Decarboxylative photorelease coupled with fluorescent up/down reporter function based on the aminophthalimide–serine system

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Received 26th January 2010, Accepted 13th April 2010
First published as an Advance Article on the web 23rd April 2010
DOI: 10.1039/c001622e

The fluorescence of caged phthalimide–serine couples 2 and 4 is up/down modulated by decarboxylative photorelease with fluorescence decrease (2) *vs.* moderate fluorescence increase (4) serving as reporter function.

Molecular fragments that are protected by covalent binding to a light-sensitive group and released from an electronically excited state are called photocaged compounds.[1] Spatial as well as timely control of the release process is crucial for the use as bioanalytical tools,[1,2] which in turn requires a fast and efficient photoliberation. Furthermore, a physical reporting function (emission) is desirable, which is able to map the region of photorelease.[3,4] Following a report on a new type of photodecarboxylative caging-system,[5] we have recently described the use of phthalimide–serine/threonine couples for photorelease of acetate with high diastereoselection in the liberation process.[6] These photocages, however, absorb light in the UV-C region, which is not advantageous for applications in biological environments, and there is no fluorescence observable, neither from the substrate nor from the separated chromophore.

From studies on PET reactions with 4,5-dimethoxyphthalimides, we became aware that this chromophore is highly fluorescent and an excellent electron acceptor in the excited state.[7] Indeed, the corresponding glycine-derived 4,5-dimethoxyphthalimide showed a 60 nm red-shift in absorption[8] (like the serine derivative in Fig. 1) and a fluorescence emission at λ_{em} = 503 nm with a fluorescence quantum yield of 0.6 in acetone/water (1 : 1) at pH = 3.5, which decreased to less than 5% at pH > 6.[8]

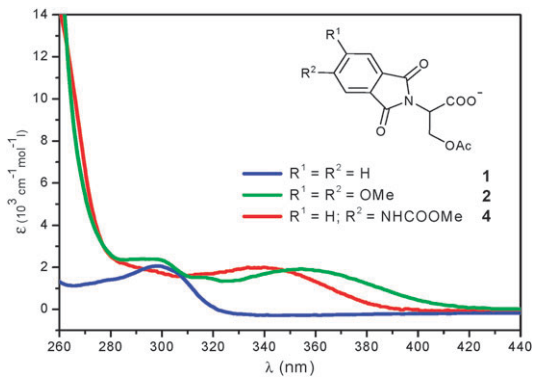
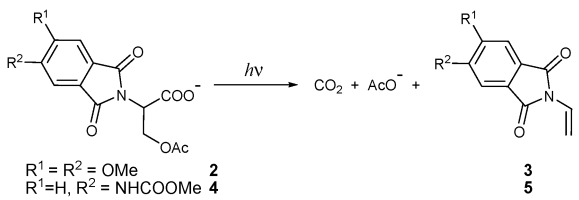


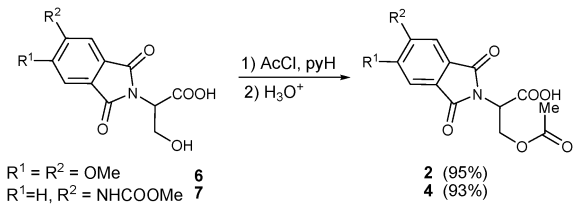
Fig. 1 Absorption spectra of donor-substituted phthalimide photocages **2** and **4** in comparison with the parent compound **1**.

In a first approach, we prepared and tested caged acetate **2**. When this photocage was irradiated at 350 nm, acetate release took place and *N*-vinylphthalimide **3** was formed (Scheme 1). This photochemical process is analogous to the previously described photorelease of acetate in phthaloyl–serine derivatives,[6] and it is related to the decarboxylation reaction of the glycine derivative.[8] In order to introduce a potential covalent peptide-binding site into the caged compound, we also investigated the 4-aminophthalimide skeleton, a highly fluorescent chromophore.[9] The corresponding caged **4** cleanly underwent the photochemical decarboxylation/release process with the formation of phthalimide **5**.

