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AN EFFICIENT ENANTIOSPECIFIC SYNTHESIS OF NEUROACTIVE GLUTAMATE ANALOGS

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Abstract – Herein we report improved enantiospecific synthesis and some structure-activity relationships of our heterotricyclic artificial glutamate analogs bearing seven-membered ring for the C-ring. Starting from readily available oxanorbornene *rac*-**3**, optically pure (2*R*)-TKM-107, (2*R*)-IKM-154, and the antipodes were synthesized in total nine steps for each. Mice in vivo assay indicated that only the (2*R*)-enantiomer was active in both cases. Behaviors phenotypes observed in the mice assay suggested that these compounds are similar in mode of action to that of IKM-159 but with discrete potency.

Excitatory synaptic neurotransmission in the mammalian central nervous system is mediated largely by ionotropic glutamate receptors (iGluRs).[1] We have previously developed artificial glutamate analog *rac*-IKM-159 (*rac*-**1**) as a subtype-selective antagonist for the (*S*)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA) type iGluR (Figure 1).[2,3] The heterotricyclic dicarboxylic acid IKM-159 (**1**) was synthesized in those studies along with a series of other analogs based on the structure of naturally derived excitatory (2*S*)-glutamate analogs, kainic acid[4] and neodysiherbaine.[5] Our subsequent synthetic study clearly showed that only (2*R*)-enantiomer **1**, but not the (2*S*)-enantiomer, exhibited the neuroactivity.[6] From the structural study of the GluA2 AMPA receptor ligand-binding domain (LBD) in complex with (2*R*)-IKM-159 (**1**),[6] the interactions were found to be analogous to those with other antagonists including (*S*)-2-amino-3-[5-*tert*-butyl-3-(phosphonomethoxy)-4-isoxazolyl]propionic acid ((*S*)-ATPO)[7] and ZK20077533.[8] However, *K*i value of *rac*-IKM-159 (*rac*-**1**), 0.21 ± 0.02 and 0.56 ± 0.07 mM for the GluA2 LBD, and for the GluA2(R)$_o$ full-length receptor, respectively, were unexpectedly high, indicating a weak interaction of these compounds to the binding pocket.[6] Our pharmacological

studies data supported that *rac*-**1** acted as a competitive antagonist at GluA2 at least in part,[2,6] although the inhibitory activity shown above suggested rather complex mechanism of action.

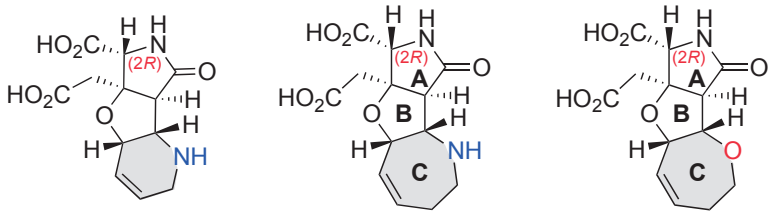
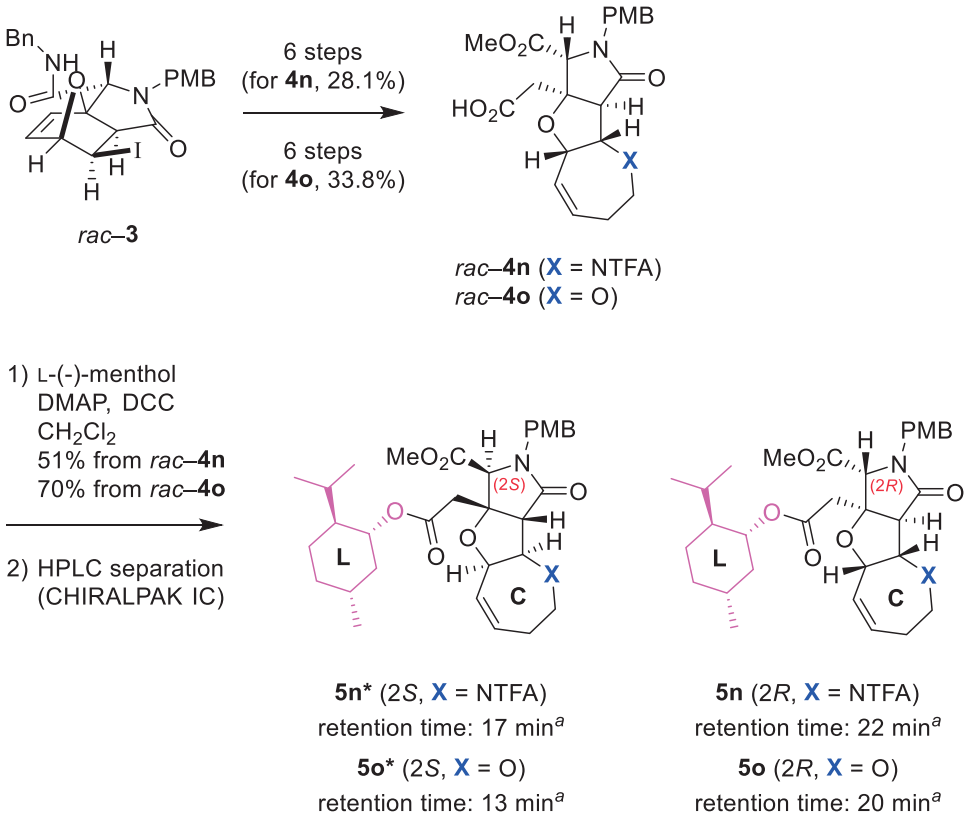


