Tetrahedron Letters 51 (2010) 1642–1647

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

1-Acyl-5-methoxy-8-nitro-1,2-dihydroquinoline: a biologically useful photolabile precursor of carboxylic acids

Naoko Obi [a], Atsuya Momotake [a], Yuya Kanemoto [b], Masanori Matsuzaki [b,c], Haruo Kasai [b], Tatsuo Arai [a,*]

[a] Graduate School of Pure and Applied Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba 305-8571, Japan
[b] Laboratory of Structural Physiology, Center for Disease Biology and Integrative Medicine, Graduate School of Medicine, University of Tokyo, Tokyo 113-0033, Japan
[c] PRESTO, Japan Science and Technology Agency, Saitama, Japan

ARTICLE INFO

Article history:
Received 15 October 2009
Revised 14 December 2009
Accepted 15 December 2009
Available online 21 December 2009

ABSTRACT

The synthesis, photochemistry, and biological application of 1-acyl-5-methoxy-8-nitro-1,2-dihydroquinoline (**MNDQ**-caged carboxylic acid) are described. Optimization experiments were carried out on three acetyl derivatives (**3a**–**c**), and the most appropriate analogue for application to the caging of glutamate was determined to be **3c**. Thus, a MNDQ-caged glutamate (**MNDQ-Glu**) was synthesized, and the photochemical release of glutamate by uncaging of **MNDQ-Glu** was confirmed by NMR, MS, and HPLC analysis. When **MNDQ-Glu** was tested with pyramidal neurons in hippocampal slices, whole-field UV illumination resulted in a large inward current due to the release of L-glutamate. A short two-photon uncaging of **MNDQ-Glu** at single dendritic spines induced a transient current that exhibited similar kinetic properties to miniature excitatory postsynaptic currents (mEPSC).

© 2010 Elsevier Ltd. All rights reserved.

1. Introduction

The photochemically triggered release (uncaging) of bioactive molecules from their photolabile precursors (caged compounds) is a valuable technique for selective stimulation at target sites in or around cells and switching on of biological processes.[1] In many caged compounds, covalent bond formation with a photoremovable-protecting group (caging group) masks an important feature for biological recognition. Before irradiation, the caged compound is biologically inert; uncaging causes rapid jumps in the concentration of active molecules. To be useful biologically, the caging group must undergo rapid and efficient photolysis upon photoexcitation at wavelengths that are not harmful to cells. In addition, a caging group with enhanced hydrophilicity enables the caged compound to be soluble in aqueous buffer at higher concentrations without the use of organic solvents. In previous studies, various molecules, including neurotransmitters such as glutamate or γ-aminobutyric acid (GABA),[2] peptides and proteins,[3] cyclic nucleotides,[4] RNAs[5] and calcium,[6] have been subjected to caging. Among neurotransmitters, caged glutamate has emerged as the most well-researched caged compound. In the preparation of caged glutamates, a γ-carboxyl group or amino group is generally the object to be caged. The widely used 4-methoxy-7-nitroindolinyl (MNI) group,[7] used for γ-carboxyl caging, displays essential properties such as light absorption at preferable wavelengths, a suitable rate of uncaging, and stability in the dark due to the presence of an amido bond between the chromophore and the glutamate. Some improvements of the MNI caging group have been carried out; for example, the quantum yield for photolysis may be dramatically increased by the introduction of an additional nitro group,[8] or by conjugation with a triplet sensitizer.[9] The development of new varieties of caging chromophore will contribute further to the advancement of caged technology.

Here, we propose a new caging chromophore, 5-methoxy-8-nitro-1,2-dihydroquinolinyl (MNDQ), for the caging of carboxylates (Fig. 1). Similarly to the MNI caging group, MNDQ contains an

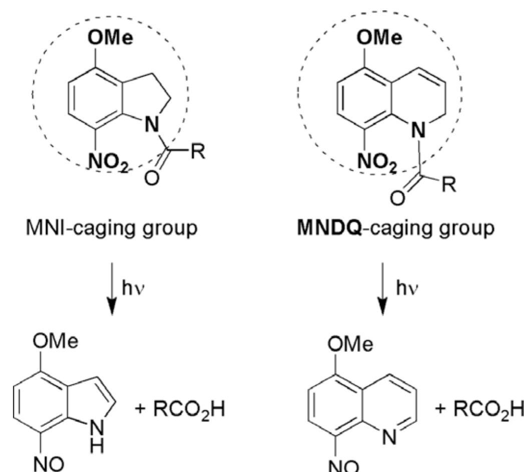
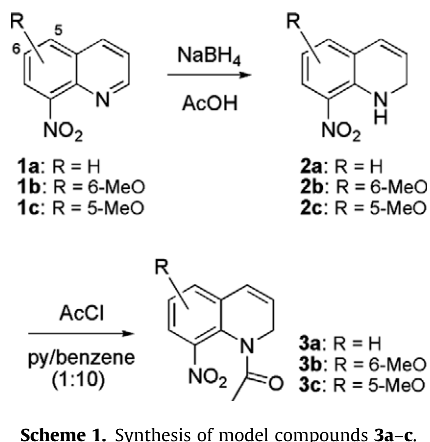


Figure 1. MNI- and MNDQ-caging group.

