Photochemical & Photobiological Sciences

Cite this: *Photochem. Photobiol. Sci.*, 2012, **11**, 493

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COMMUNICATION

N-Acyl-*N*-carboxymethyl-2-nitroaniline and its analogues: a new class of water-soluble photolabile precursor of carboxylic acids†‡

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Received 30th September 2011, Accepted 4th December 2011
DOI: 10.1039/c2pp05322e

The synthesis and photochemistry of a new class of water-soluble, photolabile protecting group, the *N*-carboxymethyl-2-nitroanilinyl (CNA) group, are described. All three CNA-caging compounds synthesized underwent clean photochemical reaction in aqueous buffer solution to produce the intended product of acetic acid, along with the corresponding nitroso aromatic.

The photochemical release of bioactive molecules from their photolabile precursors (otherwise known as caged compounds) is a useful technique for the controlled initiation of biological processes by selective stimulation at target sites.[1–6] To date, various bioactive molecules, including neurotransmitters such as glutamate or γ-aminobutyric acid (GABA),[7–10] peptides and proteins,[11] and RNAs,[12] as well as calcium ions,[13] have been subject to caging. Caged neurotransmitters are one of the most extensively researched and frequently utilized caged compounds, because the photo-release of neurotransmitters satisfies the requirements of recently developed functional imaging techniques for rapid rises in species concentration coupled with a significant temporal and spatial control in order to stimulate synapses at the single cell level. The widely utilized MNI (4-methoxy-7-nitroindolinyl) group (Fig. 1),[8] used for γ-carboxyl caging, possesses the essential property of stability in the absence of light, due to the presence of an amide bond between the chromophore and the glutamate moiety. We have previously reported the development of caging chromophores such as the 5-methoxy-8-nitro-1,2-dihydroquinolinyl group (MNDQ) (Fig. 1) which has the capacity to bind to glutamate *via* an amide linkage and has demonstrated both one- and two-photon uncaging in hippocampal slices.[14]

In this paper we report a new class of chromophore for the caging of carboxylates; the *N*-carboxymethyl-2-nitroanilinyl (CNA) protecting group (Fig. 1). CNA caging groups are characterized by the presence of an anilino nitrogen which allows the formation of an amide bond with a carboxylic acid as well as the addition of a carboxymethyl group, resulting in improved water solubility. This molecular structure also allows the introduction of various substituents onto the phenyl ring without the loss of water solubility.

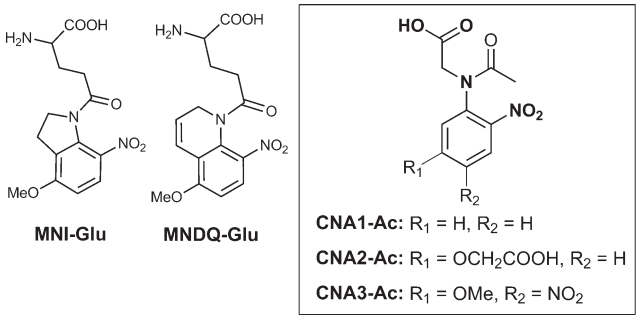

Fig. 1 Chemical structures of **MNI-Glu**, **MNDQ-Glu** and **CNA*n*-Ac**.

Since little is known at present about the photochemistry of CNA-caging chromophores, preliminary photolysis experiments with model compounds were required using some simple variants of the chromophore group. To this end, we designed three model chromophores, **CNA*n*-Ac** (Fig. 1) that we expected to undergo photolysis at the amide linkage with subsequent release of acetic acid. In this chromophore, the 2-nitro group is essential for the uncaging reaction, since the nitro compound **9** (Scheme 1) is not photoreactive. **CNA1-Ac** can be regarded as the simplest possible model of this protecting group since it has no substituents at the 3 to 6 positions. **CNA2-Ac** has a hydrophilic and electron-donating carboxymethoxy group at the 5-position. **CNA3-Ac** was designed with the expectation of high quantum yields on photocleavage (Φ_p), based on a previous study.[8d]

The synthetic pathways leading to these new caged compounds are depicted in Scheme 1. The synthesis of **CNA1-Ac** started with the *N*-acetylation of the known compound *tert*-butyl-2-(phenylamino)acetate.[15] Subsequently, nitration was carried out under mild conditions to afford **2** without decomposition of the *tert*-butyl ester. Deprotection of **2** by treatment with trifluoroacetic acid gave the final product **CNA1-Ac**. The synthesis of **CNA2-Ac** was accomplished by a somewhat circuitous process. Since treatment of 3-aminophenol with excess *tert*-butyl bromoacetate gives a complex mixture of mono-, di-, and trialkylated products and the purification of each product is problematic, the mono-*N*-alkylated product **3** was first prepared by the use of a lesser amount of the bromoacetate. Compound **3** was

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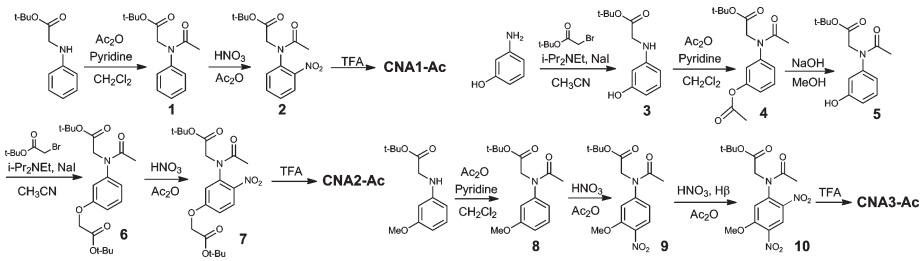
† This paper is part of a themed issue on photoremovable protecting groups: development and applications.

