Tetrahedron Letters, Vol. 34, No. 44, pp. 7035-7038, 1993
Printed in Great Britain

0040-4039/93 $6.00 + .00
Pergamon Press Ltd

Macrocyclic Renin Inhibitors: Synthesis of A Subnanomolar, Orally Active Cysteine Derived Inhibitor

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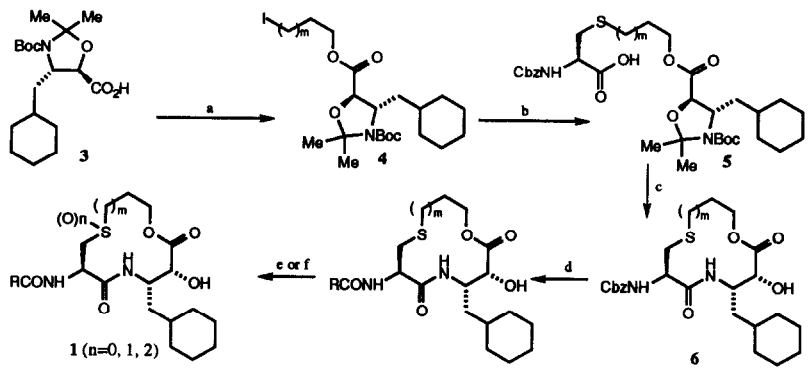
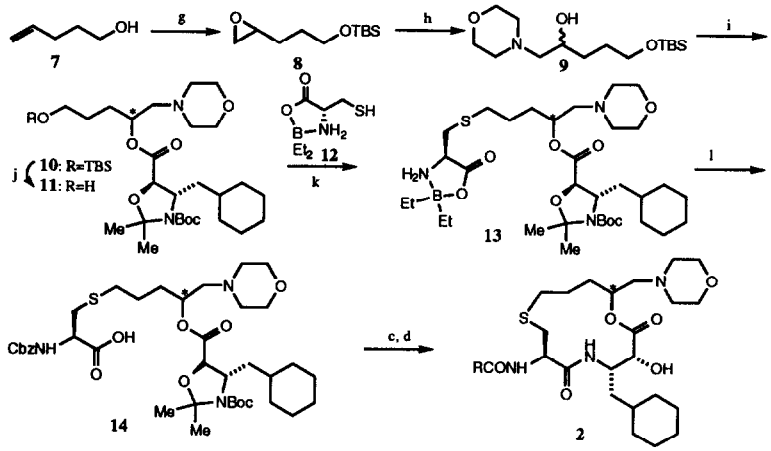
Abstract: *A class of novel thioether linked macrocyclic renin inhibitors was synthesized. A key element of the synthesis is the use of boroxazolidone for the simultaneous protection of the amino and carboxyl groups in L-cysteine.*

The renin-angiotensin system (RAS) is an important component of the interrelated homeostatic mechanisms that regulate blood pressure and electrolyte balance. The angiotensin converting enzyme inhibitors including captopril and enalapril, which intervene in the RAS, have proven effective in the treatment of hypertension and enjoy widespread medical use.[1] Nevertheless, ACE has several other substrates besides angiotensin I. In addition, recent evidence suggests that some ACE inhibitors affect other enzymes.[2] Since angiotensinogen is the only known naturally occurring substrate for renin, inhibition of renin may have advantages over ACE inhibition.[3] Although there have been considerable research activities in this area during the past decade, renin inhibitors have not been introduced into medicine. Since renin inhibitors have high molecular weight (normally mimics of hexapeptide substrate P_4 to P_2'), poor oral absorption and short duration of action plague most inhibitors.[4] We and others have reported some macrocyclic renin inhibitors, hoping they will be more stable toward proteolytic enzymes and of high potency, if their more rigid conformations fit well in the renin active site.[5] The most successful approach was to link P_2 and P_1' side chains and to utilize the (2R,3S)-3-amino-4-cyclohexyl-2-hydroxybutanoic acid (nor ACHPA) as the transition-state isostere.[5a-d] Very potent renin inhibitors have been synthesized in which the serine at P_2 was linked to P_1' by an ester bond. Studies have shown the ester bond in these compounds linking P_2 and P_1' is prone to hydrolysis.[5a] Therefore, replacing this ester bond could make the cyclic system more stable. Researchers at Pfizer reported that their renin inhibitor CP-80,794, which contains an S-methyl cysteine at P_2, is more active than the corresponding O-methyl serine derivative.[6] The incorporation of cysteine into the macrocycles, therefore, might enhance both activity and stability. Herein we report the synthesis and biological activities of a series of cysteine containing macrocyclic renin inhibitors.

