compounds, where light absorption is principally via the sensitiser, that energy transfer might not be adversely affected by the presence of a 5-substituent and therefore set out initially to prepare the conjugate **4**.



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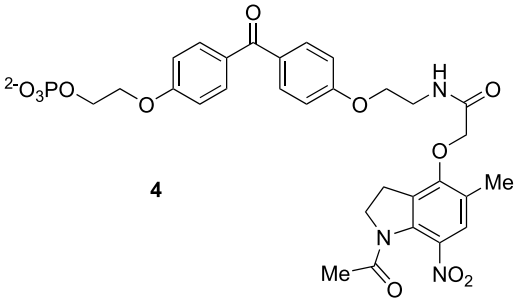

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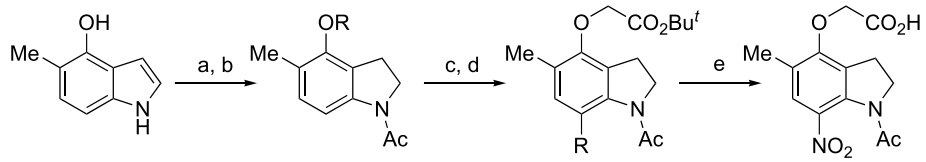
Synthesis of **4**, as outlined in Scheme 2, followed similar lines to that previously used for **2a**,**b**[4,5] but required 4-hydroxy-5-methylindole **5** as the starting material. We had previously prepared this compound by Leimgruber–Batcho synthesis but the overall yield was poor.[1b] In the present work we used an alternative, published method[6] in which 4-hydroxyindole was converted to its 5-(dimethylaminomethyl) derivative with Me_2NH–CH_2O, then hydrogenolysed over Pd–alumina. Details of the procedure in our hands are given in the Supplementary Data. With **5** available, further elaboration to the carboxylic acid **10**, as shown in Scheme 2, followed essentially the route previously used for the 5-nor analogue in our previous work,[4] except for the minor variant of a *tert*-butyl rather than a methyl ester to protect the carboxylic acid side chain. This allowed easy deprotection without the danger of partial hydrolysis of the *N*-acetyl group during saponification of the ester. In both compounds **9** and **10**, the ^{1}H NMR spectrum showed a resolved benzylic coupling (0.5 Hz) between the 5-methyl group and H-6. This was not resolved in other similar compounds within this work, although its presence could be inferred from the broadened line width of the 5-methyl and H-6 signals.

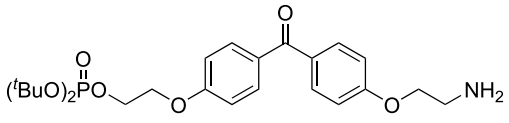
Final assembly of the conjugate **4** was by carbodiimide coupling of **10** with the previously described[4,5] amino-functionalised benzophenone **11**, followed by TFA treatment to remove the *tert*-butyl protecting groups. As described for previous conjugates of this type,[4] the calculated UV–Vis spectrum of **19**, obtained by adding the molar absorption spectra of the individual chromophores, gave an absorption maximum at 300 nm (ε 27,100 M^{-1} cm^{-1}; see Supplementary Data). This value was used to quantify solutions of **4**. Comparative irradiations of separate solutions of **4** and its nor-analogue **2b**, with the disappearance of the starting compounds being monitored by reverse-phase HPLC, showed that photolysis of **4** was ~16% more efficient. Furthermore, progressive irradiation led to very clean changes in the UV–Vis spectra, as previously reported for **2b**,[4] indicating that the photolysis reaction was not altered by the additional substituent. Although the gain in photolysis efficiency was quite modest, it contrasts markedly with the ~2-fold reduction in photoefficiency previously observed for the non-sensitised nitroindolines when a 5-methyl substituent was added.[1b] Furthermore, the presence of the 5-methyl group means that there is no problem of unwanted isomer formation when the nitro group is introduced. In our previous work, it had always been possible to separate the 5 and 7-nitro isomers of several *N*-acetyl compounds, but with a more complex side chain, such as in the precursor of the glutamate conjugate **2a**, it had been necessary to work with a mixture of isomers and to rely upon separation by HPLC of the final, water-soluble conjugate.[4,5] Obviously, avoidance of the isomer



5 **6** R = Ac, **7** R = H; **8** R = H, **9** R = NO_2; **10**

Scheme 2. Synthesis of precursor acid (**10**). Reagents and conditions: (a) $NaBH_3CN$, AcOH, Ac_2O, AcOH, Δ, 73%. (b) aq. NaOH, MeOH, 80%. (c) $BrCH_2CO_2Bu^t$, K_2CO_3, acetone, reflux, 90%. (d) claycop, Ac_2O, CCl_4, 66%. (e) TFA, 75%. Yields are given for recrystallised compounds.

problem is synthetically preferable if, as here, it can be achieved without compromising the photolysis efficiency.



