Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 2123–2128

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

PROBING THE IMPORTANCE OF SPACIAL AND CONFORMATIONAL DOMAINS IN CAPTOPRIL ANALOGS FOR ANGIOTENSIN CONVERTING ENZYME ACTIVITY

Stephen Hanessian,* Ulrich Reinhold, Michel Saulnier, and Stephen Claridge

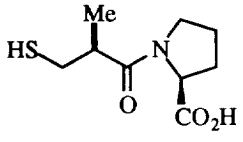
Department of Chemistry, Université de Montréal, C.P. 6128, Succursale Centre-ville, Montréal, Québec, CANADA, H3C 3J7

Received 29 April 1998; accepted 9 July 1998

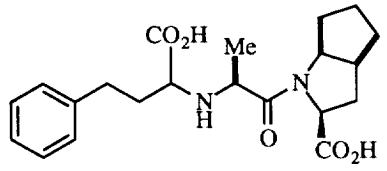
Abstract: A new synthesis of 4,5-methano-L-prolines and the enzymatic activity of the corresponding N-(3-mercapto-2-*R*-methyl-propionyl) analogs as inhibitors of angiotensin converting enzyme are described.

Target and chemistry-driven drug design based on molecular interactions with enzymes has emerged in recent years as a stimulating area of research endeavor.[1] Although there are a multitude of small-molecule enzyme inhibitors, even at nM concentrations in vitro, the process of their development into a marketable drug is arduous to say the least. One of the remarkably successful attempts at drug design based on molecular interactions with an enzyme is exemplified in captopril,[2] an inhibitor of angiotensin converting enzyme, and used for the treatment of hypertension (Figure 1).

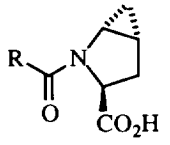
Figure 1. Captopril, Ramiprilat and new constrained analogs



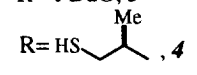
Captopril, *1*



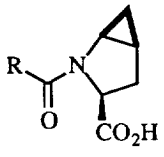
Ramiprilat, *2*



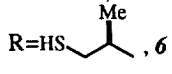
R= t-BuO, *3*



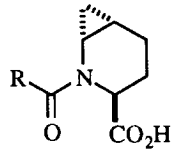
, *4*



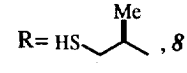
R= t-BuO, *5*



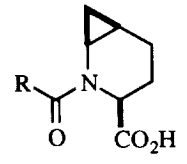
, *6*



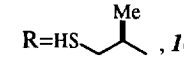
R= t-BuO, *7*



, *8*



R= t-BuO, *9*



, *10*

The discovery of captopril, an exquisitely simple molecule by today's standards of increasing molecular complexity, is a classic example of design based on a knowledge of possible interactions between the drug and

0960-894X/98/$ - see front matter
PII: S0960-894X(98)00377-1

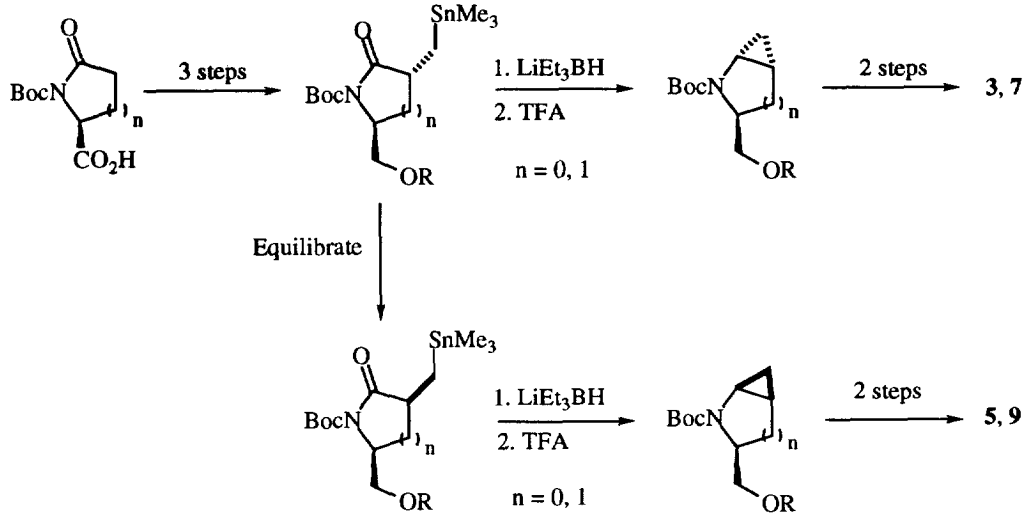
the active site of the enzyme. This was also aided by extensive analog synthesis of proline peptides, which led to the conclusion that captopril appears to offer effective interactions with the functional groups on the surface of the enzyme that involve charge, H-bonding and hydrophobic contact.[3]

