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Synthesis of 2,3- and 3,4-Methanoamino Acid Equivalents with Stereochemical Diversity and Their Conversion into the Tripeptide Proteasome Inhibitor Belactosin A and Its Highly Potent *Cis*-Cyclopropane Stereoisomer

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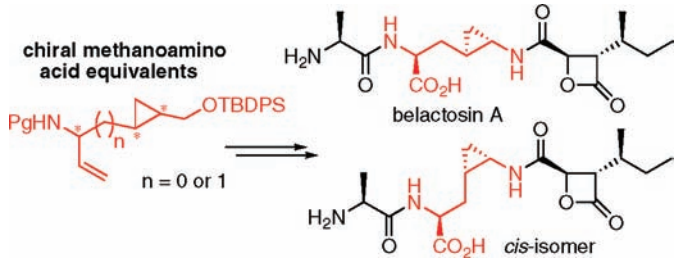
ABSTRACT



A series of chiral 2,3- and 3,4-methanoamino acid equivalents of stereochemical diversity were designed and synthesized from our chiral cyclopropane units, using a diastereoselective Grignard addition with (*R*)- or (*S*)-*t*-butanesulfinyl imines as the key step. These equivalents were converted into the proteasome inhibitor belactosin A and its *cis*-cyclopropane stereoisomer. The unnatural *cis*-isomer was shown to be more than twice as potent as belactosin A as a proteasome inhibitor.

The development of useful peptide mimetics may be achieved by replacing a key amino acid of biologically active peptides with a conformationally restricted amino acid analogue having the side chain in the bioactive conformation.[1] Because of its small, rigid structural features, a cyclopropane restricts the conformation of molecules, which can improve the biological activity of the lead molecules due to an entropic advantage.[2,3] Thus, the cyclopropane-containing amino acids have been synthesized and used

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(1) (a) Hanessian, S.; McNaughton-Smith, G.; Lombart, J.-G.; Lubell, W. D. *Tetrahedron* **1997**, *53*, 12789–12854. (b) Souers, A. J.; Ellman, J. A. *Tetrahedron* **2001**, *57*, 7431–7488.

(2) Excellent reviews of cyclopropane-containing amino acids were recently published: Brackmann, F.; de Meijere, A *Chem. Rev.* **2007**, *107*, 4493–4537, 4538−4583.

(3) (a) Kazuta, Y.; Matsuda, A.; Shuto, S. *J. Org. Chem.* **2002**, *67*, 1669–1677. (b) Kazuta, Y.; Hirano, K.; Natsume, K.; Yamada, S.; Kimura, R.; Matsumoto, S.; Furuichi, K.; Matsuda, A.; Shuto, S. *J. Med. Chem.* **2003**, *46*, 1980–1988. (c) Watanabe, M.; Kazuta, Y.; Hayashi, H.; Yamada, S.; Matsuda, A.; Shuto, S *J. Med. Chem.* **2006**, *49*, 5587–5596, and references therein.

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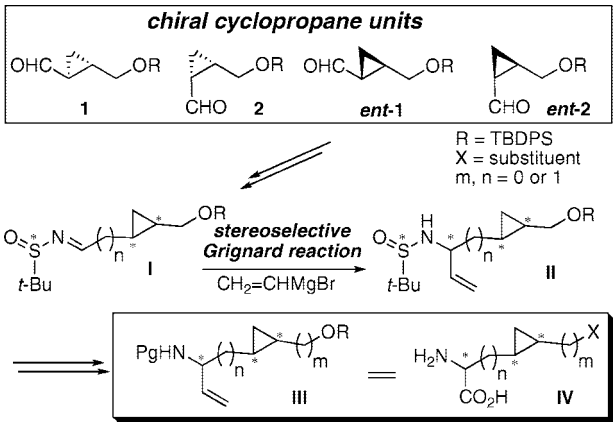
extensively for peptide mimetic studies.[2] However, the bioactive conformation of biologically active peptides, such as peptide ligands targeting a G protein-coupled receptor (GPCR), is usually unclear. In these cases, a series of cyclopropane-containing amino acids with stereochemical diversity, where the side chains are located in a variety of spatial arrangements owing to the conformational restriction, can be used to identify the bioactive conformation and to develop peptide mimetics with high potency.

