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Oxa-Michael-based divergent synthesis of artificial glutamate analogs†

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Herein we report stereoselective generation of two skeletons, 1,3-dioxane and tetrahydropyranol, by oxa-Michael reaction as the key reaction from δ-hydroxyenone. The construction of the 1,3-dioxane skeleton, achieved through hemiacetal formation followed by oxa-Michael reaction from δ-hydroxyenone, was exploited to access structurally diverse heterotricyclic artificial glutamate analogs. On the other hand, formation of a novel tetrahydro-2*H*-pyranol skeleton was accomplished by the inverse reaction order: oxa-Michael reaction followed by hemiacetal formation. Thus, this study succeeded in showing that structural diversity in a compound collection can be acquired by interchanging the order of just two reactions. Among the skeletally diverse, heterotricyclic artificial glutamate analogs synthesized in this study, a neuronally active compound named TKM-50 was discovered in the mice *in vivo* assay.

Ionotropic glutamate receptors (iGluRs) mediate the majority of the excitatory neurotransmissions such as learning, memory, and nociception in the mammalian central nervous system (CNS).[1] To study and control the function of iGluRs, specific glutamate analogs have been developed in natural product chemistry[2] and in medicinal chemistry.[3,4] IKM-159 (Fig. 1A) is an artificial glutamate analog designed and developed based on dysiherbaine[5,6] and kainic acid[7] in our laboratories as an antagonist selective to (*S*)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA)-type iGluR.[8–12] The AMPA receptor consists of four subunits: GluA1, GluA2, GluA3, and GluA4.[3] *In vitro*, IKM-159 selectively inhibits the GluA1/GluA2 heterodimer and GluA4 homodimer.[10] *In vivo*, IKM-159 inhibits voluntary action of mice for 50 min to several hours upon intracerebroventricular injection. The potency and selectivity of IKM-159 are, however, not very satisfactory to selectively modulate the function of AMPA-type iGluR. As an attempt to improve the biological profiles of IKM-159, we have been studying its structural modification.

From the first-generation studies on structure–activity relationships (SARs) of IKM-159, it had been shown that the ring size and the heteroatom of the C-ring were important for neuroactivity of IKM-159.[10,13] We then studied the second-generation SAR on the oxa analogs generated by a Prins-Ritter three-component coupling strategy, although all analogs were found to lose the original neuronal activity of IKM-159.[14] Herein, we report our continuous effort along this line employing the homoallylic alcohol such as **5** and **7** (see Scheme 2) as the common intermediates.[15]

One of the strategies in this work is the thermodynamically controlled, stereoselective formation of 1,3-dioxane (**1** in Fig. 1B) by hemiacetal formation followed by oxa-Michael reaction from δ-hydroxyenone derivative that we recently developed (Scheme 1).[16] The other strategy is the novel stereoselective formation of tetrahydropyranol (**2** in Fig. 1B) by the inverse reaction order; oxa-Michael reaction followed by hemiacetal formation (see Scheme 5). Thus, this study succeeded in showing that structural diversity in a compound collection can be acquired by interchanging the order of just two reactions; hemiacetal formation and oxa-Michael reaction. Among the skeletally diverse, heterotricyclic artificial glutamate analogs thus synthesized, a compound named TKM-50 (**1ar**) was discovered to be neuronally active in the mice *in vivo* assay.

The substrate used for the 1,3-dioxane formation was prepared from the known dimethyl ester **5** (Scheme 2).[17]

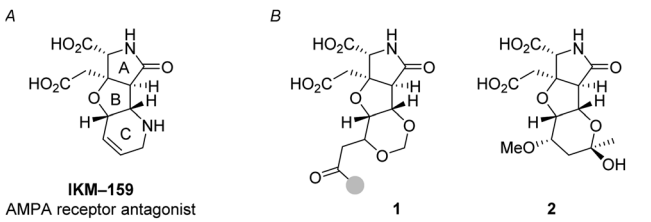

Fig. 1 Background (A) and summary (B) of this work. (A) AMPA-type iGluR antagonist IKM-159. (B) Artificial glutamate analogs **1** and **2**, generated by oxa-Michael-based transformations (this work). The gray circle in **1** denotes the position for the structural diversity.