The importance of such multiple interactions has been discussed in detail,[2] and was the basis of an intense effort directed at the discovery of analogs of captopril,[2a,b] such as enalapril,[2c] and of **2**. Since the *cis*-orientation of the cyclopentane ring in ramiprilat is important for biological activity,[4] the relevance of the hydrophobic interaction of that region of the proline moiety with the enzyme can be appreciated.

Our interest in the synthesis of conformationally constrained heterocycles, coupled with the desire to further probe the role of substituents on the enzymatic activity of captopril, instigated the research work reported in this paper.

Previous work in our laboratory[5,6] (Scheme 1) has described methods for the synthesis of Boc derivatives of *trans*-4,5-methano-L-proline **3**, *cis*-4,5-methano-L-proline **5**, *trans*-5,6-methano-L-pipecolic acid, **7**, and *cis*-5,6-methano-L-pipecolic acid, **9**. It was further observed that the proline ring in the N-Boc derivative **3** was virtually flattened (rms, 0.003 Å) compared to **5** (rms, 0.013 Å) and Boc-L-proline (rms, 0.018 Å).[5] While the reasons for this structural effect were not evident, we anticipated that such constrained proline derivatives should be explored further as surrogates for L-proline in a selected group of medicinally relevant compounds. We thus set out to prepare the (2*R*)-methyl-3-mercapto N-propionyl amides of the above mentioned ω-methano-L-prolines and L-pipecolic acids as constrained analogs of captopril, and to study their inhibitory activity on ACE (angiotensin converting enzyme).

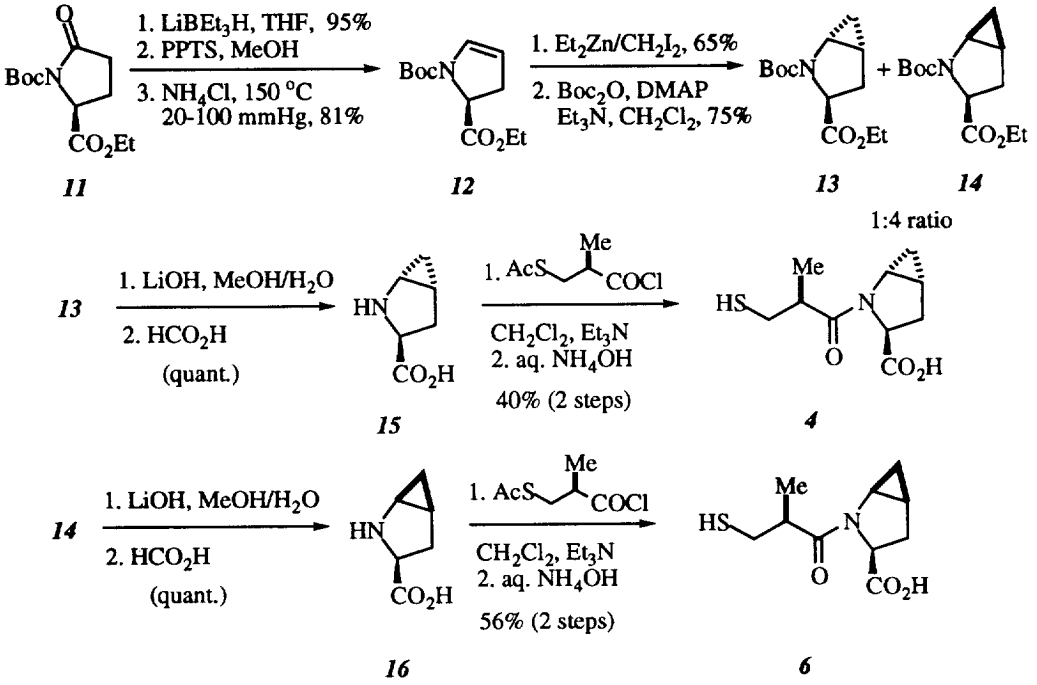
Scheme 1.