* Corresponding author.
E-mail address: arai@chem.tsukuba.ac.jp (T. Arai).

0040-4039/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2009.12.081



Scheme 1. Synthesis of model compounds **3a**–**c**.

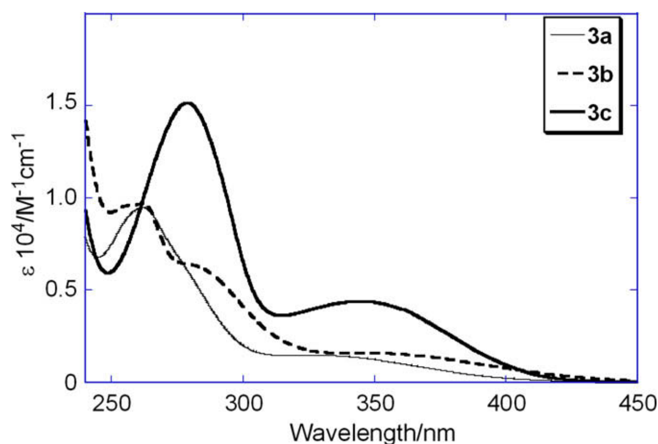


Figure 3. UV–vis absorption spectra of **3a**–**c** in MeOH/H_2O (1:1).

anilino nitrogen for amide bond formation with a carboxylic acid, providing high chemical stability under physiological conditions in the caged state. In contrast with the well-known MNI group, MNDQ has an additional C=C double bond in the neighboring six-membered ring. The distance between the nitro oxygen and the carbonyl carbon, a key reacting point, was expected to be closer than that between MNI-carboxylates. However, since the photochemistry of the MNDQ group was unknown, a preliminary photochemical study was required before the preparation of caged compounds. With this in mind, optimization experiments were carried out with three acetyl derivatives of MNDQ **3a**–**c** (Scheme 1), and the most appropriate analogue (**3c**) was chosen for application to the caging of glutamate. In this way, a new MNDQ-caged glutamate (**MNDQ-Glu**) was synthesized. The photochemical release of glutamate by uncaging of **MNDQ-Glu** was confirmed by NMR, MS, and HPLC analysis. Finally, **MNDQ-Glu** was tested on pyramidal neurons for its ability to activate glutamate ion channels upon one- and two-photon photolysis.

2. Results and discussion

The first goal was to study the effects of various substituent groups on the photochemistry of the new caging group. Thus, three test compounds **3a**–**c** were prepared, starting from reduction of 8-nitroquinolines **1a**–**c** with $NaBH_4$ in AcOH (Scheme 1). The resulting 1,2-dihydro-8-nitroquinolines **2a**[10], **2b**[11], and **2c** were not stable in air and were therefore immediately treated with acetyl chloride to give **3a**–**c**. The structures of **3a**–**c** were confirmed by ^{1}H and ^{13}C NMR and elemental analysis. In the NMR spectra of **3a**–**c**, all the proton and carbon signals were split at room temperature, indicating the presence of rotational isomers. In view of the fact

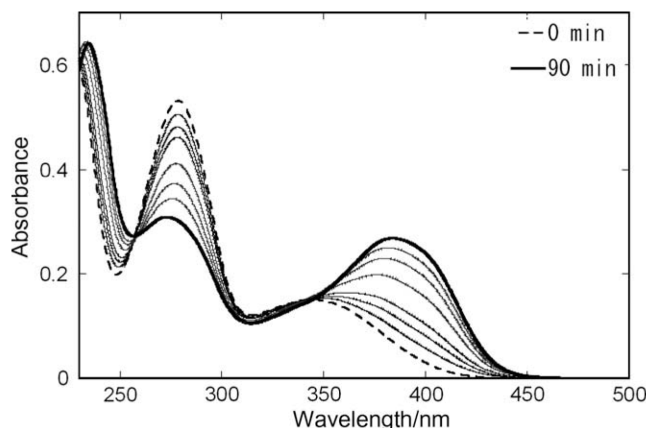


Figure 4. Change in the absorption spectrum of **3c** upon photoirradiation at 365 nm in 1:1 MeOH/H_2O.

