N-Nmoc-L-Glutamate, a New Caged Glutamate with High Chemical Stability and Low Pre-photolysis Activity*

We report the synthesis, the physicochemical characterization, and biological evaluation of a new caged glutamate, N-(o-nitromandelyl)oxycarbonyl-L-glutamic acid (Nmoc-Glu), that liberates free glutamate upon photolysis. The low affinity of certain glutamate receptors and their rapid entry into desensitization have effectively prevented the creation of an ideal caged glutamate. In the absence of an ideal compound, Nmoc-Glu was designed to resist spontaneous hydrolysis while maintaining reasonable photorelease yield and kinetics. Chemical and physiological analyses reveal that Nmoc-Glu, indeed, has exceptionally low residual activity and high chemical stability. The quantum yield of Nmoc-Glu is 0.11. Photolytic uncaging and release of free glutamate occur in two steps, consisting of an initial light-induced cleavage that proceeds on the sub-millisecond time scale, and a subsequent light-independent, pH-dependent decarboxylation step that proceeds on the millisecond time scale. The low residual activity and high chemical stability of Nmoc-Glu are important advantages in applications where pre-photolysis Glu receptor activation and desensitization must be minimized.

Non-NMDA¹ glutamate receptor (GluR) channels are the molecular entities that mediate the majority of the fast excitatory synaptic transmissions in the mammalian central nervous system (1). Studies aimed at improving understanding of the properties of synaptic non-NMDA GluR channels by direct application of glutamate are severely limited by poor access in the intact preparation. A potential solution to this problem is the use of “caged” compounds. A caged compound is an effector molecule whose activity is temporarily masked by the attachment of a photosensitive masking, or caging, group (2, 3, 16). Cleavage of the caging group by flash photolysis rapidly liberates the fully bioactive molecule to cause a “jump” in the concentration of the effector molecule. This feature, coupled with the fact that photolysis can be achieved with highly focused light beams, means that photorelease of caged molecules can afford excellent spatial and temporal control over reagent delivery to biological preparations. In situ photorelease of caged glutamate offers a potentially powerful means for studying the properties of synaptic GluRs, their distribution, and for eliciting action potentials from afar in a specifically targeted neuron (4, 5). However, a number of distinctive properties of GluRs present formidable challenges to the design of caged glutamate reagents. The non-NMDA subset of GluRs requires >1 mM glutamate for full activation, yet < 10 μM glutamate can induce significant desensitization in these same GluRs (6, 7). Furthermore, 10 μM glutamate is sufficient to activate the NMDA subset of GluRs (1). An ideal caged glutamate should, therefore, give high yield of free glutamate on photolysis and should have minimal pre-photolysis activity and high chemical stability. Moreover, because entry into desensitization occurs on the millisecond time scale, photorelease must be sufficiently rapid to outpace desensitization. Although there has been considerable effort to perfect a caged glutamate (8–11), no caged glutamate to date has fully satisfied all of these criteria. Some show high chemical stability, but very slow photorelease kinetics (9), whereas others uncage rapidly but either possess significant pre-photolysis activity or are sufficiently labile as to release glutamate slowly even in the absence of light (Refs. 10 and 11; also see below). With these concerns in mind, we have synthesized and evaluated a new caged glutamate that offers a reasonable compromise in achieving good photolysis yield, rapid kinetics, low intrinsic activity, and chemical stability.

