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Synthesis, receptor binding and activity of iso and azakainoids

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ABSTRACT

Two syntheses for the production of an unsubstituted azakainoid are described. The 1,3-dipolar cycloaddition of diazomethane with *trans*-dibenzyl glutaconate yields a 1-pyrazoline, which may be reduced directly to the pyrazolidine. An unexpected *trans-cis* isomerization is observed during Hg/Al reduction of the 1-pyrazoline N=N bond. Alternatively, when TMS diazomethane is used as the dipole, the resulting 2-pyrazoline obtained after desilylation may be reduced with $NaCNBH_3$ to provide the *trans* azakainate analog exclusively. The synthesis of an unsubstituted isokainoid via Michael addition is also described. Glutamate receptor binding assays revealed that the azakaniod has a moderate affinity for unspecified glutamate receptors. Membrane depolarization of *Aplysia* neurons upon application of the azakainoid demonstrates that it is an ionotropic glutamate receptor agonist.

L-Glutamic acid is a major excitatory neurotransmitter within the mammalian central nervous system.[1] Two major subgroups of glutamate receptors are the metabotropic and ionotropic types. Ionotropic receptors may be further divided into three subgroups according to their most potent agonist and include the NMDA (*N*-methyl-D-aspartic acid), AMPA ((*S*)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) and kainate (α-kainic acid) receptors.[2] α-Kainic acid **1** is a naturally occurring non-proteinogenic amino acid with neuroexcitatory activity towards the mammalian central nervous system.[3] It acts as a conformationally restricted analogue of L-glutamic acid. α-Kainic acid binds to and activates the ionotropic kainate and AMPA receptors with nanomolar and micromolar affinities respectively. As such, α-kainic acid has been used extensively as a probe for neuropharmacological research and kainic acid induced neurotoxicity as an experimental model for neurodegeneration.[4]

α-Kainic acid is the parent of a larger class of synthetic and naturally occurring glutamate receptor agonists known as the kainoids. All kainoids possess the pyrrolidine dicarboxylate scaffold and vary in the substituent at C-4 (Fig. 1) and the configuration of the three stereocenters. Structure–activity studies of the kainoids as well as crystal structures of the binding domain of the kainite receptor in complex with various ligands, have demonstrated the importance of the absolute configuration of the three stereocenters and unsaturation in the C-4 substituent.[5–7] The 4-unsubstituted kainoid, *trans*-2-carboxy-3-pyrrolidine-3-acetic acid (CPAA) **2** is less potent than **1** and reportedly acts on NMDA receptor subtypes.[8] Substitution at the 5-position eliminates binding affinity for the kainite receptor.[9] The syntheses, but not the biological activity of homokainoids was recently described.[10] Currently there is no information on the effect of replacing the C-5 methylene with nitrogen (azakainoids) or translocation of nitrogen relative to the ring substituents (isokainoids). Herein we report the first syntheses of unsubstituted aza **3** and isokainoid **4** as well as preliminary receptor binding and activity studies.

The 1,3-dipolar cycloaddition of diazoalkanes with alkenes yields 1-pyrazolines (Scheme 1) which may serve as precursors to azakainoids. Based on preferred Frontier Molecular Orbital (FMO) interactions[11] and the stereospecific suprafacial addition to the dipolarophile, the pyrrolidine ring of the azakainoids could be constructed with the correct relative configuration in a single step. The substituents at the 4 and 5 positions of the pyrazoline ring may favor the *cis* relative configuration if π interactions are present in the transition state.[12] Thus the 1,3-dipolar cycloaddition of *trans*-diethyl glutaconate with diazoalkanes should provide the appropriate 3,4-*trans*, 4,5-*cis* relative configurations in the cycloadducts (Scheme 2). The kainoids function as conformationally restricted glutamate receptor agonists. We reasoned that NH could serve as a bioisostere for the C-5 methylene. If this were the case, aza analogs could serve as readily accessible functional analogs of kainic acid. However, 1-pyrazolines tend to be unstable and can either become oxidized to the pyrazoles or, especially if it results in conjugation, will isomerize to the 2-pyrazolines (Scheme 1) with the direction of double bond isomerization dependent on the substituents. Thus, at least one of the newly formed stereocenters may be lost.