Figure 1. Artificial neuroactive glutamate analogs in the present study
(a) See references 2 and 3; (b) New analog

To clarify the precise mode of actions of our artificial glutamate analogs[9-11] on AMPA receptors, we planned to evaluate the neurological activity of "the enantiomers" of the analogs[3,11] in addition to IKM-159 (**1**), since all analogs except for IKM-159 had been evaluated only in the racemic forms.[2] Herein, we report enantiospecific synthesis of both enantiomers of seven-membered analogs of IKM-159 (**1**) denoted as TKM-107 (**2n** and the antipode) and IKM-154 (**2o** and the antipode)[3,11] (Figure 1), by chiral resolution. The in vivo preliminary evaluations are also reported. Among these analogs, the new TKM-107 (**2n**) analog was found to induce hypoactive behavioral phenotypes in mice for the first time in this study. For both analogs, (2*R*)-enantiomer (see Figure 1) was concluded to be responsible for the neuroactivities, as in the case of IKM-159 (**1**).

Our previous enantiospecific synthesis, reported in 2013 for (2*R*)-IKM-159 (**1**), needed total 18 steps, and the total yield was 0.70%, starting from (*R*)-2-amino-2-(4-methoxyphenyl)ethanol.[6] In this study, we planned to reduce the number of total steps in the synthesis of TKM-107 (**2n** and the antipode) and IKM-154 (**2o** and the antipode), by employing chiral resolution strategy on the racemic synthetic intermediate, using chiral auxiliary. Finally, the approach in this study allows preparation of enantiomerically pure specimens over 30 mg quantities, as follows.

Chiral resolutions of the racemic intermediates into optically pure esters **5**, for both TKM-107 (**2n** and the antipode) and IKM-154 (**2o** and the antipode), are shown in Scheme 1. Starting from *rac*-**3**[10] readily prepared by tandem Ugi/Diels-Alder reaction in a single step,[9] monocarboxylic acids *rac*-**4n** and *rac*-**4o** were synthesized as substrates for chiral resolution over six steps each in 28.1% and 33.8% yields, respectively. The monocarboxylic acids *rac*-**4n**[11] and *rac*-**4o**[10] are preferable intermediates for the racemic glutamate analogs, as the latter (*rac*-**4o**) had previously led to preparation of subgram quantities of *rac*-IKM-154 (*rac*-**2o**) by a sequence of methylation, PMB deprotection, and final acidic hydrolysis.[3,10]



Scheme 1. Chiral resolution of the synthetic intermediate toward TKM-107 and IKM-154
(a) Retention time in the preparative HPLC (CHIRALPAK IC 4.6 × 250 mm, EtOH/hexane = 1/9)