As in the case of photocage **2**, the preparation of carbamate-protected derivative **4** (Scheme 2) was accomplished by acylation of the phthalimide–serine couples (**6** and **7**, respectively). The synthesis of these reagents started with the corresponding anhydrides, 4,5-dimethoxyphthalic anhydride[10] (**8**) and 4-isocyanatophthalic anhydride[11] (**9**, Scheme 3). They were converted by methanolysis into the ring-opened phthalates **10** and **11**. DCC-coupling with *N*-hydroxysuccinimide led to the activated phthalates **12** and **13**, which subsequently were reacted with serine. The standard technique for the amino acid phthaloylation with succinimido esters,[12] using potassium carbonate as base, did not give satisfactory results in our hands when using serine. However, replacing carbonate with the stronger base, triethylamine, we were able to carry out the reaction in good yields.



Scheme 1 Decarboxylative photorelease of acetate from **2** and **4**.



Scheme 2 Preparation of caged acetates **2** and **4** by mono-acetylation of phthalimide–serine couples **6** and **7**.

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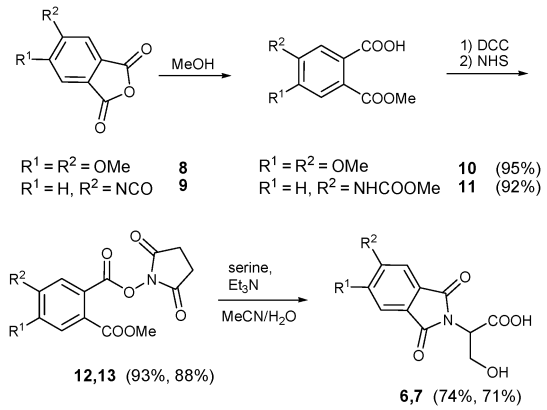

$R^1 = R^2 = OMe$ **8**
$R^1 = H, R^2 = NCO$ **9**
$R^1 = R^2 = OMe$ **10** (95%)
$R^1 = H, R^2 = NHCOOMe$ **11** (92%)
12,13 (93%, 88%)
6,7 (74%, 71%)

Scheme 3 Syntheses of caging reagents **6** and **7**.

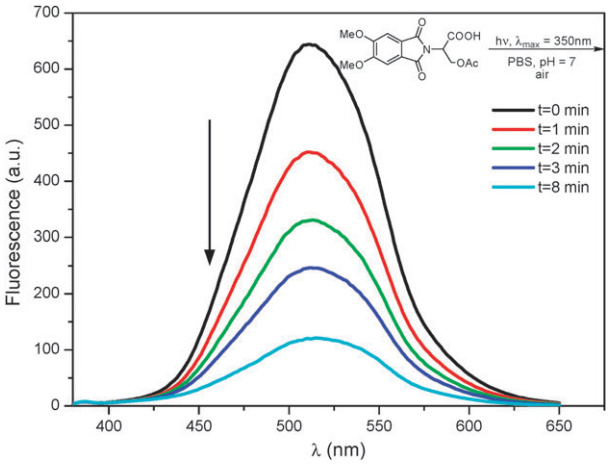

Fig. 2 Fluorescence emission spectra of **2** (λ_{exc} = 340 nm) at different irradiation times, showing fluorescence decrease and red-shift reporting the photorelease of acetate.

The UV-absorption (Fig. 1) of **2** is red-shifted by 60 nm with respect to the unsubstituted phthalimide **1** and 20 nm red-shifted with respect to the carbamate **4**. In the "armed" state of **2** in aqueous media at pH = 7, strong fluorescence is detected with λ_{max} = 513 nm. Through irradiation, **2** is transformed into **3** as the photoliberation of acetate takes place (Scheme 1), and the fluorescence decreases with a 10 nm red-shift of the emission maximum (Fig. 2). The fluorescence decrease indicates a stronger electron transfer donor capability of the enamide group in **3** in comparison with the carboxylate in the starting material **2**. The additional conjugation of the vinyl group in the enamide **3** is represented by the bathochromic shift in absorption and emission. It is striking to see that the fluorescence of **2** is more than 50 times stronger than that of the glycine derivative under the same pH conditions.[8] This indicates that the intramolecular fluorescence quenching is strongly inhibited by the presence of the acetate group in **2**.