**EXPERIMENTAL PROCEDURES**

**Synthesis**

Reagents and solvents were American Chemical Society or high pressure liquid chromatography grade and used as received from Aldrich or Fisher. Dimethylformamide and dichloromethane were stored over 3-A molecular sieves. All oxygen- and water-sensitive reactions were performed under dry argon atmosphere. For water-sensitive reactions, glassware was dried at 130°C for at least 3 h and cooled under a stream of dry argon gas or in a desiccator prior to use. Silica gel 60 (230–400 mesh, Merck) was used for column chromatography. Melting points were recorded on a Melt-temp II (Laboratory Devices) apparatus coupled to an HH23 digital thermometer (Omega Engineering) and are uncorrected. The structures of all purified products were established by NMR spectral analysis. NMR spectra were recorded on a General Electric QE-300 (300 MHz) NMR spectrometer. Samples were dissolved in CDCl₃ (0.03% tetramethylsilane) unless otherwise stated and were referenced to tetramethylsilane. Samples in solvents other than CDCl₃ were referenced to the residual solvent peak. Samples in solvents other than CDCl₃ were referenced to the residual solvent peak. Reactions are reported in the following format: NMR (solvent): chemical shift in ppm downfield from tetramethylsilane, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; b, broad), spin-spin coupling constant if appropriate, and integrated number of protons. High resolution mass spectral analysis was performed at the University of Maryland, College Park, on a model VG707E spectrometer (VG Analytical).

²-Acetoxy-2-[2-nitrophenyl] Acetic Acid (2a)—o-Nitromandelic acid (12) (7.88 g, 40 mmol) was refluxed in acetic anhydride (160 ml, 640 mmol) for 45 min. The reaction mixture was cooled to room temperature and diluted with tetrahydrofuran (50 ml) and water (50 ml). After stirring for 2 h, the aqueous layer was removed, and the organic layer was diluted with toluene (100 ml) and extracted with water (2 x 100 ml). The organic layer was dried with MgSO₄, and the solvent was

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¶These abbreviations are used: NMDA, N-methyl-d-aspartate; CI, chemical ionization (mass spectrometry); CNB, o-carboxy-2-nitrobenzyl; GluR, glutamate receptor; HRMS, high resolution mass spectrometry; Nmoc, o-nitromandeloxycarbonyl; PIPES, piperezine-N,N′-bis(2-ethanesulfonic acid).

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evaporated to give a brown oil that was carried on to 2b without further purification.

**Scheme 1. Synthesis of N-Nmoc-L-glutamic acid.**

N-acetoxy-2-[2-nitrophenyl]acetamide (2b) was treated with t-butyltrichloroacetimidate to give 2.15 g (77%) of a white solid. The reaction was stirred for 1 h, and the solid was removed by filtration. The filtrate was concentrated and chromatographed with hexane/ethyl acetate (3:2) to give 2.15 g (77%) of a white solid. The reaction was stirred for 1 h, and the solid was removed by filtration. The filtrate was concentrated and chromatographed with hexane/ethyl acetate (5:1) to give 7.41 g (63%) of a white solid.

**Cell Culture**

Experiments were performed on hippocampal neurons dissociated from 20-day-old rat embryos and plated onto 25-mm diameter number 1 coverslips that had been acid-washed and coated with collagen and maintained in culture for 2–3 weeks (18).

**Electrophysiological Recordings**

Electrophysiological measurements were performed under voltage clamp conditions using a patch clamp amplifier (Dagan 3900). Electrodes were pulled from borosilicate glass to a resistance of 3–5 MΩ for whole cell recordings and 10–40 MΩ for patch recordings. Series resistance of the electrode was compensated 80–90% during whole cell recordings. The signals were filtered at 2 kHz, sampled at 5 kHz, and analyzed with pClamp software (Axon Instruments). Membrane potential was voltage clamped at −80 mV.