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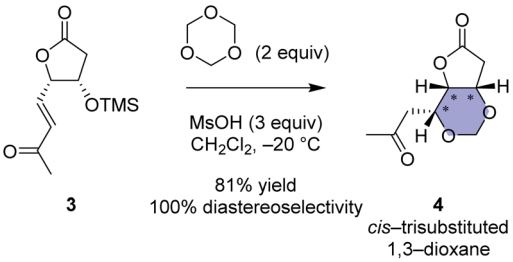
† Electronic supplementary information (ESI) available. See https://doi.org/10.1039/d2ra03744k

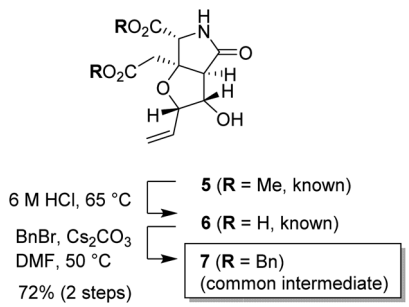


Scheme 1 Our recent work regarding stereoselective 1,3-dioxane formation.[16] For clarity and comparison, enantiomers of the reported compounds are shown in this scheme.

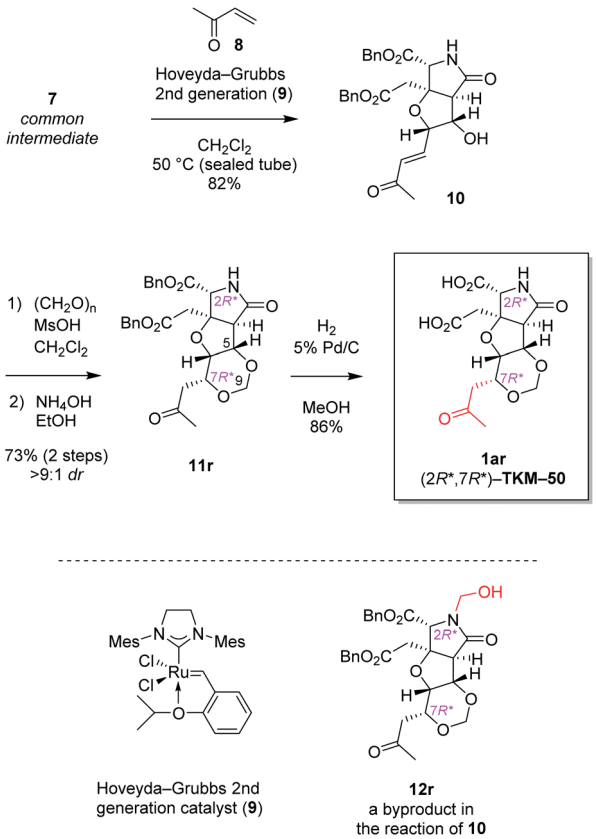
Exposure of dimethyl ester **5** to hydrochloric acid (6 M) at 65 °C provided dicarboxylic acid **6**.[17] Without purification, dicarboxylic acid **6** was treated with BnBr and Cs_2CO_3 to furnish the common intermediate **7** in 72% yield (2 steps).[18]

The alkene **7** was subjected to cross metathesis with methyl vinyl ketone (**8**) mediated by Hoveyda-Grubbs second generation catalyst (**9**)[19] to provide enone **10** in 82% yield (Scheme 3). Upon exposure to paraformaldehyde as an equivalent of formaldehyde and 1,3,5-trioxane[16] in the presence of MsOH, 1,3-dioxane ring formed smoothly by oxa-Michael reaction to give rise to desired (7*R**)-heterotricycle **11r** and the (7*S**) epimer **11s** (structure not shown) in the ratio of >9 : 1, as well as the *N*-hydroxymethylated product **12r** (see Scheme 3) and the (7*S**) epimer **12s** (structure not shown). Since we had found that alkaline hydrolysis is of use to remove the *N*-hydroxymethyl group, the mixture of hemiaminals (**12r**/**12s**) and free amides (**11r**/**11s**) was treated with ammonium hydroxide[20] to obtain free amide **11r** in 73% isolated yield (2 steps), and free amide **11s** in 10% yield (estimated by NMR, 2 steps). The formation of 1,3-dioxane ring of **11r** was determined by the HMBC correlations (Fig. 2A), and the stereochemical configuration was established by a $^3J_{H,H}$ value and NOESY correlations denoted in Fig. 2B. Both configuration and conformation of **11r** are identical to those we observed recently in the simple case (**3** → **4**, see Scheme 1),[16] showing that the 1,3-dioxane formation in this study is also thermodynamically controlled (see below for the mechanism).