Remarkably, this effect is inverted for compound **4**: this photocage shows a low fluorescence intensity at 458 nm (compared to the dimethoxy compound **2**) which is also red-shifted during photolysis but subsequently increases by a factor of 2 (Fig. 3). We have currently no explanation for this reversal in fluorescence modulation. A similar fluorescence increase has been observed in xanthone acetic acids, also

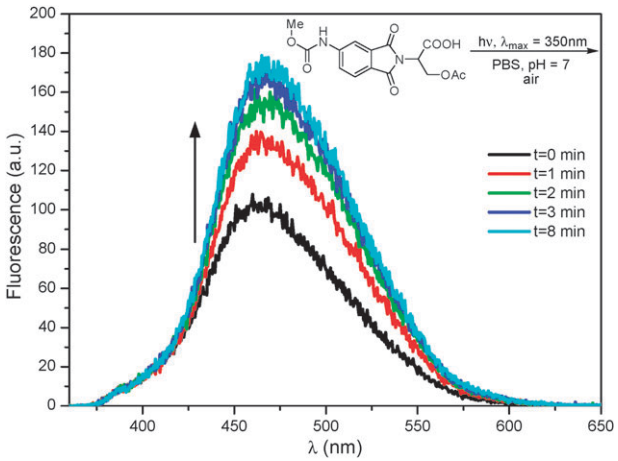

Fig. 3 Fluorescence emission spectra of **4** (λ_{exc} = 340 nm) at different irradiation times, showing fluorescence increase and red-shift reporting the photorelease of acetate.

involving a decarboxylation process but without liberation of caged molecular fragments.[13]

Mechanistically, the photodecarboxylation process initiated in the unsubstituted phthalimides most probably occurs from an upper triplet state as has been shown by time-resolved conductometry, quantum yield determination and oxygen quenching experiments.[7]

In order to examine if this is also the case for the serine-based photocages **4**, laser flash photolysis (LFP) experiments of the unsubstituted phthalimides (for the L- and D-enantiomers of **1**) were carried out in acetonitrile. Direct excitation of the L- (or D-) enantiomer at 308 nm resulted in an intense band at 340 nm (Fig. 4) which was assigned to the triplet–triplet transient absorption. This observation was in agreement with previous experimental data for other phthalimide derivatives.[14] The triplet lifetimes were found to be 1.25 μs for the L-enantiomer and 1.32 μs for the D-enantiomer. When both enantiomers were photolyzed by laser flash in pH 7.4 phosphate buffer (in order to observe a reaction transient) no absorption lines were

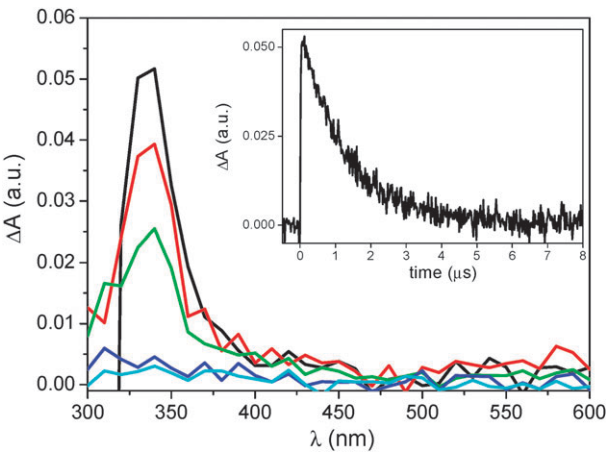

Fig. 4 Transient absorption spectra obtained upon LFP (λ_{exc} = 308 nm) of L-enantiomer of **1** ($Abs_{308\ nm}$ = 0.2) in acetonitrile, under argon. Spectra recorded at 0.1 μs (—), 0.5 μs (—), 1 μs (—), 3 μs (—) and 5 μs (—) after the laser pulse. Inset: transient decay monitored at 340 nm under argon.

observed—neither triplet–triplet absorption nor reaction intermediates. This suggests that the photodecarboxylation process is faster than a possible formation of the unsubstituted phthalimide excited triplet state under this condition and, subsequently, the intermediate carbanion lifetime is too short-lived to be detected in the nanosecond transient system.