**Preparation of Experimental Samples of Caged Compounds**

Samples of γ-O-CNB-glutamate were purchased from Molecular Probes (Eugene, OR). All caged compounds were stored as dry powders at −20 °C. Aqueous solutions of the caged compounds were kept acidic to maintain the Na+ concentration reduced to 25 mM to attenuate the whole cell current amplitude, the reduction in Na+ was compensated by replacement with choline. For working at pH values below 7, PIPES was used as buffer instead of HEPES. Tetrodotoxin (1 μM) and dl-2-amino-5-phosphonovaleric acid (100 μM) were added to the external solutions. Internal pipette solution was composed of (in mM) 150 NaCl, 3 KCl, 2 CaCl2, 1 MgCl2, 10 HEPES, pH 7.3 with NaOH. Where the Na+ concentration was reduced to 25 mM to attenuate the whole cell current amplitude, the reduction in Na+ was compensated by replacement with choline. For working at pH values below 7, PIPES was used as buffer instead of HEPES. Tetrodotoxin (1 μM) and dl-2-amino-5-phosphonovaleric acid (100 μM) were added to the external solutions. Internal pipette solution was composed of (in mM) 150 CsCl, 5 EGTA, 1 MgCl2, 2 CaCl2, 10 HEPES, titrated to pH 7.3 with CsOH.

**Photorelease by Laser Photolysis**

An argon ion laser (Coherent 190-5) configured to emit at 351–384 nm (400 milliwatts) was used for photolysis during physiological experiments. The output of the laser was gated by a laser shutter (Uniblitz LS2, Vincent Associates), controlled by the data acquisition software (pClamp, Axon Instruments). The shutter exhibited a delay to full opening of 1.5 ms (the composite result of an 800-μs induction delay and a 700-μs interval for complete movement of the shutter blade). The laser beam was steered into an inverted microscope (Diaphot, Nikon) through the epifluorescence port and was reflected by a 400-nm long-pass dichroic mirror through the back aperture of an oil immersion objective (Fluor X40, N.A. 1.3, Nikon). A divergent focused silica lens (~150 mm...
focal length) was placed 20 cm in front of the dichroic mirror so that the laser beam was imaged onto a spot ~50 μm in diameter in the image plane. The light intensity at the sample is estimated at 70 microwatts/μm². Empirically, this intensity permitted a series of 5-ms light pulses to be delivered to the cells without any evidence of cell damage during physiological recordings. During some whole cell recordings, the illuminated spot was reduced in size by closing the field diaphragm of the objective to achieve a sharply delimited spot of photolysis without decreasing light intensity. For photolysis during patch recordings, the electrode was placed in the same relative plane of focus as during whole cell recordings.

RESULTS

Simple o-nitrobenzyl carbamates are quite chemically stable to hydrolysis even under moderately strong acidic or basic aqueous conditions (19) and thus provide a good avenue for making a chemically stable caged glutamate. It has been reported, however, that the o-nitrobenzyl carbamates of glutamate photolyzes only slowly near physiological pH (τ½ = 50 ms at pH 7; Ref. 9). Hess and colleagues (10, 20–24) had shown that introducing a carboxyl group at the benzylic position of the o-nitrobenzyl system to give the α-CN8 group significantly increases the photolysis rate. Reasoning from the foregoing, we introduced the o-nitromandeloyloxy carbonyl (Nnoc) group (12), which is designed to combine the rate-enhancing effect of the benzylic carboxyl group with the known stability of carbamate linkages.

Initial attempts to synthesize N-Nmoc-t-glutamate relied on the previously prepared methyl ester of the Nnoc-imidazole caging reagent (12). When the di-t-butyl ester of l-glutamic acid was allowed to react with methyl Nnoc-imidazole, however, the major product isolated was an oxazolidinone, rather than the desired caged glutamate. This unwanted reaction could be suppressed by using the t-buty1 ester of Nnoc-imidazole instead. The preparation of t-buty1 Nnoc-imidazol and N-Nnoc-t-glutamate is summarized in Scheme 1.

Photolysis Quantum Yield and Kinetics—The spectra in Fig. 1A were taken on a sample of Nmoc-Glu before and after photolysis with UV light. The spectra illustrate the photolability of Nmoc-Glu and are consistent with the known photochemical behavior of similar nitrobenzyl systems. In particular, the post-photolysis spectrum shows increased absorbance at longer wavelengths (>340 nm), which is consistent with the nitrosoketone side-product (7 in Scheme 2) being more highly conjugated than the parent chromophore. The absorbance changes resulting from photolysis follow a simple exponential time course with a time constant (τ½) of 196 ± 40 s (Fig. 1B). The quantum yield of photolysis (φ) can thus be determined to be 0.11 (Refs. 13 and 14; also see legend to Fig. 1).