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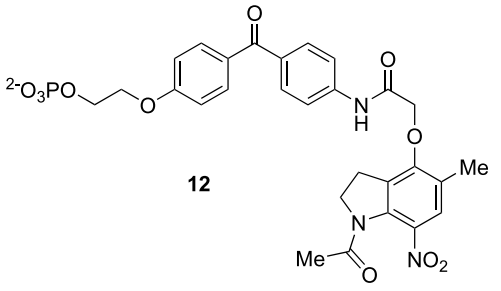
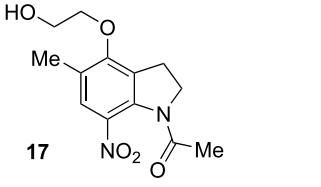
Encouraged by this initial success, we turned our attention to the combined matters of the length of the linker between the benzophenone and the nitroindoline, and establishment of an easier synthesis of a suitably functionalised benzophenone. Although it is possible that conformational relationships between the sensitiser and the nitroindoline could be influential, the more accessible strategy was to investigate shortening the flexible linker. Our first approach aimed at the conjugate **12**. Note that in each of the previous or present conjugates, the phosphate group on the side chain of the benzophenone sensitiser was present only to promote water solubility and is irrelevant to the photochemistry. Synthesis of **16**, a protected precursor of the sensitiser moiety in **12**, is shown in Scheme 3 and was straightforward from known[7] 4-hydroxy-4′-nitrobenzophenone **13**.



12

Carbodiimide-mediated coupling of **10** and **16**, followed by treatment with TFA to remove the *tert*-butyl protecting groups as described above for **4** gave the desired conjugate **12**. Its calculated UV–Vis spectrum had λ_{max} 300 nm (ε 30,500 $M^{-1}\,cm^{-1}$; see Supplementary Data). Disappointingly, irradiation of an aqueous solution of **12** resulted in no changes in the UV–Vis spectrum, even on exposure to the light source for periods up to 8 min. In contrast, irradiation of **2b** for 7 s under the same conditions resulted in ~50% photolysis (see Figure 2 of Ref. 4). We did not attempt to investigate the reasons for the lack of photoreactivity in **12**. However, there is some indication that amidobenzophenones may decay to a lower-energy triplet state than the normal n,π^* benzophenone triplet,[8] and may therefore have an insufficient energy gap to allow triplet transfer to the nitroindoline. Nevertheless, we recognise that the situation must be more complex than this effect alone, since the nitroindoline itself would have been expected to show considerable direct photolysis during such prolonged irradiation.[1] However, in practical terms there seemed little benefit in probing further into this negative result. Experimental details relating to the synthesis of **12** are reported in the Supplementary Data.

Our second approach to the combination of linker length and synthetic practicality was targeted on an ether-linked conjugate, where we envisioned assembly by a Mitsunobu coupling of a suitable ω-hydroxyalkoxy nitroindoline, exemplified by **17**, with the phenolic benzophenone **18**. The expected conjugate, **19**, would have the shortest practicable linker which maintains the principal structural features, i.e. the 4,4′-dialkoxybenzophenone and the 4-alkoxyindoline, that were present in our initial successful conjugate **2b**.



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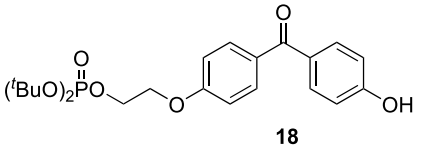

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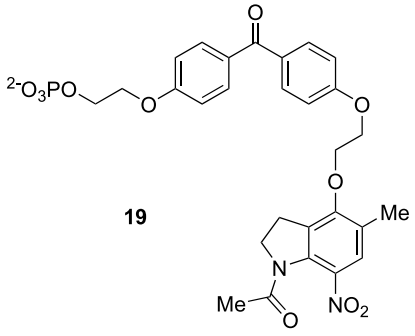

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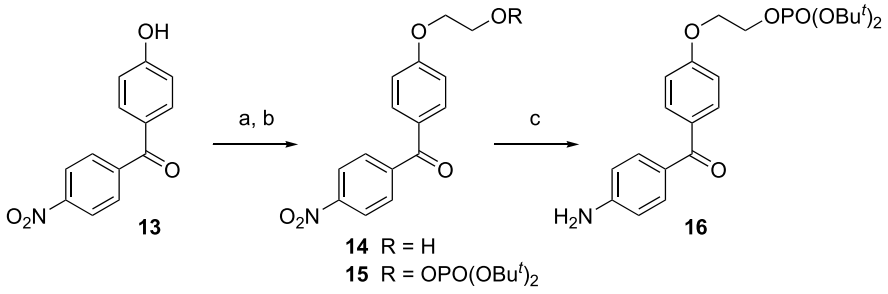
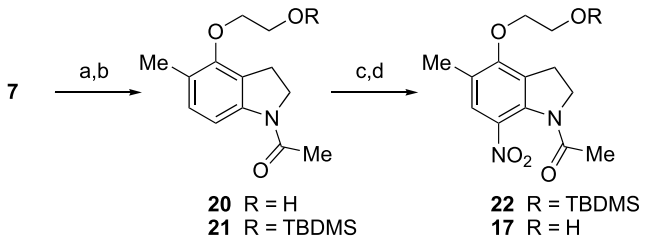
Synthesis of **17**, shown in Scheme 4, was generally straightforward but established a useful point about stability of the TBDMS protecting group (see below). Conversion of



Scheme 3. Synthesis of (**16**). Reagents and conditions: (a) $BrCH_2CH_2OH$, K_2CO_3, NaI, acetone, reflux, 81%. (b) $Et_2NP(OBu^t)_2$, 1*H*-tetrazole, THF, then MCPBA, 81%. (c) H_2, Pd–C, EtOH, ~100%.



Scheme 4. Synthesis of alcohol (**17**). Reagents and conditions: (a) ethylene carbonate, Et_4NBr, DMF, 140 °C, 74%; (b) TBDMSCl, imidazole, CH_2Cl_2, 95%; (c) claycop, Ac_2O, CCl_4, 85%; (d) TBAF, HOAc, THF, 84%.