‡ Electronic supplementary information (ESI) available: Experimental procedures and analytical and spectroscopic data. Synthetic scheme. Fluorescence decay plots. See DOI: 10.1039/c2pp05322e



Scheme 1

di-acetylated by reaction with an excess of acetic anhydride to give **4**. The following four reactions proceeded readily, resulting in **CNA2-Ac**. The methoxy-substituted analogue **CNA3-Ac** was synthesized in a similar manner except for the second nitration reaction where the activated zeolite Hβ was used.[16] All three compounds exhibited good solubility in water (>1 mM) without the use of any organic co-solvents, and were stable in phosphate buffer solution at pH 7.4, with no hydrolysis after 24 h in the dark at room temperature, as confirmed by HPLC analysis.

The UV-Vis absorption bands of the CNA-caging groups varied depending on the phenyl ring substituents. Fig. 2 shows the comparative absorption spectra of **CNA*n*-Ac** variants in phosphate buffer at pH 7.4. The main absorption peak of **CNA1-Ac** appears at 255 nm with an extinction coefficient (ε_{255}) of 4700 cm^{-1} M^{-1}. When a carboxymethoxy group is introduced at the 5-position, as in **CNA2-Ac**, the absorption maximum is red-shifted to 312 nm (ε_{312} = 7800 M^{-1} cm^{-1}). **CNA3-Ac**, with a second nitro group, exhibited an absorption spectrum similar to that of **CNA2-Ac**, but with an additional absorption peak at 270 nm. At 350 nm the extinction coefficients were 210, 3800, and 3200 M^{-1} cm^{-1} for **CNA1-Ac**, **CNA2-Ac**, and **CNA3-Ac**, respectively, suggesting that the addition of either methoxy or carboxymethoxy substituents is advantageous with regard to the uncaging of these molecules under biological conditions.

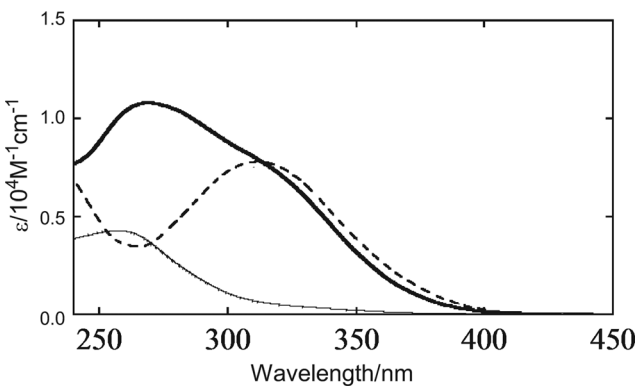


Fig. 2 Absorption spectra of **CNA1-Ac** (thin line), **CNA2-Ac** (dotted line), and **CNA3-Ac** (thick line) in aqueous phosphate buffer at pH 7.4.

Photoirradiation was performed in phosphate buffer at pH 7.4 without the exclusion of oxygen. When **CNA1-Ac** was subjected to photoirradiation, the absorption spectra changed appreciably with an isosbestic point at 276 nm (Fig. 3a), suggesting that this photochemical reaction proceeds cleanly. The absorption bands obtained during and after irradiation are likely due to a simple mixture of **CNA1-Ac** and its single photoproduct. In addition,

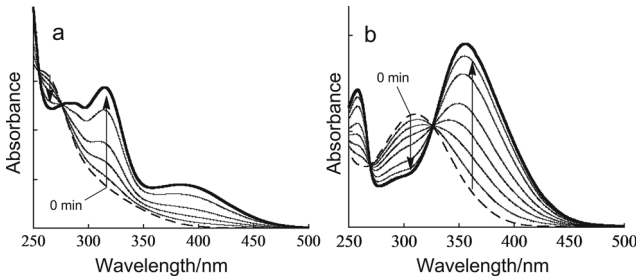
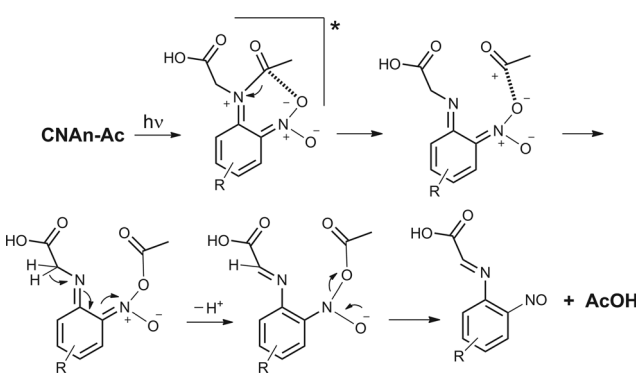