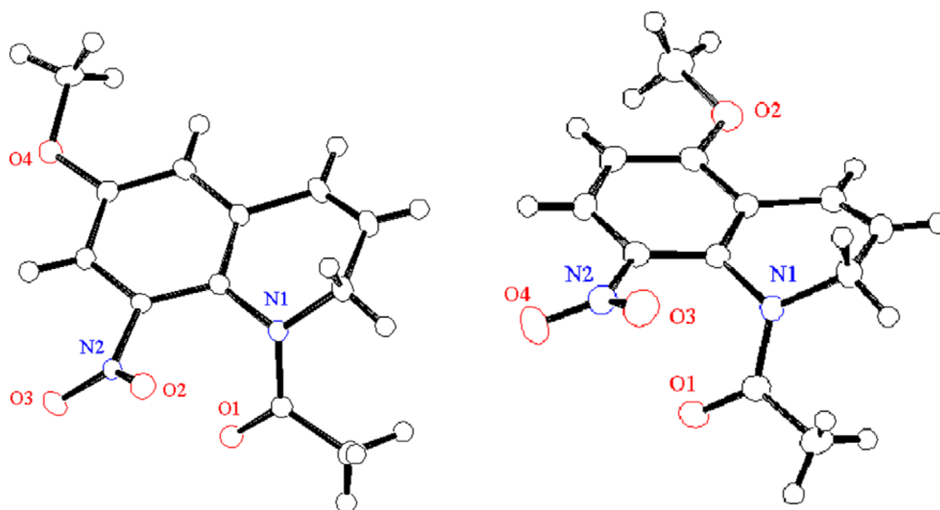


Figure 2. X-ray crystal structures of compounds **3b** (left) and **3c** (right).

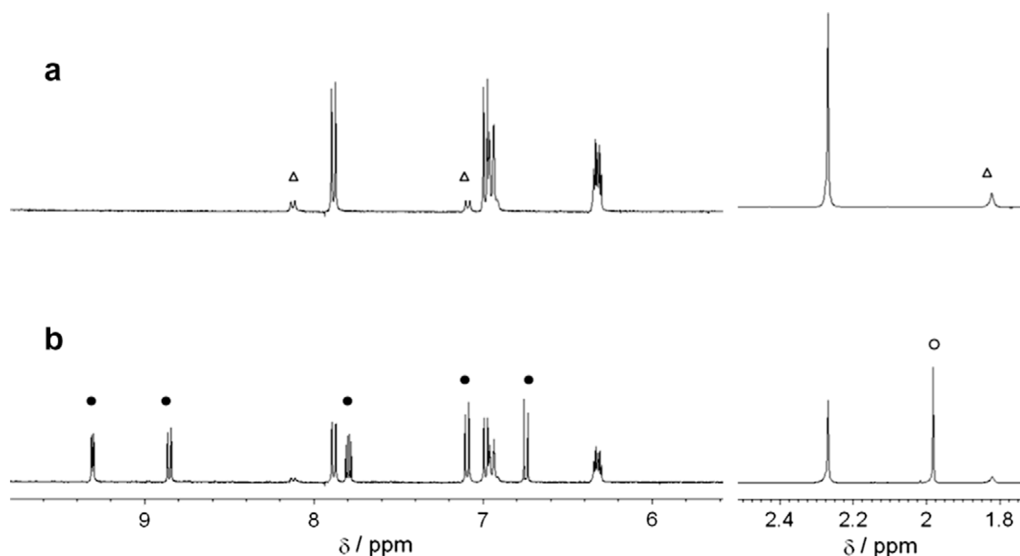
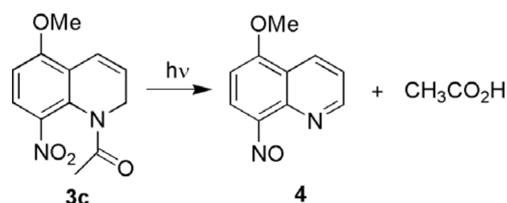


Figure 5. Comparison of ^1H NMR spectra of **3c** before (a) and after (b) irradiation in CD_3OD for 35 min. Small peaks (△) represent the rotational isomer of **3c**. New peaks appearing after photoirradiation are due to 5-methoxy-8-nitrosoquinoline **4** (●) and acetic acid (○), respectively.



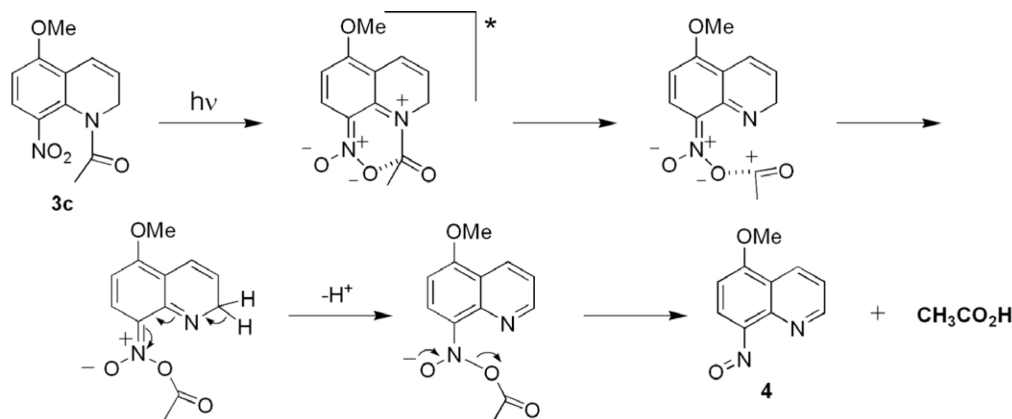
Scheme 2. Photolysis of **3c**.

