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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.

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Ionic 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 chemistry² 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 acid⁷ 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

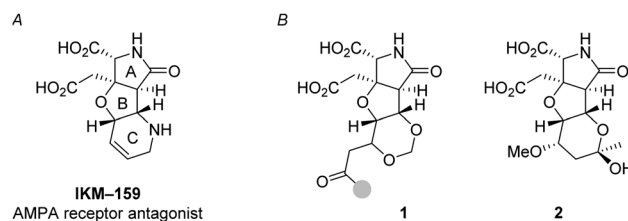


Fig. 1 Background (A) and summary (B) of this work. (A) AMPA-type iGluR antagonist IKM-159. (B) Artificial glutamate analogs **1** and **2**, generated by oxa-Michael-based transformations (this work). The gray circle in **1** denotes the position for the structural diversity.

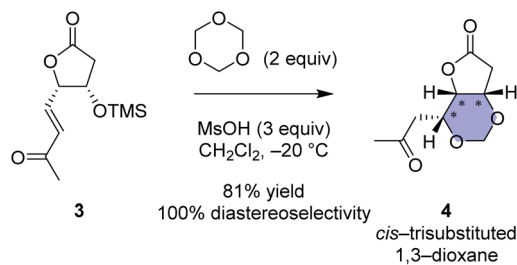
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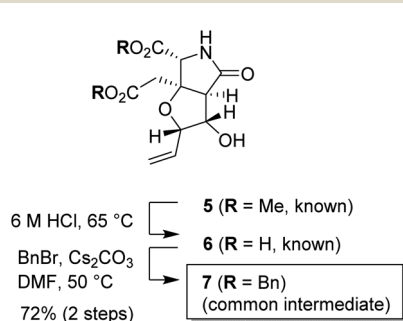


Scheme 1 Our recent work regarding stereoselective 1,3-dioxane formation.¹⁶ For clarity and comparison, enantiomers of the reported compounds are shown in this scheme.

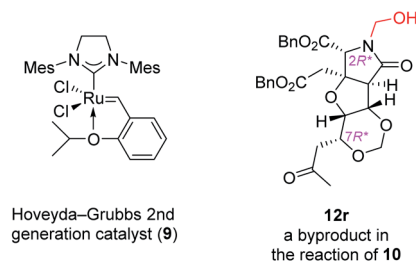
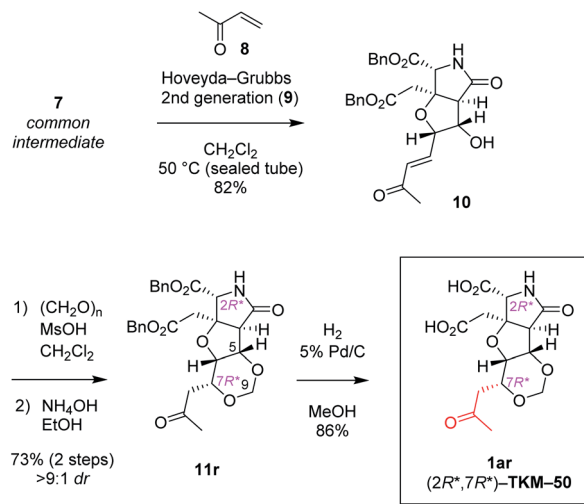
Exposure of dimethyl ester **5** to hydrochloric acid (6 M) at 65 °C provided dicarboxylic acid **6**.¹⁷ Without purification, dicarboxylic acid **6** was treated with BnBr and Cs₂CO₃ to furnish the common intermediate **7** in 72% yield (2 steps).¹⁸

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-trioxane¹⁶ 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 hydroxide²⁰ 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 ^adr 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.

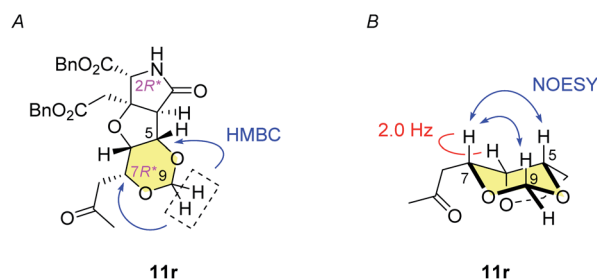
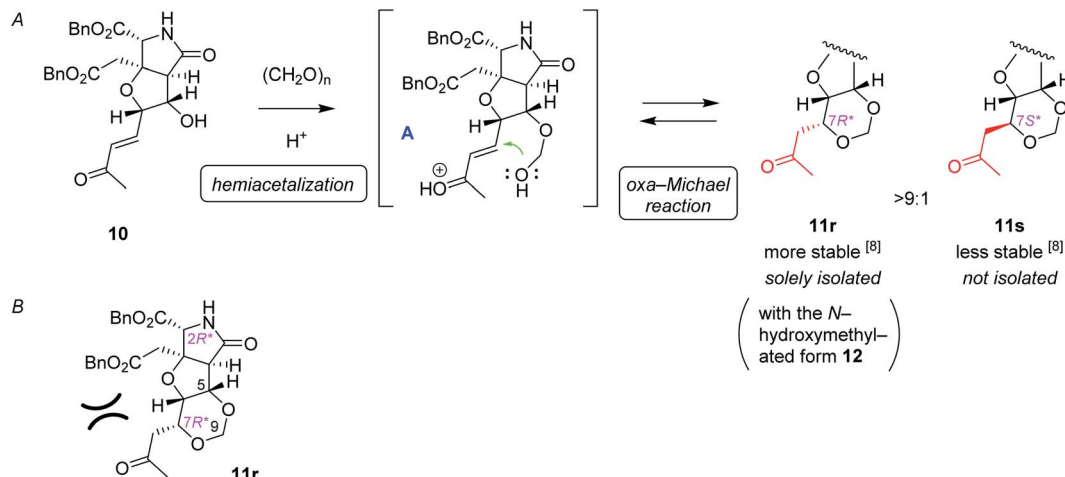