We described the synthesis of 2-pyrazolines **5**–**7** by cycloaddition of *trans*-diethyl glutaconate with phenyldiazomethane and diazomethane.[13] 1-Pyrazolines were not isolated but were

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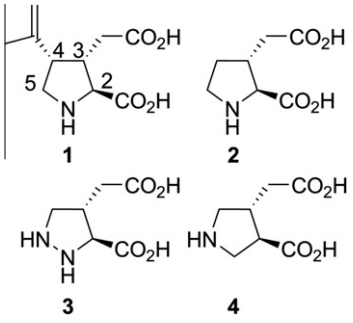
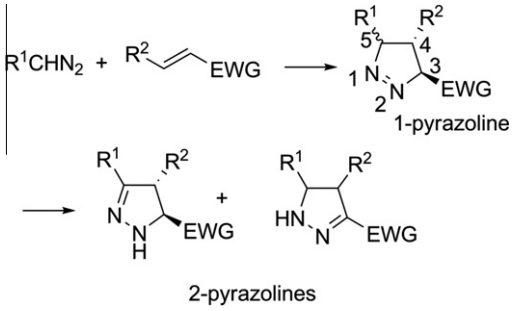


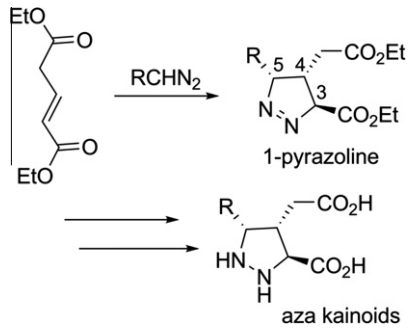
Figure 1. Representative kainoids: α-kainic acid **1**, the unsubstituted kainoid CPAA **2**, and corresponding azakainoid **3** and isokainoid **4**.



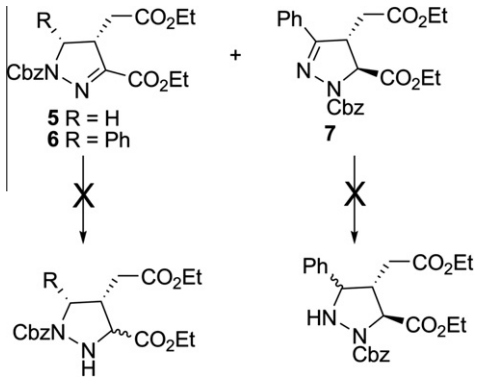
Scheme 1. 1, 3-Diploar cycloadditions with diazoalkanes produce 1-pyrazolines which readily isomerize to 2-pyrazolines.

immediately isomerized to the 2-pyrazolines and protected as the Cbz derivatives to prevent oxidation to the pyrazoles (Scheme 3). The 2-pyrazolines proved to be extremely resistant to reduction using hydride reducing agents ($NaCNBH_3$, $LiBH_4$, $LiAlH_4$, BH_3, $LiEt_3BH$, Et_3SiH, and Bu_3SiH), catalytic reduction (H_2, Pd/C) or SmI_2. Only limited success was achieved with $NaBH_4$ in refluxing methanol. We therefore sought to reduce the 1-pyrazolines in situ prior to isomerization which, we anticipated, would have the added benefit of preserving the relative stereochemistry of the 1-pyrazoline. Again, we found the 1-pyrazolines to be resistant to a variety of reducing agents. However, the use of a mercury/aluminum almagam[14] proved to be effective and provided the protected pyrazolidine **8** in 95% yields after quenching the reaction with Cbz chloride (Scheme 4).

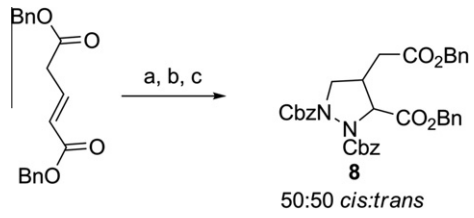
We were surprised to find that pyrazolidine **8** was isolated as a 50:50 mixture of *cis* and *trans* isomers. Our initial thought was that double bond isomerization to the conjugated 2-pyrazoline had occurred prior to the reduction. However, hydrogenolysis of the protected 2-pyrazoline **5**, followed by treatment with the Hg/Al almagam failed to produce any pyrazolidine (Scheme 5). The Hg/Al amalgam reduction is performed under slightly basic conditions which apparently causes the isomerization of the C-3 stereocenter. *cis*-**8** and *trans*-**8** were readily separated chromatographically.



