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Three-Dimensional Structural Diversity-Oriented Peptidomimetics Based on the Cyclopropylic Strain

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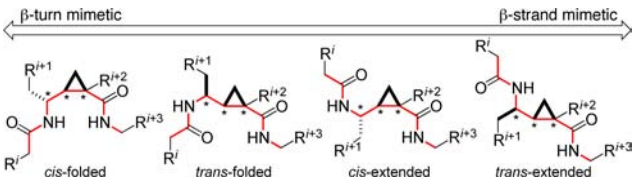
ABSTRACT



Conformationally restricted peptidomimetics comprising eight stereoisomeric scaffolds with three-dimensional structural diversity were designed based on the structural features of cyclopropane, that is, cyclopropylic strain, which mimic wide-ranging tetrapeptide conformations covering β-turns through β-strands. Stereoselective synthesis of the designed peptidomimetics led to the identification of nonpeptidic melanocortin-4 receptor ligands.

A number of peptidomimetic scaffolds have been reported to date.[1] Peptide conformations are highly flexible and dynamically changeable; therefore, the bioactive conformations of bioactive peptides are diverse and often unidentified.[2] Accordingly, the development of nonpeptidic leads using peptidomimetics is often unsuccessful because most of the known peptidomimetic scaffolds mimic only one of the diverse peptide secondary structures, such as β-turns,[3] γ-turns,[4] or β-strands.[5] We here report conformationally restricted peptidomimetics with three-dimensional (3D) structural diversity covering a wide range of peptide secondary structures based on the characteristic structural features of cyclopropane, which can be effectively used to identify nonpeptidic leads in various cases.

Cyclopropane can fix the substituents into the *trans*- or *cis*-configuration, which is a widely applied structural feature of cyclopropane.[6] Furthermore, the *cis*-configurated substituents exert significant mutual steric repulsion, restricting the conformation of the adjacent sp^3-carbon (C1′) so that the repulsion is minimal, as indicated in Figure 1, which we previously confirmed by the X-ray crystal structure of NMDA receptor ligand **1**, and termed "cyclopropylic strain".[7] Wipf et al. later reported peptidomimetic

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molecule **2**,[8] and the X-ray crystal structure shows that the cyclopropylic strain effectively works to restrict the conformation.

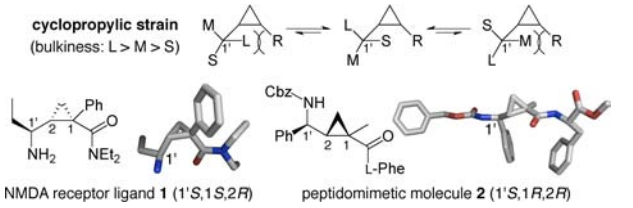

Figure 1. Diagram of the cyclopropylic strain, and the X-ray crystal structures of **1** and **2**.

We hypothesized that peptidomimetics with 3D structural diversity could be developed using the cyclopropylic strain. We first designed the tetrapeptide-mimetic scaffolds derived from the structures of **1** and **2**, which comprise eight stereoisomers **I**–**IV** and ***ent*-I**–***ent*-IV** in Figure 2. In each stereoisomer, the backbone (indicated in red) is not only restricted into the "*trans*" or "*cis*" configuration by the cyclopropane ring itself, but is also further constrained to the "folded" or "extended" form due to the cyclopropylic strain, depending on the stereochemistry of C1′. Accordingly, the "*cis*-folded" (**I**) and "*trans*-extended" (**IV**) scaffolds mimic the β-turns and β-strands of the tetrapeptides, respectively, while the "*trans*-folded" (**II**) and "*cis*-extended" (**III**) scaffolds mimic the conformations halfway between the β-turns and β-strands. In addition, the absolute 3D positioning of the side-chain functional groups in each of the scaffolds **I**–**IV** differs from those of the enantiomeric scaffolds ***ent*-I**–***ent*-IV** (up-series vs down-series, based on the direction of the cyclopropane ring), which would be effective for searching a broad chemical space to determine the bioactive conformation, thereby identifying nonpeptidic leads.

