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Practical Synthesis of Kainoids: A New Chemical Probe Precursor and a Fluorescent Probe

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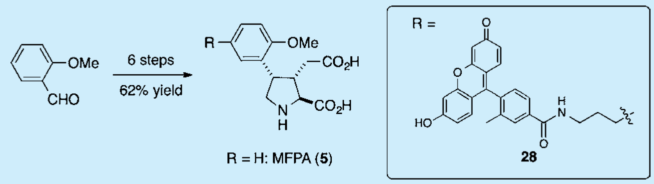
Supporting Information

ABSTRACT: A practical total synthesis of kainoid MFPA (**5**) was achieved in only six steps, via a novel Ni-catalyst-mediated asymmetric conjugate addition reaction. Furthermore, a fluorescein-based fluorescent ionotropic glutamate receptor probe **28** was efficiently synthesized from a precursor derived from a synthetic intermediate of **5**.



Kainoids, as represented by kainic acid (**1**), have been studied for more than 40 years due to their selective binding to ionotropic glutamate receptors (iGluRs), which are central neurotransmitters in the brain.[1] Because **1** is a selective ligand for kainate receptors (KARs), a subset of iGluRs, it has been widely used as a standard tool in neuropharmacological research. Since KARs are involved in the transmission of pain, especially neuropathic pain, compounds that interact with KARs are of particular interest as research tools or even potential therapeutic agents. Natural (**1**,[2] **2**,[3] **3**−**4**[4]) and synthetic (**5**,[5] **6**−**9**[6]) kainoids have been used to investigate neuropathic pain transmission, and it was reported that allodynia induced by acromelic acids (**3** and **4**) was attenuated by 3-carboxymethyl-4-(4-methylphenylthio)pyrrolidine-2-carboxylic acid (PSPA, **7**) (Figure 1).[6] Therefore, we wished to develop a practical synthetic route to prepare various kainoids in a small number of steps. We first validated our strategy by preparing a potent kainoid (MFPA, **5**)[5a] bearing an acromelic acid motif in six steps from nitrostyrene derivative **15**.[7b] We then turned to the preparation of fluorescein-conjugated kainoid **28**. Such a fluorescent probe molecule with a high affinity for KA receptors could be useful for characterizing the behavior of synaptic KAR in live cells. Herein, we report a practical total synthesis of MFPA (**5**). We also describe its biological activity, as well as adaption of our synthesis to obtain a fluorescent iGluR probe molecule, **28**.

We recently reported an efficient organometal-catalyzed construction of three consecutive chiral centers in a pyrrolidine ring, leading to the total synthesis of MFPA (**5**).[7a,b] However, a shorter, more practical synthesis of **5** was needed for efficient preparation of iGluR probes. Thus, we set out to improve our synthetic procedures.[7b]

The heart of our synthetic plan is illustrated in Scheme 1. Our reported synthesis of **5** utilized nitroalkene **15** and α-ketoester **13** to allow for the stepwise oxidation sequence at the C2 position at a later stage. We envisioned that starting from α-ketoester **14**, which possesses two carboxylic acid moieties, would allow a shorter synthetic route with retention of the oxidation state. Furthermore, construction of the pyrrolidine ring from α-ketoester **14** could be achieved by reduction of the nitro group and subsequent intramolecular reductive amination in a single step.

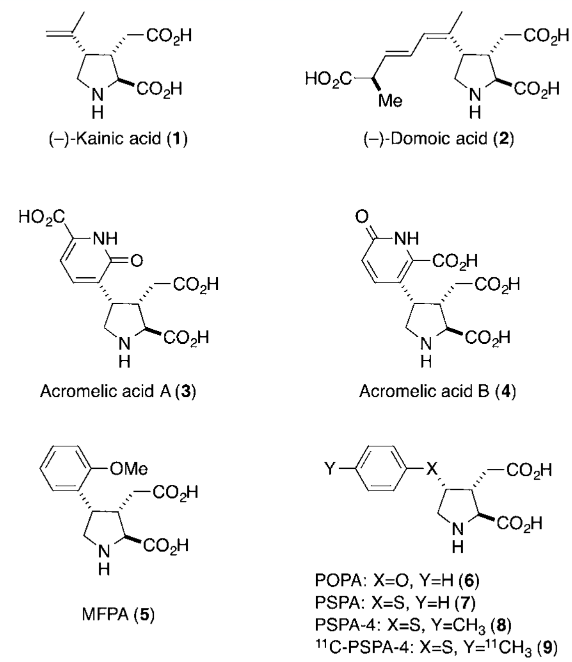


Figure 1. Structure of kainoids **1**−**9**.