The photocleavage kinetics of the caging group were examined by monitoring the transient absorbance changes in the 400–450-nm region characteristic of the short-lived aci-nitro intermediate (6a and 6b in Scheme 2) (25–28). The decay of the transient aci-nitro absorbance is common to be concomitant with cleavage of the caging group (16, 17). The time course for the appearance and decay of the transient absorbance following laser pulse photolysis of Nnoc-Glu is shown in Fig. 1C. The decay is dominated by a component with an exponential time constant of 550 ± 2 μs, i.e. removal of the nitromandeloyloxy moiety of the caging group occurs with rate constant k = 1820 s⁻¹ (τ½ = 380 μs). Photolytic removal of the nitromandeloyloxy group leaves the carbamate of glutamate (8 in Scheme 2). Subsequent loss of CO₂ (decarbonylation) liberates

2 A minor fast component contributes to the early part of the decay. It has a time constant of 103 ± 2 μs and accounts for ~20% of the total decay amplitude. The biexponential decay of the aci-nitro intermediate observed in flash photolysis is likely due to the presence of cis and trans structures of the intermediate (6a and 6b), as shown in Scheme 2.
free glutamate. The rate of decarboxylation to yield free glutamate could not be determined easily by spectroscopic means, but it could be estimated indirectly through electrophysiological measurements, as described below.

**Physiological Characterization of Photorelease**—The quantum yield, \( \phi \), represents the probability that an absorbed photon will lead to photorelease. The quantum yield, therefore, does not in itself allow one to estimate actual yield of glutamate photoreleased under physiological experimental conditions, which also depends on the extinction coefficient of the caged compound at the wavelength of irradiation, the concentration of the caged reagent used, and the incident light intensity. In an actual experiment, the most important empirical parameter is the duration of light exposure that is required to achieve a desired concentration of free glutamate. The results shown in Fig. 2 demonstrate the procedure we used to determine the minimal UV pulse duration that is required under our typical experimental conditions. In these experiments, the current response from an excised membrane patch was monitored in response to a sequence of three 5-ms UV light pulses delivered in the presence of caged glutamate. GluR desensitization was blocked with 100 \( \mu \)M cyclothiazide (29). If photolysis was incomplete after the first UV pulse, one would expect the current response to increase further after subsequent light flashes caused more uncaging. We found that when the UV pulse duration was \( \geq 5 \) ms, flashes subsequent to the first caused no further increase in current response, which suggests that a 5-ms UV pulse was sufficient to effect maximal release of caged glutamate within the irradiated volume. To verify that the failure of the second and third UV flashes to produce increases in current response was not due to receptor saturation by photorelease from 300 \( \mu \)M Nmoc-Glu (Fig. 2, top trace), the experiment was repeated with 1 mM Nmoc-Glu (Fig. 2, middle trace). Increasing the Nmoc-Glu concentration increased the absolute amplitude of the current response but not the relative magnitude of responses elicited by the three pulses. These results verified that the receptors were not saturated by photorelease of 300 \( \mu \)M Nmoc-Glu and that photolysis was complete within the irradiated volume after a single 5-ms UV pulse.

One way to characterize the kinetics of glutamate release from Nmoc-Glu is through comparison with the behavior of \( \gamma \)-CNB-Glu, a caged glutamate known to exhibit fast photorelease kinetics (10). Fig. 2 compares the current responses of an excised outside-out membrane patch to glutamate photorelease from 1 mM Nmoc-Glu (middle trace) and from 1 mM \( \gamma \)-CNB-Glu (bottom trace), in the presence of 100 \( \mu \)M cyclothiazide to block desensitization. Two differences between the compounds are apparent. First, the current induced by photorelease from Nmoc-Glu showed delayed onset and exhibited sigmoidal character. Second, the rate of rise of the inward current was slower for Nmoc-Glu than for \( \gamma \)-CNB-Glu (10–90% rise time of 5.4 versus 1.9 ms, respectively). The delayed onset and the sigmoidal shape of the inward current response are consistent with the two-step uncaging process outlined in Scheme 2. The slower rate of rise of the current response to Nmoc-Glu photorelease is consistent with the rate-limiting decarboxylation step before release of free glutamate.