2,3-Methano-prolines have been previously reported by a number of groups.[7] *cis*- and *trans*-3,4-Methano-prolines were described by Witkop and coworkers[8] in 1971. We were aware of one synthesis of racemic 4,5-methano-proline amides[9] which was based on a Beckmann rearrangement of *cis*-bicyclo[3.1.0]hexan-2-one to a δ-lactam followed by chlorination and ring contraction.

Our synthesis of the analogs **3**, **5**, **7**, and **9**, relied on the intramolecular cyclopropanation of α-trimethylstannylmethyl lactams, via the corresponding iminium salts.[5,6] The *cis*- or *trans*-orientation of the cyclopropane ring could be controlled by the β- or α-disposition of the trimethylstannylmethyl group respectively vis-à-vis the stereocontrolling substituent in the corresponding derivatives (Scheme 1).

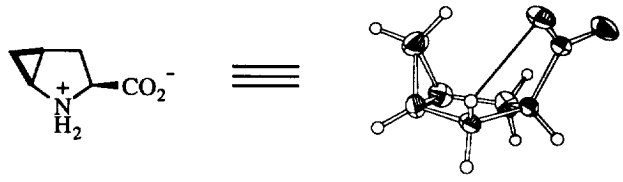
Scheme 2.



We have now devised an expedient route to 4,5-methano-prolines that affords the enantiopure derivatives **3** and **5** in essentially eight steps from commercially available L-pyroglutamic acid (Scheme 2). Thus reduction of the lactam carbonyl in **11** with lithium triethylborohydride followed by transformation to the methoxy hemiaminal carbamate and elimination of methanol in the presence of ammonium chloride[10] led to the enecarbamate analog **12** in good yield.[11] Originally, we had used other published methods[12] to effect the dehydration, but in our hands, yields were modest and reproducibility depended on the scale of the reaction. Application of the modified Simmons–Smith cyclopropanation[13] reaction to the enecarbamate **12** followed by protection of the amine with Boc anhydride, led to a mixture of the *trans*-4,5-methano-L-proline analog **13** and the corresponding *cis*-4,5-isomer **14** in a ratio of 1:4 in a combined yield of 75%.[14] This expedient method based on a well-known reaction,[13] proceeds in good overall yield and it is 3 steps shorter than our previous synthesis[5] which utilized an organotin reagent. It has allowed us to prepare gram quantities of both 4,5-methano-L-prolines which were easily separable by column chromatography as their N-Boc derivatives **13** and **14**. Hydrolysis with LiOH in aq. methanol gave the known respective 4,5-methano-N-Boc-L-prolines[5] as crystalline solids. Treatment of **13** with aq. base followed by formic acid gave the free acid **15** as a white solid in quantitative yield. Analogous hydrolysis of **14** gave the isomeric free acid **16** as a crystalline solid. The

X-ray structure and solid state conformational characteristics of **16** revealed considerable flattening of the pyrrolidine ring (rms 0.09 Å) compared to L-proline (rms 0.181 Å). A H-bond was evident between the protonated amine and the carboxylate group. (Figure 2).

Figure 2.



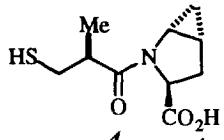
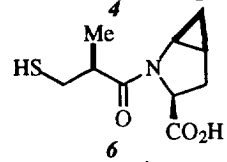
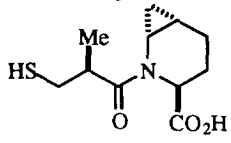
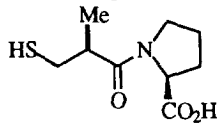
The predominance of the *cis*-4,5-methano isomer **14** in the cyclopropanation reaction is of interest, since the tin-mediated iminium ion cyclization protocol[5] (Scheme 1) favored the formation of the *trans*-isomer due to steric factors imposed by a bulky resident group. Most probably, the *cis*-cyclopropanation is the result of an anchoring effect of the zinc species with the ester group in **12**, thus delivering the nucleophile from the same side to give **14**.[15]

Acylation of the free amino acids with the readily available S-acetyl-2-(*R*)-methyl propionyl chloride and saponification afforded the captopril analogs **4** and **6**. The L-pipecolic acid analog **8** was similarly prepared from the precursor amino acid.[5]