7 (Scheme 2) to its 2-hydroxyethyl ether **20** was readily achieved by heating with ethylene carbonate in DMF in the presence of catalytic tetraethylammonium bromide.[9] This procedure was much more effective in this case than alkylation with 2-bromoethanol (K_2CO_3-butanone), although the latter method was effective when the phenol was more acidic, as with 4-hydroxybenzophenones, for example in synthesis of **14** (Scheme 3). The next significant step in the synthesis was introduction of the 7-nitro group, for which the previously established use of claycop–Ac_2O as the nitrating reagent[10] was our preferred option. However, it was obviously necessary to protect the primary alcohol to avoid side reaction(s) under the oxidative conditions of this reaction. Protection as a silyl ether was attractive, since its subsequent deprotection was expected to be achievable without perturbing other parts of the molecule but we had some concern as to whether it would survive the conditions: previously we have reported partial loss (presumably acid-catalysed) of a Boc group from a $(Boc)_2N$-moiety during claycop-mediated nitration.[5] However, we were gratified to find that the TBDMS ether **21** underwent claycop nitration to give an 86% yield of purified product **22**, implying that there was no significant cleavage of the silyl ether during the reaction. This useful observation is particularly relevant to proposed future work with more complex acyl groups (such as protected amino acid residues) attached to the indoline nitrogen. Normal TBAF deprotection (buffered with acetic acid) then gave the required alcohol **17**.

Our second required compound was the benzophenone **18**, and a desirable goal was to establish a direct route from a readily available benzophenone precursor. In our previous work, the unsymmetrical benzophenone used as the sensitiser had been prepared from mono-aryl starting materials in order to establish different substituents on the two rings.[4] In the present work (Scheme 5), we started from the symmetrical dipivalate ester **23** of 4,4′-dihydroxybenzophenone. Controlled alkaline hydrolysis gave the monopivalate **24** in purified yield of 73% after simple recrystallisation of the crude hydrolysis mixture. Previous preparations by partial esterification of 4,4′-dihydroxybenzophenone required chromatography for isolation of the pure monoester.[11] Conversion of **24** via **25** to the phosphate ester **26** was uneventful and final alkaline hydrolysis of the pivalate ester gave **18** in excellent yield. The overall yield for the five-stage sequence from commercial 4,4′-dihydroxybenzophenone was 29%.

Mitsunobu coupling of the alcohol **17** and the phenol **18** (Ph_3P, diisopropyl azodicarboxylate)[12] proceeded in high yield and subsequent TFA treatment to remove the *tert*-butyl protecting groups gave conjugate **19**. Solutions of **19** were quantified using the same molar absorbance coefficient (ε_{300} 27,900 $M^{-1}\,cm^{-1}$) calculated for **4**, since the chromophores of the two conjugates are essentially identical. Progressive photolysis of **19** in air-saturated, neutral aqueous solution showed a clean transition between the initial and final UV–Vis spectra (Fig. 1), very similar to that previously reported[4] for photolysis of **2b**. Comparative 300 nm irradiation of separate solutions of **2b** and **19** showed that the two compounds had very similar efficiency of photolysis (Scheme 5).

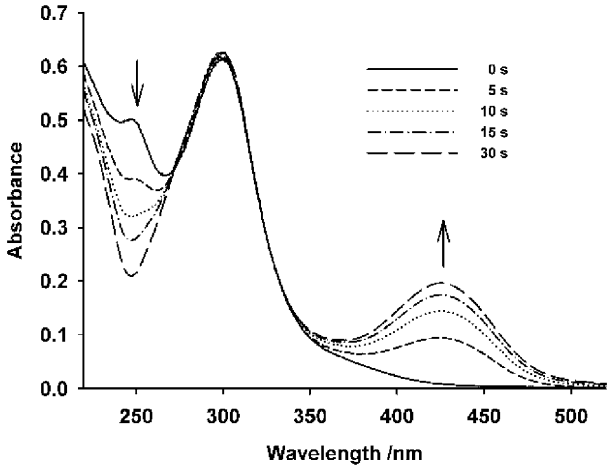
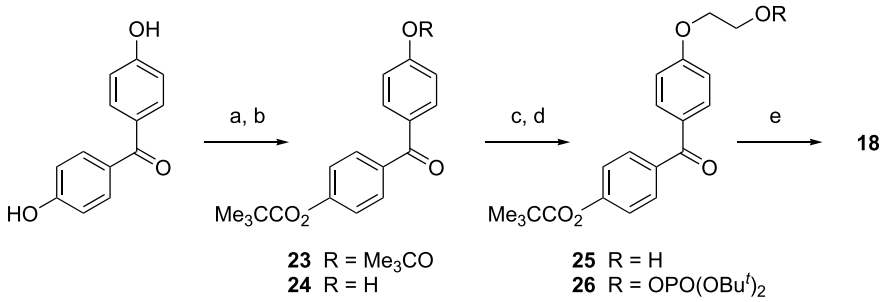


Figure 1. UV–Vis absorption spectra for an aqueous solution of **19** upon 300-nm irradiation for the cumulative time periods indicated. The arrows indicate the direction of absorbance changes with increasing irradiation time.

