Dual Function Glutamate-Related Ligands: Discovery of a Novel, Potent Inhibitor of Glutamate Carboxypeptidase II Possessing mGluR3 Agonist Activity

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The metabotropic glutamate receptors (mGluRs) are a heterogeneous family of G-protein-linked receptors that couple to multiple second messengers. These include the negative modulation of adenylate cyclase, activation of phosphoinositide-specific phospholipase C, and modulation of ion channel currents.[1] Three types of mGlu receptors have been identified: group I receptors couple to phosphoinositide hydrolysis and include mGluR1 and mGluR5; group II receptors couple to the inhibition of cyclic adenosine 5′-monophosphate (cAMP) formation and include mGluR2 and mGluR3; group III receptors (mGluR4, mGluR6, mGluR7, and mGluR8) are negatively coupled to cAMP. Each subtype is distinguished on the basis of its pharmacology and sequence homology. Excessive activation of glutamate receptors or disturbances in the cellular mechanisms that protect against the adverse consequences of physiological glutamate receptor activation have been implicated in the pathogenesis of a host of neurological disorders. These disorders include epilepsy, ischemia, central nervous system trauma, neuropathic pain, and chronic neurodegenerative diseases. Because of the ubiquitous distribution of glutamatergic synapses, mGluRs have the potential to participate in a wide variety of functions in the CNS. In addition, because of the wide diversity and heterogeneous distribution of the mGluR subtypes, the opportunity exists for the development of highly selective drugs that affect a limited number of CNS functions. The mGluRs therefore provide novel targets for the development of therapeutic agents that could have a dramatic impact on the treatment of CNS disorders.

To date, almost all of the commonly used agonists and antagonists employed in biological studies of the mGluRs are amino acids, often embodying a structurally rigidified glutamate-like core.[2] During our efforts to identify potent and selective ligands acting at these receptors, we have discovered an mGluR3-selective agonist that contains only acid groups and that acts simultaneously as a potent inhibitor of NAAG peptidase.

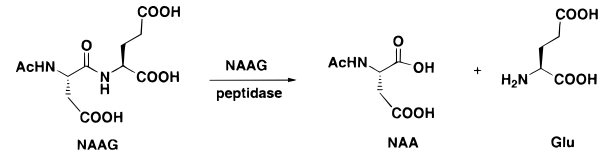

Figure 1. Catabolism of NAAG by the peptidase.

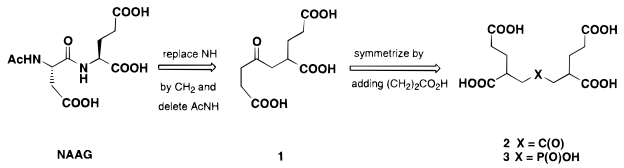

Figure 2. Design strategy for NAAG-based mimics.

The starting point of our studies is *N*-acetyl-L-aspartyl-L-glutamate (NAAG), which is a peptide neurotransmitter that is widely distributed in the mammalian nervous system.[3] This peptide is inactivated by an extracellular peptidase (NAAG peptidase or glutamate carboxypeptidase II) producing glutamate and *N*-acetylaspartate (Figure 1).[4] The peptidase is concentrated, if not exclusively localized, in glia. NAAG is a low-potency agonist and may act as a partial antagonist at some NMDA receptors.[5] Additionally, in studies using cell lines transfected with mGluR1−6, NAAG was found to selectively activate the mGluR3 with an EC_{50} value in the range of 65 ± 20 μM.[6] NAAG therefore provides a starting point for the design of new therapeutic drugs that may selectively inhibit glutamate carboxypeptidase II and/or act as mGluR3 agonists or antagonists.

Certain phosphonate analogues of NAAG, such as 2-(phosphonomethyl)pentanedioic acid, have been reported to act as potent inhibitors of NAAG peptidase.[7] Interestingly, while this compound was reported to show no activity at glutamate receptors, we found that it does, in fact, act as a weak antagonist at mGluR3. Upon the basis of this unexpected result, we chose to explore the activity of other NAAG analogues, the subject of the present report.