To demonstrate the utility of the cyclopropylic strain-based peptidomimetics with 3D structural diversity, we identified nonpeptidic melanocortin receptor (MCR) ligands. The common core sequence His-Phe-Arg-Trp in endogenous MCR ligands is essential for their biological

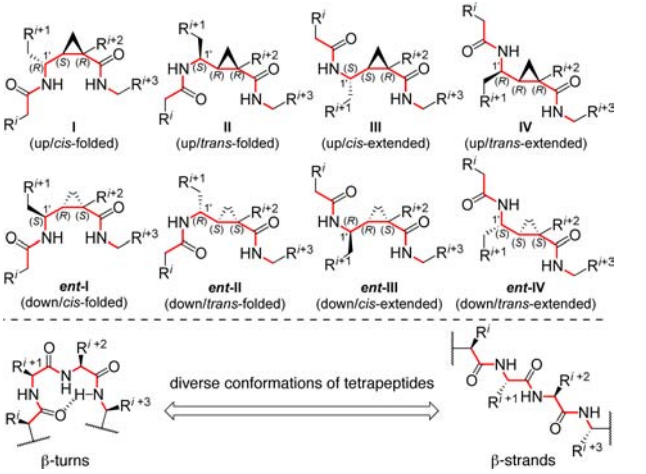

Figure 2. Designed tetrapeptide-mimetic scaffolds.

activities;[9] therefore, we designed peptidomimetics **3**–**6** and ***ent*-3**–***ent*-6** (Figure 3) by allocating the functional groups corresponding to the core tetrapeptide sequence side chains into R^{i}–R^{i+3}. The 2-naphthyl moiety (Nap) was used as bioisostere for the indole ring of Trp.[9c]

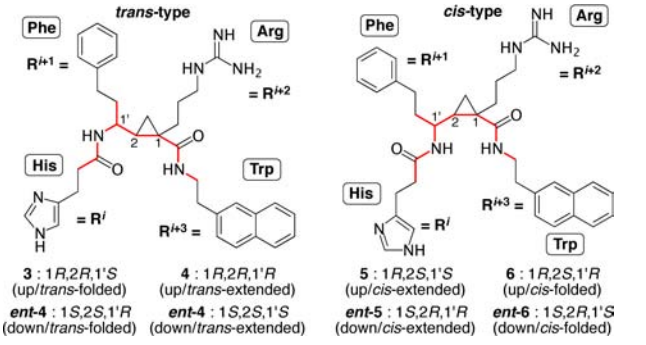

Figure 3. Designed MCR ligands.

In the previous synthesis of **1** and **2**, corresponding to the scaffolds ***ent*-I** and **IV**, respectively, both the stereochemistry and functional groups introduced were limited. This study, however, required a common procedure for synthesizing all eight scaffolds **I**–**IV** and ***ent*-I**–***ent*-IV** stereoselectively, where the side-chain substituents R^{i}–R^{i+3} must be potentially replaceable. Our general synthetic scheme is shown in Scheme 1. We planned to prepare both the *trans*- and *cis*-type mimetics from known chiral cyclopropane derivative **7** or ***ent*-7**, which are readily prepared in high optical purity from (*R*)- or (*S*)-epichlorohydrin, respectively.[10] The R^{i+2} and R^{i+3} moieties, respectively, would be regioselectively introduced into the two ester moieties of **7** (***ent*-7**).

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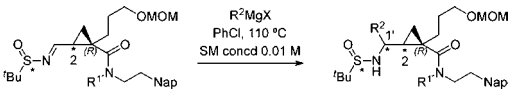
(10) Sekiyama, T.; Hatsuya, S.; Tanaka, Y.; Uchiyama, M.; Ono, N.; Iwayama, S.; Oikawa, M.; Suzuki, K.; Okunishi, M.; Tsuji, T. *J. Med. Chem.* **1998**, *41*, 1284.