Inhibition of ACE obtained from rabbit lung and partially purified, was studied using hippuryl-His-Leu as a substrate following the procedure of Cushman and Cheung.[16] The results shown in Table 1 indicate that the *cis*- and *trans*-5-methano analogs of L-proline **4**,**6**, and the *trans*-L-pipecolic acid analog **8** are highly potent inhibitors, even surpassing captopril. The *cis*-4,5-carbocyclic analog of captopril, ramiprilat **2**, is much more active than the corresponding *trans*-isomer. In this respect it is of interest that the *cis*-analog **6** is equally as active as the *trans*-analog **4**. Clearly, this study has shown that small rings can be tolerated at the 4,5-position of captopril with *cis*- or *trans*-orientations. It also appears that the degree of ring flattening in these derivatives relative to captopril is not having an adverse effect on enzyme inhibitory activity, and it could even be an advantage.

Analogs **4**, **6**, and **8**, were inactive against neutral endopeptidase 24.11 enzyme at 10 μM as well as against endothelin converting enzyme at 1 μM. The selectivity described herein exhibited by captopril and the methano analogs **4**, **6**, and **8** towards ACE is obviously of interest. Future work will focus on the incorporation of 4,5-methano prolines and 5,6-methano pipecolic acids into strategic positions as replacements of the corresponding natural amino acids in pharmacologically relevant molecules.

Table 1 Inhibition Tests on Angiotensin Converting Enzyme (ACE)[a]

Compound	IC_{50}
 *4*	7.6 (± 0.7 nM)
 *6*	6.6 (± 0.3 nM)
 *8*	5.3 (± 0.2 nM)
 *1*, Captopril	13.0 (± 1.0 nM)

a. For assay method, see ref 16

Acknowledgements. We gratefully acknowledge financial assistance from NSERC under the Medicinal Chemistry Chair program. We are indebted to Dr. Arco Y. Jeng (Novartis, Summit, N.J.) for the enzymatic assays. We thank Dr. Michel Simard of the X-ray crystallography laboratory at the Université de Montréal for structure determinations.

References and Notes.

1. For excellent summaries, see Burger's Medicinal Chemistry and Drug Discovery, Wiley-Interscience, N. Y., 1995. Muscate, A.; Kenyon, G. L., p 739; Cannon, J. G., p 783.
2. (a) Ondetti, M. A.; Cushman, D. W. *J. Med. Chem.* **1981**, *24*, 355; (b) Cushman, D. W.; Ondetti, M. A. *Prog. Med. Chem.* **1980**, *17*, 41. (c) Patchett, A. A.; Harris, E.; Tristram, E. W.; Wyvratt, M. J.; Wu, M. T.; Taub, D.; Peterson, E. R.; Ikeler, T. J.; ten Broeke, J.; Payne, L. G.; Ondeyka, D. L.; Thorsett, E. D.; Greenlee, W. J.; Lohr, N. S.; Hoffsommer, R. D.; Joshua, H.; Ruyle, W. V.; Rothrock, J. W.; Aster, S. D.; Maycock, A. L.; Robinson, F. M.; Hirschmann, R.; Sweet, C. S.; Ulm, E. H.; Gross, D. M.; Vassil, T. C.; Stone, C. A. *Nature* **1980**, *288*, 280.
3. Ondetti, M. A.; Rubin, B.; Cushman, D. W. *Science* **1977**, *196*, 441; Cushman, D. W.; Cheung, H. S.; Sabo, F. C.; Ondetti, M. A. *Biochemistry* **1977**, *16*, 5484.
4. Urbach, H.; Henning, R. *Tetrahedron Lett.* **1985**, *26*, 1839; Henning, R.; Urbach, H. *Tetrahedron Lett.* **1983**, *24*, 5339; Teetz, V.; Geiger, R.; Henning, R.; Urbach, H. *Arzneim. Forsch./Drug Res.* **1984**, *34*, 1399.