The proposed mechanism for the 1,3-dioxane formation is shown in Scheme 4A. Reaction of alcohol **10** and paraformaldehyde would form hemiacetal intermediate **A** under acidic conditions, which, then undergoes intramolecular oxa-



Scheme 2 Preparation of the common intermediate **7** (racemate).



Scheme 3 Stereoselective 1,3-dioxane formation leading to heterotricyclic artificial glutamate analog **1ar**.[a] [a]dr denotes the diastereoselectivity in the 1,3-dioxane formation.

Michael reaction to give **11r** and **11s**. Since the second conjugate addition is generally a thermodynamically controlled, reversible process, production of more stable (7*R**) isomer **11r** predominated over the (7*S**) epimer **11s**, as discussed also in our preliminary study.[16] It should be also noted here that, in that preliminary study employing a simple substrate, the (7*S*) epimer had not been obtained.[16] Generation of the less stable (7*S**) epimer **11s** in this study would be due to unfavorable steric interactions between the acetyl group and the benzyl ester on the near side in **11r** (Scheme 4B), that make the energy difference between the two diastereomers (**11r** and **11s**) smaller.

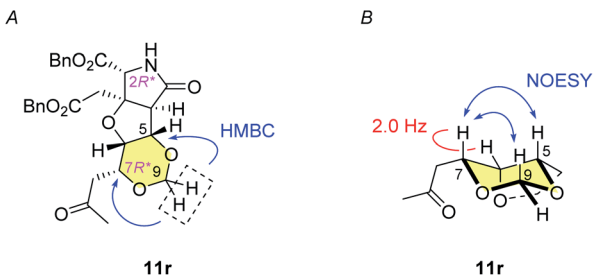
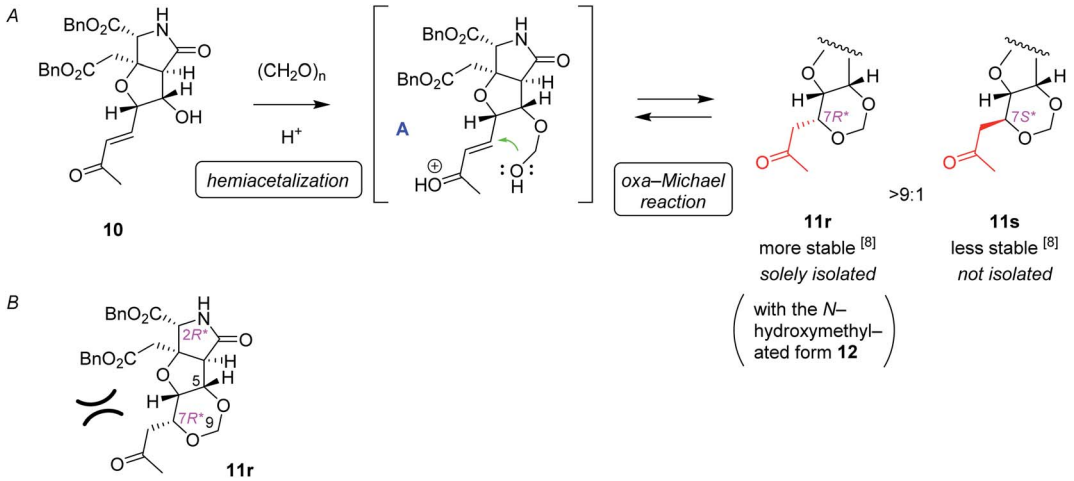


Fig. 2 Structure analysis of 1,3-dioxane **11r**. (A) HMBC correlations in **11r** indicates formation of the 1,3-dioxane ring. (B) Small $^3J_{H,H}$ value and NOESY correlations show the configuration and the conformation of **11r**.



Scheme 4 The plausible mechanism of 1,3-dioxane ring formation. (A) Stepwise mechanism that consists of hemiacetal formation followed by intramolecular oxa-Michael reaction. (B) The steric repulsion included in the stable isomer **11r**.