Table 1. Conditions for the Diastereoselective Grignard Reaction

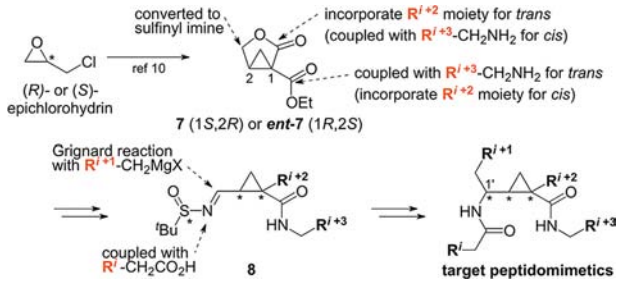


entry	*trans*/*cis*	substrate	R^1	R^2 MgX	products[a]
1	*trans* (2*R*)	**17*R*** (R_S)	H	$PhCH_2CH_2MgCl$	**18*RS*** (1′*S*, 89%), **18*RR*** (1′*R*, 5%)
2	*trans* (2*R*)	**17*S*** (S_S)	H	$PhCH_2CH_2MgCl$	**18*SR*** (1′*R*, 30%)[b]
3	*trans* (2*R*)	**17*S*** (S_S)	H	PhC≡CMgBr	**19*SS*** (1′*S*, 82%), **19*SR*** (1′*R*, 10%)
4	*cis* (2*S*)	**28*R*** (R_S)	allyl	$PhCH_2CH_2MgCl$	**29*RS*** (1′*S*, 3%), **29*RR*** (1′*R*, 78%)
5[c]	*cis* (2*S*)	**28*R*** (R_S)	allyl	$PhCH_2CH_2MgCl$	**29*RS*** (1′*S*, 66%), **29*RR*** (1′*R*, 22%)
6	*cis* (2*S*)	**28*S*** (S_S)	allyl	$PhCH_2CH_2MgCl$	**29*SR*** (1′*R*, 88%)[d]

[a] Stereochemistry of C1′ and isolated yields are shown in parentheses. [b] Diastereomeric ratio was not determined. [c] 10 equiv of HMPA was added. [d] Single isomer.

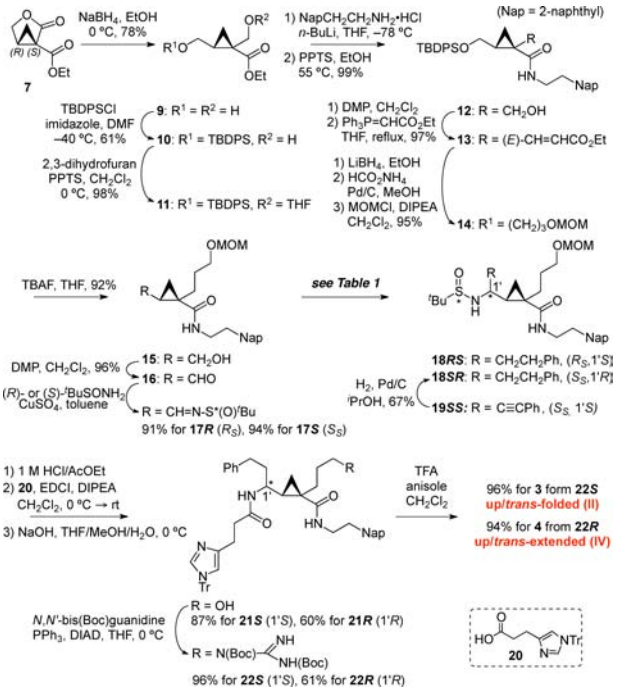
The asymmetric center C1′ would be constructed by a diastereoselective Grignard addition[11] of the R^{i+1} moiety to the (*R*)- or (*S*)-*N*-*tert*-butanesulfinyl imine **8**. Finally, the resulting amino group of the Grignard product would be coupled with the R^i moiety to afford the target peptidomimetics.

Scheme 1. General Synthetic Scheme for the Designed Mimetics



The synthetic schemes for the up/*trans*-type mimetics **3** and **4**, and for the up/*cis*-type mimetics **5** and **6** are shown in Schemes 2 and 3, respectively. The conditions of the key diastereoselective Grignard reaction are summarized in Table 1. For the *trans*-type mimetics **3** and **4** (scaffolds **II** and **IV**), after functional group manipulation of **7**, 2-(2-naphthyl)-ethylamine (R^{i+3}) was introduced into the ethyl ester moiety of **11**. After two-carbon elongation in the R^{i+2} chain, the product was converted to the *N*-*tert*-butanesulfinyl imines **17*R*** and **17*S***,[12] corresponding to **8** in Scheme 1. For the diastereoselective Grignard addition of $PhCH_2CH_2MgCl$ (R^{i+1}) to the imine **17*R*** (Table 1, entry 1), low substrate concentration/high temperature conditions proved effective, consistent with our previous results.[13] Thus, the reaction using 0.01 M solution of **17*R*** in PhCl at 110 °C gave the desired diastereomer **18*RS*** with high diastereoslectivity (dr = 18:1). Although the Grignard reaction of imine **17*S*** under the same conditions gave the desired product **18*SR*** in low yield (entry 2), reaction with more sterically accessible PhC≡CMgBr effectively provided the Grignard adduct **19*SS*** in an 82% yield (dr = 8:1; entry 3), which was then converted to **18*SR*** by hydrogenation. The sulfinyl and MOM groups of **18*RS*** and **18*SR*** were removed under acidic condition, and the resulting amino groups of the products were condensed with carboxylic acid **20**[14] (R^i). Finally, nucleophilic substitution at the primary alcohol moieties of **21*S*** and **21*R*** with *N*,*N*′-bis(Boc)guanidine,[15] followed by deprotection, gave the *trans*-type mimetics **3** and **4**, respectively.