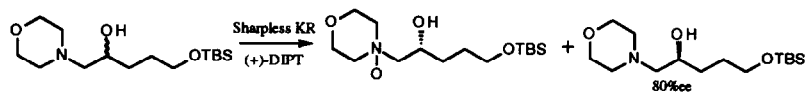
The synthesis of compounds of general structure **1** is illustrated in **Scheme 1**. Esterification of Boc-norACHPA acetonide **3** [5d] with an alkyl diiodide gave iodoalkyl ester **4**. Alkylation of L-cysteine with iodide **4** afforded an amino acid which was protected with a Cbz group to yield acid **5**. Removal of the Boc and acetonide protecting groups by TFA followed by the Keck macrocyclization conditions[7] furnished compound **6**. Removing the Cbz group by hydrogenolysis (H_2/Pd/C) was unsuccessful; however, treatment of the compound with TFA and DMS (3:1) removed it cleanly. The resulting amine was then coupled with the appropriate acid to afford compound **1** (n=0). Oxidation of the sulfide with sodium periodate or oxone[8] gave the sulfoxide or sulfone, respectively.



Scheme 1: (a) $I(CH_2)_mI$, K_2CO_3/DMF, RT., 2 h; 90%; (b) i) L-cysteine, i-Pr_2NEt, EtOH-H_2O, RT., 12 h; ii) CbzCl, $NaHCO_3$, H_2O-dioxane, RT., 2 h; 80% for 2 steps; c) i) TFA, RT., 1/2 h; ii) EDC, DMAP, DMAP•HCl, CH_2Cl_2, reflux, infuse amino acid in THF via a syringe pump; 30-50% for 2 steps; d) i) DMS/TFA (1/3), RT., overnight; ii) EDC, HOBT, NMM, RCO_2H, 0°, 4 h; 80% for 2 steps; e) $NaIO_4$/EtOH, RT., 2 h, n=1; 90%, f) oxone/MeOH-H_2O, RT., 1 h, n=2; 90%.



Scheme 2: (g) i) TBSCl, TEA, DMAP, CH_2Cl_2, reflux, 3 h; ii) MCPBA/hexane, RT., 4 h; 72% for 2 steps; h) morpholine, neutral Al_2O_3, ether, RT., 2 days, 87%; (i) **3**, 2 eqv. **9**, EDC, DMAP, CH_2Cl_2, RT., 4 h, 55% d1 (less mobile on tlc) and 38% d2; 90% d1 when 80% ee (R)-**9** is used; (j) HF-Py/THF, RT., 3 h, 90%; (k) i) MsCl, TEA/CH_2Cl_2; ii) **12**, LiHMDS/THF, Mesylate, -78° to RT. overnight; 70% 2 steps; (l) i) $NaHCO_3$/MeOH reflux, 1 h; ii) CbzCl, $NaHCO_3$, H_2O-CH_2Cl_2, RT., 2 h; 80% for 2 steps.



