Photorelease of Carboxylic Acids from 1-Acyl-7-nitroindolines in Aqueous Solution: Rapid and Efficient Photorelease of L-Glutamate[1]

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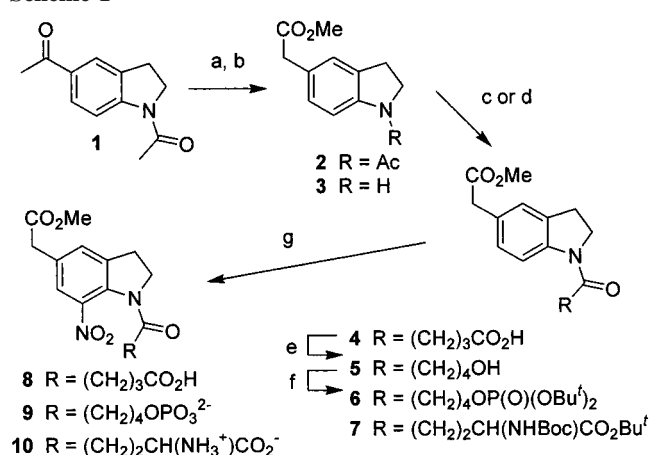Photorelease of biologically active compounds from photocleavable (caged) precursors is a useful tool to study biological processes[2] but rapid, efficient release of neuroactive amino acids has been elusive. We and others[3] have approached the problem with various photolabile protecting groups. Among the better reagents are *p*-hydroxyphenacyl and 2,2′-dinitrobenzhydryl esters of Givens[4a] and Hess,[4b] respectively. A recent report[5] describes photorelease from 7-hydroxycoumarin-4-ylmethyl carbamates, but these are rate-limited by decarboxylation of the carbamate salt ($k \approx 150$ s^{-1}, pH 7, 21 °C).[6] We now describe stable 1-acyl-7-nitroindolines that rapidly and efficiently photorelease carboxylates, including L-glutamate, in neutral aqueous solution. Related reagents undergo clean photolysis in dioxane-CH$_2$Cl$_2$ with ~1% water to yield a carboxylic acid and nitroindoline.[7a] Photosolvolysis by the water was shown, but no reaction mechanism was given.[7] We found the reaction takes a different course in aqueous solution.

In compounds **8**−**10** a 5-substituent ensured nitration at C-7. Previous work[7] used 5-bromo compounds, but a heavy atom might lower the photolysis efficiency. We used a CH$_2$CO$_2$Me group that was also expected to enhance aqueous solubility. Indoline **3** was prepared by Tl(NO$_3$)$_3$ oxidation[8] of 1,5-diacetylindoline **1** and acidic methanolysis of **2** (Scheme 1). Acylation and further transformation of the introduced acyl group as required (**4** → **6**) followed by nitration[9] concurrently removed *tert*-butyl protecting groups (in **6** and **7**) to give **8**−**10**. The route avoids a difficult acylation of 7-nitroindolines,[7b] especially with sensitive side chains as in glutamic acid. 5-Bromo compound **11** was prepared by a related route from 1-acetyl-5-bromoindoline.

Photolysis of **8** and **11** in neutral aqueous solution (without excluding O$_2$) showed **11** was converted ~2.5-fold less efficiently

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(1) Patent Appl. GB 99/06192.1, filed 18 March 1999 covers this work.
(2) Corrie, J. E. T.; Trentham, D. R. In *Bioorganic Photochemistry*; Morrison, H., Ed.; Wiley: New York, 1993; Vol. 2, pp 243−305.
(3) See refs 3−7 of Papageorgiou, G.; Corrie, J. E. T. *Tetrahedron* **1999**, *55*, 237 for a comprehensive list.
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(10) 7-Nitrosoindole itself has been described from an unrelated photolysis of a 7-nitroindole derivative.[11]
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Scheme 1[a]

[a] Reagents: (a) Tl(NO$_3$)$_3$−MeOH−HClO$_4$; (b) HCl−H$_2$O−MeOH; (c) glutaric anhydride; (d) L-HO$_2$C(CH$_2$)$_2$CH(NHBoc)CO$_2$Bu^t−EDC−DMAP; (e) BH$_3$−THF; (f) Et$_2$NP(OBu^t)$_2$−1H-tetrazole, then MCPBA; (g) NaNO$_3$−Tfa.