Scheme 2. Synthesis of *trans*-type Mimetics **3** and **4**



For the synthesis of *cis*-type mimetics **5** and **6** (scaffolds **III** and **I**), 2-(2-naphthyl)ethylamine (R^{i+3}) was introduced

(11) (a) Robak, M. T.; Herbage, M. A.; Ellman, J. A. *Chem. Rev.* **2010**, *110*, 3600. (b) Pierry, C.; Cahard, D.; Couve-Bonnaire, S.; Pannecoucke, X. *Org. Biomol. Chem.* **2011**, *9*, 2378.

(12) In Schemes 2 and 3, and Table 1, the absolute stereochemistry of the chiral sulfur atom is described as R_S or S_S.

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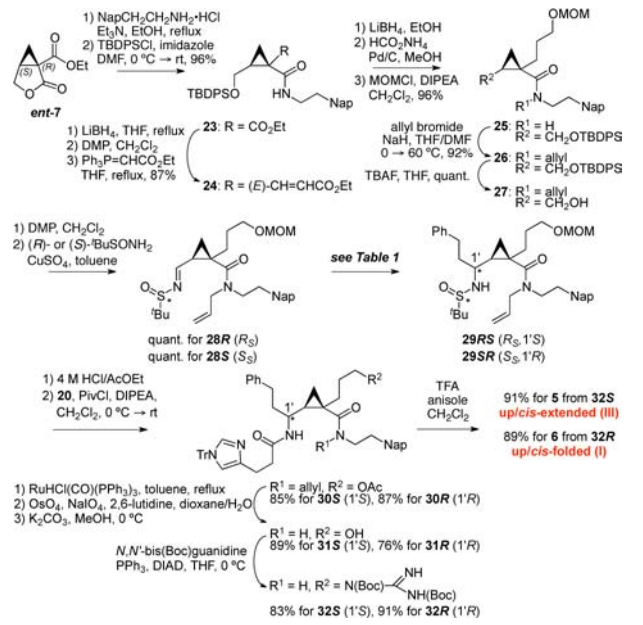
Scheme 3. Synthesis of *cis*-type Mimetics **5** and **6**



by the lactone ring-opening of ***ent*-7**. The *N*-*tert*-butanesulfinyl imines **28*R*** and **28*S*** were prepared using similar procedures as for preparing **17*R*** and **17*S***, while allyl protection of amide **25** was needed to prevent cylization between the *cis*-configured substituents. The Grignard reaction of **28*R*** was carried out under the same conditions used for the synthesis of **18*RS*** (Table 1 entry 4). However, the undesired diastereomer **29*RR*** was formed with high diastereoselectivity (dr = 1:26), and the stereochemical outcome was inconsistent with the six-membered chelated transition state proposed by Ellman et al.[11a] We assumed that the neighboring amide moiety might have prevented the formation of the expected transition state, and the addition of 10 eq. of HMPA in the reaction system afforded the desired stereoisomer **29*RS*** as the major product (dr = 3:1; entry 5). The Grignard reaction of imine **28*S*** gave the desired product **29*SR*** as a single stereoisomer without any additives (entry 6). After introduction of the imidazolyl moiety (R^i), the *N*-allyl groups of **30*S*** and **30*R*** were removed by the olefin isomerization-oxidation method that we recently developed[16] to give **31*S*** and **31*R***. Finally, the *cis*-type mimetics **5** and **6** were synthesized via the same conditions as used for the *trans*-type mimetics.[17]

The binding affinities of the synthesized mimetics **3**–**6** and ***ent*-3**–***ent*-6** for the three human MCR subtypes (hMC3R, hMC4R, hMC5R) are summarized in Table 2. The *trans*-type mimetics generally showed higher affinities

(16) Kajihara, K.; Arisawa, M.; Shuto, S. *J. Org. Chem.* **2008**, *73*, 9494. Although we previously used ozone to oxidize an enamide moiety, a combination of OsO_4 and $NaIO_4$ was effectively used in this case to avoid the undesired oxidation of the naphthalene ring by ozone.

