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A new synthesis of caged GABA compounds for studying $GABA_A$ receptors

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ABSTRACT

A short and convergent synthetic approach to new photoactivatable precursors of γ-aminobutyric acid (GABA) is described. When photolyzed, the 'caged' GABA precursor efficiently releases GABA, as judged by depolarization measurements on the mammalian $GABA_A$ receptor.

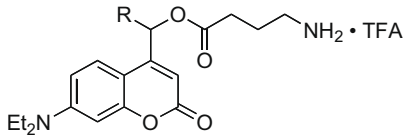
The primary inhibitory neurotransmitter receptors in the mammalian central nervous system (CNS) are γ-aminobutyric acid type A ($GABA_A$) receptors.[1] These receptors exist as heteropentameric, transmembrane, ligand-gated ion channels composed of diverse subtypes that exhibit distinct kinetic and pharmacological profiles.[2] Mutations in the various $GABA_A$ subunits are associated with dysfunctional neuronal transmission.[3] Mutations of $GABA_A$ receptors have been linked to several genetically heritable forms of epilepsy, a disease that in the US affects approximately 2.5 million people at an estimated annual cost of $12.5 billion.[4]

To investigate the kinetics and mechanism of wild-type $GABA_A$ receptor function, and how mutations affect these properties, we required a biologically inert ('caged') carboxyl-linked GABA precursor that can release free GABA by photoactivation using visible light, which causes less damage to biological systems than UV light. Such caged neurotransmitters have proven to be powerful tools in probing signal transduction within the CNS.[5]

Of the various caging groups for carboxylic acids, the 7-(*N*,*N*-diethylamino)-4-(hydroxymethyl)coumarin (DECM) moiety is particularly effective, since it undergoes rapid and efficient photolytic ester cleavage to release the carboxylic acid using visible light. The DECM group has recently been developed to protect the γ-carboxyl group of the neurotransmitter glutamic acid.[6] Besides releasing glutamate efficiently at 400 nm, both DECM–glutamate and its photolytic byproducts were shown to be biologically inert. The corresponding caged GABA precursor **1** (Fig. 1), which was recently synthesized,[7] also released GABA upon photolysis. Unfortunately, however, **1** itself inhibited various $GABA_A$ receptors.

To circumvent this unwanted activity, we devised an alternative and convergent route to a family of carboxy-substituted DECM–GABA derivatives, exemplified by structure **2**. This caged GABA derivative exhibits all the requisite properties of a desirable GABA precursor (decaging at 400 nm with good quantum yield), but with the particular advantage that both **2** and its photolysis byproducts are inert towards the GABA receptor tested.

The synthetic approach we developed took advantage of the well-known Passerini 3-component condensation, which produces α-acyloxyesters from the reaction of simple carbonyl compounds with carboxylic acids and isonitriles.[8] In this instance, the known[9] aldehyde **3**, prepared by selenium dioxide oxidation of 7-(*N*,*N*-diethylamino)-4-methylcoumarin, underwent a smooth 3-component condensation with ethyl isocyanoacetate and BOC-protected GABA using concentrated reaction conditions to afford the acyloxydiester **4** (Scheme 1) in 96% yield. Similar condensations of **3** and BOC–GABA with *t*-butyl isonitrile and *o*-tolylisonitrile afforded caged GABA derivatives **5** and **6**, respectively. Although sensitive to light, compounds **4**–**6** could be purified by flash chromatography (darkroom) for full structural characterization.



1 R= H
2 R= $CONHCH_2CO_2Et$

Figure 1. DECM-caged GABA compounds of interest.

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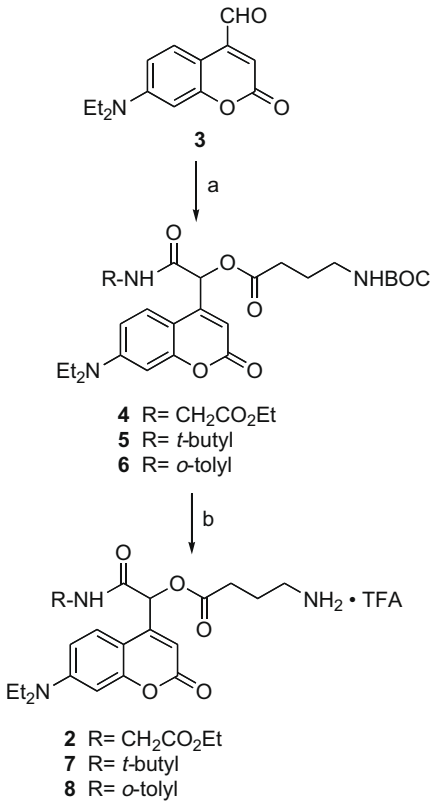