3. Conclusions

At its outset, a hoped-for outcome this work was to obtain enhanced photolysis efficiency over that of our previous conjugates of general structure **2**. In the event, the improvements have been modest (for **4**) or absent (for **19**), while the amidobenzophenone conjugate **12** was inert. Thus variation in length of the linker between the sensitiser and the nitroindoline, at least over the range explored here, has little effect on the photolysis efficiency. On the other hand, changes in the benzophenone substituents can have major negative consequences. The principal benefit to have emerged is a synthesis of **19** that is more practicable in several respects than that of the initial conjugates **2**. These improvements are (a) in the use of a 5-methyl substituent to block nitration other than at the 7-position of the indoline, (b) a short, effective route to preparation of the sensitiser moiety and (c) efficient chemical coupling of the sensitiser and the nitroindoline. An effective synthesis of conjugates of this general type will be important if the sensitised nitroindoline photochemistry is to become widely adopted. Future work will be to adapt the present synthesis of **19** to an L-glutamate analogue and to perform detailed assessment of the utility of such a conjugate (and perhaps of the corresponding GABA and glycine analogues) in



Scheme 5. Synthesis of benzophenone (**18**). Reagents and conditions: (a) Me_3CCOCl, pyridine, 88%. (b) NaOH (2 equiv), aq. THF, 73%. (c) $BrCH_2CH_2OH$, K_2CO_3, NaI, butanone, reflux, 58%. (d) $Et_2NP(OBu^t)_2$, 1*H*-tetrazole, THF, then MCPBA, 72%. (e) NaOH (1 equiv), aq. MeOH, 91%.

neurophysiological applications. This chemistry and its biological sequelae will be reported in due course.

4. Experimental

4.1. 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 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. Details of mobile phases are given at relevant points in the text. Detection for analytical and preparative work was at 254 nm. Organic solvents were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. Hexane solvent (bp 40–60 °C) was redistilled before use. Photolysis experiments were performed in a Rayonet RPR-100 photochemical reactor fitted with 16×300 nm lamps.

4.2. Synthesis of 4-{2-[(1-acetyl-5-methyl-7-nitroindolin-4-oxy)acetamido]ethoxy}-4′-[2-(dihydroxyphosphoryloxy) ethoxy]benzophenone (4)

4.2.1. 4-Acetoxy-1-acetyl-5-methylindoline (5). $NaBH_3CN$ (3.58 g, 57 mmol) was added portionwise over 0.5 h to a solution of 5-methylindol-4-ol[6] (2.80 g, 19 mmol) in acetic acid (90 mL), keeping the temperature at ~15 °C by intermittent cooling. The mixture was then stirred at rt for 1 h and water (3 mL) was added and the solvent was evaporated. The residue was dissolved in EtOAc (50 mL) and washed with saturated aq. $NaHCO_3$ and brine, dried and evaporated to give 5-methylindolin-4-ol as pale foam (2.83 g, 100%); ^{1}H NMR (90 MHz) δ 6.78 (d, J=8 Hz, 1H), 6.20 (d, J=8 Hz, 1H), 3.98 (s, 2H, exchanges with D_2O), 3.54 (t, J=8 Hz, 2H), 2.93 (t, J=8 Hz, 2H), 2.14 (s, 3H). The crude indoline was dissolved in a mixture of acetic acid (20 mL) and acetic anhydride (20 mL) and heated under reflux for 1 h. The resulting dark solution was diluted with water (5 mL) and the solvents were evaporated. The residue was dissolved in EtOAc (100 mL) and washed with saturated aq. $NaHCO_3$ and brine, dried and evaporated to give **6** as pale fawn crystals (3.22 g, 73%), mp 103–104 °C (EtOAc–hexanes); ^{1}H NMR (500 MHz) δ 7.98 (d, J=8.1 Hz, 1H), 7.06 (d, J=8.1 Hz, 1H), 4.08 (t, J=8.5 Hz, 2H), 3.04 (t, J=8.5 Hz, 2H), 2.32 (s, 3H), 2.20 (s, 3H), 2.13 (s, 3H). Anal. calcd for $C_{13}H_{15}NO_3$: C, 66.94; H, 6.48; N, 6.00; found: C, 66.51; H, 6.07; N, 5.97.

4.2.2. 1-Acetyl-5-methylindolin-4-ol (7). A solution of **6** (3.15 g, 13.5 mmol) in MeOH (95 mL) was treated with 1 M aq. NaOH (14.85 mL, 14.85 mmol) and stirred at rt for 0.75 h, then diluted with water (100 mL) and concentrated. The residue was acidified to pH 3 with dilute HCl and the precipitated solid was filtered off, washed with water and dried. The filtrate was washed with EtOAc and the organic phase was washed with saturated aq. $NaHCO_3$ and brine, dried and evaporated to give additional solid. The combined solid was recrystallised (MeOH–EtOAc) to give **7** as white crystals (2.06 g, 80%), mp 242 °C; ^{1}H NMR (500 MHz, $CDCl_3$+DMSO-d_6) δ 8.04 (s, 1H), 7.57 (d, J=8.1 Hz, 1H), 6.88 (d, J=8.1 Hz, 1H), 4.05 (t, J=8.5 Hz, 2H), 3.13 (t, J=8.5 Hz, 2H), 2.91 (s, 3H), 2.19 (s, 3H). Anal. calcd for $C_{11}H_{13}NO_2$: C, 69.09; H, 6.85; N, 7.32; found: C, 68.93; H, 6.97; N, 7.29.

