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Development of carbamate-tethered coumarins as phototriggers for caged nicotinamide

Pauline Bourbon [a], Qian Peng [a], Guillermo Ferraudi [a,b], Cynthia Stauffacher [c], Olaf Wiest [a], Paul Helquist [a,*]

[a] Department of Chemistry and Biochemistry, University of Notre Dame, 251 Nieuwland Science Hall, Notre Dame, IN 46556, USA
[b] Notre Dame Radiation Research Laboratory, Notre Dame, IN 46556, USA
[c] Department of Biological Sciences, 927 S. Martin Jischke Street, West Lafayette, IN 47907, USA

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ABSTRACT

The syntheses of 7-diethylaminocoumarin- or modified DEACM-nicotinamide and 6-bromo-7-methoxycoumarin- or BMCM-nicotinamide have been accomplished by reaction of nicotinoyl isocyanate with the corresponding coumarin allylic alcohol derivatives. The resulting compounds contain an *N*-acyl *O*-alkyl carbamate as a new type of linkage for the caging of nicotinamide with a coumarin phototrigger, which undergoes cleavage upon photolysis. Our design of specific caged-nicotinamides was based upon NBO and TD-FT calculations to predict absorption wavelengths and photocleavage potential. This work provides a potentially general method for the caging of amides with coumarin photolabile protecting groups.

For the purpose of studying biochemical processes, controlled release of bioactive molecules is frequently required. This need is often met in solution, crystals, or cells by cleavage of photoactivatable protecting groups. These protected biomolecules are known as caged compounds.[1–4] Upon irradiation with light, photolysis occurs irreversibly, providing the reactive molecule for which subsequent biochemical reactions can be monitored by any of several techniques. Of utmost importance for this process is the choice of the caging group, which should completely inhibit the normal function of the bioactive molecule. The photocleavage should be achieved with useful conversions rates, which can be achieved even for quantum yields of 2% or less if high-intensity lasers, standard lamps or LEDs are used. However, the use of high-powered lasers or non-laser based illumination techniques in biological systems is only feasible at wavelengths at which absorptions by other species that may be present do not lead to competing processes. This requirement suggests the need for wavelengths above ca. 350 nm, well outside the absorption window of most interfering cellular components.

Nicotinamide adenine dinucleotide (NAD^+) is an important cofactor for many enzymes catalyzing biological redox reactions. In comparison with other caged cofactors such as ATP,[5] GTP,[6] and cAMP,[7] caged NAD^+ or $NADP^+$ derivatives have not been widely developed. Salerno et al. and Cohen et al. have reported *N*-nitrobenzyl caged derivatives of NAD^+, but a major problem with these derivatives is that the absorption maximum (λ_{max}) of the nitrobenzyl group occurs at ca. 260 nm, which overlaps with the absorption of cellular tryptophan residues at ca. 270 nm.[8,9] To avoid this problem, these investigators developed various substituted nitrobenzyl cages having much longer wavelengths of absorption (>300 nm) but poorer quantum yields than the parent nitrobenzyl derivatives. As an alternative, 7-diethylaminocoumarin (DEACM) and 6-bromo-7-hydroxycoumarin (BHC) groups have been shown to have λ_{max} values in the cellular transparent region.[10,11] Coumarin-caged compounds that release alcohols,[12] carboxylic acids,[13] amines,[13] diols,[14] ketones[15] and aldehydes[15] upon irradiation are known. Previously reported caged compounds bearing a carbonate linkage can undergo cleavage releasing CO_2 as a basis for caging of compounds having an oxygen atom as a point of attachment.[12,13] In contrast, the use of an *N*-acyl *O*-alkyl carbamate linkage would in principle permit amide nitrogen atoms to be the point of attachment.

In a previous study,[16] we stated our objective of pursuing this further need. Our aim was to develop coumarin-based phototriggers for nicotinamide as a model for NAD^+ that can be cleaved readily at higher wavelengths (>350 nm). We synthesized BHC-nicotinamide (**1**) and DEACM-nicotinamide (**2**), but we found that they did not undergo the desired C–N bond cleavage at their λ_{max} values. In comparison, BHC-acetate[17,18] (**3**) was found by others to undergo cleavage of its corresponding C–O bond (Fig. 1).

We explained these results by Natural Bond Orbital (NBO) calculations, which indicate in a quantitative fashion the expectation that the C–N antibonding orbital σ^* in **1** and **2** is higher in energy

* Corresponding author.
E-mail address: phelquis@nd.edu (P. Helquist).