Scheme 1. Passerini route to DECM-caged GABA compounds. Reagents and conditions: (a) $BOCNH(CH_2)_3CO_2H$ (1 equiv), $CNCH_2CO_2Et$ (1 equiv), CH_2Cl_2 rt, 12 h, dark (96%); (b) CF_3CO_2H (5 equiv), CH_2Cl_2, rt, 18 h, dark (90%).

To remove the BOC group, freshly prepared samples of **4** were exposed to TFA at rt, affording the desired caged DECM–GABA **2**. In like fashion, BOC–GABA compounds **5** and **6** were also deprotected to afford caged GABA salts **7** and **8**, respectively.

Electrophysiological measurements with caged GABA **2** were conducted as shown in Figure 2. A borosilicate pipette fitted with an electrode (Fig. 2A) was attached to an HEK 293T cell transiently expressing a $GABA_A$ receptor (alpha1, beta2, delta subunits). The cell membrane was ruptured, exposing the cytosol to the pipette buffer. The cell was lifted from the substratum and suspended in front of a U-tube having a small (150 μm) opening for flowing solutions of **2** over the cell surface. Solutions of **2** were kept on ice; photolysis measurements were made at rt. An optical fiber positioned perpendicular to the U-tube directed light from a Rapp SP-20 Xe flash-lamp onto the cell and surrounding buffer. A pulse of 385–450 nm light released free GABA, activating the opening of $GABA_A$ receptor-channels in the cell membrane and increasing the permeability of the cell membrane to chloride ions. This resulted in a rapid change in the current amplitude across the membrane as measured by the pipette electrode (Fig. 2B).

Like other coumarin-based caged compounds, GABA precursor **2** proved resistant to hydrolysis in the dark. Moreover, initial biological experiments demonstrated that **2** was sufficiently soluble in the assay buffer[10] and was stable to hydrolysis. Upon photolysis at 400 nm (Fig. 2B), **2** smoothly released GABA with a quantum yield of 0.1. Moreover, no current was observed in the presence of **2** or its photolysis byproduct(s). These characteristics suggest that **2** and its congeners may prove useful in transient kinetic measurements of $GABA_A$ neurotransmitter receptors on cell surfaces without causing damage to key cellular constituents.

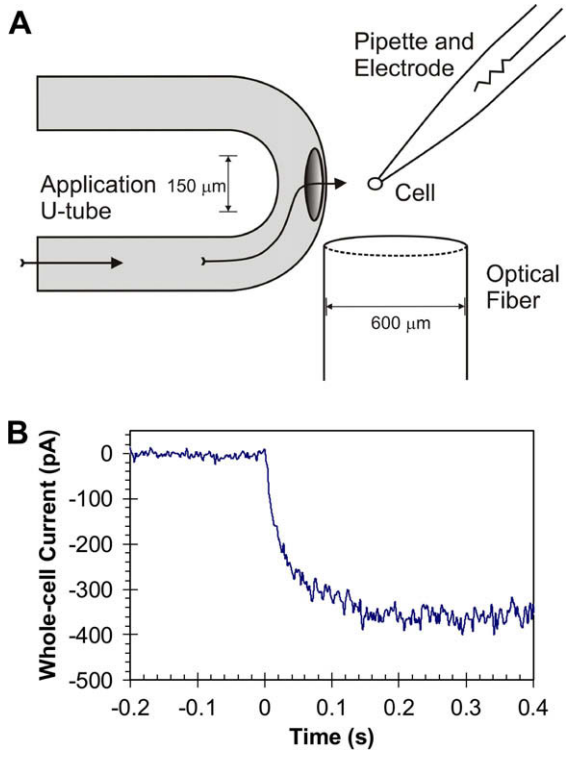

Figure 2. Electrophysiological measurements using caged GABA **2**. (A) Diagram of the system used to measure $GABA_A$ receptor activity; (B) a representative current trace in which the flash lamp was pulsed at the zero time point to induce photolysis.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.03.065.

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