Fig. 3 Change in the absorption spectra of **CNA1-Ac** (a) and **CNA2-Ac** (b) upon irradiation at 365 nm in aqueous phosphate buffer at pH 7.4.

the photoproduct is evidently photo-stable, since subsequent photoreaction, as normally evident by a loss of the isosbestic point, was not observed. The absorption spectrum after irradiation of **CNA1-Ac** indicates that the photoproduct is a nitroso aromatic which exhibits an absorption band at a longer wavelength. The other photoproduct should be the intended acetic acid. Changes in the absorption spectra of **CNA2-Ac** upon irradiation also indicate a clean reaction with the development of a pronounced band at 360 nm (Fig. 3b). This band is similar to those observed in the photolysis of the NMI and MNDQ caging groups, likely due to the production of nitroso aromatics.

Changes in the ^1H NMR spectrum of **CNA2-Ac** during irradiation are shown in Fig. S1 (see ESI‡). It was observed that new peaks, increasing in intensity with irradiation time, developed at 2.09 ppm in D_2O due to the release of acetic acid. New peaks were also seen in the aromatic region due to the photoproduct of the caging group, again increasing with irradiation time.

Scheme 2 shows a plausible mechanism for the photolysis of **CNA*n*-Ac**, based on our observations of clean reactions resulting in acetic acid and 2-((5-(carboxymethoxy)-2-nitrosophenyl)



Scheme 2

imino)acetic acid. Due to the presence of both electron-donating and accepting groups, the first step of photolysis is likely to be a charge transfer from the anilino nitrogen to the neighboring nitro group. Cleavage of the N–C bond then takes place to form acetic acid and the nitroso product. The quantum yields of this photolysis (Φ_p) in aqueous buffer were determined to be 0.06, 0.07, and 0.07, for **CNA1-Ac**, **CNA2-Ac**, and **CNA3-Ac**, respectively, and these values are equivalent to the quantum yield for the production of acetic acid due to the clean reaction. The photochemical efficiency of caged compounds has often been defined as the product of Φ_p and the molar extinction coefficient ε ($\varepsilon \cdot \Phi_p$). At the 350 nm wavelength the $\varepsilon_{350} \cdot \Phi_p$ values were calculated to be 13, 266, and 224 for **CNA1-Ac**, **CNA2-Ac**, and **CNA3-Ac**, respectively. A suitably large $\varepsilon \cdot \Phi_p$ value is important in a caged compound in order to reduce the likelihood of cell phototoxicity.

Interestingly, the uncaging quantum yield of the three CNA-caging chromophores was only slightly affected by their substituent functional groups, even though the absorption spectra of the chromophores varied substantially with the addition of the different substituents, indicating that the electronic structures of all three compounds are different. A previous study of substituent effects on the photocleavage of 1-acyl-7-nitroindolines, including MNI-Glu, showed that both the introduction of an alkoxyl group at the 4-position and a nitro group at the 5-position dramatically enhanced the photochemical efficiency of the uncaging reaction.[8] In addition, the quantum yield for the classical *o*-nitrobenzyl caging group has been shown to be significantly affected by the addition of substituents.[7] In contrast to such well-established caging groups as these, substituent effects on the photochemical reactions of CNA-caging groups are still not clearly understood. However, the present findings that **CNA*n*-Ac** exhibit clean photoreactions with moderate efficiency and demonstrate both stability in solution and water-solubility, even in the case of the dinitro derivative **CNA3-Ac**, indicate that CNA-caging groups show promise with regard to the rapid photorelease of bioactive molecules. The photochemistry of these chromophores is substituent-independent, with the formation of acetic acid and nitroso aromatics as the sole byproducts in aqueous solution. Future detailed photochemical studies will define the time scale of the photorelease more precisely. We are also in the process of investigating additional CNA-caged amino acid derivatives.

Conclusions

In summary, a new class of water-soluble, photolabile CNA-caging chromophore has been developed and the effects of substituent functional groups were examined. Both water-solubility (>1 mM) and chemical stability in aqueous solution at room temperature were confirmed for all derivatives. Upon photoirradiation, each derivative underwent a clean uncaging reaction with moderate quantum yield to produce the desired product of acetic acid. The uncaging quantum yield was only slightly affected by substituent groups, whereas the absorption spectra of the chromophores varied dramatically according to the particular substituents appended. Further investigation of the excited state and ground state reaction dynamics of this compound as well as studies on the modification of its quantum yield are in progress.

Acknowledgements

This work was supported by a Grant-in-Aid for Scientific Research in a Priority Area "New Frontiers in Photochromism" (No. 471) and a Grant-in-Aid for Scientific Research (B) (No. 23350075) and for young scientists (B) (No. 18750141 to AM), from the Ministry of Education, Culture, Sports, Science, and Technology (MEXT), Japan.

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