4.2.3. *tert*-Butyl (1-acetyl-5-methylindolin-4-yloxy)acetate (8). A suspension of anhydrous K_2CO_3 (1.24 g, 9 mmol) in acetone (60 mL) was treated with **7** (1.15 g, 6 mmol). After 15 min, *tert*-butyl bromoacetate (2.34 g, 12 mmol) was added and the mixture was heated under reflux for 4 h. The solid was filtered off, washed with acetone and the filtrate was evaporated. The residue was dissolved in EtOAc (50 mL), washed with brine, dried and evaporated to give **8** as white crystals (1.64 g, 90%), mp 95–96 °C (EtOAc–hexanes); ^{1}H NMR (500 MHz) δ 7.87 (d, J=8.1 Hz, 1H), 7.00 (d, J=8.1 Hz, 1H), 4.38 (s, 2H), 4.05 (t, J=8.4 Hz, 2H), 3.24 (t, J=8.4 Hz, 2H), 2.26 (s, 3H), 2.20 (s, 3H), 1.50 (s, 9H). Anal. calcd for $C_{17}H_{23}NO_4$: C, 66.86; H, 7.59; N, 4.59; found: C, 67.07; H, 7.64; N, 4.60.

4.2.4. *tert*-Butyl (1-acetyl-5-methyl-7-nitroindolin-4-yloxy)acetate (9). Claycop (3.2 g; prepared as described)[13] was added to a solution of **8** (1.53 g, 5 mmol) in a mixture of CCl_4 (40 mL) and acetic anhydride (20 mL) and the mixture was stirred at rt for 4 h. The solid was filtered off, 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 **9** as yellow needles (1.15 g, 66%), mp 97.5–98.5 °C (Et_2O–hexanes with charcoal); ^{1}H NMR (500 MHz) δ 7.54 (q, J=0.5 Hz, 1H), 4.47 (s, 2H), 4.22 (t, J=8.0 Hz, 2H), 3.24 (t, J=8.0 Hz, 2H), 2.31 (d, J=0.5 Hz, 3H), 2.24 (s, 3H), 1.49 (s, 9H).

Anal. calcd for $C_{17}H_{22}N_2O_6$: C, 58.28; H, 6.33; N, 7.99; found: C, 58.20; H, 6.33; N, 7.96.

4.2.5. (1-Acetyl-5-methyl-7-nitroindolin-4-yloxy)acetic acid (10). A solution of **9** (1.05 g, 3 mmol) in TFA (10 mL) was stirred at rt for 1 h, concentrated and re-evaporated from toluene (2×10 mL) to give **10** as light brown crystals (0.66 g, 75%), mp 188–190 °C (EtOAc); UV: λ_{max} (EtOH)/nm 250 (ε/M^{-1} cm^{-1} 25,400), 287 (9300) 329sh (4000); λ_{max} [EtOH–25 mM Na phosphate, pH 7.0 (1:40)]/nm 247 (ε/M^{-1} cm^{-1} 19,700), 338 (3600); ^{1}H NMR (500 MHz, $CDCl_3$+DMSO-d_6) δ 7.51 (q, J= 0.5 Hz, 1H), 4.57 (s, 2H), 4.23 (t, J=8.0 Hz, 2H), 3.27 (t, J=8.0 Hz, 2H), 2.33 (d, J=0.5 Hz, 3H), 2.24 (s, 3H). Anal. calcd for $C_{13}H_{14}N_2O_6$: C, 53.06; H, 4.80; N, 9.52; found: C, 53.16; H, 4.79; N, 9.40.

4.2.6. 4-{2-(1-Acetyl-5-methyl-7-nitroindolin-4-yloxy)acetamido]ethoxy]-4′-[2-(dihydroxyphosphoryloxy)ethoxy]-benzophenone (4). A solution of 4-(2-azidoethoxy)-4′-{2-[di(*tert*-butoxyphosphoryloxy]ethoxy}benzophenone[4] (312 mg, 0.6 mmol) was reduced with Ph_3P in moist THF as previously described,[5] to give the crude amine **11**, which was dissolved in $CHCl_3$ (30 mL), dried and evaporated. The residue was then dissolved in dry MeCN (20 mL) and treated with the acid **10** (206 mg, 0.7 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (161 mg, 0.84 mmol). The mixture was stirred at rt under nitrogen 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 di-*tert*-butyl ester of **4** (393 mg, 85%) as a yellow viscous oil which was used in the next step without further purification; ^{1}H NMR (90 MHz) δ 7.79 (d, J=8 Hz, 4H), 7.42 (s, 1H), 6.98 (d, J=8 Hz, 4H), 4.42 (s, 2H), 4.04–4.36 (m, 8H), 3.76–3.98 (m, 2H), 3.12 (t, J=8 Hz, 2H), 2.23 (s, 3H), 2.18 (s, 3H), 1.50 (s, 18H). This ester (393 mg, 0.51 mmol) was dissolved in TFA (10 mL), stirred at rt for 1 h and concentrated in vacuo. The residue was dissolved in water (65 mL) and adjusted to pH 7.08 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—MeCN (100:45 v/v), t_R 4.4 min. The solution was lyophilised, dissolved in 25 mM Na phosphate, pH 6.0 (110 mL) and pumped onto the preparative HPLC column. The column was first 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 v/v). Fractions containing the product were analysed by HPLC as above, combined and concentrated in vacuo. The residue was dissolved in water, filtered through a 0.2 μm membrane, lyophilised and the remaining yellow powder was dissolved in water (10.5 mL) and quantified by UV–Vis spectroscopy at 300 nm (see above) to give a solution of **4** (Na^+ salt) (27.5 mM, 289 μmol, 48% from **11**); ^{1}H NMR (500 MHz, D_2O, acetone ref.) δ 7.53 (d, J=8.5 Hz, 4H), 7.10 (s, 1H), 6.97 (d, J=8.5 Hz, 2H), 6.85 (d, J=8.5 Hz, 2H), 4.41 (s, 2H), 4.21–4.24 (m, 2H), 4.12–4.19 (m, 4H), 3.91 (t, J= 7.7 Hz, 2H), 3.68–3.76 (m, 2H), 2.92 (t, J=7.4 Hz, 2H), 2.10 (s, 3H), 1.93 (s, 3H); LRMS (ESI) calcd for $(C_{30}H_{30}N_3O_{12}P+H)^-$: 656.2; found: 656.4.

