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2-(*N*-Acyl-*N*-carboxymethylamino)-1-nitrodibenzo[*b*,*d*]thiophene: A New Class of Caging Group for Environmental Selective Photorelease of Carboxylic Acid

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The new environment sensitive photolabile protecting group, *N*-carboxymethyl-1-nitrodibenzo[*b*,*d*]thiophen-2-ylamino (CNDT) group, is described. CNDT-caging compound underwent efficient photochemical reaction to produce desired acetic acid in the presence of zwitterionic detergent, CHAPS.

Photochemical precursors of bioactive molecules (Caged compounds) have been used as an essential tool for switching on of biological processes by selective stimulation at target sites.[1–5] In most caged compounds, covalent bond formation with a photoremovable protecting group masks important features for biological recognition. Particularly, caged carboxylic acid is one of the most chemically established and frequently-used compounds especially in neuroscience research, because amino acids including glutamate or γ-aminobutyric acid (GABA)[6–8] are one of the most abundant neurotransmitters in the vertebrate central nervous system.[9–12]

So far many photolabile protecting groups for carboxylic acids have been developed. As an improved version of classical *o*-nitrobenzyl photolabile caging groups (Figure 1, NB), we have reported caging chromophores such as 5-methoxy-8-nitro-1,2-dihydroquinolinyl (MNDQ) group[13] or *N*-carboxymethyl-2-nitroanilinyl (CNA) group.[14] Apart from these, many caged glutamates and GABAs have also been reported so far. Even in such a situation, further exploration to find new photolabile chromophores is still considered to be important for development of a variety of caged compounds, especially to correspond to recent imaging techniques in diversified research areas. In this paper we report a novel environment sensitive photolabile protecting group that undergoes uncaging reaction efficiently in hydrophobic environment but is almost photochemically inert in buffer.

2-(*N*-Carboxymethylamino)-1-nitrodibenzo[*b*,*d*]thiophene (CNDT) is a new candidate for the caging of carboxylic acids (Figure 1) for spatially selective photorelease. So far, dibenzothiophene skeleton has not been used as a photolabile protecting group. The CNDT caging group is characterized to have a hydrophobic dibenzothiophene unit and carboxylic acid arm, which may lead to formation of aggregates in water. The expected environmental sensitivity may be caused by self-quenching in water but not in hydrophobic environment such as in a membrane. Methylene spacers between a carboxy group and anilino nitrogen would be helpful to avoid photoreaction of carboxy group. In addition, parent dibenzothiophene is known to undergo intersystem crossing efficiently to give the excited triplet state ($\Phi_{\mathrm{isc}} = 0.97$).[15] The effect of the triplet state formation on the photoreactivity of uncaging reaction also would be of interest, because the enhancement of photochemical efficiency of nitroindoline-caged compound have been observed by connecting the triplet sensitizer.[7b,16]

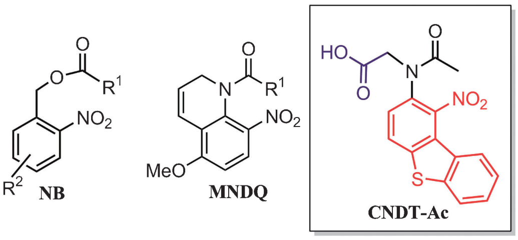
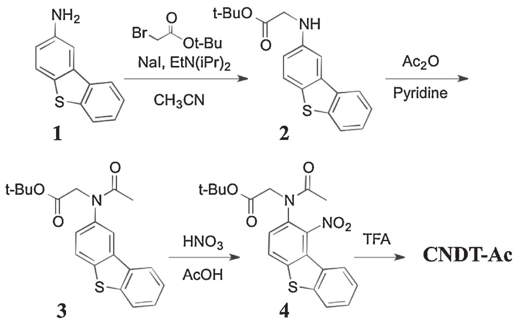


Figure 1. Chemical structures of photolabile NB group, MNDQ group, and **CNDT-Ac**.

Since the CNDT group has been newly prepared and its photochemistry is unknown, preliminary photolysis experiments are required with a simple model. Thus, we designed **CNDT-Ac** (Figure 1), that we expected to undergo photocleavage at the amide-linkage and to release acetic acid. In this compound, a 2-nitro group is essential for initiating uncaging reaction: compound **3** (Scheme 1) is not photoreactive.

The synthesis of **CNDT-Ac** started with *N*-alkylation of known compound 2-aminodibenzo[*b*,*d*]thiophen (**1**)[17] with *tert*-butyl bromoacetate. Compound **2** was acetylated by excess acetic anhydride to give **3**. The following nitration was carried out under HNO_3–AcOH to afford its 1-nitro derivative **4** without decomposition of *tert*-butyl ester. When the reaction was carried out with $NaNO_3$–trifluoroacetic acid, nitration took place at the 8-position and the product did not undergo photolysis. The final deprotection of **4** by treatment with trifluoroacetic acid gave **CNDT-Ac**.

