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Synthesis and photochemical reactivity of caged glutamates with a π-extended coumarin chromophore as a photolabile protecting group

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ABSTRACT

'Caging' and 'uncaging' bioactive substrates are key techniques in studying a wide variety of biological processes. In the present study, two-types of novel caged glutamates with a two-photon absorption (TPA) core, that is, π-extended coumarin, were synthesized and their photochemical release of glutamate was analyzed. The high yields of glutamate (>92%) were observed in the photolysis of compounds **1** and **10**, respectively.

Introduction

'Caging' bioactive substrates (protection) and 'uncaging' them under photolysis conditions (deprotection) are key techniques in studying a great variety of biological processes (Fig. 1).[1] Glutamate (glu, $CO_2^-(CH_2)_2CH(NH_2)(CO_2^-)$) is known to be an important neurotransmitter that plays a key role for learning and memory.[2] Several caged glutamates have been so far synthesized and used for physiological studies. Uncaging (photo-releasing) glutamate using two-photon (TP) excitation process is a most recent challenging subject in this field.[3] The advantage of the TP excitation process includes the following two points:[4] (1) the reduced scattering of the near IR photons in biological tissues gives an increase in depth compared with UV (one-photon: OP) excitation, and (2) TP excitation gives better definition of the focal spot than OP excitation. Thus, an appropriate molecular design of chromophores is crucial in studying the role of the bioactive compound. Molecular design, synthesis, and photochemical reactivity of new caged compounds are of task for chemists.

Several chromophores for TP absorption (TPA) have been designed and synthesized in the last decade (Chart 1), for example, bromohydroxycoumarin (Bhc)[5] core (>1 GM[6]), 7-substituted coumarin derivatives (~0.1–0.2 GM), nitroindolinyl (MNI, CDNI)[7] unit (~0.06 GM), nitrodibenzofuran (NDBF)[8] unit (0.6 GM), 4-nitrobiphenyl unit (PMNB,[9] up to 11 GM), and a quadrupolar structure of fluorine unit (BNSF).[10] In the present study, caged glutamates **1** and **2** with a π-extended coumarin core were designed and synthesized. The photochemical release of glutamate was also conducted in this Letter. Photo-induced S_N1 type of bond cleavage[11] of C-glu was expected to release glutamic acid.

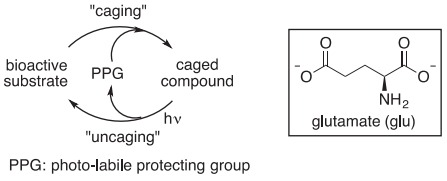

Figure 1. 'Caging' and 'uncaging' bioactive substrates.

Results and discussion

Computational prediction of TPA

First of all, the OP absorption (OPA) and TPA spectra of the parent chromophore unit **3** with a π-extended coumarin core were

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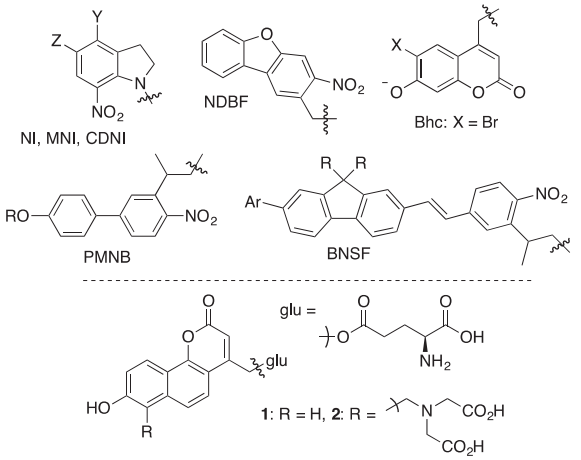


Chart 1. TPA chromophores.

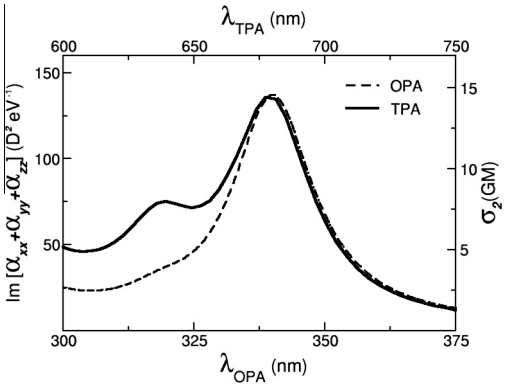
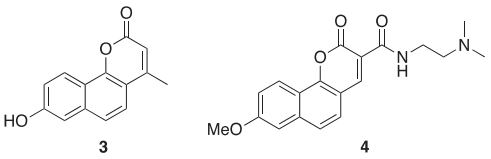