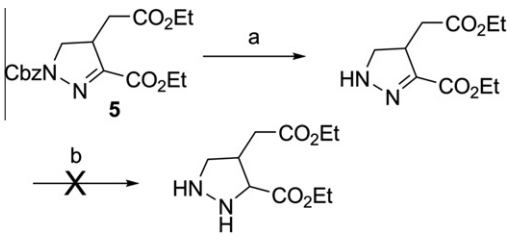
Scheme 2. Proposed synthesis of aza-kainoids by reduction of 1-pyrazolines.



Scheme 3. 2-Pyrazolines proved resistant to reduction (see text).

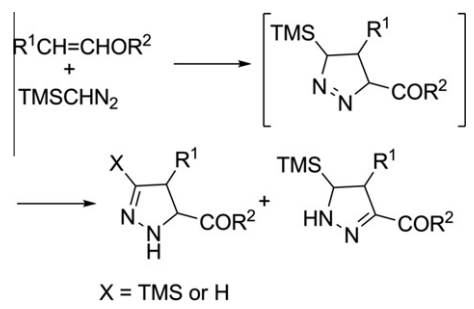


Scheme 4. Reagents and conditions: (a) CH_2N_2, Et_2O, rt, 2 h; (b) Al/Hg, MeOH/ H_2O, 60 °C; (c) CbzCl, CH_2Cl_2, $NaHCO_3$ (aq), rt, overnight, 95% (three steps).



Scheme 5. Reagents and conditions: (a) H_2, Pd/C, EtOH (100%); (b) Al/Hg, MeOH/ H_2O, 60 °C.

The production of both *cis* and *trans* isomers of **8** prompted us to seek an alternate route to the unsubstituted aza-kainoid precursor, *trans*-**8**. Carreira and co-workers described 1,3-dipolar cycloadditions with TMS diazomethane as a key step in the synthesis of aza-proline analogs.[15–17] The initially formed 1-pyrazolines from TMS diazomethane may yield three different types of products,



Scheme 6. 1,3-Dipolar cycloadditon with TMS diazomethane may yield three products.

arising from double bond isomerization in either direction or desilylation (Scheme 6).[18] Carreira reported that products arising from desilylation could be formed exclusively under certain conditions.[19] Similarly, the 1,3-dipolar cycloaddition of TMS diazomethane with *trans*-dibenzyl glutaconate, followed by desilylation could provide *trans*-**8** after reduction of the C=N double bond and Cbz protection. While we previously had difficulties in reducing 2-pyrazolines (Scheme 3), we reasoned that the unconjugated C=N double bond would be more readily reduced.

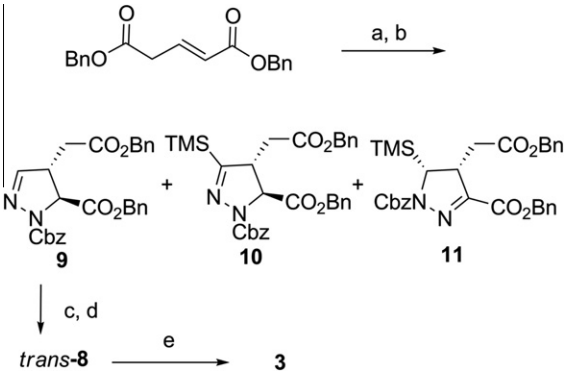
TMS diazomethane reacts with dibenzyl glutaconate which, upon quenching with Cbz-Cl, yields a mixture of two 2-pyrazolines **9** and **11** (Scheme 7). Under the conditions employed, **10** was not observed. After flash chromatography, the desilylated 2-pyrazoline **9** was obtained in 28% yield for the two steps. 2-Pyrazoline **9** was reduced using $NaCNBH_4$ followed by an additional Cbz protection step to yield *trans*-**8** in 58% yield for the two steps. A one step-4-group-deprotection by hydrogenolysis in the presence of 5% palladium on carbon in methanol provided a quantitative yield of **3**.

We reasoned that the unsubstituted isokainoid **4**, could serve as a conformationally restricted glutamate agonist with selectivity distinct from the kainoids. It was prepared in five steps as outlined in Scheme 8. The reductive alkylation of β-alanine ethyl ester with benzaldehyde provided *N*-benzyl-β-alanine ethyl ester. Alkylation with ethyl-4-bromocrotonate provided the precursor **12**, which was cyclized via a Michael addition of the enolate affected by treatment with LiHMDS to provide pyrrolidine **13**. Acid catalyzed hydrolysis of the ethyl ester followed by hydrogenolysis provided the isokainoid, *trans*-4-(carboxymethyl)pyrrolidine-3-carboxylic acid **4**. NOESY correlations confirmed the anticipated *trans* relative configuration of **4** (Fig. 2).