4.3. Synthesis of 4-[2-(1-acetyl-5-methyl-7-nitroindolin-4-oxy)ethoxy]-4′-[2-(dihydroxyphosphoryloxy)ethoxy] benzophenone (19)

4.3.1. 4-Hydroxy-4′-(trimethylacetoxy)benzophenone (24). A solution of 4,4′-bis(trimethylacetoxy)benzophenone **23** (7.65 g, 20 mmol; see Supplementary Data) in THF (40 mL) was diluted with MeOH (360 mL) and rapidly mixed with 1 M aq. NaOH (40 mL, 40 mmol). The solution was stirred at rt for 1.5 min, then neutralised with 1 M aq. citric acid (40 mL, 40 mmol) and concentrated. The residue was diluted with water and washed with EtOAc and the combined organic phases were washed with brine, dried and evaporated to give **24** as white crystals (4.38 g, 73%), mp 171–173 °C (EtOAc), mp 171–173 °C (lit.[11a] 171–173 °C).

4.3.2. 4-(2-Hydroxyethoxy)-4′-(trimethylacetoxy)benzophenone (25). To a solution of **24** (3.88 g, 13 mmol) in butanone (260 mL) was added anhydrous K_2CO_3 (3.59 g, 26 mmol), NaI (1.3 g) and 2-bromoethanol (8.12 g, 65 mmol), and the mixture was heated under reflux. The progress of the reaction was followed by TLC [EtOAc–hexanes (1:1)]. Further amounts of 2-bromoethanol (8.12 g), NaI (1.3 g) and anhydrous K_2CO_3 (3.59 g,) were added after each of 2 h and 5 h and reflux was continued for a total of 7 h. The solid was filtered off, washed with acetone and the filtrate was evaporated. The residue was taken up in EtOAc (80 mL) and washed with water and brine, dried and evaporated. Flash chromatography [EtOAc–hexanes (1:1)] gave two fractions. The unreacted starting phenol **24** (0.64 g, 16%) eluted first, followed by **25** as white crystals (2.69 g, 60%), mp 115–116 °C (EtOAc–hexanes); ^{1}H NMR (500 MHz) δ 7.82 (dt, J=8.6, 2.0 Hz, 2H), 7.80 (dt, J=8.6, 2.0 Hz, 2H), 7.18 (dt, J=8.6, 2.2 Hz, 2H), 6.99 (dt, J=8.6, 2.2 Hz, 2H), 4.18 (t, J=4.4 Hz, 2H), 4.03 (dt, J=4.4, 6.2 Hz, 2H), 2.00 (t, J=6.2 Hz, 1H, exchanges with D_2O), 1.38 (s, 9H). Anal. calcd for $C_{20}H_{22}O_5$ ¼H_2O: C, 69.25; H, 6.54. Found: C, 69.51; H, 6.33; HRMS (ESI): calcd for $(C_{20}H_{22}O_5+H)^+$: 343.1540. Found: 343.1552.

4.3.3. 4-{2-[Di(*tert*-butoxy)phosphoryloxy]ethoxy}-4′-(trimethylacetoxy)benzophenone (26). A solution of **25** (2.40 g, 7 mmol) in dry THF (50 mL) was treated under nitrogen with 1*H*-tetrazole (1.96 g, 28 mmol) and di-*tert*-butyl *N*,*N*-diethylphosphoramidite (93% purity; 3.75 g, 14 mmol) and the mixture was stirred at rt overnight. The solution was cooled to 0 °C and treated dropwise with a solution of *m*-chloroperbenzoic acid (55% peracid; 6.59 g, 21 mmol) in CH_2Cl_2 (50 mL). The solution was stirred at 4 °C for 1 h, diluted with CH_2Cl_2 (100 mL), washed with 10% aq. $Na_2S_2O_5$ and the organic phase was washed with saturated aq. $NaHCO_3$ and brine, dried and evaporated. Flash chromatography [EtOAc–hexanes (4:1)] gave **26** as white crystals (3.18 g, 85%), mp 80–81 °C (Et_2O–hexanes); ^{1}H NMR (500 MHz) δ 7.81 (dt, J=8.6, 2.0 Hz, 2H), 7.80 (dt, J=8.6, 2.0 Hz, 2H), 7.18 (dt, J=8.6, 2.0 Hz, 2H), 6.97 (dt, J=8.6, 2.0 Hz, 2H), 4.31–4.34 (m, 2H), 4.25–4.28 (t, J=5 Hz, 2H), 1.50 (s, 18H), 1.38 (s, 9H). Anal. calcd for $C_{28}H_{39}O_8P$: C, 62.91; H, 7.35; found: C, 63.00; H, 7.38.