The results of previous studies suggest that the rate of decarboxylation to yield free glutamate should increase with decreased pH (9, 30). Physiological studies confirmed this prediction. Fig. 3A displays whole cell current responses to uncaging Nmoc-Glu at three different pH values. As anticipated, the rate of current activation increases with lowered pH, reflecting acceleration of the decarboxylation reaction. The 10–90% rise times were 1.8, 3.0, and 4.8 ms at pH 6.2, 6.7, and 7.2, respectively. These results suggest that glutamate photorelease from Nmoc-Glu is at least an order of magnitude faster than from \( N \)-1-(2-nitrophenyl)ethoxycarbonyl-\( \gamma \)-glutamate (9). A full record of the whole cell current response from a different neuron to photorelease of Nmoc-Glu was presented in Fig. 3B.

**“Residual” Activity and Chemical Stability**—The pre-photolysis bioactivity and resistance to spontaneous hydrolysis of Nmoc-Glu were assessed by measuring whole cell current responses to applications of Nmoc-Glu in the absence of light. For these experiments, solutions of caged compounds were freshly prepared from solid samples, stored on ice in a dark container, and used within 30 min of preparation. Caged reagent solutions were delivered to each tested neuron via a light-protected pipette, in 10-\( \mu \)l aliquots. Each aliquot was sufficient to blanket the entire visible surface of the cell. As control, \( \gamma \)-CNB-Glu was applied in an identical manner. The results are shown in Fig. 4A. In the absence of light, Nmoc-Glu was essentially inert, whereas \( \gamma \)-CNB-Glu still activated an inward current response. Using the same stock solutions, the comparison was repeated over a period of 2.5 h. The data, summarized in Fig. 4B, show that whereas Nmoc-Glu remained biologically inert throughout, the pre-photolysis bioactivity of the \( \gamma \)-CNB-Glu solution progressively increased with time.

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3 Because GluR desensitization was blocked by cyclothiazide in these experiments, the estimated rise times are indicative of the relative kinetics of photorelease from the two caged compounds but may not represent absolute measures of the rates of photorelease.

4 One potential concern in these photorelease experiments is the potential bioactivity of photochemical by-products. Photolysis of the nitromandelyl cage (the photosensitive moiety of Nmoc-Glu), at concentrations and under conditions identical to those used in glutamate photorelease experiments, elicited no current response whatsoever. Moreover, the fast inward current response to photolysis of Nmoc-Glu is completely abolished by 100 \( \mu \)M 6-cyano-7-nitroquinoxaline-2,3-dione, a competitive antagonist at non-NMDA GluRs. The observed responses are thus truly due to photoreleased free glutamate and do not arise from nonspecific effects of photochemical by-products or light exposure.
The amplitude of the response to photorelease of Nmoc-Glu (1 mM, top trace) at pH 6.3. The 10–90% rise times are 0.98 ms.

DISCUSSION

Nmoc-Glu was created as a caged glutamate that would be resistant to spontaneous hydrolysis while maintaining reasonable quantum yield and photorelease kinetics. Such a compound could complement reagents that have fast photorelease kinetics but exhibit high residual activity in biological applications. The most commonly used caged glutamate, γ-CNBB-Glu, developed by Hess and colleagues (10), exhibits photorelease rate kinetics more clearly.