that MNI-acetate does not have a rotational isomer,[7a] the distance between the nitro group and the carbonyl group in **3a**–**c** was concluded to be closer than that in MNI-based caged compounds. The structures of **3b** and **3c** were determined unequivocally by X-ray crystallography, and their ORTEP representations are shown in Figure 2.

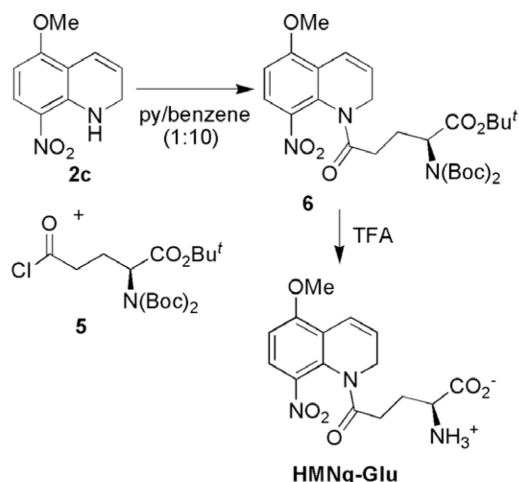
The absorption spectra of compounds **3a**–**c** varied depending on the position of the methoxy substituent. Figure 3 shows the comparative absorption spectra of **3a**–**c** in 1:1 $MeOH/H_2O$. The longest wavelength absorption peak of **3a** appears at 325 nm, with an extinction coefficient at 325 nm (ε_{325} of 1490 cm^{-1} M^{-1}. When a methoxy group was substituted at the 6-position (**3b**), the band was red-shifted to 345 nm, but the extinction coefficient at that peak (ε_{345} = 1620 cm^{-1} M^{-1}) was similar to that of **3a**. An increase in the extinction coefficient as well as spectral red-shift was observed for **3c**, which has a 5-methoxy group (λ_{max} = 345 nm, ε_{345} = 4400 cm^{-1} M^{-1}). At the same wavelength (365 nm), the extinction coefficients of **3a**, **3b,** and **3c** were 900, 1500, and 3700 cm^{-1} M^{-1}, respectively. This suggests that the protecting group in **3c** is advantageous from the point of view of its use as a caging group because near-UV light is generally used for uncaging in biological conditions.

Table 1
Photochemical properties of **3a**–**c** in $MeOH/H_2O$ (1:1) and **MNDQ-Glu** in buffer at pH 7.2

	$\varepsilon_{\lambda max}$ (M^{-1} cm^{-1}) (nm)	ε_{365} (M^{-1} cm^{-1})	Φ_p	$\varepsilon_{365}\cdot\Phi_p$ (M^{-1} cm^{-1})
3a	1490 (325)	900	0.01	9
3b	1650 (345)	1500	0.006	9
3c	4200 (345)	3500	0.05	175
MNDQ-Glu	4200 (350)	4000	0.04	160, 168 ($\varepsilon_{350}\cdot\Phi_p$)
MNI-Glu[8a]	4300 (350)		0.085	366 ($\varepsilon_{350}\cdot\Phi_p$)



Scheme 3. Plausible mechanism for photolysis of **3c**.



Scheme 4. Synthesis of **MNDQ-Glu**.

Photolysis in 1:1 $MeOH/H_2O$ (without exclusion of O_2) also gave different results for the three test compounds. When **3a** and **3b** were subjected to photoirradiation at 365 nm, the absorption spectra changed without showing an isosbestic point. Irradiation of **3a** in an NMR tube (solvent: CD_3OD) revealed that acetate was released, but the photoreaction of the protecting group led to complicated spectra, probably due to the occurrence of side reactions or immediate decomposition of the photoproduct. In contrast, clean photolysis of **3c** was confirmed by the observation of isosbestic points at 257 and 340 nm and the development of a band at 384 nm (Fig. 4). This band, which is similar to those observed in the photolysis of the MNI caging group,[7a] is probably due to the production of nitroso aromatics. Figure 5 shows the change in the ^{1}H NMR spectrum during irradiation of **3c**, in which new peaks due to released acetate and the photoproducts of the caging group appear around 6.5–8.5 ppm, increasing with irradiation time. This set of signals in the aromatic region was equivalent to five protons. High-resolution ESI-MS analysis after irradiation of **3c** strongly indicated that the photoproduct was not 1,2-dihydro-5-methoxy-8-nitrosoquinoline but 5-methoxy-8-nitrosoquinoline **4** (Scheme 2), produced by oxidation of 1,2-dihydroquinoline during photolysis.