Then two benzyl groups of **11r** were removed by hydrogenolysis[21] to cleanly provide glutamate analog **1ar** ((2*R**,7*R**)-TKM-50) in 86% yield (Scheme 3).

With the same reaction sequences for **1ar** (Scheme 3), two more analogs **1br** and **1cr** were furthermore synthesized (Fig. 3). The marked decrease in diastereoselectivity in these oxa-Michael reactions (see Fig. 3) suggests that the steric repulsion between the pentyl/methoxyphenyl group and the benzyl ester on the near side is extremely large. The minor (7*S**) diastereomers obtained in these oxa-Michael reactions were also isolated and deprotected to give **1bs** and **1cs** (see the ESI†), which were subjected to *in vivo* assay (see below).

We also found that another skeleton can be constructed from δ-hydroxyenone being used for 1,3-dioxane formation, under alkaline hydrolytic conditions. Thus, as shown in Scheme 5, the δ-hydroxyenone **13** derived from homoallylic alcohol **5** by cross metathesis was selectively transformed into cyclic hemiacetal **2** in 53% yield (1 M LiOH in water, MeOH, rt). In this transformation, dimethyl ester and δ-hydroxyenone moiety independently suffer hydrolysis and cyclization, respectively, to generate glutamate analog **2** efficiently. The configuration of **2** was determined by combined analysis of NMR and DFT calculation (see the ESI†).[22]

The plausible mechanism for the hemiacetal formation is shown in Scheme 6. In view of the fact that the hydroxy and carbonyl groups are located apart in **13**, the six-membered-ring formation should take place after saturation of the *trans*-alkene. It is, therefore, supposed that oxa-Michael reaction of MeOH to enone **13** first generates saturated ketone **C** *via* enolate **B**.[23] Under alkaline conditions, the alkoxide **C** intramolecularly attacks carbonyl group to give rise to hemiacetal **2**. Considering the fact that oxa-Michael reaction and the acetalization are thermodynamically controlled, reversible processes, energetically favorable diastereomer **2** would have been obtained predominantly (see the ESI† for discussions on thermodynamic stability of **2**). A related example had been reported in 1992 by Shing *et al.*[24]

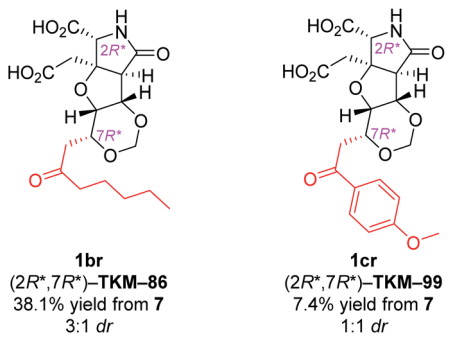
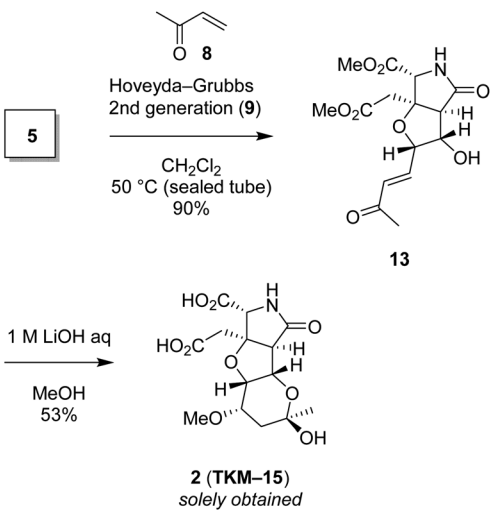
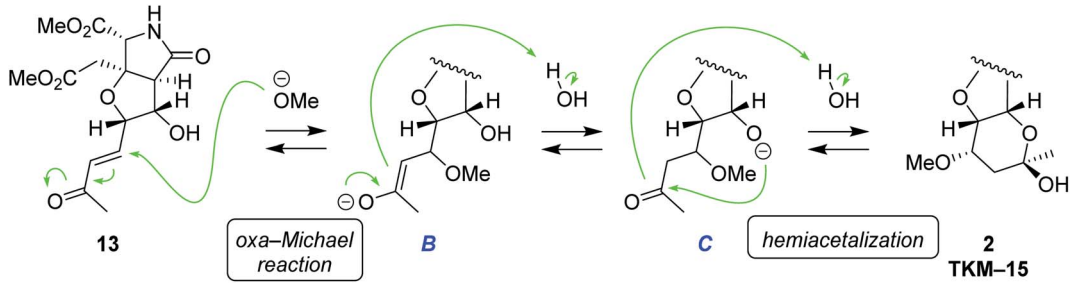


