Tetrahedron Letters 57 (2016) 5750–5752

Contents lists available at ScienceDirect

Tetrahedron Letters

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

A photocaged, cyclopropene-containing analog of the amino acid neurotransmitter glutamate

Pratik Kumar, David Shukhman, Scott T. Laughlin *

Department of Chemistry, Stony Brook University, Stony Brook, New York 11794-3400, USA

ARTICLE INFO

Article history:
Received 5 October 2016
Revised 24 October 2016
Accepted 27 October 2016
Available online 11 November 2016

Keywords:
Cyclopropene
Bioorthogonal
Glutamate
Neurotransmitter
Tetrazine ligation

ABSTRACT

Substituted cyclopropenes serve as compact biorthogonal appendages that enable analysis of biomolecules in complex systems. Neurotransmitters, a chemically diverse group of biomolecules that control neuron excitation and inhibition, are not among the systems that have been studied using biorthogonal chemistry. Here we describe the synthesis of cyclopropene-containing analogs of the excitatory amino acid neurotransmitter glutamate starting from a Garner's aldehyde-derived alkyne. The deprotected cyclopropene glutamate was stable in solution but decomposed upon concentration. Appending a light-cleavable group improved the stability of the cyclopropene while simultaneously caging the neurotransmitter. This strategy has the potential to permit deployment of cyclopropene-modified glutamate as a bioorthogonal probe of the neurotransmitter glutamate *in vivo* with spatiotemporal precision.

Cyclopropenes have become popular biorthogonal functional groups due to their small size, inertness to biological functionality, and reactivity with tetrazines[1] and 1,3-dipoles.[2] For example, cyclopropene installation on monosaccharides[3–6] and lipids[7] has promoted these biomolecules' characterization in challenging *in vivo* environs, and their use as chemical handles in proteomics applications has facilitated analysis of proteomes with improved spatiotemporal precision.[8–10] Additionally, in efforts to employ cyclopropene tags in studies of protein function, several groups have synthesized amino acid analogs bearing a cyclopropene moiety at the alpha carbon[11] or terminal positions on amino acid side chains, resulting in novel non-proteinogenic amino acid structures.[2] However, there has been little exploration of cyclopropenes and bio-orthogonal chemistry to analyze neurotransmitters, an important and diverse class of biomolecules that modulate neuron excitation and inhibition to control brain function.

The only documented synthesis of a cyclopropene-containing neurotransmitter was performed by Reissig and coworkers, who synthesized cyclopropene-containing analogs of the neurotransmitter gamma-aminobutyric acid (GABA).[12] However, there are numerous neurotransmitters that have structures conducive to modification with a reactive cyclopropene handle. For example, glutamate, the subject of this study and an amino acid neurotransmitter that leads to neuron excitation in most situations, has had a complete panel of saturated, unreactive cyclopropane-containing analogs synthesized.[13] Several of these structures were confirmed as substrates for metabotropic glutamate receptors,[14,15] which detect glutamate release from synaptic vesicles at the neural synapse to propagate neural signals, as well as glutamate transporters that package glutamate into synaptic vesicles or remove it from the synaptic cleft.[13] These findings suggest that cyclopropene modified analogs of glutamate might serve as chemically traceable neurotransmitter mimics. Here we describe the synthesis of a light-activatable cyclopropene-containing glutamate analog (Nvoc-cpGlu, Compound **1**, Fig. 1), which holds promise to extend the reach of biorthogonal chemistry into the realm of neurotransmitter biology.

Initially, our strategy for the synthesis of cyclopropene glutamate analogs employed Rh-catalyzed cyclopropenation of ethyl diazoacetate to protected alkynyl glycine analogs (**3a**/**b**, Scheme 1). Interestingly, our attempts at the preparation of an *N*-benzoyl alkynyl glycine by addition of a tributyl tin acetylene to α-chloroglycine **2b** according to Aldous and coworkers[16,17] did not result in the desired alkyne **3b**. Instead, we observed a rapid cycloisomerization-elimination affording oxazole **4b**, similar to that reported for other *N*-propargyl amides by Ciufolini and coworkers.[18] Swapping the amine protecting group from benzoyl to methyl carbamate, compound **2a**, allowed the formation of the required alkynyl glycine **3a**. Subsequent Rh-catalyzed cyclopropenation of ethyl diazoacetate produced a reaction mixture whose analysis by mass spectrometry suggested the formation of cyclopropene **4a**. However, our extensive efforts to purify the cyclopropene were ultimately unsuccessful. The purification challenge resulted from

* Corresponding author.
E-mail address: scott.laughlin@stonybrook.edu (S.T. Laughlin).