than **8**, confirming a deleterious effect of the bromo substituent. Clean photolysis of **8** was shown by an isosbestic point at 365 nm in spectra of a solution photolyzed for increasing times (≤65% conversion). The photoproduct (λ_{max} 412 nm) of the protecting
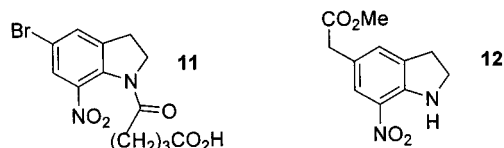
group was not the 7-nitroindoline **12** (λ_{max} 450 nm) but the 7-nitrosoindole **13**[10] (Scheme 2). Reaction with a thiol abolished the 412 nm chromophore, presumably by converting the nitroso to the corresponding hydroxylamine.[12,13] Quantitative amino acid analysis of part-photolyzed solutions of **10** (±dithiothreitol to react with released **13**) and HPLC assay of starting material consumption showed glutamate release at 1:1 stoichiometry. Photolysis of **8** in CH$_2$Cl$_2$−dioxane−H$_2$O (2:3:0.05) cleanly gave nitroindoline **12**, confirming previous data.[7a] Photolysis in the organic solvent was at least 2-fold more efficient than in water.

Formation of different products implies a changed mechanism. Different photoreactivity of nitroaryl compounds in aqueous or organic solvents has been explained by formation of a highly polarized π,π^* triplet state in water and an n,π^* state in organic solvents.[14] We performed some mechanistic studies, but full details of the reaction mechanism will require additional work. First, flash photolysis of **9** (that has no carboxylate group to interfere with measurements on the photolytically released carboxylate) coupled with FTIR difference spectroscopy[13] showed antisymmetric stretch of the released carboxylate at 1553 cm^{-1} in normal or [^{18}O]water (97% isotopic abundance). Thus, the oxygen atom introduced to form the carboxylate is not from solvent. Second, we measured product formation kinetics, specifically of the released proton and carboxylate. Proton release was studied by flash photolysis (320 nm laser, 1 µs pulse, 20 °C) coupled with time-resolved absorption spectroscopy (615 nm) of a pH 7 solution containing **8** and a pH indicator (bromothymol blue), as described for other examples.[15]

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The absorbance change of the indicator revealed biphasic acidification of the solution. The major signal ($\sim$85%) was complete within the laser pulse ($k > 10^5$ s^{-1}), and the minor component had $k \approx 9$ s^{-1}. Time-resolved IR measurements[13] at 1557 cm^{-1} upon flash photolysis of **9** (351 nm laser, 9 ns pulse, spectral resolution 25 cm^{-1}, 25 °C, pH 7) to monitor carboxylate formation showed "instantaneous" decrease in absorption from the nearby disappearing nitro absorption (1539 cm^{-1}), followed by biphasic absorption increase with major ($\sim$80%) and minor components at $k \approx 2700$ and $\sim$10 s^{-1}, respectively. The latter process may correspond with the slow phase of proton release. An overlapping band on the high-frequency side of the 1553 cm^{-1} band makes complete interpretation uncertain, but the demonstrated release of carboxylates and initial biological tests (see below) suggest the faster process principally represents carboxylate release ($t_{1/2} \approx 0.26$ ms). Future work will investigate if the slower process also forms carboxylate. Scheme 2 shows a provisional mechanism to accommodate most of the data. Oxygen transfer from the nitro to the acyl group has been shown for photolysis of related 1-acyl-8-nitrotetrahydroquinolines in various organic solvents, but the byproduct of the heterocycle was not characterized.[16] A feature of the scheme is that H$^+$ loss precedes release of the carboxylate, as required by the kinetic data. The mechanism does not consider the slower, minor processes seen for the H$^+$ and carboxylate signals. Further experiments are needed to substantiate the proposal, account for the minor signals, and understand the different photochemistry in organic solvent.

Scheme 2. Possible Photolysis Mechanism for 1-Acyl-7-nitroindolines in Aqueous Solution
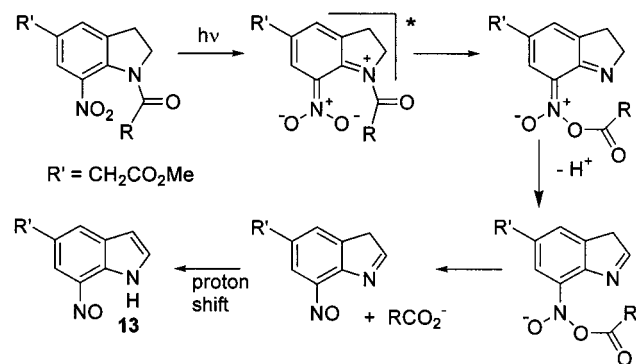
As well as release rates of bioeffector species upon flash irradiation, important properties of caged compounds include stability in aqueous solution and extent of photolysis by a single flash. The amide bond of 1-acylnitroindolines was stable at pH 7; at pH 12, 30 °C, half-times for amide hydrolysis of **9** and **10** were 29 and 6 h, respectively.[17] Faster hydrolysis of the glutamate derivative **10** indicated that its free amino group competes with external base but hydrolysis can be disregarded during purification, storage, and use of these compounds near neutral pH. The product quantum yield (Q_p) for **10** was determined by laser flash irradiation (347 nm) of a solution containing **10** and 1-(2-nitrophenyl)ethyl phosphate.[18] Conversions by a single flash ($\sim$90 mJ) were 9.7 and 23%, respectively (reverse-phase HPLC), leading to $Q_p = 0.043$ by comparison with the known value[18] (0.54) for the caged phosphate and after correction for the different extinction coefficients.