Fig. 2 Structure analysis of 1,3-dioxane **11r**. (A) HMBC correlations in **11r** indicates formation of the 1,3-dioxane ring. (B) Small ³J_{H,H} value and NOESY correlations show the configuration and the conformation of **11r**.





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 hydrogenolysis²¹ 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**

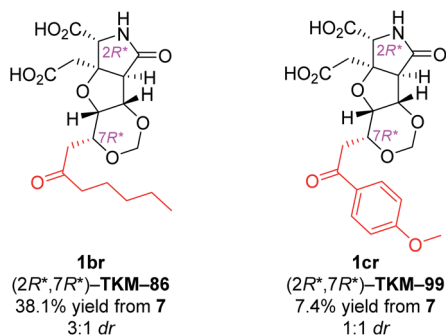
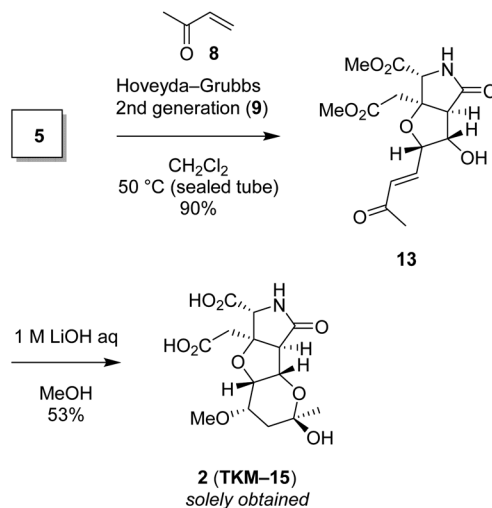


Fig. 3 Other 1,3-dioxane analogs synthesized by the intramolecular oxa-Michael reaction.^a *dr* denotes the diastereoselectivity in the 1,3-dioxane formation.

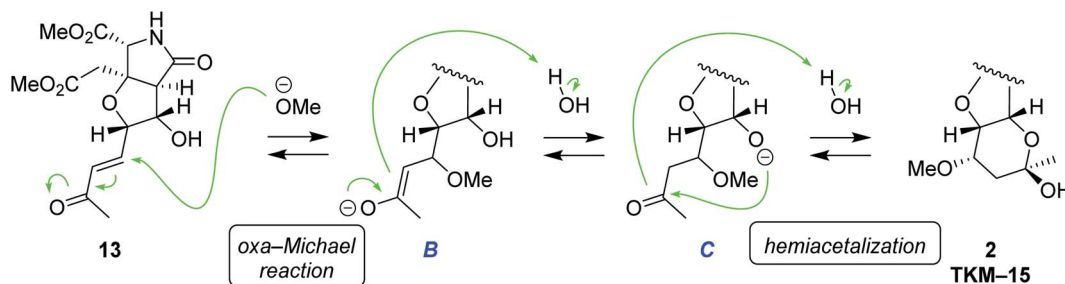
was determined by combined analysis of NMR and DFT calculation (see the ESI†).²²

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



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 <i>R</i> *,7 <i>R</i> *)	(2 <i>R</i> *,7 <i>S</i> *)
methyl class	<p>1ar (2<i>R</i>*,7<i>R</i>*)-TKM-50 <i>hypoactive</i></p>	not available
pentyl class	<p>1br (2<i>R</i>*,7<i>R</i>*)-TKM-86 <i>inactive</i></p>	<p>1bs (2<i>R</i>*,7<i>S</i>*)-TKM-86 <i>inactive</i></p>
methoxyphenyl class	<p>1cr (2<i>R</i>*,7<i>R</i>*)-TKM-99 <i>inactive</i></p>	<p>1cs (2<i>R</i>*,7<i>S</i>*)-TKM-99 <i>inactive</i></p>

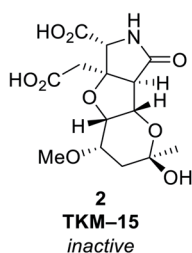


Fig. 4 The *in vivo* activities on mice.

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.