The pre-photolysis activity of a solution of Nmoc-Glu in Scheme 3. The benzylic carboxylate first attacks the ester linkage to the γ-carboxyl of glutamate to generate an anhydride. The anhydride is then hydrolyzed by water to yield the intact CNB cage and free glutamate. Such a mechanism involving participation by the neighboring carboxylate on CNB is consistent with the fact that generation of pre-photolysis activity increases with time near neutral pH (Fig. 4B) but is inhibited at rather low pH (see “Experimental Procedures”), because at low pH, the critical carboxylate would become protonated and thus lose its ability to engage in nucleophilic attack on the ester linkage.

In designing Nmoc-Glu, we wished to retain the kinetic advan-
Solid lines are least-square fits to the data points. Arrowheads mark the time of reagent de- livery. Nmoc-Glu was essentially inert, but γ-O-CNB-glutamate definitely evoked an inward current. These experiments were performed within 30 min of preparing 1 mM reagent solutions (from solid samples), which were kept on ice and in the dark until use. B, pre-photolysis activity as a function of time in solution at room temperature. Activity is expressed as the peak inward current evoked by direct application of caged reagent and plotted as mean data point is the average peak current from a group tested. Data are times after the reagent solutions had been at room temperature. Each workers (10) stated that spontaneous hydrolysis of Nmoc-Glu (9), would be significantly accelerated. We thus reasoned that such a design would dramatically increase the hydrolytic stability of Nmoc-Glu relative to γ-CNB-Glu, with only a modest sacrifice in photorelease quantum yield and speed. Indeed, the data gathered in our studies validated this conjecture. In the paper that introduced γ-CNB-Glu, Wieboldt and co-workers (10) stated that spontaneous hydrolysis of γ-CNB-Glu was less than 2% after 2 days at physiological pH, as assayed by high pressure liquid chromatography analysis of fluorescent conjugates of free glutamate. Spontaneous hydrolysis may thus seem insignificant. We note, however, that even if contamination due to background hydrolysis is low by the standards of chemical analysis, it could still be quite significant biologically (1/10 of 1% spontaneous hydrolysis in 10 min caged glutamate gives 10 μM free glutamate, which is quite sufficient to activate NMDA-GluRs and desensitized non-NMDA GluRs (1, 6, 7)). Although vulnerability to spontaneous hydrolysis can largely account for the differences in pre-photolysis activity between Nmoc-Glu and γ-CNB-Glu, there may be other contributing factors. For example, attachment of a caging group may not completely abolish the biological activity of an effector molecule (discussed in Ref. 2; for the specific case of caged ATP, see refs. 32 and 33). Whether Nmoc-Glu and γ-CNB-Glu possess differing levels of residual intrinsic activity is difficult to determine with certainty, since differences in pre-photolysis activity between these two reagents were detected even at the earliest possible time of testing. Wieboldt et al. (10) stated that in their tests, γ-CNB-Glu did not activate, enhance, or inhibit Glu- activated currents. The apparent intrinsic bioactivity we ob- serve even at the earliest times may thus be due to low levels of free glutamate contamination in commercial samples.

The carbamate linkage between the Nmoc group and the α-amino group of glutamate effectively eliminated hydrolytic instability but at the cost of introducing an additional rate-limiting, pH-dependent decarboxylation step, which slows the final release of free glutamate. Glutamate photorelease from Nmoc-Glu thus occurs on the millisecond time scale, which is slower than from γ-CNB-Glu. As expected from the design of Nmoc-Glu, however, it is still close to 2 orders of magnitude faster than from N-1-(2-nitrophenyl)ethoxycarbonyl-caged glutamate (9), which also incorporates a carbamate linkage.