In summary, an efficient and fast photoremovable protecting group in two substituent-modified chromophores has been described, which also includes a simple but effective fluorescent reporting function associated with a photorelease process. Depending on the substitution pattern at the aromatic ring, fluorescence up/down reporter function was observed. These modifications on previously described photocaging systems substantially improve phthalimide-photocages to a state of usability in real applications. Moreover, these photocages include a stereogenic center, readily available from a convenient source (serine), opening the gate for the study of photoliberation of selected molecules in combination with chiral recognition and enantiodifferentiation processes similar to the phthalimide–urea couples we have reported recently.[15]

Notes and references

1 (*a*) R. S. Givens, P. G. Conrad and A. L. Yousef, *CRC Handbook of Organic Photochemistry and Photobiology*, ed. W. M. Horspool, F. Lenci, CRC Press, Boca Raton, FL, 2nd edn, 2004, ch. 69; (*b*) A. P. Pelliccioli and J. Wirz, *Photochem. Photobiol. Sci.*, 2002, **1**, 441.

2 J. A. McCray and D. R. Trentham, *Annu. Rev. Biophys. Biophys. Chem.*, 1989, **18**, 239.

3 (*a*) J. R. R. Majjigapu, A. N. Kurchan, R. Kottani, T. P. Gustafson and A. G. Kutateladze, *J. Am. Chem. Soc.*, 2005, **127**, 12458; (*b*) J.-P. Pellois, M. E. Hahn and T. W. Muir, *J. Am. Chem. Soc.*, 2004, **126**, 7170; (*c*) W. F. Veldhuyzen, Q. Nguyen, G. McMaster and D. S. Lawrence, *J. Am. Chem. Soc.*, 2003, **125**, 13358.

4 Y. Zhao, Q. Zheng, K. Dakin, K. Xu, M. L. Martinez and W.-H. Li, *J. Am. Chem. Soc.*, 2004, **126**, 4653.

5 (*a*) G. Cosa, M. Lukeman and J. C. Scaiano, *Acc. Chem. Res.*, 2009, **42**, 599; (*b*) M. Lukeman and J. C. Scaiano, *J. Am. Chem. Soc.*, 2005, **127**, 7698.

6 A. Soldevilla and A. G. Griesbeck, *J. Am. Chem. Soc.*, 2006, **128**, 16472.

7 K.-D. Warzecha, H. Görner and A. G. Griesbeck, *J. Phys. Chem. A*, 2006, **110**, 3356.

8 A. G. Griesbeck and S. Schieffer, *Photochem. Photobiol. Sci.*, 2003, **2**, 113.

9 (*a*) T. G. Kim, M. F. Wolford and M. R. Topp, *Photochem. Photobiol. Sci.*, 2003, **2**, 576; (*b*) G. Saroja, B. Ramachandram, S. Saha and A. Samanta, *J. Phys. Chem. B*, 1999, **103**, 2906.

10 J. Gawroński, K. Gawrońska and M. Brzostowska, *Tetrahedron Lett.*, 1999, **40**, 1191.

11 H. Ulrich and R. Richter, *J. Org. Chem.*, 1973, **38**, 2557.

12 J. R. Casimir, G. Guichard and J.-P. Briand, *J. Org. Chem.*, 2002, **67**, 3764.

13 J. A. Blake, E. Gagnon, M. Lukeman and J. C. Scaiano, *Org. Lett.*, 2006, **8**, 1057.

14 H. Görner, A. G. Griesbeck, T. Heinrich, W. Kramer and M. Oelgemöller, *Chem.–Eur. J.*, 2001, **7**, 1530.

15 R. Pérez-Ruiz, Y. Diaz, B. Goldfuss, D. Hertel, K. Meerholz and A. G. Griesbeck, *Org. Biomol. Chem.*, 2009, **7**, 3499.