Figure 2. Calculated OPA (dashed lines) and TPA (lines) spectra of compound **3** in vacuum at TD-B3LYP/6-31+G(d) level of theory.

computed at the TD-B3LYP/6-31+G(d) level of theory in vacuum.[12] Actually, 15 GM was calculated for the first TPA maximum at 680 nm (Fig. 2). This first peak shows a good correspondence with twice the wavelength of the first OPA band as was found for related chromophores.[13] Environmental effects are expected to lead to both sizeable increase and red-shift of both OPA and TPA bands. Indeed, a similar structure **4** was found to be 57 GM at 750 nm in EtOH/buffer.[14] The computed TPA spectrum of **3** with a sizeable TPA cross section (GM) prompted us to synthesize the caged-glutamates **1**,**2** and the photochemical reactivity was investigated.

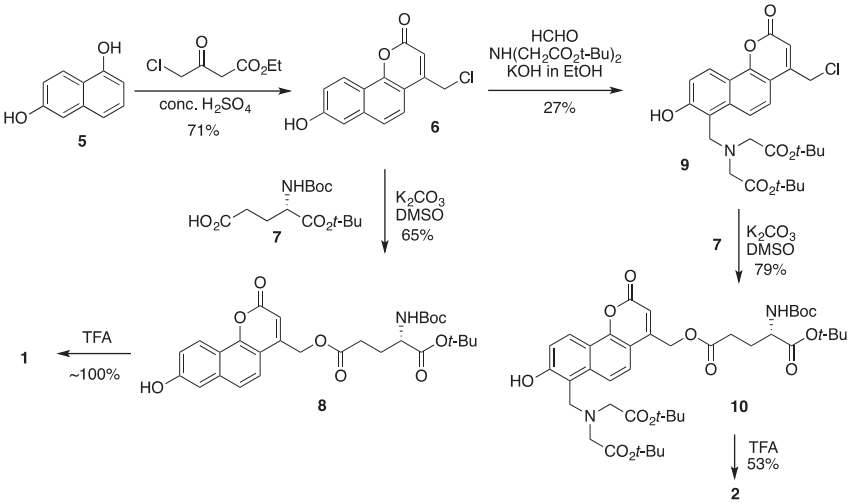


3 **4**

'Caging' of glutamate, synthesis of 1 and 2

Caged glutamates **1** and **2** were synthesized from the commercially available naphthalene-1,6-diol (**5**), Scheme 1. The condensation of **5** with ethyl 4-chloro-3-oxobutanoate produced a π-extended coumarin derivative **6** in 71% isolated yield. Slow addition of concd H_2SO_4 at 0 °C, for ca. 30 min, was found to be indispensable for obtaining **6** in high yields. The nucleophilic substitution reaction of **6** with protected glutamic acid **7**[15] gave the protected caged glutamate **8** in 65% isolated yield. The reaction conditions were tricky. The use of 0.5 equiv of K_2CO_3 in dry DMSO was required for the high yield synthesis of **8**. Other bases such as DBU and KF were found to be not appropriate for the substitution reaction. The caged glutamate **1** (λ_{max} 360 nm in DMSO, ε 9463) was obtained as TFA salts after deprotection of the Boc group and *t*-Bu group using trifluoroacetic acid (TFA). The compound **1** was thermally stable in methanol, thus, the study on the photochemical reactivity was possible. The results are shown in Figure 3, vide infra.

Water soluble group, that is, $-N(CH_2CO_2H)_2$,[11d] was introduced at C(7) position of **6** to give compound **9**. The water solubility is necessary for the biological test. After the introduction of the glutamate unit **7** the deprotection of **10** gave compound **2** (1.9 mM watersolubility in pH 7.4 HEPES buffer, λ_{max} 362 nm in DMSO, ε 9854). Although the protected glutamate **10** was stable in methanol solution, the deprotected compound **2** was slowly decomposed in water and methanol. Thus, the chemical yield of **2** was tentative. The photochemical release of glutamate derivative was tested in the photolysis of compound **10** (Fig. 4, vide infra).



Scheme 1. Synthesis of caged glutamates **1** and **2**.