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Notes and references

- 1 G. Riedel, B. Platt and J. Micheau, *Behav. Brain Res.*, 2003, **140**, 1–47.
- 2 G. T. Swanson and R. Sakai, in *Marine Toxins as Research Tools*, ed. N. Fusetani and W. Kem, Springer Berlin Heidelberg, Berlin, Heidelberg, 2009, pp. 123–157.
- 3 P. Stawski, H. Janovjak and D. Trauner, *Bioorg. Med. Chem.*, 2010, **18**, 7759–7772.
- 4 S. K. Bagal, A. D. Brown, P. J. Cox, K. Omoto, R. M. Owen, D. C. Pryde, B. Sidders, S. E. Skerratt, E. B. Stevens, R. I. Storer and N. A. Swain, *J. Med. Chem.*, 2013, **56**, 593–624.
- 5 R. Sakai, H. Kamiya, M. Murata and K. Shimamoto, *J. Am. Chem. Soc.*, 1997, **119**, 4112–4116.
- 6 R. Sakai, T. Koike, M. Sasaki, K. Shimamoto, C. Oiwa, A. Yano, K. Suzuki, K. Tachibana and H. Kamiya, *Org. Lett.*, 2001, **3**, 1479–1482.
- 7 A. F. Parsons, *Tetrahedron*, 1996, **52**, 4149–4174.
- 8 M. Ikoma, M. Oikawa, M. B. Gill, G. T. Swanson, R. Sakai, K. Shimamoto and M. Sasaki, *Eur. J. Org. Chem.*, 2008, **2008**, 5215–5220.
- 9 M. Oikawa, M. Ikoma, M. Sasaki, M. B. Gill, G. T. Swanson, K. Shimamoto and R. Sakai, *Eur. J. Org. Chem.*, 2009, **2009**, 5531–5548.
- 10 M. B. Gill, S. Frausto, M. Ikoma, M. Sasaki, M. Oikawa, R. Sakai and G. T. Swanson, *Br. J. Pharmacol.*, 2010, **160**, 1417–1429.
- 11 M. Oikawa, M. Ikoma, M. Sasaki, M. B. Gill, G. T. Swanson, K. Shimamoto and R. Sakai, *Bioorg. Med. Chem.*, 2010, **18**, 3795–3804.
- 12 L. Juknaite, Y. Sugamata, K. Tokiwa, Y. Ishikawa, S. Takamizawa, A. Eng, R. Sakai, D. S. Pickering, K. Frydenvang, G. T. Swanson, J. S. Kastrop and M. Oikawa, *J. Med. Chem.*, 2013, **56**, 2283–2293.
- 13 M. Oikawa, Y. Kasori, L. Katayama, E. Murakami, Y. Oikawa and Y. Ishikawa, *Synthesis*, 2013, **45**, 3106–3117.
- 14 M. Chiba, Y. Ishikawa, R. Sakai and M. Oikawa, *ACS Comb. Sci.*, 2016, **18**, 399–404.
- 15 Racemate synthesis was studied in this study, however (2*R*)-enantiomer has been shown to be neuronally active in IKM-159. See ref. 12.
- 16 O. Hlokoane, S. Tsukamoto, R. Irie and M. Oikawa, *Chem. Lett.*, 2021, **50**, 1464–1466.
- 17 M. Chiba, C. Fujimoto, R. Sakai and M. Oikawa, *Bioorg. Med. Chem. Lett.*, 2015, **25**, 1869–1871.
- 18 J. C. Lee, Y. S. Oh, S. H. Cho and J. D. Lee, *Org. Prep. Proced. Int.*, 1996, **28**, 480–483.
- 19 S. B. Garber, J. S. Kingsbury, B. L. Gray and A. H. Hoveyda, *J. Am. Chem. Soc.*, 2000, **122**, 8168–8179.
- 20 Z. Galla, E. Forró and F. Fülöp, *Tetrahedron: Asymmetry*, 2016, **27**, 729–731.
- 21 W. H. Hartung and R. Simonoff, *Org. React.*, 1953, **7**, 263–326.
- 22 In our preliminary experiments, this transformation can be realized also in the presence of other alcohols such as EtOH or BnOH instead of MeOH.
- 23 When monitoring the progress of the reaction **13** → **2** by LCMS and TLC, oxa-Michael product which is a protonated form for intermediated C (see Scheme 6) was detected as the reaction intermediate. Finally, the reaction product was converged to **2** via the oxa-Michael product, supporting the reaction is based on a stepwise mechanism we proposed in Scheme 6. This observation will be summarized and discussed in detail in a separate paper in the future.
- 24 T. K. M. Shing, Z.-H. Zhou and T. C. W. Mak, *J. Chem. Soc., Perkin Trans. 1*, 1992, 1907–1910.
- 25 H. Uchimasu, K. Matsumura, M. Tsuda, K. Kumagai, M. Akakabe, M. J. Fujita and R. Sakai, *Tetrahedron*, 2016, **72**, 7185–7193.
- 26 C. J. Gerry and S. L. Schreiber, *Nat. Rev. Drug Discovery*, 2018, **17**, 333–352.