We proposed a reaction mechanism for **3c** based on the observation of a clean reaction forming acetate and 5-methoxy-8-nitrosoquinoline (Scheme 3). The first step of photolysis is likely to be intramolecular charge transfer from the anilino nitrogen to the 8-nitro group. The reaction starts with N–C bond cleavage from the excited singlet state, followed by formation of ground-state nitronic anhydride, which undergoes unimolecular cleavage of the N–O bond to give acetate and **4**. The quantum yield for photolysis (Φ_p) of **3c** in 1:1 $MeOH/H_2O$ was 0.05, which, due to the clean reaction, is equivalent to the quantum yield for production of acetate. The photochemical efficiency of caged compounds is defined as the product of Φ_p and the molar extinction coefficient ε ($\varepsilon \cdot \Phi_p$). For compound **3c** this was calculated as 175, which is much greater than the values calculated for **3a** and **3b** (Table 1). Since light can be phototoxic to cells, a large $\varepsilon \cdot \Phi_p$ value is essential.[6e,8a]

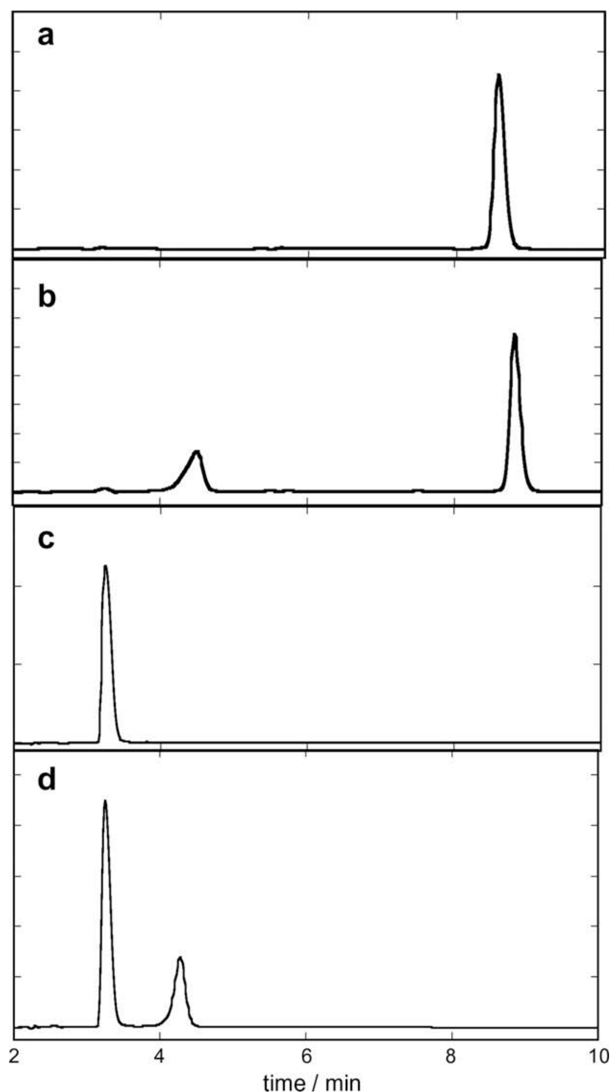


Figure 6. HPLC chromatogram of **3c** before irradiation (a), **3c** after irradiation for 5 min (b), **MNDQ-Glu** before irradiation (c), and **MNDQ-Glu** after irradiation for 5 min. (d).

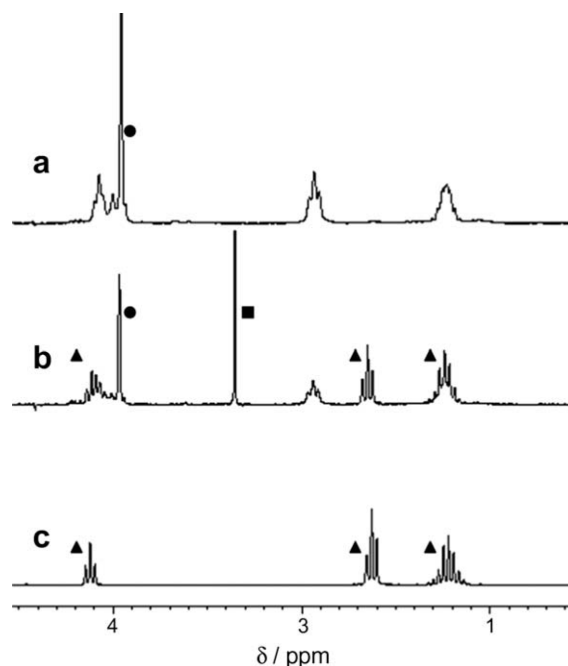


Figure 7. ^{1}H NMR spectra in D_2O of the amino acid portion of **MNDQ-Glu** before (a) and after (b) photolysis and authentic sample of L-glutamate (c). On irradiation for 80 min. (b), the caging chromophore (●) decreases, L-glutamate (▲) is released, and 5-methoxy-8-nitroso quinoline (■) is simultaneously produced.