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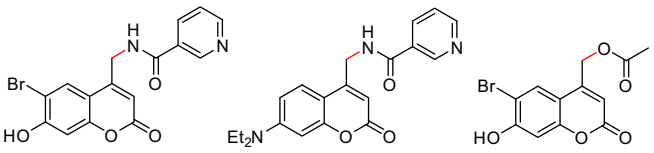


BHC-nicotinamide, **1**
$E(\sigma^*_{C\text{-}N})$= 0.323 Ha

DEACM-nicotinamide, **2**
$E(\sigma^*_{C\text{-}N})$= 0.325 Ha

BHC-acetate, **3**
$E(\sigma^*_{C\text{-}O})$= 0.230 Ha

Figure 1. Structure of **1**, **2** and **3**.

than the C–O antibonding orbital σ^* of **3**. On the other hand, we envisioned that successful cleavage may be more likely for compounds bearing an *N*-acyl *O*-alkyl carbamate linkage between the nicotinamide and the coumarin as depicted in the previously unexplored derivatives **4** and **5** (Fig. 2). Initial calculations indicated that if these compounds could be obtained, they would have relevant bond energies more comparable to the photocleavable acetate **3** than the recalcitrant amides **1** and **2**. In this Letter, we now report that this prediction has been successfully borne out in practice. We describe herein that we have been able to synthesize the desired compounds and that they provide an alternative to the previously reported nitrobenzyl-caged nicotinamide derivatives.

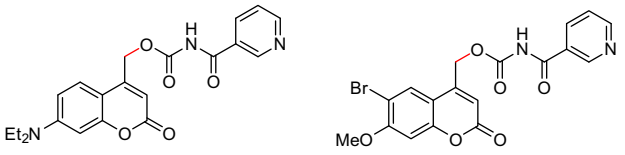
Prior to investing efforts in the synthesis of the envisioned caged candidates **4** and **5**, we chose to pursue additional computational studies to predict their utility as photocleavable nicotinamide derivatives. The M06L/6-311+G(d,p) level of theory was used to predict the absorption wavelengths and estimate the ease of cleavage based on the energies of the bond orbitals as described previously.[16] The calculations (see Table S1 in the Supplementary data) show that the linkage in compounds **4** and **5** has a lower energy C–O σ^* antibonding orbital and that these compounds are therefore predicted to be better candidates compared to **1** and **2** in which the C–N bonds have higher energy σ^* antibonding orbitals. The predicted λ_{max} values for **4** and **5** were 382 and 332 nm, respectively, based upon TD-DFT calculations with the CPCM solvation model.

With these positive indications of suitable photophysical characteristics, we designed a synthetic route to produce the desired *N*-acylated carbamates **4** and **5** based upon reaction of an amide with oxalyl chloride to form the corresponding acyl isocyanate, followed by reaction of this intermediate with a coumarin bearing a free alcohol. This overall conversion proved to be straightforward with the use of benzamide as a simple substrate, but upon switching to nicotinamide, the task proved to be more difficult due to a side reaction producing an undesired salt as a precipitate. This observation is consistent with a report in 2005 that 2-chloro-4,6-dimethylnicotinamide underwent rapid reaction with oxalyl chloride to give the corresponding nicotinamide·HCl salt.[19] We believe that in our reaction this side reaction is also happening. To minimize salt formation, the previous workers conducted the reaction under dilute conditions whereby the nicotinamide was added slowly to a solution of oxalyl chloride. We have found similarly that treatment of a cold, dilute solution of oxalyl chloride in DCE with nicotinamide (**6**) followed by increasing the temperature to 60 °C over one hour and then addition of DEACM-OH **8** resulted in the formation of modified DEACM-nicotinamide **4** in 20% yield (Scheme 1). The substrate **8** was obtained in one step from commercially available 7-dimethylamino-4-methylcoumarin.[20]

The synthesis of 6-bromo-7-methoxycoumarin-nicotinamide (BMCM-nicotinamide, **5**) started from the chloromethylcoumarin **9**, which was converted into the alcohol **10** in 87% yield (Scheme 2).[18] The phenolic hydroxy group was etherified using methyl iodide and potassium carbonate to provide **11** in 35% yield.[10] Coupling with nicotinoyl isocyanate (**7**) furnished BMCM-nicotinamide **5** in 38% yield.

The λ_{max} of the newly synthesized modified DEACM-nicotinamide **4** in methanol is 379 nm, which is in good agreement with the value of 382 nm predicted by the TD-DFT calculations, and has an extinction coefficient of 14,027 M^{-1} cm^{-1}. Continuous photolysis of **4** using a high pressure Xe–Hg lamp (Schoeffel, model LPS255HR) followed by a Bausch & Lomb monochromator, was done for 15 min at a concentration of 50 μM in methanol at its λ_{max}, and the photolytic reaction was followed by LC/MS at different time intervals (Fig. 3).