We describe here an efficient synthetic method for a series of chiral 2,3- and 3,4-methanoamino acid equivalents **III** (Scheme 1) with *cis*/*trans*, D/L, and *syn*/*anti* stereochemical

Scheme 1



diversity. Using these equivalents, we synthesized belactosin A (**3**),[4,5] a tripeptidic proteasome inhibitor containing a 3-(*trans*-2-aminocyclopropyl)-L-alanine (*trans*-3,4-methano-L-ornithine), and its *cis*-cyclopropane stereoisomer **4** (Figure 1). The biological evaluation showed that the unnatural *cis*-isomer **4** is a more potent proteasome inhibitor than the natural belactosin A having the *trans*-cyclopropane structure.

The stereoselective synthesis of cyclopropane derivatives with a desired stereochemistry is often troublesome.[2,3,6] To solve this problem, we recently developed chiral units composed of four stereoisomeric cyclopropanes, **1** and **2**, and their enantiomers ***ent*-1** and ***ent*-2** (Scheme 1), for preparing various cyclopropane derivatives bearing adjacent carbon substituents in a *cis* or a *trans* relationship.[3a] These units were employed as synthons in this study.

We designed the cyclopropane derivatives **III** with a CH_2═CHC*H(NHPg)− side chain (Scheme 1) as versatile

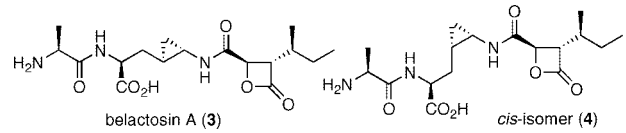
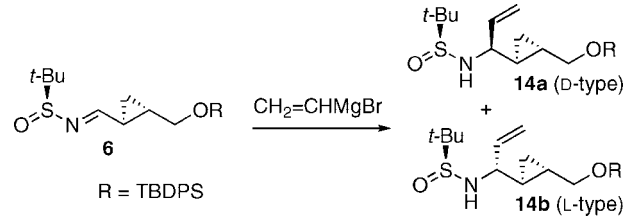


Figure 1. Structures of belactosin A (**3**) and its *cis*-cyclopropane stereoisomer (**4**).

equivalents of L- or D-2,3- and -3,4-methanoamino acids **IV**, since a vinyl group is stable under various reaction conditions and can be converted easily into a carboxyl group.[7] Compounds **III** would be derived from compounds **II**, the symmetric CH_2═CHC*H(NH)− moiety of which would be constructed via the diastereoselective Grignard addition to the (*R*)- and (*S*)-*t*-butanesulfinyl imines **I**.[8] Grignard additions to the *t*-butanesulfinyl imines are known to occur highly stereoselectively, where the stereochemical outcome is dependent on the configuration at the sulfinyl moiety.[8]

Table 1. Grignard Reaction of (*S*)-*t*-Butanesulfinyl Imine **6**



entry[a,b]	temp (°C)	conc (M)[c]	yield (%)	D/L[d]
1	0	0.1	quant	1:4.6
2	rt	0.1	quant	1:6.7
3	rt	0.1	quant	1:5.1
4	rt	0.1	97	1:7.4
5	rt	0.01	98	1:10
6	110	0.1	94	1:8.1
7	110	0.01	quant	1:16

[a] Solvent: entries 1 and 2, CH_2Cl_2; entry 3, THF; entries 4−7, toluene. [b] Equivalent of Grignard reagent: entries 1−5, 3 equiv; entry 6, 1.5 equiv; entry 7, 1.1 equiv. [c] Concentration of the substrate. [d] D or L indicates the stereochemistry of the cyclopropane amino acid by replacing the vinyl group of the Grignard reaction product with a carboxyl, and the ratio was determined by HPLC.

We investigated the Grignard reaction using a series of (*R*)- and (*S*)-*t*-butanesulfinyl imines **6**−**13** with a *cis*- or *trans*-cyclopropane structure (Tables 1 and 2).[9] The *trans*-cyclopropane *t*-butanesulfinyl imines **6**−**9** were prepared from the chiral *trans*-cyclopropane unit **1** (Scheme 2). Similarly, the *cis*-

(4) (a) Asai, A.; Hasegawa, A.; Ochiai, K.; Yamashita, Y.; Mizukami, T. *J. Antibiot.* **2000**, *53*, 81–83. (b) Asai, A.; Tsujita, T.; Sharma, S. V.; Yamashita, Y.; Akinaga, S.; Funakoshi, M.; Kobayashi, H.; Mizukami, T. *Biochem. Pharmacol.* **2004**, *67*, 227–234.