(17) The stereochemistry of C1′ was confirmed by Mosher's method, and the corresponding enantiomers ***ent*-3**–***ent*-6** were synthesized by the same synthetic routes as Schemes 2 and 3.

(18) Koikov, L. N.; Ebetino, F. H.; Solinsky, M. G.; Cross-Doersen, D.; Knittel, J. J. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2647.

Table 2. Binding Affinities for Human MCR Subtypes (K_i, μM)[a]

mimetic	scaffold	hMC3R	hMC4R	hMC5R
3	**II**	4.96 ± 0.27	0.88 ± 0.09	1.78 ± 0.06
4	**IV**	7.07 ± 1.19	0.68 ± 0.10	4.22 ± 0.24
***ent*-3**	***ent*-II**	4.90 ± 0.27	0.38 ± 0.05	2.09 ± 0.11
***ent*-4**	***ent*-IV**	6.49 ± 0.82	0.37 ± 0.04	2.32 ± 0.25
5	**III**	41.6%[b]	4.02 ± 0.60	70.5%[b]
6	**I**	36.6%[b]	3.25 ± 0.13	61.8%[b]
***ent*-5**	***ent*-III**	3.64 ± 1.14	0.84 ± 0.07	2.67 ± 0.02
***ent*-6**	***ent*-I**	50.7%[b]	4.55 ± 0.35	74.2%[b]
Ac-His-D-Phe-Arg-Trp-NH_2[c]	-	0.77 ± 0.30	0.28 ± 0.17	n.d.

[a] Mean value ± SE (μM, n = 3). [b] K_i values for hMC3R and hMC5R were determined only for the mimetics showing high affinity (K_i, < 1 μM) for hMC4R. For the other mimetics, the inhibition rates of ^{125}I-labeled α-MSH binding at 10 μM concentration of mimetics are shown. [c] Cited from reference 18.

for the MCR receptors than the corresponding *cis*-type mimetics, and the *trans*-type mimetics bound more strongly to hMC4R (K_i = 0.37–0.88 μM) than to the other two subtypes (K_i = 4.90–7.07 μM for hMC3R; K_i = 1.78–4.22 μM for hMC5R). Among the *trans*-type mimetics, the down/*trans*-folded mimetic ***ent*-3** (K_i = 0.38 μM) and the down/*trans*-extended mimetic ***ent*-4** (K_i = 0.37 μM) were the most potent hMC4R ligands in this series, which would mimic the hMC4R-binding conformation of the core sequence. Their potencies were comparable to that of the reported core sequence-derived tetrapeptide Ac-His-D-Phe-Arg-Trp-NH_2,[18] and the subtype selectivity of ***ent*-3** and ***ent*-4** for hMC4R was substantially better than that of the tetrapeptide. Furthermore, these nonpeptidic hMC4R ligands ***ent*-3** and ***ent*-4** were highly stable in human serum ($t_{1/2}$ > 24 h), while the corresponding natural tetrapeptide (Ac-His-Phe-Arg-Trp-NH_2) was rapidly degradated ($t_{1/2} \approx$ 1.7 h).

In conclusion, we designed cyclopropylic strain-based peptidomimetic scaffolds mimicking wide-ranging tetrapeptide conformations, and established a procedure for their stereoselective synthesis. The selective hMC4R ligands ***ent*-3** and ***ent*-4** were successfully developed based on the designed peptidomimetic scaffolds to demonstrate their usefulness for searching the chemical space to identify nonpeptidic leads of bioactive peptides.

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Supporting Information Available. Experimental procedures for the synthesis of all new compounds, binding assays of the MCR subtypes, and stability test of the mimetics in human serum. Spectral data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.