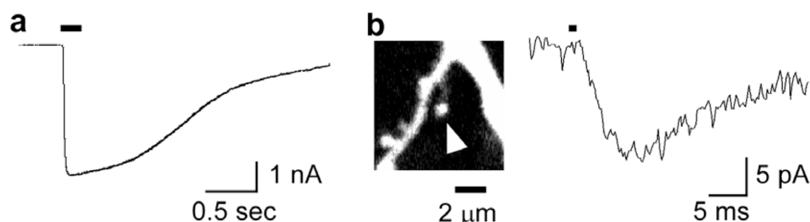


Figure 8. (a) Whole-field UV illumination of 0.8 mM **MNDQ-Glu** induced a large inward current in a CA1 pyramidal neuron in an acutely isolated hippocampal brain slice. The illumination time was 0.2 s (black bar) and the laser intensity was 14 mW/mm^2. **MNDQ-Glu** was locally perfused onto the recording neuron through an application pipette. (b) Two-photon uncaging of 10 mM **MNDQ-Glu** at a dendritic spine (arrowhead) induced a small inward current similar to a postsynaptic current. The illumination time was 1 ms (black bar) and the laser intensity was 9.8 mW.

Based on these experiments, we concluded that the substituent pattern in **3c** was the most appropriate among **3a**–**c** for efficient and clean photolysis, and therefore we chose the 1,2-dihydro-5-methoxy-8-nitroquinolinyl group (MNDQ) as a caging group for glutamic acid. The new caged glutamate **MNDQ-Glu** was synthesized as shown in Scheme 4. A key intermediate in this synthesis was the acid chloride of protected glutamate **5**. Indeed, acylation of **2c** using α-*tert*-butyl *N*-Boc glutamate with a peptide-coupling reagent such as DCC or DMT-MM did not proceed under various conditions. Finally, treatment with neat TFA removed all *tert*-butyl-protecting groups in precursor **6** to leave the water-soluble product **MNDQ-Glu.** After reverse-phase HPLC purification, **MNDQ-Glu** was identified by ^{1}H-NMR and UV spectroscopy. **MNDQ-Glu** showed good solubility in water (>10 mM) without the use of any organic solvents, and stability in D_2O and HEPES buffer solution at pH 7.2, with no hydrolysis after 2 days in the dark at room temperature.

The photochemical properties of **MNDQ-Glu** are shown in Table 1. Irradiation of **MNDQ-Glu** in buffer solution at pH 7.2 resulted in the release of glutamate accompanied by quantitative rearrangement of the MNDQ moiety to 5-methoxy-8-nitrosoquinoline **4**. This was confirmed based on the change in the UV absorption spectrum and HPLC analysis (Fig. 6). The absorption change upon irradiation of **MNDQ-Glu** in buffer solution at pH 7.2 (see SI) was quite similar to that of **3c** shown in Figure 3. Furthermore, NMR analysis of the photolysis solution revealed that glutamate was released essentially quantitatively from **MNDQ-Glu** (Fig. 7). The quantum yield for glutamate release from **MNDQ-Glu** was determined to be 0.04 in buffer solution at pH 7.2, which is a similar value to that for MNI-glu.[8a]

MNDQ-Glu was tested on CA1 pyramidal neurons in acutely isolated hippocampal brain slices for its ability to activate glutamate ion channels upon photolysis. The current response of the cell evoked by light flashes was recorded in whole cell voltage-clamp mode, as shown in Figure 8. On whole-field UV illumination, a large inward current was measured (Fig. 8a). This response was clearly the result of light-induced release of L-glutamate, because no response was evoked in slices containing no caged glutamate. The rapid kinetics of the currents produced by photolysis of **MNDQ-Glu** was comparable with those previously reported for MNI-Glu. This result suggests that **MNDQ-Glu** does not act as a partial antagonist for AMPA receptors. In addition, the fact that the holding current did not change before UV irradiation of **MNDQ-Glu** indicates that the compound remains caged under physiological conditions.

Two-photon uncaging of **MNDQ-Glu** at a dendritic spine was also carried out using a femtosecond laser with a wavelength of 720 nm. Based on the previously developed protocol for MNI-Glu, glutamate-induced currents were recorded upon two-photon uncaging of either **MNDQ-Glu** (Fig. 8b) or MNI-Glu (data not shown). Uncaging of **MNDQ-Glu-**induced currents with similar kinetic properties to those induced by MNI-Glu; however, the amplitude of the current elicited by **MNDQ-Glu** was smaller (relative amplitude 0.68 ± 0.06, mean ± S.E.M., $n = 6$ spines in 3 cells) than that of the current elicited by MNI-Glu in the same spines. Thus, the two-photon action cross section of **MNDQ-Glu** may be slightly smaller than that of MNI-Glu.