Scheme 3: (+)-diisopropyl tartrate, $Ti(Oi\text{-}Pr)_4$, *t*-BuOOH, CH_2Cl_2, -20°

Results of the in vitro plasma renin assay (PRA) [9]of cyclic inhibitors **1a-h** are summarized in **Table 1**. Thirteen membered ring compounds proved to be more active than their fourteen and twelve-membered ring counterparts. Oxidation of the sulfide to sulfoxide or sulfone decreases activity. In our previously reported macrocyclic renin inhibitors, the introduction of a substituent such as morpholinomethyl at a position corresponding to P_2' enhances activity by 5-25 fold over that of the unsubstituted analog;[5a,c] therefore, the

incorporation of a P_2' moiety to the thirteen-membered ring compounds could result in more potent inhibitors in this series as well. The synthesis of such inhibitors is shown in **Scheme 2**. 4-Pentene-1-ol **7** was converted to epoxide **8** by silylation and epoxidation. Reaction of the epoxide with morpholine yielded the racemic alcohol **9**. EDC coupling of alcohol **9** with acid **3** gave a diastereomeric mixture, which was separated by MPLC to afford **10a** and **10b**. Both isomers were carried though the synthesis for SAR studies. Deprotection of the TBS group with TBAF caused extensive intramolecular trans-esterification, while treatment with excess pyridinium hydrofluoride afforded the desired alcohol **11**. Mesylation of the alcohol **11** gave its mesylate. When subjected to the previously described alkylation conditions (**Scheme 1**), it gave a quaternary ammonium salt through intramolecular displacement of the mesylate by the morpholine nitrogen . In fact this process is spontaneous. Quaternary salt formation was noticeble after standing at room temperature for a few hours and the conversion was complete in one day. It became apparent that a more nucleophilic, organic-soluble cysteine equivalent was necessary in order for the nucleophilic reaction of the thiol to compete with the intramolecular displacement. The requirement for the cysteine protecting group is rather stringent. Because of the presence of thio, ester and Boc in intermediates such as **14**, procedures using oxidative, strongly alkaline, or acidic conditions for deprotection were not considered. In 1983, Zwanenburg reported the use of boroxazolidones to provide simultaneous protection of the amino and carboxyl group in α-amino acids.[10] The benzylation of a boroxazolidone cysteine derivative was reported in this paper, however, to our knowledge no subsequent use has been reported in the literature. The boroxazolidone could be converted to the amino acid under mild acidic or basic conditions. It turned out to be the perfect protecting group for our purpose. The mesylate, when treated with the thiolate of diethylborane protected cysteine, gave compound **13** in 80% yield from the alcohol **11**. Deblocking the borane complex in refluxing methanol in the presence of sodium bicarbonate, and reprotecting the resulting amino acid with a Cbz group afforded acid **14**. After this point, the synthesis was completed as described in Scheme 1. The desired R-enantiomer of compound **9** could be enriched (10:1) through a Sharpless kinetic resolution[11] using (+)-DIPT as the catalyst (**Scheme 3**). Since the minor isomer could be easily separated by flash column after coulpling with acid **3**, no attempt was made to optimize the kinetic resolution.

Comp'd	m	n	R	IC_{50} (nM)	Comp'd	isomer	R	IC_{50} (nM)
1a	1	0	A	108	**2a**	(R)	A	1.25
1b	2	0	A	42	**2b**	(S)	A	20000
1c	3	0	A	96	**2c**	(R)	B	0.35
1d	3	1	A	136	**2d**	(S)	B	2863
1e	3	2	A	217				
1f	3	1	B	45				
1g	2	2	B	28				
1h	2	0	B	3.1				

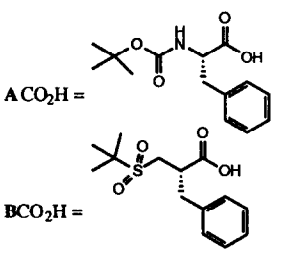


Table 1 Human Plasma Renin Inhibition by Macrocycles **1** and **2**

Incorporation of the P_2' morpholinomethyl group increases the potency by 10-30 fold. As observed before, the R-isomers at this center are 10^4 times more potent than the S-isomers.[5a,c] Replacement of the BocPhe at the N-terminus with (2R)-t-butylsulfonylmethyl-3-phenylpropionic acid effects a 4-10 fold increase in activity. The most potent compound reported here is **2c** with an IC_{50} of 0.35 nM. When tested in vivo, it lowered blood pressure about 20 mm Hg (17-23 mm Hg) for six hours (lenghth of the experiment) in conscious

sodium-depleted rhesus monkeys following an oral dose of 10 mg/kg. PRA was fully inhibited (>90%) during the experiment.[12]