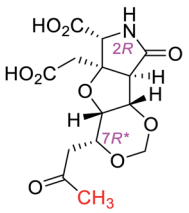
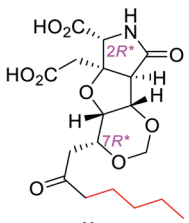
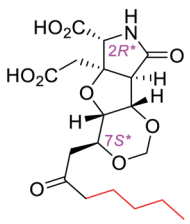
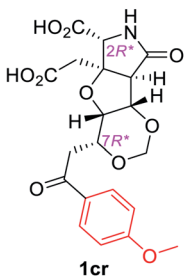
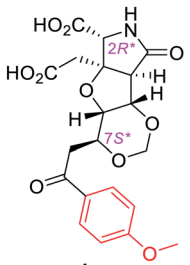
Fig. 3 Other 1,3-dioxane analogs synthesized by the intramolecular oxa-Michael reaction.[a] [a]dr denotes the diastereoselectivity in the 1,3-dioxane formation.



Scheme 5 The heterotricyclic artificial glutamate analog **2**, constructed by intermolecular oxa-Michael reaction of MeOH followed by acetalization.



Scheme 6 The plausible mechanism for hemiacetal formation under alkaline conditions.

	(2*R**,7*R**)	(2*R**,7*S**)
methyl class	 **1ar** (2*R**,7*R**)–**TKM–50** *hypoactive*	not available
pentyl class	 **1br** (2*R**,7*R**)–**TKM–86** *inactive*	 **1bs** (2*R**,7*S**)–**TKM–86** *inactive*
methoxyphenyl class	 **1cr** (2*R**,7*R**)–**TKM–99** *inactive*	 **1cs** (2*R**,7*S**)–**TKM–99** *inactive*

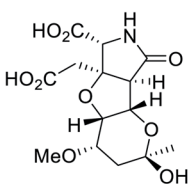

2
TKM–15
inactive

Fig. 4 The *in vivo* activities on mice.

Behavioral activities of all six compounds upon intracerebroventricular (i.c.v.) injection were evaluated in mice (Fig. 4).[25] Injection of **1ar** (TKM-50, 50 μg per mouse) resulted in loss of voluntary motor activity for 10 min after injection and then ataxia-like motions were recorded, thus annotated as hypoactive. The hypoactivity observed for **1ar** (TKM-50) is thus somewhat weaker than IKM-159 which causes loss of mice spontaneous activity for up to 4 h.[12] Other congeners, however, did not cause any noticeable behavioral changes at the same dose tested.

Conclusions

In this paper, we reported synthesis of skeletally diverse artificial glutamate analogs from a common precursor. Since we employed thermodynamically controlled, reversible process for the key cyclizations, most of the reactions proceeded stereoselectively. The cases that were less selective (**1br** and **1cr** in Fig. 3) could even be reasonably explained, supporting the origin of the stereoselectivity we proposed in Scheme 4.[16]

It is of interest to note that the formed skeleton changes significantly, just by interchanging the order of the oxa-Michael reaction and the hemiacetalization (see Schemes 4 and 6). Therefore, it is expected that our methodology is generally of use for discovery of biologically active small molecules.[26] In fact, we succeeded in identifying neuroactive compound (**1ar**, TKM-50) in this study.

We are currently working on the construction of a larger compound library using this methodology and the development of alternative methodology for generation of other skeletons. The results will be reported in due course.

Author contributions

ST: investigation, writing the first draft and editing; OH: investigation and editing; KM: investigation; RI: formal analysis and editing; RS: funding acquisition, investigation and writing the first draft; MO: conceptualization, formal analysis, funding acquisition, project administration, supervision, writing the first draft and final editing.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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