In order to establish the full potential of **MNDQ-Glu**, further physiological examinations are required. However, its high extinction coefficient (around 350 nm), moderate quantum yield for photolysis, high stability in the dark, biological inertness before uncaging, and clean photolysis may extend the utility of the new MNDQ-caging platform.

3. Conclusion

A new photolabile-protecting group for carboxylic acids is described. The 5-methoxy compound **3c** was shown to release acetate by clean and efficient photolysis. Caged glutamate **MNDQ-Glu** was prepared using this compound as a caging group, and was shown to release L-glutamate with a quantum yield of 0.04 on irradiation with light at 365 nm. When **MNDQ-Glu** was tested with pyramidal neurons, whole-field UV illumination resulted in a large inward current due to the release of L-glutamate. A short two-photon-uncaging reaction of **MNDQ-Glu** at single dendritic spines induced a transient current that exhibited similar kinetic properties to those induced by MNI-Glu. Further investigation of the excited state and ground state reaction dynamics of this compound as well as studies on the modification of quantum yield are in progress.

Acknowledgements

This work was supported by Grants-in-Aid for Scientific Research in a Priority Area 'New Frontiers in Photochromism (No. 471 to T.A.)', for young scientists (B) (No. 18750141 to A.M.), for specially promoted research (No. 16002021 to H.K.), for scientific research on priority areas (Elucidation of neural network function in the brain) (No. 20021008 to M.M.), and for young scientists (A) (No. 19680020 to M.M.) from the Ministry of Education, Culture, Sports, Science, and Technology (MEXT), Japan.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.12.081.

References and notes

1. (a) Kaplan, J. H.; Forbush, B., III; Hoffman, J. F. *Biochemistry* **1978**, *17*, 1929–1935; (b) Lester, H. A.; Nerbonne, J. M. *Ann. Rev. Biophys. Bioeng.* **1982**, *11*, 151–175; (c) Adams, S. R.; Tsien, R. Y. *Annu. Rev. Physiol.* **1993**, *55*, 755–784; (d) Marriott, G.. In *Methods in Enzymology*; Academic Press: San Diego, 1998; Vol. ; (e) Goeldner, M.; Givens, R. *Dynamic Studies in Biology*; Wiley-VCH: Weinheim, 2005; (f) Mayer, G.; Heckel, A. *Angew. Chem., Int. Ed.* **2006**, *45*, 4900–4921.
2. (a) Rossi, F. M.; Margulis, M.; Tang, C.-M.; Kao, J. P. Y. *J. Biol. Chem.* **1997**, *272*, 32933–32939; (b) Givens, R. S.; Jung, A.; Park, C.-H.; Weber, J.; Wenzel Bartlett,

W. *J. Am. Chem. Soc.* **1997**, *119*, 8369–8370; (c) Furuta, T.; Wang, S. S.-H.; Dantzker, J. L.; Dore, T. M.; Bybee, W. J.; Callaway, E. M.; Denk, W.; Tsien, R. Y. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 1193–1200; (d) Fedoryak, O. D.; Dore, T. M. *Org. Lett.* **2002**, *4*, 3419–3422; (e) Shembekar, V. R.; Chen, Y.; Carpenter, B. K.; Hess, G. P. *Biochemistry* **2005**, *44*, 7107–7114; (f) Senda, N.; Momotake, A.; Arai, T. *Bull. Chem. Soc. Jpn.* **2007**, *80*, 2384–2388.