After several trial experiments for chiral resolution, we found esterification with L-(-)-menthol was practical in this study. Thus, the carboxylic acids *rac*-**4n** and *rac*-**4o** were independently subjected to esterification with L-(-)-menthol mediated by DCC and DMAP[12] to give menthyl esters **5n***/**5n** and **5o***/**5o** in 51% and 70% combined yields, respectively. The low yields would be due to the low reactivity of the activated ester derived from the carboxylic acids. Conceptually the yield can be improved as the reaction of *rac*-**4n** proceeded in a higher yield (93%) under Shiina esterification conditions (MNBA, DMAP, Et_3N)[13] in a smaller-scale experiment (not shown). The diastereomeric ratio was determined from ^1H NMR analyses as 1:1 for both **5n***/**5n** and **5o***/**5o**. Although the diastereomers were difficult to separate by silica-gel column chromatography in both cases, however, the separation was possible using HPLC (CHIRALPAK IC 4.6 × 250 mm, EtOH/hexane = 1/9), as denoted in Scheme 1. The diastereomerically pure menthyl esters (**5n***, **5n**; and **5o***, **5o**) were thus obtained.

Unfortunately, none of the compounds in this work was obtained as crystals. The stereochemistries of the menthyl esters were, therefore, determined on the basis of NOESY analysis and conformational analysis. Briefly, as for **5n***, the NOESY cross peaks observed between C-ring olefinic protons and isopropyl protons in the menthyl residue were consistent well with the top four stable conformers (total population: 72.8%) of the (2*S*)-isomer generated by molecular mechanics calculation (MM2, CONFLEX) (Figure 2,

see also the Supplementary data). On the other hand, the NOESY spectrum of the diastereomer **5n** was accounted well by the top two stable conformers (total population: 50.7%) of the (2*R*)-isomer generated by CONFLEX (Figure 3). Thus, the configuration of two menthyl ester diastereomers (2*S*)-**5n*** (retention time t_R: 17 min) and (2*R*)-**5n** (t_R: 22 min), being denoted in Scheme 1, was determined.

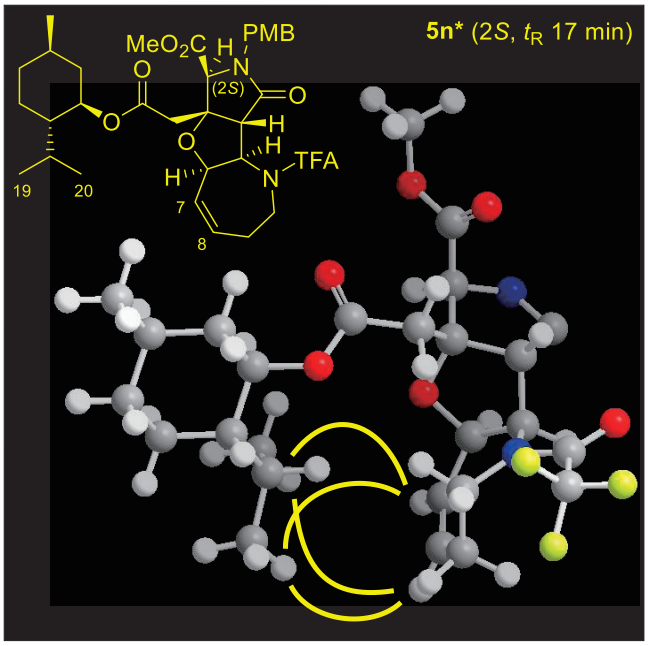


Figure 2. The NOESY cross peaks (yellow lines) observed for **5n*** (t_R 17 min) are consistent well with the most stable conformer (MM2, CONFLEX) for (2*S*)-enantiomer. PMB group is omitted for clarity.