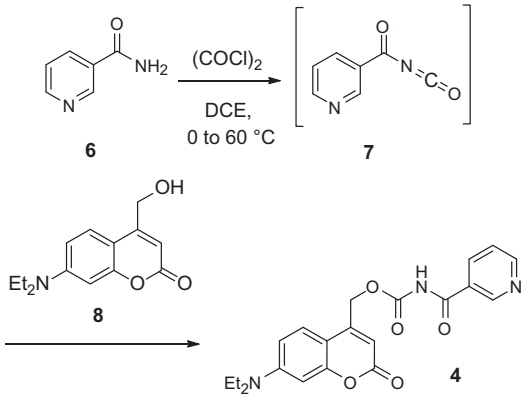
In the HPLC trace, the peak at t_R 5.35 min corresponded to modified DEACM-nicotinamide **4**, which steadily diminished during the course of the photolysis. In conjunction with the disappearance of **4**, a peak at t_R 6.35 min started to appear. The *m*/*z* of this compound was 262.15, which corresponds to the coumarin methyl ether **12**. The solvent methanol could participate in the reaction. To confirm this hypothesis, the photolysis was repeated in trideuterated methanol. The product showed an increase of *m*/*z* by three mass units, which corresponds to incorporation of the three deuterium atoms (Scheme 3, Fig. 4) suggesting a cationic pathway for the cleavage of this new *N*-acylated carbamate linkage in contrast to



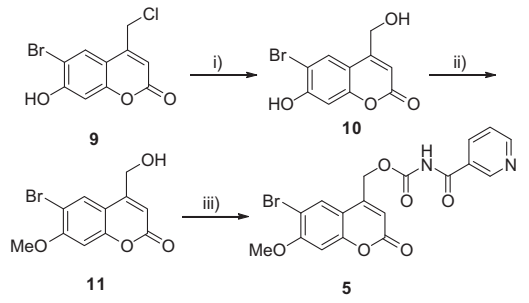
Modified DEACM-nicotinamide, 4
$E(\sigma^*_{C\text{-}O})$= 0.217 Ha

BMCM-nicotinamide, 5
$E(\sigma^*_{C\text{-}O})$= 0.215 Ha

Figure 2. New type of caged nicotinamide.



Scheme 1. Synthesis of modified DEACM nicotinamide **4**.



Scheme 2. Synthesis of BMCM-nicotinamide **5**. Reagents and condions: (i) H_2O, reflux; (ii) MeI, K_2CO_3, DMF; (iii) **7**, DCE.

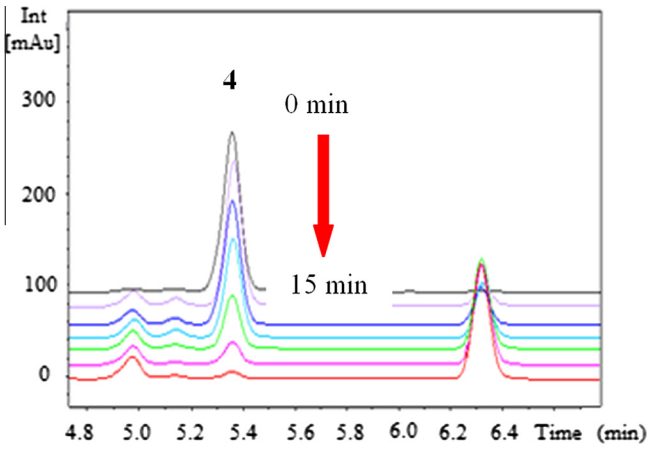
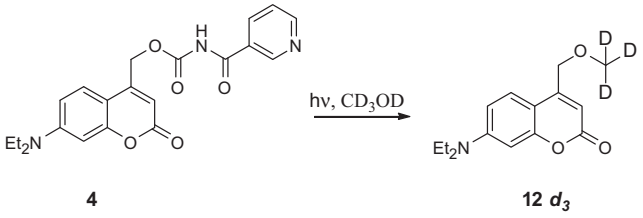


Figure 3. HPLC traces during the photolysis of **4** at different times (0, 1, 2, 3, 7, 10 and 15 min, respectively), (λ of irradiation 380 nm).



Scheme 3. Photolysis of **4** in CD_3OD.

Table 1
Properties of **4** and **5**

	λ_{max} (nm)	ε (M^{-1} cm^{-1})	I_0 (E.min^{-1})	Φ
4	379	14027	1.075×10^{-3}	0.010
5	330	11442	5.63×10^{-3}	0.016[a]
			5.06×10^{-3}	0.012[b]

[a] At 330 nm.
[b] At 350 nm.

the more commonly proposed radical pathway for nitrobenzyl groups.[21]

In addition, the appearance of a peak in the extracted ion chromatogram (EIC) at m/z 123.05 (data not shown) corresponds to formation of nicotinamide (**6**) during the photolysis. Taken together, these data showed that modified DEACM-caged nicotinamide **4** undergoes cleavage at 380 nm, releasing the coumarin methyl ether **12** and nicotinamide **6** (Scheme 4).