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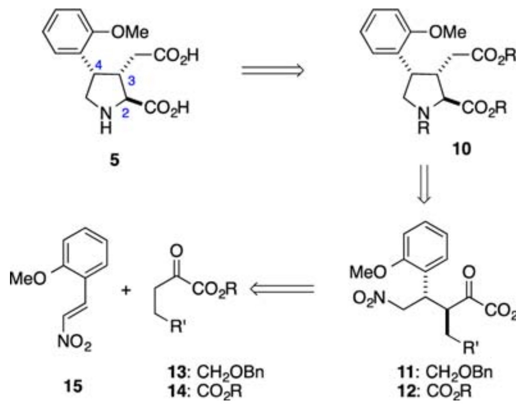
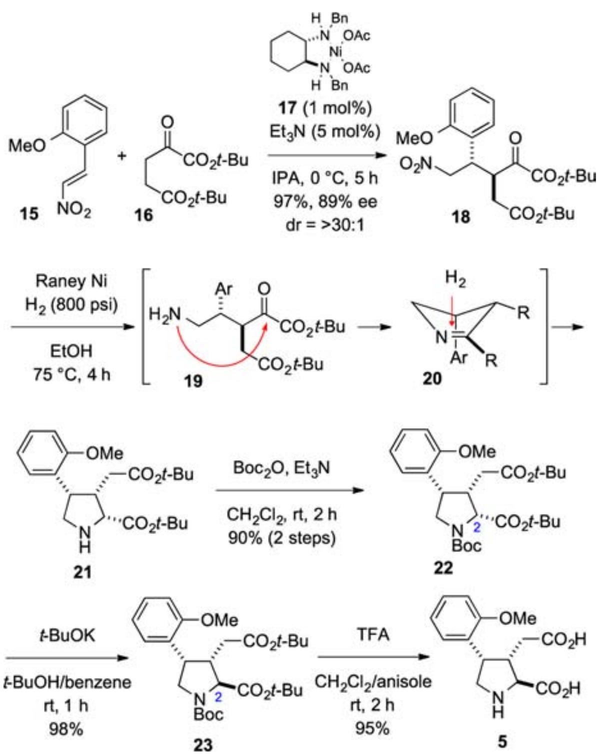
Scheme 1. Retrosynthetic Analysis of 5



The two chiral centers of **12** would be constructed by Ni-catalyzed diastereo- and enantioselective conjugate addition of α-ketoesters **14** to nitroalkene **15**, as developed by one of the authors (Y. Hamashima).[7b]

As shown in Scheme 2, our total synthesis of **5** was achieved in six steps, starting from nitrostyrene derivative **15**.[7b] Reaction

Scheme 2. Synthesis of MFPA (5)

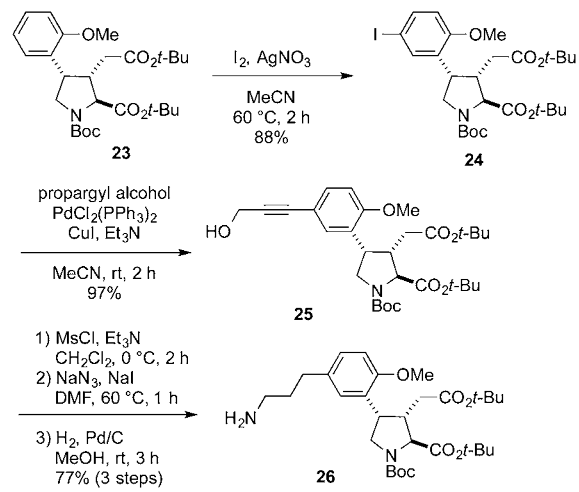


of α-ketoester **16**[8] and **15**[9] in the presence of 1 mol % of our Ni catalyst **17** resulted in a smooth asymmetric conjugate addition reaction to afford **18** exclusively in 97% yield and 89% ee. Sequential construction of the pyrrolidine ring of **21** was accomplished by treatment of **18** with hydrogen (800 psi) and Raney Ni. Tandem reduction of the nitro group, intramolecular imine formation of **19**, and reductive amination of **20** proceeded smoothly to provide the pyrrolidine **21**. In the reduction step, hydride attack occurred from the less hindered β-face of the pyrrolidine ring of **20** to provide a single diastereomer with α-stereochemistry. After protection of the secondary amine of **21** with a Boc group, the pyrrolidine derivative **22** was isolated in 90% yield from **18** in two steps. According to our reported procedure, epimerization of the C-2 position could be accomplished by treatment with a combination of *t*-BuOK and *t*-BuOH/benzene to give **23**. Finally, simultaneous cleavage of the Boc group and the *t*-Bu esters of **23** was carried out by treatment with TFA in the presence of anisole to afford the desired MFPA (**5**). Its spectral data (^{1}H NMR, ^{13}C NMR, IR, and HRMS) were in full agreement with reported values.[3a,b]

With the enantioselective total synthesis of **5** having been accomplished in six steps from *o*-anisaldehyde (overall yield: 62%), we next assessed the affinity of **5** for ionotropic glutamate receptor sites and its excitatory toxicity in mice. As described previously,[5c−e] **5** significantly inhibited [^{3}H]KA binding (K_d 1.8 ± 0.3 nM), while its displacement of [^{3}H]AMPA binding was weaker (K_d 322 ± 80 nM). Intracerebroventricular (i.c.v.) injection in mice resulted in the dose-dependent toxicity typically observed in excitatory amino acids including generalized convulsion at higher doses and catalepsy, stereotyped behaviors, and Straub tail response at lower doses. The ED_{50} value was determined to be 0.046 nmol/mouse. Thus, **5** is more potent than kainic acid (ED_{50} 0.28 nmol/mouse).