http://dx.doi.org/10.1016/j.tetlet.2016.10.106
0040-4039/

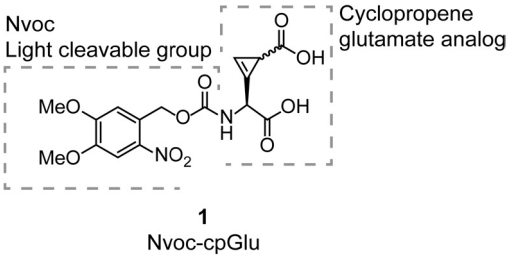
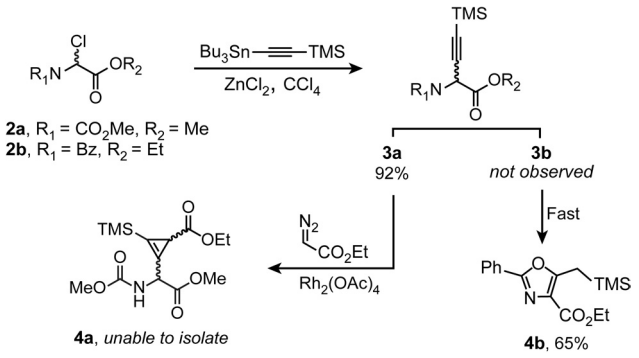


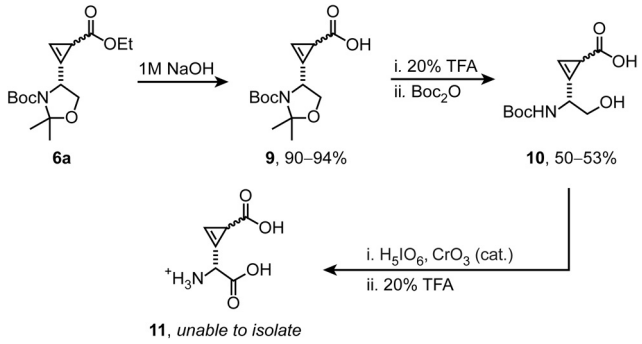
Fig. 1. Target photo-activatable cyclopropene containing analog of the excitatory neurotransmitter glutamate.



Scheme 1. Efforts to synthesize cpGlu via a alkynyl glycine were unsuccessful.

unreacted alkynyl glycine starting material in the reaction mixture. This alkynyl glycine is notoriously unstable by virtue of its propensity to form allenes.[19] During our attempts at separation via SiO_2 Flash chromatography, reversed phase HPLC, and distillation, the remaining alkynyl glycine in the reaction mixture decomposed into multiple species that coeluted with the product and precluded its purification.

We reasoned that avoiding the alkynyl glycine in our synthetic strategy would be possible if we performed the ethyl diazoacetate cyclopropenation to alcohol substrates instead of carboxylic acids, thereby lowering the acidity of the alpha carbon proton and inhibiting allene formation (Table 1). Alkyne **5**, derived from Garner's aldehyde, provided an ideal chiral building block for this strategy. The addition of ethyl diazoacetate to alkyne **5** proceeded in good yield (55–60%, 85% when calculated based on recovered starting material) and opening the dimethyloxazolidine followed



Scheme 2. Accessing the cyclopropene via a Garner's aldehyde derived synthon.

by reinstallation of the Boc protecting group proceeded in quantitative yield.

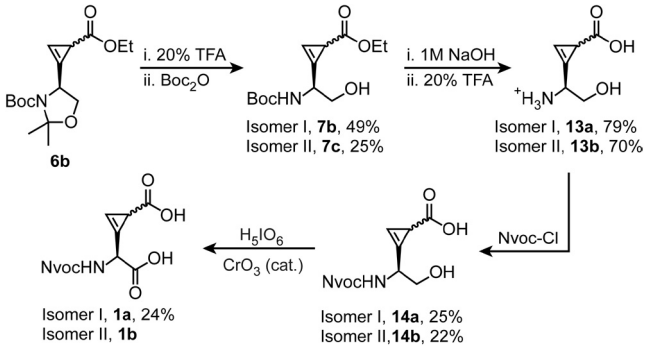
The subsequent oxidation of **7a** is a key step in the synthesis of these cyclopropene-containing glutamate analogs, considering that oxidations on cyclopropene scaffolds are not well precedented in the literature. Thus, we sought an effective oxidation strategy for this scaffold (See conditions tested in Table 1). Our literature search found successful oxidations of cyclopropene alcohols using Dess-Martin periodinane (DMP).[20] Consistent with these reports, our attempts to oxidize alcohol **7a** with DMP did produce an aldehyde. However, DMP oxidation also resulted in the opening of the cyclopropene ring and formation of an allene at the alpha carbon (**S4** in SI), and our efforts to oxidize this aldehyde to the carboxylic acid using Pinnick oxidation conditions were unsuccessful. Another report employed a TEMPO-based oxidation to transform alcohol to carboxylic acid in a cyclopropenone-containing fatty acid analog.[21] However, multiple efforts to employ TEMPO or 4-acetamido-TEMPO oxidations on cyclopropene **7a** were unsuccessful. Chromium based oxidation gave the desired product in very low yields and produced complex reaction mixtures that precluded satisfactory purification. Thankfully, periodic acid-based oxidations with sub-stoichiometric amounts of Chromium, first described by Reider and coworkers,[22] produced the desired product with few apparent side products, although in poor yield. Unfortunately, attempts at ester removal at this stage were unsuccessful, presumably due to deprotonation of the now acidic alpha carbon proton and subsequent decomposition.