5. Hanessian, S.; Reinhold, U.; Gentile, G. *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1881.
6. Hanessian, S.; Reinhold, U.; Ninkovic, S. *Tetrahedron Lett.* **1996**, *37*, 8967; 8971.
7. For the synthesis of racemic and enantiopure 2,3-methano proline, see (a) Hercouet, A.; Bessières, B.; Le Corre, M. *Tetrahedron: Asymmetry* **1996**, *7*, 1267. (b) Switzer, F. L.; van Halbeek, H.; Holt, E. M.; Stammer, C. H. *Tetrahedron* **1989**, *45*, 6091. For recent accounts on 2,3-methano amino acids, see: Stammer, C. H. *Tetrahedron* **1990**, *46*, 2231; Burgess, K.; Ho, K. K.; Moyl-Sherman, D. *Synlett* **1994**, 575; Burgess, K.; Ke, C.-Y. *J. Org. Chem.* **1996**, *61*, 8627. Jiménez, J. M.; Ortuno, R. M. *Tetrahedron: Asymmetry* **1996**, *7*, 3203, and references cited therein.
8. For the synthesis of *cis*- and *trans*- 3,4-methano prolines, see (a) Fujimoto, Y.; Irrevere, F.; Karle, J. M.; Karle, I. L.; Witkop, B. *J. Am. Chem. Soc.* **1971**, *93*, 3471; for a recent discussion of cyclopropyl pyrrolidines, see Harvey, D. F.; Sigano, D. M. *J. Org. Chem.* **1996**, *61*, 2268; Brighty, K. E.; Castaldi, M. J. *Synlett* **1996**, 1097.
9. For the synthesis of racemic 4,5-methano proline amides, see a) H. Urbach, R. Henning, R. Becker, Ger. Offen. DE 3,324,263 (Cl. C07D209/2); *Chem. Abstr.* **1985**, 103: P 54461q; for a related example involving mixtures of diastereomers, see also R. Pellicciari, L. Arenare, P. De Caprariis, B. Natalin, M. Marinozzi, A.Galli, *J. Chem. Soc. Perkin I.* **1995**, 1251.
10. Shono, T.; Matsumura, Y.; Tsubata, K.; Sugihara, Y.; Yamane, S. I.; Kanazawa, T.; Aoki, T. *J. Am. Chem. Soc.* **1982**, *104*, 6697.
11. Typical procedure: A mixture containing N-Boc-5-methoxy-(2*S*)-pyrrolidine-1-carboxylic acid ethyl ester (1.2 g, 4.4 mmol) and ammonium chloride (0.044 g, 0.15 equiv) was heated under reduced pressure (20–100 mm Hg) in a flask equipped with a reflux condenser. The reaction mixture was allowed to cool to room temperature, and the resultant oil was purified by flash chromatography on silica gel (hexanes-ethyl acetate, 9:1) to afford the product **12** (0.854 g, 81%) as a colorless oil.
12. Dieter, R.K.; Sharma, R.R. *J. Org. Chem.* **1996**, *61*, 4180; Cossy, J.; Cases, M.; Pardo, D.G. *Syn. Commun.* **1997**, *27*, 2769.
13. Furukawa, J.; Kawabata, N.; Nishimura, J. *Tetrahedron* **1968**, *24*, 53.
14. Typical procedure: To a cooled solution of **12** (0.5708 g, 2.366 mmol) in dry 1,2-dichloroethane under nitrogen was added dropwise 1.1 equiv of diethyl zinc (Et_2Zn, 1.0M solution in hexane, 2.603 mmol, 2.6 mL) and 1.5 equiv. of diiodomethane (3.55 mmol, 0.29 mL) at 0 °C. The reaction mixture was stirred for 30 min at 0 °C then 6 h at room temperature and quenched with dilute $NaHCO_3$ (7 mL). Water was added (40 mL), and the aqueous layer was extracted with dichloromethane (4 x 50 mL) and processed as usual. The residue after evaporation was purified by flash chromatography (methanol-hexanes-ethyl acetate, 1:5:13) to afford the isomeric mixture of products (0.332 g, 65%) as a pale-yellow oil that was transformed into the N-Boc derivatives (Boc_2O, DMAP, Et_3N, CH_2Cl_2), and separated by chromatograhy to give **13** and **14** (75% yield, 1:4 ratio).
15. For related examples of directed cyclopropanations see, Charette, A. B.; Marcoux, J.-F. *Synlett* **1995**, 1197; For examples of proximity effects, see Beak, P.; Meyers, A. I. *Acc. Chem. Res.* **1986**, *19*, 356.
16. Cushman, D. W.; Cheung, H. S. *Biochem. Pharmacol.* **1971**, *20*, 1637.