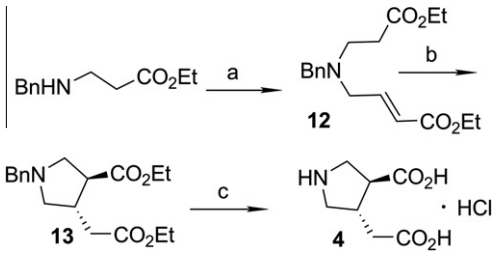
Finally, the unsubstituted kainoid, *trans*-CPAA **2**, was prepared in racemic form (Scheme 9). The cyclization precursor **14** was prepared as previously described[20] and cyclized according to the procedure described by Karoyan et al.[21] followed by hydrolysis of the ester and nitrile groups, and hydrogenolysis provided *cis*-CPAA. Heating *cis*-CPAA in water at 190 °C for 12 h provided a separable 80:20 mixture of *cis* and *trans* CPAA (**2**).[21]

Azakainoid **3**, isokainoid **4** and *trans*-CPAA **2** were tested in receptor binding assays in rat brain against [^{3}H]NMDA, [^{3}H]AMPA, [^{3}H]kainic acid and [^{3}H]glutamic acid. With the single exception of azakainoid **3**, none of the compounds demonstrated affinity for glutamate receptors. Azakainoid **3** competitively inhibited unidentified [^{3}H] L-Glu binding in rat brain with an EC_{50} of 12 μM. (Fig. 3).

Agonist activity of **3** was tested in neurons of the marine neural model organism, *Aplysia californica*. Figure 4 shows cell responses in patch clamp recordings from buccal S cluster neurons dissoci-



Scheme 7. Reagents and conditions: (a) $TMSCH_2N_2$, benzene:hexane, reflux, 8 h; (b) CbzCl, CH_2Cl_2, $NaHCO_3$ (aq), rt, overnight (36% **9** and **11**, two steps); (c) $NaCNBH_3$ (6 equiv), HOAc, rt, 5 h; (d) CbzCl, CH_2Cl_2, $NaHCO_3$ (aq), rt, overnight, 58% (two steps); (e) H_2, Pd/C, MeOH (100%).



Scheme 8. Reagents and conditions: (a) $BrCH_2CH{=}CHCO_2Et$, K_2CO_3, CH_3CN, rt, overnight (61%); (b) LiHMDS, THF, −78 °C to rt 25 min (79%); (c) (i) 1 N HCl, reflux overnight; (ii) H_2 (50 psi), Pd/C, 26 h; (75%, two steps).

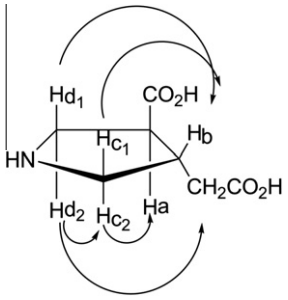
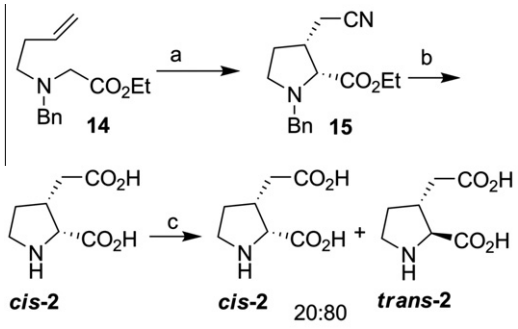


Figure 2. NOESY correlations for determination of relative configuration of isokainoid **4**.



Scheme 9. Reagents and conditions: (a) (i) LDA, THF, −20 °C to −100 °C; (ii) $ZnBr_2$, −100 °C to rt 2 h; (iii) CuCN/2LiCl, 0 °C, TsCN, rt overnight (33%); (b) (i) HCl 6 N, reflux; (ii) H_2/Pd then Dowex 50-X8 (45% yield); (c) H_2O, 190 °C, 12 h; (79% after purification as the Cbz derivative)

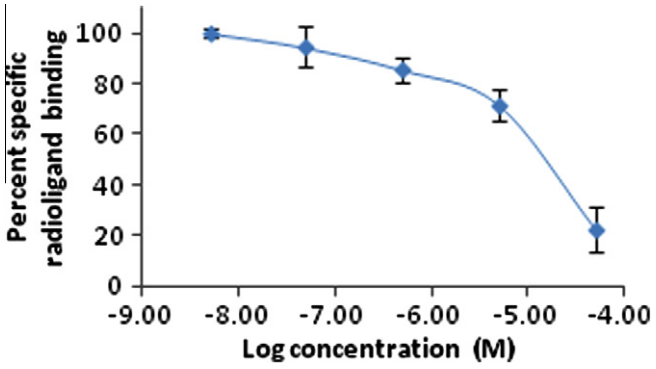


Figure 3. Competitive displacement receptor binding assay for **3**. Error bars represent the range of values from two trials.