Figure 1. Current response of a cultured rat cerebellar granule neuron to photolytic release of L-glutamate from **10**. The arrow marks the start of the 1-ms flash from a xenon arc flash lamp[21,22] through a Schott UG11 filter and focused to a 200 μm diameter spot at the preparation.[23]

L-Glutamate precursor **10** was tested in primary cultures of rat cerebellar granule neurons for its pharmacological properties and ability to activate glutamate ion channels upon photolysis. Cells were voltage-clamped at -65 mV with whole-cell patch clamp,[19,20] and glutamate sensitivity was established by responses to 20-ms pulses of L-glutamate, applied at 0.25 Hz by iontophoresis from a 1-μm tipped glass pipet. Replacing the bath solution with one containing **10** (1 mM) caused no activation of current nor diminished the peak response to iontophoretic pulses of L-glutamate. Furthermore, when phosphate **9** was used instead of the glutamate precursor **10** ($\pm$iontophoretic application of L-glutamate), it showed no effect before, during, or after photolysis. The control experiments during and after photolysis confirm that byproduct **13** has no adverse effect on glutamate receptors or resting membrane properties of granule neurons. Flash photolysis of **10** resulted in a fast rise of inward current (Figure 1) with $t_{1/2}$ 0.74 ms (SD 0.13 ms, $n = 8$), followed by a decline comprising fast (mean $t_{1/2}$ 2.9 ms) and slow time courses. The latter likely arises from glutamate diffusion away from the photolyzed region. The time course of initial decline may represent desensitization of non-NMDA glutamate channels. During the slow decline there was an increase in low-frequency noise of the glutamate-activated current, consistent with gating of glutamate-activated ion channels. Our data indicate that **10** is inactive as an agonist or antagonist at glutamate ion channels of both NMDA- and non-NMDA-activated types, and that release of L-glutamate by flash photolysis of **10** is on the same time scale as the light pulse.

To conclude, electrophysiological characterization indicates that **10** is a promising reagent for rapid photorelease of L-glutamate. The photochemistry is markedly solvent-dependent, with formation of the nitrosoindole **13** as the principal byproduct in aqueous solution. Future detailed mechanistic studies will define more precisely the time course of the photorelease. We are extending the range of amino acids derivatized with the nitroindoline.

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(17) Hydrolytic stability is of the amide bond. In all of these compounds, the ester group in the side chain hydrolyzed slowly at pH 7, but in separate experiments **10** and the salt of its corresponding free acid (isolated as a minor byproduct) were found to photorelease glutamate with equal efficiency. Ester hydrolysis is thus unimportant. The stabilities quoted are from experiments on the free acids of **9** and **10** and were determined from reverse-phase HPLC measurements of disappearance of starting compounds. The free acids avoided analytical complications from fast cleavage of the ester group.

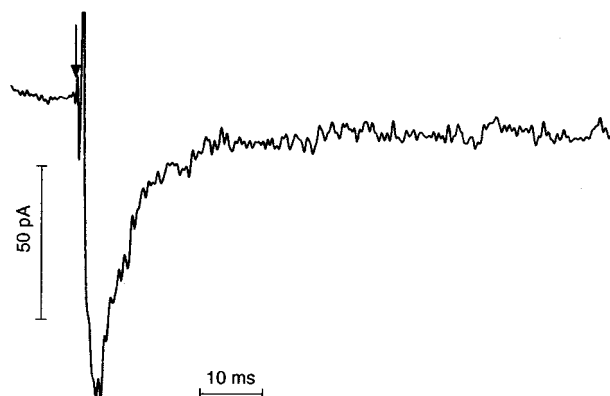(18) Kaplan, J. H.; Forbush, B.; Hoffman, J. F. *Biochemistry* **1978**, *17*, 1929.

(19) All electrophysiological experiments were in Mg^{2+}-free mammalian Ringer's solution containing 100 μM glycine.

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(22) Full lamp output converted 40% of caged ATP in a 1-ms pulse. From relative Q_p values and extinction coefficients, $\sim$15% conversion of the 1 mM solution of **10** is expected.

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