(5) Total synthesis of belactosin A: (a) Armstrong, A.; Scutt, J, N. *Chem. Commun.* **2004**, 510–511. (b) Larionov, O. V.; de Meijere, A. *Org. Lett.* **2004**, *6*, 2153–2156.

(6) For examples see: (a) Stammer, C. H. *Tetrahedron* **1990**, *46*, 2231–2254. (b) Kodama, H.; Shimohigashi, Y. *J. Synth. Org. Chem. Jpn.* **1994**, *52*, 180–191. (c) Shimamoto, K.; Ohfune, Y. *J. Med. Chem.* **1996**, *39*, 407–423. (d) Demir, A. S.; Sesenoglu, Ö *Helv. Chim. Acta* **2004**, *87*, 106–119, and references therein.

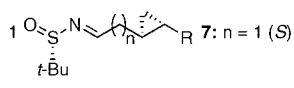
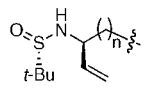
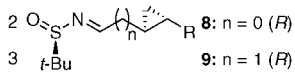
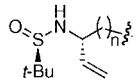
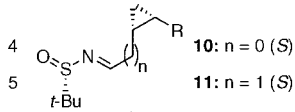
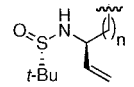
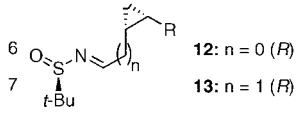
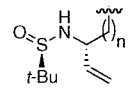
(7) Cooper, T. S.; Laurent, P.; Moody, C. J.; Takle, A. K. *Org. Biol. Chem.* **2004**, *2*, 265–267.

(8) (a) Cogan, D. A.; Liu, G.; Kim, K.; Backes, B. J.; Ellman, J. A. *J. Am. Chem. Soc.* **1998**, *120*, 8011–8019. (b) Cogan, D. A.; Liu, G.; Ellman, J. A. *Tetrahedron* **1999**, *55*, 8883–8904. (c) Davis, F. A.; McCoull, W. *J. Org. Chem.* **1999**, *64*, 3396–3397. (d) Senanayake, C. H.; Krishnamurthy, D.; Lu, A.-H.; Han, Z.; Gallou, I. *Aldrichimica Acta* **2005**, *38*, 93–104.

(9) Stereochemistries of the Grignard reaction products were determined by the modified Mosher method.

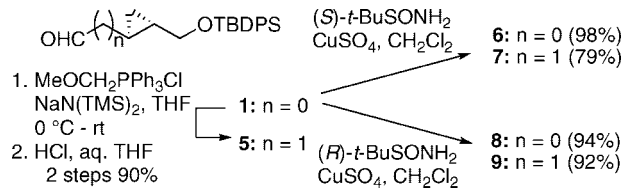
(10) (a) Adamas, J. *Nat. Rev. Cancer* **2004**, *4*, 349–360. (b) Ciechanover, A. *Angew. Chem., Int. Ed.* **2005**, *44*, 5944–5996.

Table 2. Grignard Reaction of the (*S*)- and (*R*)-*t*-Butanesulfinyl Imines **7**−**13** under the Low Substrate Concentration/High Temperature Conditions[a,b]

entry	substrate (*t*-BuS*O)[a]	major product	yield (D/L)[b]
1	 **7:** n = 1 (*S*)		96% (1 : 8.4)
2	 **8:** n = 0 (*R*)		95% (26 : 1)
3	**9:** n = 1 (*R*)		96% (9.4: 1)
4	 **10:** n = 0 (*S*)		quant (1 : 18)
5	**11:** n = 1 (*S*)		93% (1 : 8.1)
6	 **12:** n = 0 (*R*)		94% (8.9 : 1)
7	**13:** n = 1 (*R*)		quant (40 : 1)

[a] R = $CH_2OTBDPS$. [b] D or L indicates the stereochemistry by replacing the vinyl group with a carboxyl, and the ratio was determined by ^1H NMR (entries 1−4) or HPLC (entries 5−7).

Scheme 2



cyclopropane *t*-butanesulfinyl imines **10**−**13** were prepared from the chiral *cis*-cyclopropane unit **2**.