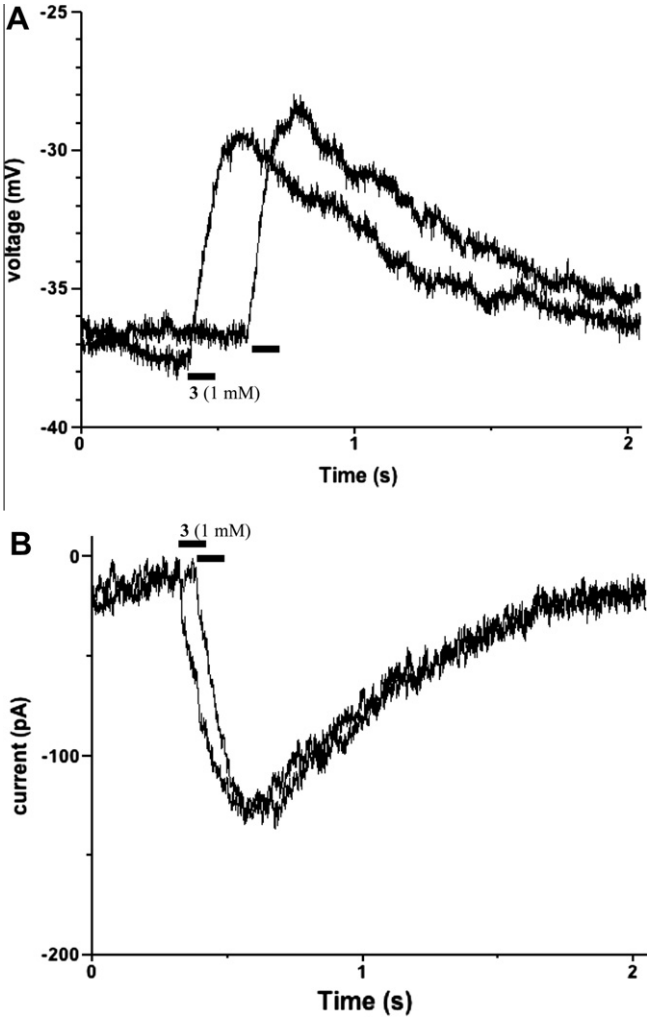


Figure 4. Cell responses to **3** (1 mM; 100 ms) in a cultured buccal ganglion neuron of *Aplysia*. Paired applications of agonist are separated by 2 s (bars). A. Current clamp recording showing depolarization in response to **3**. B. −30 mV voltage clamp recording showing inward current in response to application of **3**.

ated from sexually mature *Aplysia* and cultured.[22] These neurons have ionotropic L-glutamate receptors including NMDA-like,[23] and others sensitive to both L-glutamate and D-aspartate that may be unique receptors.[24] In Figure 4A, **3** applied repetitively at 2 s intervals evoked 8–10 mV depolarizations of the cell membrane from its resting membrane potential of ∼−37 mV. Figure 4B demonstrates that an excitatory inward current underlies the depolarization. The current showed no decrease in amplitude when it was repetitively elicited by multiple applications of **3**. Trains of ⩾6 responses were evoked without appreciable decrease in current amplitude in seven experiments (data not shown), suggesting that receptor desensitization to agonist actions of **3** on this time scale did not occur.

Azakainate and isokainate analogs **3** and **4** were synthesized in order to test the hypothesis that the pyrazolidine and pyrrolidine analogs of kainic acid could serve as glutamate receptor agonists. Compound **3** exhibited an affinity for unspecified glutamate receptors in rat brain and an excitatory effect on *Aplysia* neurons that may result from its action on L-glutamate receptors. Interestingly, the agonist actions of this kainate derivative are non-desensitizing, much like the actions of L-glutamate on these cells.[25] By analogy with the kainoids, it is highly likely that azakainoids which are appropriately substituted at the C-4 position will be significantly more potent glutamate agonists than **3**. Alternate syntheses of such compounds are currently being explored.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.02.046.

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