4.3.4. 4-{2-[Di(*tert*-butoxy)phosphoryloxy]ethoxy}-4′-hydroxybenzophenone (18). A solution of **26** (2.94 g, 5.5 mmol) in MeOH (220 mL) was treated with water

(16.5 mL) and 1 M aq. NaOH (5.5 mL, 5.5 mmol) and stirred at rt for 40 min, then neutralised with 1 M aq. citric acid (5.5 mL, 5.5 mmol) and concentrated. The residue was diluted with water and washed with EtOAc and the combined organic phases were washed with brine, dried and evaporated to give **18** as white crystals (2.24 g, 90%), mp 104–106 °C (EtOAc–hexanes); ^{1}H NMR (500 MHz) δ 7.73 (dt, J=8.6, 2.0 Hz, 2H), 7.69 (dt, J=8.6, 2.0 Hz, 2H), 6.95 (dt, J=8.6, 2.0 Hz, 2H), 6.91 (dt, J=8.6, 2.0 Hz, 2H), 4.31–4.34 (m, 2H), 4.23 (t, J=4.7 Hz, 2H), 1.51 (s, 18H). Anal. calcd for $C_{23}H_{31}O_9P$: C, 61.33; H, 6.94; found: C, 61.13; H, 7.05.

4.3.5. 1-Acetyl-4-(2-hydroxyethoxy)-5-methylindoline (20). A solution of **7** (1.53 g, 8 mmol) and ethylene carbonate (1.41 g, 16 mmol) in dry DMF (40 mL) was treated with tetraethylammonium bromide (0.17 g, 0.8 mmol) and the mixture was heated at 140 °C for 20 h. The solvent was then evaporated under reduced pressure and the residue, dissolved in a mixture of EtOAc (50 mL) and MeOH (10 mL), was washed with 1 M aq. NaOH and brine, dried and evaporated to give **20** as white crystals (1.39 g, 74%), mp 112–113 °C (EtOAc–hexanes); ^{1}H NMR (500 MHz) δ 7.87 (d, J=8.2 Hz, 1H), 7.01 (d, J=8.2 Hz, 1H), 4.04 (t, J=8.4 Hz, 2H), 3.97 (t, J=4.4 Hz, 2H), 3.90–3.93 (m, 2H), 3.19 (t, J=8.4 Hz, 2H), 2.24 (s, 3H), 2.20 (s, 3H). Anal. calcd for $C_{13}H_{17}NO_3$: C, 66.36; H, 7.28; N, 5.95; found: C, 66.38; H, 7.38; N, 6.03.

4.3.6. 1-Acetyl-4-[2-(*tert*-butyldimethylsilyloxy)ethoxy]-5-methylindoline (21). To a solution of **20** (1.29 g, 5.5 mmol) in dry CH_2Cl_2 (55 mL) was added imidazole (0.56 g, 8.25 mmol) and *tert*-butyldimethylsilyl chloride (0.99 g, 6.6 mmol) and the mixture was stirred at rt under nitrogen overnight. The precipitated white solid was filtered off, washed with CH_2Cl_2 and the filtrate was washed with 0.5 M aq. HCl, saturated aq. $NaHCO_3$ and brine, dried and evaporated to give **21** as white crystals (1.82 g, 95%), mp 101–102 °C (Et_2O–hexanes); ^{1}H NMR (500 MHz) δ 7.84 (d, J=8.1 Hz, 1H), 6.99 (d, J=8.1 Hz, 1H), 4.05 (t, J=8.4 Hz, 2H), 3.89–3.97 (m, 4H), 3.20 (t, J=8.4 Hz, 2H), 2.24 (s, 3H), 2.20 (s, 3H), 0.91 (s, 9H), 0.10 (s, 6H). Anal. calcd for $C_{19}H_{31}NO_3Si$: C, 65.29; H, 8.94; N, 4.01; found: C, 65.27; H, 9.09; N, 3.96.

4.3.7. 1-Acetyl-4-[2-(*tert*-butyldimethylsilyloxy)ethoxy]-5-methyl-7-nitroindoline (22). A solution of **21** (1.75 g, 5 mmol) in a mixture of acetic anhydride (15 mL) and CCl_4 (30 mL) was treated with claycop (3.20 g) and the mixture was stirred at rt for 3 h. The solid was filtered off, washed with CCl_4 and the filtrate was evaporated. The residue was dissolved in EtOAc (50 mL) and washed with saturated aq. $NaHCO_3$ and brine, dried and evaporated. Flash chromatography [EtOAc–hexanes (1:1)] gave **22** as yellow crystals (1.51 g, 86%), mp 79–80 °C (Et_2O–hexanes); ^{1}H NMR (500 MHz) δ 7.54 (s, 1H), 4.21 (t, J=8 Hz, 2H), 4.02 (t, J=4.7 Hz, 2H), 3.91 (t, J=4.7 Hz, 2H), 3.21 (t, J=8 Hz, 2H), 2.28 (s, 3H), 2.23 (s, 3H), 0.90 (s, 9H), 0.09 (s, 6H). Anal. calcd for $C_{19}H_{30}N_2O_5Si$ ¼H_2O: C, 57.19; H, 7.70; N, 7.02; found: C, 57.18; H, 7.79; N, 7.14.

4.3.8. 1-Acetyl-4-(2-hydroxyethoxy)-5-methyl-7-nitroindoline (17). A solution of **22** (1.46 g, 3.7 mmol) and acetic acid (0.45 g, 7.4 mmol) in THF (37 mL) was treated at 0 °C with TBAF (1 M in THF; 7.4 mL, 7.4 mmol) and the mixture was stirred at rt overnight. The solvent was evaporated and the residue was taken up in EtOAc (40 mL) and washed with saturated aq. $NaHCO_3$ and brine, dried and evaporated to give **17** as yellow crystals (0.87 g, 84%), mp 171–173 °C (EtOAc–MeOH); ^{1}H NMR (500 MHz; $CDCl_3$+DMSO-d_6) δ 7.50 (s, 1H), 4.30 (t, J=5.5 Hz, 1H, exchanges with D_2O), 4.23 (t, J=8 Hz, 2H), 4.07 (t, J=4.8 Hz, 2H), 3.85–3.88 (m, 2H), 3.26 (t, J=8 Hz, 2H), 2.30 (s, 3H), 2.24 (s, 3H). Anal. calcd for $C_{13}H_{16}N_2O_5$: C, 55.71; H, 5.75; N, 9.99; found: C, 55.67; H, 5.78; N, 9.83.