3. (a) Petersson, E. J.; Brandt, G. S.; Zacharias, N. M.; Dougherty, D. A.; Lester, H. A. *Methods in Enzymology, 360: Biophotonics*; Academic Press: New York, 2003. pp 258–273; (b) Marriott, G.; Roy, P.; Jacobson, K. *Methods in Enzymology, 360: Biophotonics*; Academic Press: New York, 2003. pp 274–289; (c) Lawrence, D. S. *Curr. Opin. Chem. Biol.* **2005**, *9*, 570–575; (d) Li, H.; Hah, J.-M.; Lawrence, D. S. *J. Am. Chem. Soc.* **2008**, *130*, 10474–10475.
4. (a) Furuta, T.; Torigai, H.; Sugimoto, M.; Iwamura, M. *J. Org. Chem.* **1995**, *60*, 3953–3956; (b) Furuta, T.; Momotake, A.; Sugimoto, M.; Hatayama, M.; Torigai, H.; Iwamura, M. *Biochem. Biophys. Res. Commun.* **1996**, *228*, 193–198; (c) Schade, B.; Hagen, V.; Schmidt, R.; Herbrich, R.; Krause, E.; Eckardt, T.; Bendig, J. *J. Org. Chem.* **1999**, *64*, 9109–9117; (d) Hagen, V.; Bendig, J.; Frings, S.; Eckardt, T.; Helm, S.; Reuter, D.; Kaupp, U. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 1046–1048; (e) Hagen, V.; Frings, S.; Bendig, J.; Lorenz, D.; Wiesner, B.; Kaupp, U. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 3625–3628; (f) Hagen, V.; Dekowski, B.; Nache, V.; Schmidt, R.; Geißler, D.; Lorenz, D.; Eichhorst, J.; Keller, S.; Kaneko, H.; Benndorf, K.; Wiesner, B. *Angew. Chem., Int. Ed.* **2005**, *44*, 7887–7891.
5. (a) Ando, H.; Furuta, T.; Tsien, R. Y.; Okamoto, H. *Nat. Genet.* **2001**, *28*, 317–325; (b) Wenter, P.; Fürtig, B.; Hainard, A.; Schwalbe, H.; Pitsch, S. *Angew. Chem., Int. Ed.* **2005**, *44*, 2600–2603; (c) Hobartner, C.; Silverman, S. K. *Angew. Chem., Int. Ed.* **2005**, *44*, 7305–7309.
6. (a) Ellis-Davies, G. C. R.; Kaplan, J. H. *J. Org. Chem.* **1988**, *53*, 1966–1969; (b) Adams, S. R.; Kao, J. P. Y.; Grynkiewicz, G.; Minta, A. V.; Tsien, R. Y. *J. Am. Chem. Soc.* **1988**, *110*, 3212–3220; (c) Adams, S. R.; Lev-Ram, V.; Tsien, R. Y. *Chem. Biol.* **1997**, *4*, 867–868; (d) Ellis-Davies, G. C. R.; Kaplan, J. H. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 187–191; (e) Momotake, A.; Lindegger, N.; Niggli, E.; Barsotti, R. J.; Ellis-Davies, G. C. R. *Nat. Methods* **2006**, *3*, 35–40; (f) Ellis-Davies, G. C. R. *Chem. Rev.* **2008**, *108*, 1603–1613.
7. (a) Papageorgiou, G.; Corrie, J. E. T. *Tetrahedron* **2000**, *56*, 8197–8205; (b) Matsuzaki, M.; Ellis-Davies, G. C.; Nemoto, T.; Miyashita, Y.; Iino, M.; Kasai, H. *Nat. Neurosci.* **2001**, *4*, 1086–1092; (c) Canepari, M.; Ogden, D. *J. Neurosci.* **2003**, *23*, 4066–4071; (d) Smith, M. A.; Ellis-Davies, G. C. R.; Magee, J. C. *J. Physiol.* **2003**, *548*, 245–258; (e) Canepari, M.; Auger, C.; Ogden, D. *J. Neurosci.* **2004**, *24*, 3563–3573; (f) Bernardinelli, Y.; Haeberli, C.; Chatton, J.-Y. *Cell Calcium* **2005**, *37*, 565–572; (g) Maier, W.; Corrie, J. E. T.; Papageorgiou, G.; Laube, B.; Grewer, C. *J. Neurosci. Methods* **2005**, *142*, 1–9; (h) Noguchi, J.; Matsuzaki, M.; Ellis-Davies Graham, C. R.; Kasai, H. *Neuron* **2005**, *46*, 609–622; (i) Araya, R.; Eisenthal Kenneth, B.; Yuste, R. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 18799–18804; (j) Nikolenko, V.; Poskanzer, K. E.; Yuste, R. *Nat. Methods* **2007**, *4*, 943–950; (k) Mim, C.; Tao, Z.; Grewer, C. *Biochemistry* **2007**, *46*, 9007–9018.
8. (a) Fedoryak, O. D.; Sul, J.-Y.; Haydonb, P. G.; Ellis-Davies, G. C. R. *Chem. Commun.* **2005**, 3664–3666; (b) Ellis-Davies, G. C. R.; Matsuzaki, M.; Paukert, M.; Kasai, H.; Bergles, D. E. *J. Neurosci.* **2007**, *27*, 6601–6604.
9. (a) Papageorgiou, G.; Lukeman, M.; Wan, P.; Corrie, J. E. T. *Photochem. Photobiol. Sci.* **2004**, *3*, 366–373; (b) Papageorgiou, G.; Ogden, D.; Corrie, J. E. T. *J. Org. Chem.* **2004**, *69*, 7228–7233; (c) Papageorgiou, G.; Corrie, J. E. T. *Tetrahedron* **2005**, *61*, 609–616; (d) Papageorgiou, G.; Ogden, D.; Corrie, J. E. T. *Photochem. Photobiol. Sci.* **2008**, *7*, 423–432.
10. Rao, K. V.; Jackman, D. *J. Heterocycl. Chem.* **1973**, *10*, 213–215.
11. Carroll, F. I.; Blackwell, J. T.; Philip, A.; Twine, C. E. *J. Med. Chem.* **1976**, *19*, 1111–1119.