Conversely, removing the ester prior to opening the dimethyloxazolidine proceeded in excellent yield (9, 90–94%, Scheme 2), and ester deprotection after ring opening proceeded in slightly

Table 1
Oxidation conditions for accessing cpGlu via a Garner's aldehyde derived alkyne.

Entry	Oxidant	Solvent	Temp. (°C)	Time	Yield (%)[a]	Comment
1	H_5IO_6, CrO_3 (cat.)	MeCN, H_2O	0	20 min	16	
2	Dess-Martin periodinane	DCM	rt	15 min	5	Decomposed during later $NaClO_2$ oxidation
3	Jones (CrO_3, H_2SO_4)	Acetone	0 → rt	20 min	<5	Unable to purify to >95%
4	Pyridinium dichromate	DMF	0	3 d	0	No reaction
5	TEMPO, $NaClO_2$, NaOCl	MeCN, PBS	35	120 min	0	No reaction
6	Acetamido TEMPO, NaOCl	MeCN, H_2O	rt	240 min	0	Decomposition
7	Ruthenium Cl, $NaIO_4$	CCl_4, MeCN, H_2O	rt	4 d	0	No reaction

[a] Isolated yields.



Scheme 3. Generation of a photoactivatable cyclopropene-containing analog of glutamate.

lower yield but enabled separation of diastereomers via Flash chromatography (7b/c, Scheme 3). Oxidation of alcohol 10 using the previously identified periodic acid/Jones reagent mixture proceeded in poor yield to produce the penultimate compound S5. Removal of the Boc group to produce 11 proceeded as expected based on mass spectrometry analysis of the reaction mixture, and purification via HPLC was uneventful. However, this molecule decomposed when concentrated and could not be satisfactorily characterized. This result is consistent with our and others' observations of cyclopropene decomposition upon concentration, likely through polymerization.[7,23,24]

Interestingly, we noted that deprotected **11** remained present in crude reaction mixtures after workup and HPLC fractions after purification, suggesting that it is stable for days in aqueous solutions. Additionally, we observed that Boc-protected compound **S5** was stable in solution and after concentration. These observations suggested a mechanism for deploying cpGlu using a light-activated prodrug strategy. Essentially, the cpGlu analog could be endowed with a light cleavable moiety that would facilitate characterization and storage, while also permitting precise spatiotemporal control of its release in solution. Similar strategies have been used for controlling the release of natural neurotransmitters, including peptide neurotransmitters,[25] glutamate,[26–28] GABA,[28,29] dopamine,[30] and serotonin.[31]

To explore this strategy, we synthesized a cpGlu variant with an Nvoc moiety, a photo labile protecting group, appended to the amine (Scheme 3). We chose Nvoc due to its solubility, ease of synthetic addition, and popularity as a caging group for neurotransmitters. Treatment of diastereomers 13a/b with Nvoc-Cl produced alcohol diastereomers 14a/b in poor yield, and subsequent oxidation proceeded in similar yield to the Boc-protected derivative to produce Nvoc-cpGlu 1a. The minor diastereomer 1b was observed by mass spectrometry and HPLC, but it was produced at levels too low to obtain full characterization. Notably, these molecules were stable at 20 mM in methanol at rt for at least 1 month and did not decompose upon concentration. Experiments are currently ongoing to evaluate these molecule's utility as bioorthogonal probes for glutamatergic vesicles and synapses *in vivo*.

In conclusion, neurotransmitters are a class of biomolecules whose analysis *in vivo* would benefit from bio-orthogonal approaches, but they have been largely neglected. We have synthesized a cyclopropene-containing analog of the excitatory neurotransmitter glutamate with a pendant photocleavable protecting group, which promotes stability for storage and characterization as well as precise spatiotemporal control of the molecule's deployment *in vivo*. When coupled with pro-fluorescent tetrazine[7] or tetrazole[32,33] reaction partners, these glutamate mimics have the potential to reveal the anatomy of glutamatergic vesicles in cultured neurons or small transparent models such as drosophila or zebrafish to promote a more detailed understanding of brain function. Additionally, the glutamate analogs we described add to the list of cyclopropene-containing amino acids that could serve as monomers in the *in vitro* synthesis of unnatural peptides.