4.3.9. 4-[2-(1-Acetyl-5-methyl-7-nitroindolin-4-oxy)-ethoxy]-4′-[2-(dihydroxy-phosphoryloxy)ethoxy]benzophenone (19). A solution of **17** (0.21 g, 0.75 mmol) in dry THF (14 mL) was treated with **18** (0.38 g, 0.85 mmol) and triphenylphosphine (0.24 g, 0.9 mmol) and cooled to 0 °C under nitrogen. Diisopropyl azodicarboxylate (95% purity; 0.19 g, 0.9 mmol) was added and the mixture was stirred at rt under nitrogen for 24 h. The solvent was evaporated, the residue was dissolved in EtOAc (30 mL) and washed with 0.5 M aq. NaOH, 0.5 M aq. HCl and brine, dried and evaporated. Flash chromatography [EtOAc, then EtOAc–MeOH (95:5)] followed by trituration with ($CHCl_3$–hexanes) gave the di-*tert*-butyl ester of **23** as a pale foam (0.47 g, 88%) which was used in the next step without further purification; ^{1}H NMR (500 MHz) δ 7.80 (dt, J=9.0, 2.0 Hz, 2H), 7.78 (dt, J=9.0, 2.0 Hz, 2H), 7.57 (s, 1H), 6.98 (dt, J=9.0, 2.0 Hz, 2H), 6.97 (dt, J=9.0, 2.0 Hz, 2H), 4.35 (s, 4H), 4.31–4.35 (m, 2H), 4.27 (t, J=4.9 Hz, 2H), 4.22 (t, J=8 Hz, 2H), 3.22 (t, J=8 Hz, 2H), 2.31 (s, 3H), 2.25 (s, 3H), 1.50 (s, 18H). This ester (0.46 g, 0.65 mmol) was dissolved in TFA (10 mL), stirred at rt for 1 h and concentrated in vacuo. The residue was dissolved in water (72 mL) and adjusted to pH 7.1 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—MeCN (100:55 v/v)], t_R 4.6 min. The solution was lyophilised, dissolved in 25 mM Na phosphate, pH 6.0 (100 mL) and pumped onto the preparative HPLC column. The column was first washed with 25 mM Na phosphate, pH 6.0 for 1 h, then with water for 2 h and product was eluted with water–MeOH (3:2 v/v). 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, lyophilised and the yellow powder obtained was dissolved in water (15 mL), quantified by UV–Vis spectroscopy at 300 nm (see above), to give **19** (Na^+ salt) (22.1 mM, 331 μmol, 51%); ^{1}H NMR (500 MHz, D_2O, acetone ref.) δ 7.57 (d, J=8.5 Hz, 2H), 7.54 (d, J=8.5 Hz, 2H), 7.24 (s, 1H), 7.00 (d, J=8.9 Hz, 2H), 6.82 (d, J=8.5 Hz, 2H), 4.22–4.28 (m, 6H), 4.12–4.17 (m, 2H), 4.08 (t, J=7.5 Hz, 2H), 3.01 (t, J=7.5 Hz, 2H), 2.17 (s, 3H), 2.01 (s, 3H); LRMS (ESI) calcd for $(C_{28}H_{27}N_2O_{11}P+H)^-$: 599.1; found: 599.2.

4.4. Photolysis experiments

4.4.1. Comparative irradiation of (2b) and (4). Separate solutions of **2b** and **4** (each 0.3 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 of 25 mM Na phosphate, pH 6.0—MeCN (100:40 v/v) for **2b**, t_R 4.6 min and 25 mM Na phosphate, pH 6.0—MeCN (100:45 v/v) for **4**, t_R 5.6 min. The extent of photolysis of each solution was determined by comparison of peak heights with those of unphotolysed controls. After 5 s irradiation, conversions for **2b** and **4** were 39.0% and 45.1%, respectively, indicating that photolysis of **4** was ~1.16-fold more efficient than that for **2b**.

4.4.1.1. Attempted photolysis of (12). A solution of **12** (0.22 mM in 25 mM Na phosphate pH 7.0) was irradiated in a 1 mm path length cell for increasing times up to 8 min and monitored by UV–Vis spectroscopy. No change in the spectrum was observed throughout the irradiation time course.

4.4.1.2. Progressive photolysis of (19). A solution of **19** (0.23 mM in 25 mM Na phosphate, pH 7.0) was irradiated in a 1 mm path length cell for increasing times in the range of 0–35 s. The extent of photolysis was monitored by UV–Vis spectroscopy. Conversion was ~50% after 5.5 s and the spectral evolution was complete after 30 s.

4.4.1.3. Relative photolysis efficiencies of (19) and (2b). Separate solutions of **2b** and **19** (each 0.3 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 **2b**, t_R 4.0 min and 25 mM Na phosphate, pH 6.0—MeCN (100:55 v/v) for **19**, t_R 5.1 min. The extent of photolysis of each solution was determined by comparison of peak areas with those of unphotolysed controls and quantification was by measurement of peak heights. After 5 s irradiation, conversions for **2b** and **19** were 49.0% and 49.3%, respectively, indicating that the two compounds photolysed with essentially equal efficiency.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2004.10.094

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