The Grignard reaction was first investigated with the *trans*-cyclopropane (*S*)-*t*-butanesulfinyl imine **6** as the substrate. As shown in Table 1, the reaction gave a mixture of the diastereomers **14a** with the D-amino acid stereochemistry and **14b** with the L-amino acid stereochemistry in excellent yield under all of the reaction conditions examined, where the diastereomer **14b** was always the major product (entries 1−7). The reaction was carried out with 0.1 M substrate concentration in CH_2Cl_2 at 0 °C and gave the addition products in a D/L ratio of 1:4.6, while 3.0 equiv of the reagent was needed to complete the reaction (entry 1). The stereoselectivity increased in a similar reaction at room temperature (entry 2, D/L = 1:6.7). Although the stereoselectivity decreased with THF as solvent (entry 3, D/L = 1:5.1), it increased somewhat with toluene as solvent (entry 4, D/L = 1:7.4). Although the Grignard reactions with the *t*-butanesulfinyl imines usually occur highly stereoselectively under low-temperature conditions,[8] we found that the stereoselectivity clearly improved when the reaction was performed with a lower substrate concentration of 0.01 M (entry 5, D/L = 1:10) or at a higher temperature (entry 6, D/L = 1:8.1). Thus, the diastereomer **14b** was obtained highly selectively (entry 7, D/L = 1:16) under 0.01 M substrate concentration conditions in toluene at 110 °C, in which the reaction was complete with only 1.1 equiv of the Grignard reagent.

By employing the low substrate concentration (0.01M)/high temperature (110 °C) reaction conditions, we carried out the Grignard reactions with a variety of the (*S*)-*t*-butanesulfinyl imines and the (*R*)-*t*-butanesulfinyl imines as the substrates (Table 2). In all of these cases, the addition products equivalent to an L-amino acid were selectively formed from the (*S*)-*t*-butanesulfinyl imines (entries 1, 4, and 5), and the products equivalent to a D-amino acid were selectively formed from the (*R*)-*t*-butanesulfinyl imines (entries 2, 3, 6, and 7), respectively, regardless of the stereochemistry of the cyclopropane backbone or the side-chain length of the substrates. The stereochemical outcome is in accord with the chelated transition state model reported by Ellman.[8a,b]

Thus, we have successfully developed a general procedure for the preparation of a series of 2,3- and 3,4-methanoamino acid equivalents with *cis*/*trans*, D/L, and *syn*/*anti* stereochemical diversity. This is, to our knowledge, the first synthetic method for systematically providing chiral 2,3- and 3,4-methanoamino acids with stereochemical diversity.[2,6]

Using the 3,4-methanoamino acid equivalents obtained by the Grignard reaction as the key units, we next planned to synthesize belactosin A (**3**) and its *cis*-cyclopropane stereoisomer **4**.

Belactosin A, a tripeptide consisting of L-Ala, 3-((1*R*,2*S*)-2-aminocyclopropyl)-L-Ala, and a chiral carboxy-β-lactone, was isolated by Asai. Belactosin A may be an effective anticancer drug lead since it prevents tumor cell cycle progression due to proteasome inhibition.[4,10] The cyclopropane part of **3** would restrict the orientation of the L-Ala and the β-lactone moieties to determine the three-dimensional structure of the molecule, which might be critical for its interaction with the proteasome. Thus, our interest derives from the biological activity of the *cis*-cyclopropane isomer **4**, in which the L-Ala and the β-lactone moieties can be restricted in a spatial arrangement different from that in **3** with the *trans*-cyclopropane structure.

These two target compounds would be obtained by condensation between L-Ala (**A**), *trans*- or *cis*-3,4-methanoamino acid (**B**), and the chiral carboxy-β-lactone (**C**) (Figure 2). The chiral β-lactone can be prepared from L-Ile, according to the method reported by Armstrong,[5a] and the Grignard reaction products **15** and **16** (Figure 2) would be equivalents of the *trans*- and *cis*-3,4-methanoamino acids (**B**).