At the onset of this work, we explored the activity of NAAG-like analogues that were missing the amide bond between the Asp and Glu residues (the standard ketomethylene isosteric replacement). Furthermore, the acetylamino group was deleted (**1**), as this particular group was reported not to be an absolute requirement for binding to NAAG peptidase.[8] Among the compounds synthesized, the symmetric compound **2,** comprising an acetone moiety flanked by the two pentanedioic acid groups, proved to be the most interesting, as it retained some mGluR3 activity albeit at high micromolar concentrations. Following this observation, we chose to explore the activity of the compound in which the central carbonyl group of **2** was replaced by P(O)OH (**3**, 4,4′-phosphinicobis(butane-1,3-dicarboxylic acid)) with the idea that this compound might not only act as an mGluR3-selective ligand but also function as an NAAG peptidase inhibitor (Figure 2).

The synthesis of **1** and **2** is outlined in Scheme 1. Compound **4** was prepared using a literature method.[7] Conjugate addition of nitromethane to **4** in the presence of Triton B[9] afforded a mixture of compounds **5** and **6**

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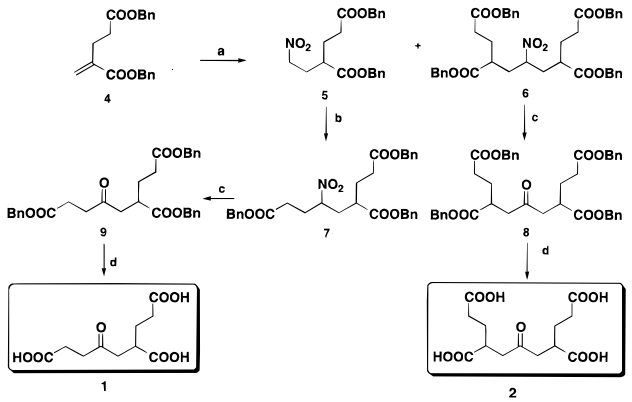
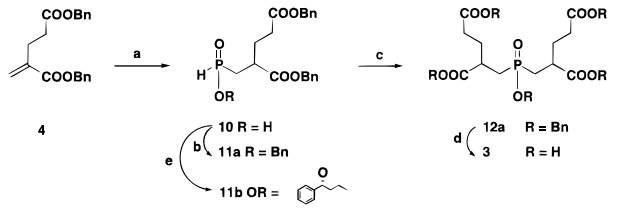
Scheme 1[a]



[a] Reagents and conditions: (a) CH_3NO_2, Triton B, rt, 24 h; (b) benzyl acrylate, Triton B, CH_2Cl_2, rt, 24 h; (c) cetyltrimethylammonium permanganate, Et_3N, CH_2Cl_2, rt, 4 h; (d) H_2 (1 atm), 20% $Pd(OH)_2/C$, rt, 12 h.

Scheme 2[a]



[a] Reagents and conditions: (a) NaH_2PO_2, TMSCl, Et_3N, then **4**, rt, 24 h; (b) PivCl, BnOH, CH_2Cl_2/py (10:1), rt, 2 h; (c) NaH, THF, 0 °C, then **4**, rt, 2 h; (d) 70 psi H_2, 20% $Pd(OH)_2/C$, rt, 24 h; (e) PivCl, (*R*)-(+)-1-phenyl-1-butanol, CH_2Cl_2/py (10:1), rt, 2 h.

in a ratio of 2:1.5. After separation, **5** underwent a second conjugate addition with benzyl acrylate to afford compound **7**. Intermediates **6** and **7** were converted to carbonyl compounds **8** and **9**,[10] respectively, which were finally transformed to **1** and **2** by hydrogenolysis.

The synthesis of **3** is outlined in Scheme 2. Conjugate addition of sodium hypophosphite to dibenzyl α-methyleneglutarate **(4)** afforded the phosphonate **10**, which was converted to the benzyl-protected compound **11a** with benzyl alcohol in the presence of trimethylacetyl chloride.[11] Deprotonation of **11a** with NaH followed by conjugate addition to **4** provided the completely protected pentaester **12a**, which was finally transformed to **3** by hydrogenolysis (Scheme 2).

As the above synthesis proceeds in a stereorandom fashion, we also prepared the three optically pure isomers of **3** through the use of the chiral alcohol (*R*)-(+)-1-phenyl-1-butanol (see Supporting Information).