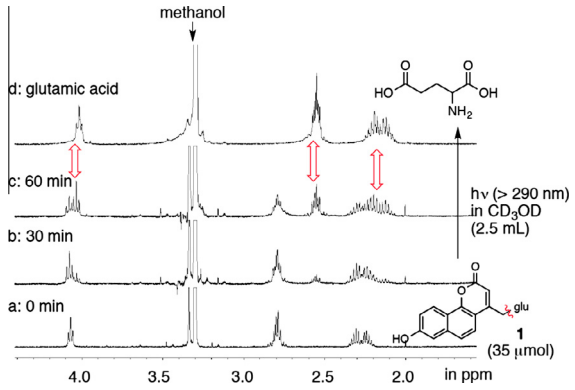


Figure 3. (a) ^{1}H NMR of caged glutamate **1** in CD_3OD; (b) ^{1}H NMR spectrum after 30 min irradiation of **1** using high-pressure Hg lamp through a Pyrex filter; (c) ^{1}H NMR spectrum after 60 min irradiation of **1**; (d) ^{1}H NMR spectrum of glutamic acid (TFA salt) in CD_3OD.

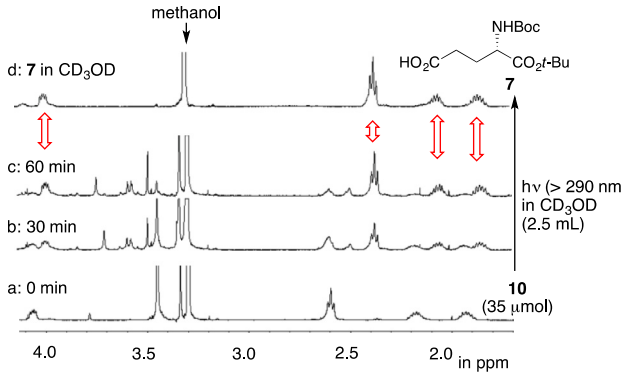


Figure 4. (a) ^{1}H NMR of caged glutamate **10** in CD_3OD; (b) ^{1}H NMR spectrum after 30 min irradiation of **10** using high-pressure Hg lamp through a Pyrex filter; (c) ^{1}H NMR spectrum after 60 min irradiation of **10**; (d) ^{1}H NMR spectrum of **7** in CD_3OD.

'Uncaging' of glutamate, photochemical reaction of 1 and 10

Photochemical release of glutamic acid ('uncaging') from compound **1** (35 μmol) was conducted in CD_3OD (2.5 mL) through a Pyrex filter using a high-pressure Hg lamp (>290 nm), (Fig. 3). The photochemical reaction was monitored using ^{1}H NMR (400 MHz) spectroscopic analysis. As shown in Figures 3a–c, the release of glutamic acid ('uncaging') was clearly observed. The quantitative uncaging of glutamic acid at 60% conversion of **1** was proved by comparing the ^{1}H NMR spectrum (Fig. 3c) with that of the authentic sample (Fig. 3d). The photo-induced S_N1 type of the C–O bond cleavage[11] was proposed for the mechanism of the photochemical release of glutamic acid. The quantum yield of the formation of glutamic acid from **1** (ε_{355} = 10634) was determined to be 0.006 at 355 nm irradiation using Nd: YAG laser (10 Hz, ~7 mg, 4–5 ns pulse-width). The photochemical formation of acetophenone (Φ = 0.33) from valerophenone was used as a chemical actinometer reaction.[16] Thus, ε_{355}xΦ value was calculated to be 63.8. Although the formation of the glutamic acid was clearly observed in the photolysate, white precipitation was detected during the photolysis. The white precipitate is supposed to be the products from the coumarin part. But, the structure was not determined because of the broadening of the ^{1}H NMR spectrum.

The release of the glutamate unit was also tested in the photolysis of compound **10** under the similar conditions for compound **1** (Fig. 4). The clean formation of protected glutamate **7** was observed in the photolysate of **10** after 60 min, compare Figure 4c with Figure 4d. The chemical yield of compound **7** was determined to be 92% using Ph_3CH as an internal standard. The quantum yield for the formation of **7** from **10** (ε_{355} = 8741, $\varepsilon_{355}\Phi$ = 96.2) was determined to be 0.011 using the similar method determined for **1**.

Summary

New types of caged-glutamates with sizable TPA core were designed and synthesized with good chemical yields. The photochemical reaction was proved to release glutamic acids in high yields. Femtosecond laser system is necessary to test the TP excitation reaction. In the present study, the OP excitation was investigated to see whether glutamate was released under photolysis conditions.

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Supplementary data

Supplementary data (computational details, experimental section including ^{1}H and ^{13}C NMR for all new compounds **1**, **8**, **9**, **10**) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2013.10.107.

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