From the plot of the concentration of **4** as a function of the photolysis time, (dC/dt), the rate of disappearance of **4** was determined to be 9×10^{-6} mol min^{-1} (Supplementary data). The quantum yield ϕ of the photolysis of **4** was determined to be 0.010 using the relationship $\phi = (dC/dt)/I_0(1-10^{-A})$, where I_0 is the irradiation intensity determined either by potassium reineckate actinometry or bromopentaamminecobalt^{2+} actinometry (Table 1).[22]

For BMCM-nicotinamide **5**, the λ_{max} was determined to be 330 nm, again in excellent agreement with the TD-DFT prediction of 332 nm, with an ε of 11,442 M^{-1} cm^{-1}. The photolysis of **5** was done at both 330 nm and 350 nm over 20 min in a solution of 10% DMSO in methanol during which the EIC peak for **5** decreased over time with a quantum yield in the range of

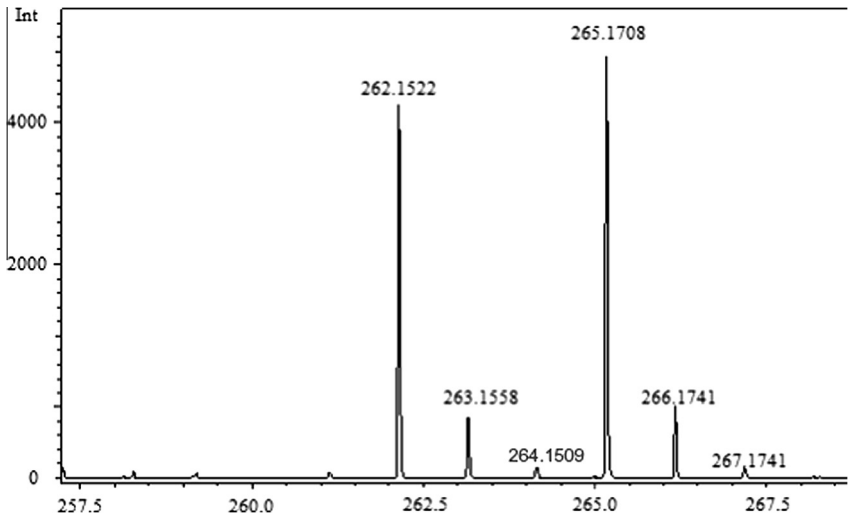
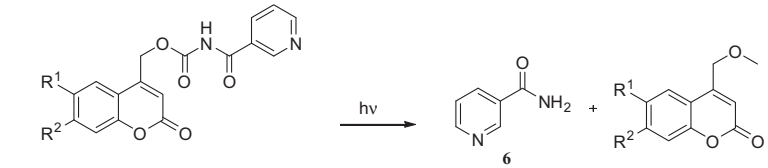


Figure 4. Mass spectrum of the product from photolysis of **4** in CD_3OD at 380 nm.



Modified DEACM-nicotinamide, **4**, R^1= H, R^2= NEt_2
BMCM-nicotinamide, **5**, R^1= Br, R^2= OMe

12, R^1= H, R^2= NEt_2
13, R^1= Br, R^2= OMe

Scheme 4. Photolysis reaction of **4** and **5**.

0.012–0.016. Concomitant with this decrease of the starting material, the EIC peaks for both the coumarin methyl ether **13** and nicotinamide (**6**) increased.

In summary, a key finding of this work is that the performance of phototriggers for caged compounds can be predicted by use of appropriate computational methods. Their λ_{max} absorption values can be calculated with good accuracy, and their potential for photocleavage can also be assessed in advance of obtaining the compounds by synthesis. As an outcome of using this approach, modified DEACM-nicotinamide (**4**) and BMCM-nicotinamide (**5**) bearing *N*-acyl *O*-alkyl carbamate linkages between the nicotinamide and the coumarins have been designed with the aid of NBO and TD-DFT calculations and have been synthesized experimentally. These compounds undergo cleavage upon photolysis in methanol, releasing nicotinamide (**6**) and the coumarins as methyl ethers. Based upon these observations, the BMCM group in particular is a candidate as a phototrigger for biologically relevant amides with absorption wavelengths and extinction coefficients appropriate for study of many cellular pathways. Although the quantum yields for photocleavage of the new caged nicotinamides **4** and **5** are low, they are adequate for applications using high-power lasers such as time-resolved Laue crystallography that forms the basis of our ongoing uses of caged compounds to study enzyme mechanisms in the solid state,[23] and they form the basis for further use of the computational methods described in this work.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.09.067.

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