One can compensate for the slower kinetics of glutamate release from Nmoc-Glu in two ways. First, one can accelerate the rate of glutamate generation by increasing the concentration of Nmoc-Glu.5 This compensatory approach to increasing the rate of free glutamate photorelease is possible in the case of

\[
\begin{align*}
    I & \rightarrow C \\
    C & \rightarrow G
\end{align*}
\]

where \( I \) is the first intermediate species, \( C \) is the carbamate of glutamate, \( G \) is free glutamate product, and \( k_1 \) and \( k_2 \) are unimolecular rate constants. For such a sequential reaction scheme, in accordance with Ref. 34, the time course of glutamate release is given by Equation 2.

\[
G(t) = I_0 \left[ \frac{k_{\text{obs}} - k_2}{k_2 - k_1} \right] \left[ 1 + \frac{k_{\text{obs}} - k_2}{k_2 - k_1} \right]^{k_2 - k_1}
\]

where \( G(t) \) is the concentration of free glutamate as a function of time, and \( I_0 \) is the initial concentration of the intermediate produced by photolysis. This integrated rate equation shows that the rate of glutamate generation is directly proportional to the concentration of intermediate produced initially by photolysis, which is, in turn, directly proportional to the concentration of caged compound used. Therefore, increasing the concentration of caged glutamate will always result in faster accumulation of free glutamate after photolysis.
Nmoc-Glu, because the pre-photolysis activity of Nmoc-Glu is negligible. Thus, increasing the Nmoc-Glu concentration will always lead to faster accumulation of free glutamate without causing unwanted activation or desensitization of GluRs. Second, because the rate-limiting decarboxylation to release free glutamate is pH-dependent, one can accelerate photorelease by lowering pH.

Nmoc-Glu finds utility in experiments where its major advantages (low pre-photolysis activity and high hydrolytic stability) are most useful. Such applications include 1) studies where the caged glutamate needs to be kept in aqueous solution near neutral pH for extended periods of time; 2) studies where background activation of neurons needs to be minimized (particularly important for NMDA GluRs, which require only micromolar levels of free glutamate for activation); 3) studies where desensitization of non-NMDA GluR channels needs to be minimized; 4) studies where the absolute amplitude of the non-NMDA GluR response is important; and 5) studies where the concentration of photoreleased free glutamate must be high (in the mM range, which means high initial concentration of caged glutamate must be used).

In view of the foregoing, one potential application of Nmoc-Glu might be its use in brain slices, such as in studies that use photostimulation to analyze brain circuitry (5, 35). Residual pre-photolysis activity, even if low by chemical measures (e.g., <1% free glutamate), may still cause significant distortion of neuronal circuit properties. Micromolar levels of free glutamate may activate NMDA GluRs on non-targeted neurons (1) and may desensitize non-NMDA GluRs of the relevant neuronal circuit (6). Therefore, in such studies, the low pre-photolysis activity of Nmoc-Glu may help minimize background activation of non-targeted neurons and maintain optimal responsiveness of the stimulated circuit.

Another potential application of Nmoc-Glu may be in experiments where it is advantageous to isolate the response of the non-NMDA subset of GluRs to the exclusion of other membrane conductances. For example, to quantitatively map the distribution of functional non-NMDA GluRs on the dendrite, it would be necessary to achieve saturating concentrations of free glutamate following photorelease without triggering receptor desensitization before photolysis. It would also be necessary to eliminate activation of NMDA GluR channels and voltage-gated calcium channels on the dendrite. Decreasing extracellular pH will increase the rate of glutamate photorelease from Nmoc-Glu without significantly affecting the gating properties of non-NMDA GluRs (36). Decreasing extracellular pH would also down-regulate voltage-gated calcium channels (37) and NMDA GluR channels (38) (two conductances that may be inadvertently recruited with strong dendritic stimulation). These considerations, combined with the low residual activity of Nmoc-Glu, suggest that rapid focal photolysis of Nmoc-Glu at millimolar concentrations in an acidic environment may provide the ideal means for quantitatively mapping the distribution of non-NMDA GluRs.

In summary, we have synthesized, characterized, and demonstrated biological utility of N-Nmoc-L-glutamate, a new caged glutamate. Nmoc-Glu exhibits low pre-photolysis agonist activity and exceptional chemical stability with reasonably fast photorelease kinetics. These characteristics are expected to make Nmoc-Glu suitable for many neurophysiological studies.

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