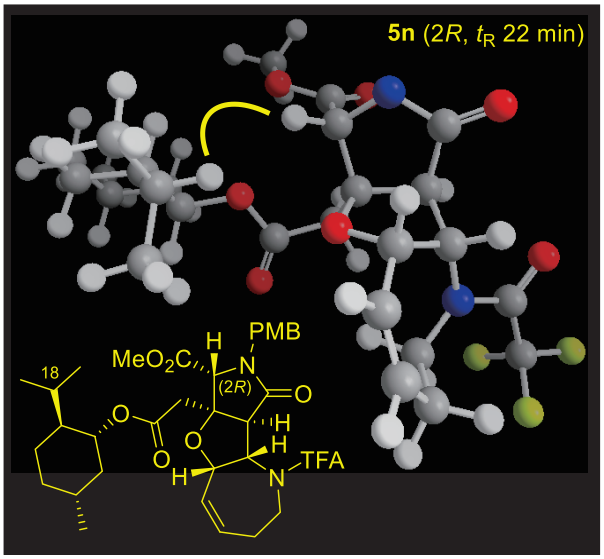
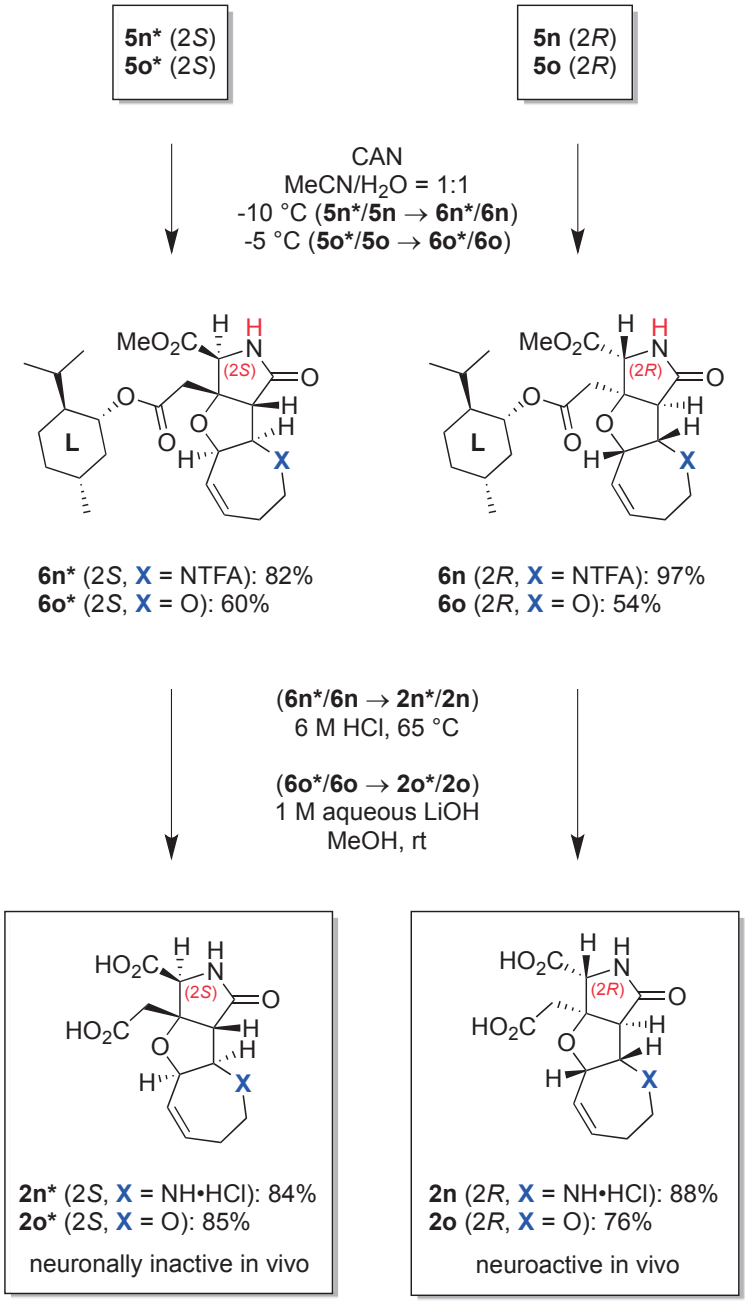


Figure 3. The NOESY cross peak (yellow line) observed for **5n** (t_R 22 min) is consistent well with the most stable conformer (MM2, CONFLEX) for (2*R*)-enantiomer. PMB group is omitted for clarity.

Stereochemistries of the seven-membered ether diastereomers **5o*** (retention time: 13 min) and **5o** (retention time: 20 min) were also determined to be 2*S* and 2*R*, respectively, by the same procedures for **5n***/**5n** described above (see the Supplementary data for details).