Compound **15**, including some of the minor diastereomer, was treated with HCl to remove the *N*- and *O*-protecting groups, and the resulting free amino group was protected with a Fmoc group to give the diastereomerically pure **17** after silica gel column chromatography. Oxidation of the hydroxymethyl moiety of **17**, followed by treatment with diphenyl phosphoryl azide (DPPA)/Et_3N, gave the corresponding acid azide, which was heated in *t*-BuOH to afford the Curtius rearrangement product **18**. After removal of the Fmoc group of **18**, the product was condensed with the mixed anhydride Cbz-L-Ala-OPiv to form the dipeptide **19** in 72%

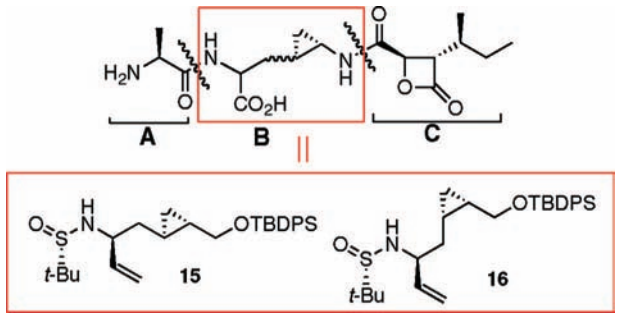


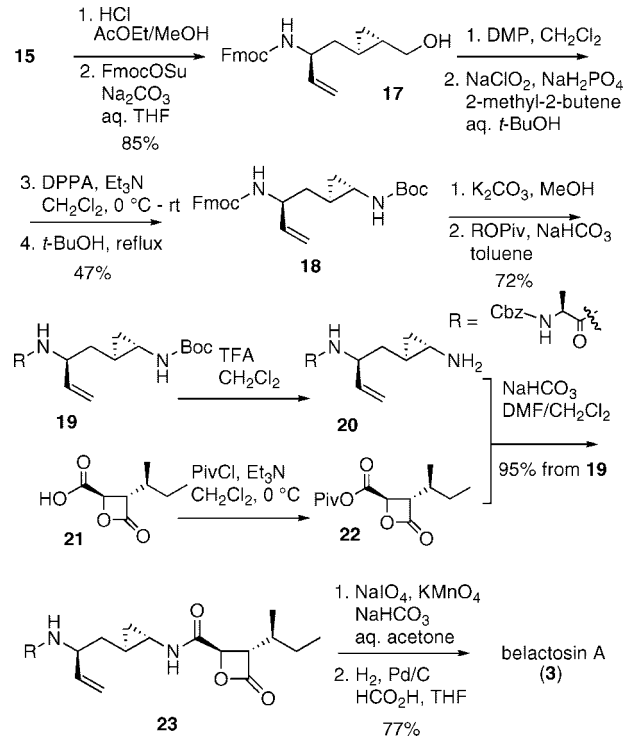
Figure 2. Representation of the synthetic targets **3** and **4**.

yield. After removal of the Boc group of **19**, the resulting amine **20** was condensed with the mixed anhydride **22**, prepared from the known β-lactone **21**,[5a] in the presence of $NaHCO_3$ in DMF/CH_2Cl_2 to form the tripeptide **23** in excellent yield. Oxidation of the vinyl group to a carboxyl group with a $NaIO_4/KMnO_4/NaHCO_3$ combination in aqueous acetone, followed by removal of the Cbz group, finally produced belactosin A (**3**) in 77% yield (Scheme 3). By the same procedure, the *cis*-cyclopropane isomer **4** was synthesized from the *cis*-3,4-methanoamino acid equivalent **16**.

In measuring the inhibitory effect of belactosin A (**3**) and its *cis*-cyclopropane stereoisomer **4** on the chymotrypsin-like activity of the purified human 20S proteasome, it was noted that, while the synthesized belactosin A showed significant inhibitory effect ($IC_{50} = 304 \pm 153$ nM), as reported previously,[4a] the isomer **4** ($IC_{50} = 150 \pm 47$ nM) was about twice as potent as belactosin A.

In summary, we designed and synthesized a series of chiral 2,3- and 3,4-methanoamino acid equivalents of stereochemical diversity. Two of these equivalents were effectively used for the synthesis of belactosin A (**3**) and its *cis*-cyclopropane isomer **4**. The isomer **4** was identified as a more potent proteasome inhibitor than belactosin A itself. These results suggest that the 2,3- and 3,4-methanoamino acid equivalents

Scheme 3



with stereochemical diversity can be used effectively as the key units to identify biologically active peptidic compounds.

Acknowledgment. We are grateful to Dr. Y. Kanda (Kyowa Hakko Kogyo) for providing an ^1H NMR chart of natural belactosin A.

Supporting Information Available: Experimental procedures and spectroscopic data of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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