These compounds were assayed for their ability to inhibit rat NAAG peptidase stably expressed in Chinese hamster ovary (CHO) cells using conditions identical to those reported previously.[4d] Of the compounds tested, compound **3** proved to be the most potent and inhibited NAAG peptidase with an EC_{50} of 21.7 ± 2.1 nM. The ketone **2** showed no ability to inhibit this enzyme, while **1** was only weakly active, inhibiting about 35% of enzyme activity at 100 μM. The individual stereoisomers of **3** show some differences for the inhibition of NAAG peptidase compared to the isomeric mixture. However, the isomeric mixture is only 3-fold less potent than the best of the optically pure isomers.

The mGluR activity of compound **3** was tested in cell lines expressing the individual mGluRs using conditions

Table 1. EC_{50} Values for **3** and Its Stereoisomers at NAAG Peptidase and mGluR3

compound	NAAG peptidase inhib (EC_{50}, nM)	mGluR3 agonism (EC_{50}, μM)
1	35% inhib at 100 μM	NA[b]
2	NA[b]	>300
3 (mixture of isomers)	21.7 ± 2.1	39 ± 8
(*R,R*)/(*S,S*)-**3**[a]	84.0 ± 20.8	33 ± 9
(*S,S*)/(*R,R*)-**3**[a]	6.9 ± 0.7	45 ± 7
meso-**3**	22.3 ± 5.8	51 ± 10

[a] Absolute stereochemistry not determined. [b] NA = no activity.

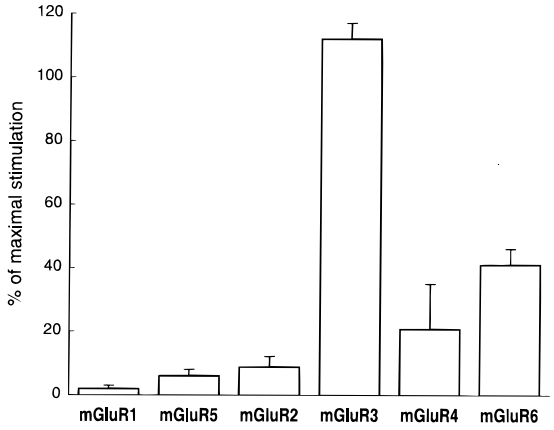


Figure 3. Effect of **3** (1000 μM) at mGluRs. Results are means ± SEM from 4−8 experiments.

as previously described.[12] In brief, group I receptors (mGluR1a and mGluR5a) are coupled to phospholipase C, and their activity was determined by accumulation of inositol phosphates. Group II receptors included the cAMP-coupled mGluR2, as well as the chimeric receptor mGluR3/1a which, as shown previously,[6] combines the pharmacological properties of mGluR3 with the activation of phospholipase C. Group III receptors were represented by mGluR4a and mGluR6, both coupled to the inhibition of cAMP formation. As is apparent from Table 1 and Figure 3, compound **3** exhibits good potency and selectivity as an agonist for mGluR3 in comparison to the other mGluR subtypes. Upon the basis of its EC_{50} value, it is roughly 2-fold more potent than NAAG.

In summary, we describe the discovery of a dually acting ligand that offers a novel approach for the generation of possible neuroprotective agents for the treatment of neurodegenerative disorders. As the tetraacid **3** acts both as an mGluR3-selective agonist and as a potent inhibitor of the enzyme involved in the cleavage of NAAG to glutamate, it may simultaneously be able to control levels of glutamate and to activate an mGluR subtype known to confer neuroprotection.[13] Although the pharmacological profiles of the mGluR2 and mGluR3 subtypes are very similar, compound **3** proved to be more potent for mGluR3 than for mGluR2, indicating that it is possible to develop selective drugs that are able to discriminate between these two receptors. The mGluR selectivity of **3** may make it a useful pharmacological agent for defining mGluR3 function at identified synapses. Further efforts aimed at improving compound potency as well as selectivity are in progress.

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Supporting Information Available: Characterization data for compounds **1**, **2**, **5**−**9**, **11a**, **11b**, **12a**, **12b$_1$**, **12b$_2$**, **12b$_3$**, **12b$_4$**, **3,** and its three stereoisomers. This material is available free of charge via the Internet at http://pubs.acs.org.

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