We next turned to design a useful kainoid-based precursor molecule that allows incorporation of various probes or attachment to affinity media. Because, in our previous study,[10,11] terminal amine or azide groups extending from the phenolic ring proved useful in incorporating probe functionality, we designed a precursor **26** bearing a linker at the *para*-position of the methoxy group of **5**, as a versatile platform for chemical biological work on the kainoids. Though a bulky fluorophore might interfere with binding of the ligand to the relatively small binding cavity of the kainite type glutamate receptors,[12] we thought that the fluorescence probe **28** might still provide useful data. As shown in Scheme 3, our synthesis of

Scheme 3. Synthesis of Key Intermediate 26



the probe precursor **26**, possessing an amino group, was commenced with protected MFPA **23**. For incorporation of the linker unit, we employed the Sonogashira reaction.[13] Regioselective iodination of the *para*-position of **23** was accomplished by treatment with I_2 and $AgNO_3$ to afford coupling precursor **24** in 88% yield. The reaction of iodide **24** and propargyl alcohol in the presence of catalytic quantities of $PdCl_2(PPh_3)_2$ and CuI in MeCN proceeded smoothly to give

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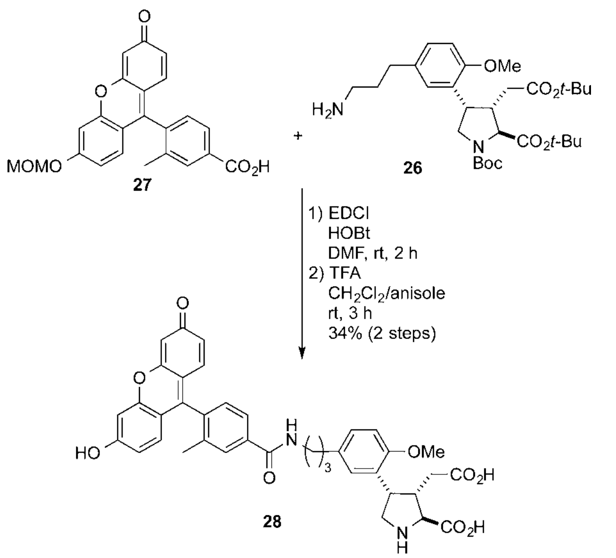
the desired cross-coupling product **25** in high yield.[13] The propargyl alcohol moiety of **25** was converted to propyl amine **26** in three steps via mesylation, displacement with an azido group, and a simultaneous hydrogenation reaction of the azido and alkyne groups.

With the desired probe precursor **26** in hand, we finally focused on incorporation of a fluorescent moiety to obtain the desired probe molecule **28**. For this purpose, we selected a reliable photophore, Tokyo-Green (TG, **27**).[14] As shown in Scheme 4, condensation of the probe precursor **26** and TG **27** with EDCI and HOBt provided **28**. In our preliminary study, an i.c.v. injection of **28** (0.03 μmol/mouse) induced convulsant behaviors in mice followed by death, suggesting that **28** can interact with iGluRs, though much more weakly than **5**. Further investigations of the biological properties including binding affinity and selectivity are ongoing with **28** and will be reported in due course.

Scheme 4. Synthesis of Fluorescent Probe 28



In conclusion, a practical total synthesis of **5** and the design and efficient synthesis of the kainoid probe precursor **26** have been accomplished by applying our Ni-catalyst-mediated asymmetric conjugate addition reaction, together with the efficient construction of the pyrrolidine ring by a tandem reduction reaction. We have also prepared **28**, a new fluorescent probe with a kainoid core that possesses some excitotoxicity properties. The precursor **26** should be suitable for easy incorporation of a range of probe moieties, as well as linker groups, and is expected to afford a range of probe molecules for iGluR-related research.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details and spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(8) Nitroalkene **15** was synthesized from commercially available *o*-anisaldehyde in one step. See the Supporting Information.

(9) Since we performed the enantioselective conjugate addition with α-ketoester **16**, which possessed the same oxidation state for **5**, the improvement of the total synthesis was accomplished in this report. α-Ketoester **16** was synthesized from commercially available dicarboxylic acid in one step. See the Supporting Information.

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