Acknowledgments

We thank Alyssa Preston and Sining Li for helpful discussions and critical reading of the manuscript, Dr. Bela Ruzsicska and the Stony Brook University Institute for Chemical Biology and Drug Discovery for Mass Spectrometry resources and analyses, Dr. James Marecek and Francis Picart for NMR analysis. This material is based upon work supported by the National Science Foundation under CHE – 1451366.

A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2016.10.106.

References

1. Yang J, Liang Y, Šečkutė J, Houk KN, Devaraj NK. *Chemistry*. 2014;20:3365–3375.
2. Yu Z, Pan Y, Wang Z, Wang J, Lin Q. *Angew Chem Int Ed Engl*. 2012;51:10600–10604.
3. Patterson DM, Nazarova LA, Xie B, Kamber DN, Prescher JA. *J Am Chem Soc*. 2012;134:18638–18643.
4. Cole CM, Yang J, Šečkutė J, Devaraj NK. *ChemBioChem*. 2013;14:205–208.
5. Patterson DM, Jones KA, Prescher JA. *Mol BioSyst*. 2014;10:1693–1697.
6. Xiong D-C, Zhu J, Han M-J, et al. *Org Biomol Chem*. 2015;13:3911–3917.
7. Yang J, Šečkutė J, Cole CM, Devaraj NK. *Angew Chem Int Ed Engl*. 2012;51:7476–7479.
8. Elliott TS, Townsley FM, Bianco A, et al. *Nat Biotechnol*. 2014;32:465–472.
9. Sachdeva A, Wang K, Elliott T, Chin JW. *J Am Chem Soc*. 2014;136:7785–7788.
10. Li Z, Wang D, Li L, et al. *J Am Chem Soc*. 2014;136:9990–9998.
11. Zhang F, Fox JM. *Org Lett*. 2006;8:2965–2968.
12. Paulini K, Reissig H-U. *Synlett*. 1992;1992:505–506.
13. Shigeri Y, Seal RP, Shimamoto K. *Brain Res Brain Res Rev*. 2004;45:250–265.
14. Nakagawa Y, Saitoh K, Ishihara T, Ishida M, Shinozaki H. *Eur J Pharmacol*. 1990;184:205–206.
15. Huynh THV, Erichsen MN, Tora AS, et al. *J Med Chem*. 2016;59:914–924.
16. Williams RM, Aldous DJ, Aldous SC. *J Org Chem*. 1990;55:4657–4663.
17. Williams RM, Aldous DJ, Aldous SC. *J. Chem. Soc. Perkin Trans.*. 1990;1:171.
18. Chau J, Zhang J, Ciufolini MA. *Tetrahedron Lett*. 2009;50:6163–6165.
19. Casara P, Metcalf BW. *Tetrahedron Lett*. 1978;19:1581–1584.
20. Fisher LA, Smith NJ, Fox JM. *J Org Chem*. 2013;78:3342–3348.
21. Kogen H, Kiho T, Tago K, et al. *J Am Chem Soc*. 2000;122:1842–1843.
22. Zhao M, Li J, Song Z, et al. *Tetrahedron Lett*. 1998;39:5323–5326.
23. Carter FL, Frampton VL. *Chem Rev*. 1964;64:497–525.
24. Dowd P, Gold A. *Tetrahedron Lett*. 1969;10:85–86.
25. Banghart MR, Sabatini BL. *Neuron*. 2012;73:249–259.
26. Kramer RH, Fortin DL, Trauner D. *Curr Opin Neurobiol*. 2009;19:544–552.
27. Olson JP, Kwon H-B, Takasaki KT, et al. *J Am Chem Soc*. 2013;135:5954–5957.
28. Kantevari S, Matsuzaki M, Kanemoto Y, Kasai H, Ellis-Davies GCR. *Nat Methods*. 2010;7:123–125.
29. Amatrudo JM, Olson JP, Lur G, Chiu CQ, Higley MJ, Ellis-Davies GCR. *ACS Chem Neurosci*. 2014;5:64–70.
30. Araya R, Andino-Pavlovsky V, Yuste R, Etchenique R. *ACS Chem Neurosci*. 2013;4:1163–1167.
31. Rea AC, Vandenberg LN, Ball RE, et al. *Chem Biol*. 2013;20:1536–1546.
32. Ramil CP, Lin Q. *Curr Opin Chem Biol*. 2014;21:89–95.
33. An P, Yu Z, Lin Q. *Org Lett*. 2013;15:5496–5499.