Environmental dependence of photolysis was examined by use of 3-[(3-cholamidopropyl)dimethylammonio]propanesulfonate (CHAPS), which is a mild, stable, and zwitterionic detergent.[18] Upon irradiation at 365 nm in the presence of 20 mM of CHAPS in phosphate buffer (pH 7.4), uncaging reaction took place smoothly with the quantum yield (Φ_{p}) of 0.27, while in the absence of CHAPS, the spectral change due to the uncaging reaction was not significant (Figure 2) and the



Scheme 1.

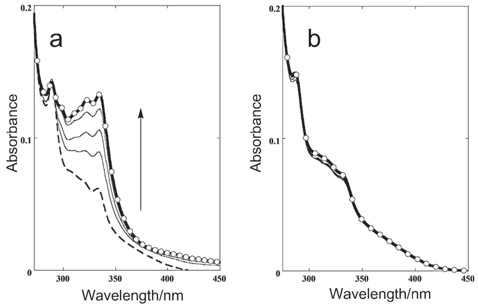


Figure 2. Change in the absorption spectra of **CNDT-Ac** (3×10^{-5} M) upon irradiation at 365 nm in the presence (a) and in the absence (b) of CHAPS (20 mM) in phosphate buffer at pH 7.4.

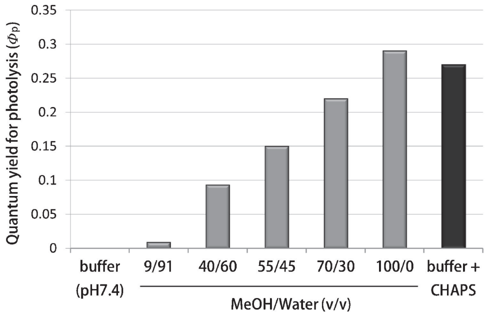
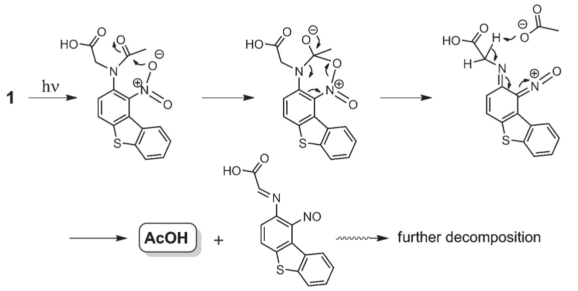


Figure 3. Quantum yield for photolysis of **CNDT-Ac** under different conditions.



Scheme 2.

quantum yield of uncaging reaction was much lower ($\Phi_p < 0.001$). This low reactivity may be partially explained by self-quenching of the **CNDT-Ac** in water due to its amphiphilicity. To gain more insight into the environmental effect on the reactivity, photoirradiation was performed in a series of solutions with different MeOH/water proportions. When **CNDT-Ac** was subjected to photoirradiation in 100% methanol, the absorption spectra changed obviously (Figure S1[19]). The Φ_p value in methanol was determined to be 0.29 which is much higher than those for well-known **NMI-glu** ($\Phi_p = 0.08$) and **MNDQ-glu** ($\Phi_p = 0.04$). As predicted, the quantum yields decreased with increasing the water proportion, and the Φ_p value was only 0.01 in methanol/water (10/90; v/v), which is a thirtieth of that in 100% methanol (Figure 3).

Changes in the ^{1}H NMR spectrum of **CNDT-Ac** during irradiation are shown in Figure S2.[19] It was observed that new peaks, increasing in intensity with irradiation time, developed at 1.92 ppm in CD_3OD due to the release of acetic acid. Scheme 2 shows a plausible mechanism for the photolysis of **CNDT-Ac**. Cleavage of the N–C bond takes place to form acetic acid, and the following thermal reaction gives a nitroso product. However the photoproduct of **CNDT-Ac** was not photostable and its structure is still unclear. From a mechanistic point of view, the photoreaction certainly releases the acetic acid, and therefore the Φ_p value is equivalent to that for the production of acetic acid.

In summary, new photolabile CNDT-caging chromophore has been developed and its photoreaction was examined. In the presence of CHAPS, **CNDT-Ac** underwent a clean uncaging reaction with high quantum yield to produce the desired product of acetic acid, while the quantum yield was much lower in the absence of CHAPS. Further investigation of environmental sensitivity and substituent effect of CNDT caging group as well as preparation of its glutamate or GABA derivaties are in progress.

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