The stereochemical analyses carried out in the present study were possible owing to the conformational rigidity of the four consecutive single bonds between heterotricycle and menthyl group, which has been supported well by the crystallographic analysis of another menthyl ester synthetic intermediate directed toward optically active MC-27,[14] an oxa analog of IKM-159 (see Figure 1). The X-ray structural data were recently obtained, and the work will be separately reported soon.



Scheme 2. Final elaboration toward optically active TKM-107 (**2n***, **2n**) and IKM-154 (**2o***, **2o**)

Four diastereomers **5n***/**5n** and **5o***/**5o** were then independently subjected to PMB deprotection by CAN in moderate to excellent yields (Scheme 2). It should be also noted here that, to reduce the number of steps after the chiral resolution, we examined PMB deprotection prior to the chiral resolution, which, however, was found to be impractical, since separation of the *des*-PMB products **6o***/**6o** by both silica-gel column chromatography and HPLC was unsuccessful.

Finally, hydrolytic deprotections of esters and the TFA amide of **6n***/**6n** and **6o***/**6o** were investigated. After several experiments, it was found that acidic hydrolysis (6 M HCl, 65 °C)[3] is suitable for removal of TFA group and two esters of **6n***/**6n** to furnish (2*S*)-**2n*** and (2*R*)-**2n** in good yields: 84% and 88%, respectively. An acidic hydrolysis[3] of the diesters **6o***/**6o**, however, resulted in incomplete reaction. We therefore employed alkaline hydrolysis conditions using aqueous LiOH (MeOH, rt). Under this condition, the reaction went smoothly to completion with good reproducibility to furnish (2*S*)-**2o*** and (2*R*)-**2o** in 85% and 76% yields, respectively (Scheme 2). The chromatographic and spectroscopic data for (2*S*)-**2o***/(2*R*)-**2o** were in good agreement with those reported for the racemate.[3] The purities were >95% for all four final products, as judged from ^{1}H NMR and HPLC. Starting from *rac*-**3**, all syntheses were thus performed in total nine steps, and total yields were 5.0% for (2*S*)-**2n***, 6.1% for (2*R*)-**2n**, 6.1% for (2*S*)-**2o***, and 4.9% for (2*R*)-**2o**, which are large improvement from our previous enantiospecific synthesis of (2*R*)-IKM-159 (**1**, see above).

Behavioral activity of **2n** and **2n*** was assessed using mouse assay as reported previously.[6] An intracerebroventricular injection of **2n** (50 μg/mouse) resulted in suppression of voluntary motion (hypoactivity) immediately after the injection, but the animal became flaccid in ten minutes after injection. This behavioral consequence was similar to that observed in **1**. The enantiomer **2n*** did not show any noticeable behavioral change. These observations support that **2n** has biological activity similar to **1**, suppression of excitatory neurotransmission by interacting with subset of AMPA-type iGluR. The activity of **2o** was much weaker than that of **2n** as injection of 100 μg caused hypoactivity accompanying with moderate ataxia-like movement. This observation is consistent with that observed in the 6-membered analog IKM-159 (**1**).[6] The antipodal **2o*** did not show noticeable effects on the mice volunteer action at the highest dose tested (100 μg/mouse). Further SAR to reveal detailed mechanism of action of the IKM compounds are in progress.

In summary, the synthetic analogs of the heterotricyclic iGluR ligand prepared in the present study clearly demonstrated that the (2*R*)-enantiomer is the one that interacts with the target protein regardless of the heteroatom in the C-ring. It is also evident form the results of the present and previous[2,3,6] studies that the C-ring amines are more active than the C-ring ether. Since the nitrogen atom forms a salt bridge between the distal carboxylate as shown in the crystallographic analysis,[6] it might stabilize active conformation,

especially orientation of the methylenecarboxylate, whose interaction with Ser675 inside the ligand binding domain of the receptor is important in the binding state.[6]

ACKNOWLEDGEMENTS

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SUPPORTING INFORMATION

Supplementary (^{1}H and ^{13}C NMR spectra, HPLC chromatograms, etc.) data associated with this article can be found, in the online version, at URL: https://www.heterocycles.jp/newlibrary/downloads/PDFsi/26220/101/1.

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