In summary, we have developed a highly convergent synthesis of the potent cysteine incorporated macrocyclic renin inhibitors. Of the three ring sizes (12, 13, 14) in this series, thirteen is the most active. Oxidation of the sulfide to sulfoxide or sulfone decreases activity. The replacement of a serine ester linkage with a cysteine thio ether lead to very potent renin inhibitors. Incorporation of the morpholinomethyl (P_2') and appropriate P4-P3 subunits resulted in the subnanomolar inhibitor **2c**, which showed good oral activity and duration of action. The inhibitor design described here may be useful for the design of other aspartic proteinase inhibitors, and the synthetic strategy utilized here could be applicable for the synthesis of other thio ether linked macrocycles.

Acknowledgment: We thank Professor Dave A. Evans for helpful discussions. We gratefully acknowledge the bioassay support provided by Mr. John J. Doyle, Ms. Terry W. Schorn and Dr. Peter K.S. Siegl.

References

1 Wyvratt, M. J.; Patchett, A. A. *Med. Res. Rev.* **1985**, *5*, 483.

2 Zusman, R. M. *J. Kidney Dis.*, **1987**, X, suppl. 1, 13.

3 Boger, J. In *Annual Reports in Medicinal Chemistry*; Bailey, D. M., Ed., Academic Press: New York, 1985; Vol. 20, pp. 257.

4 For recent reviews see: Ocain, T. D.; Abou-Gharbia, M. *Drugs of the Future* **1991**, *16*, 37; b) Greenlee, W. J. *Med. Res. Rev.* **1990**, *10*, 173 and references cited therein.

5 (a) Weber, A. E,; Steiner, M. G.; Krieter, P. A.; Colletti, A. E.; Tata, J. R.; Halgren, T. A.; Ball, R. G.; Doyle, J. J.; Scheorn, T. W.; Stearns, R. A.; Miller, R. R.; Siegl, P. K. S.; Greenlee, W. J.; Patchett, A. A. *J. Med. Chem.* **1992**, 35, 3755-3733. (b) Weber, A. E.; Steiner, M. G.; Yang, L.; Dhanoa, D. S.; Tata, J. R.; Halgren, T. A.; Siegl, P. K. S.; Parsons, W. H.; Greenlee, W. J.; Patchett, A. A. In *Peptides. Chemistry and Biology. Proceedings of the Twelfth American Peptide Symposium* Smith, J. A.; Rivier, J. E., Eds., ESCOM: Leiden, 1992; pp 749. (c) Dhanoa, D. S., Parsons, W. H., Greenlee, W. J., Patchett, A. A. *Tetrahedron Lett.* **1992**, *33*, 1725. (d) Weber, A. E.; Halgren, T. A.; Doyle, J. J.; Lynch, R. J.; Siegl, P. K. S.; Parsons, W. H.; Greenlee, W. J.; Patchett, A. A. *J. Med. Chem.* **1991**, *34*, 2692. (e) Wittenberger, S. J.; Baker, W. R.; Donner, B. G.; Hutchins, C. W. *Tetrahedron Lett.* **1991**, *32*, 7655-7658. (f) Rivero, R. A.; Greenlee, W. J. *Tetrahedron Lett.* **1991**, *32*, 2453. (g) Thaisrivongs, S.; Blinn, J. R.; Pals, D. T.; Turner, S. R. *J. Med. Chem.* **1991**, *34*, 1276. (h) Sham, H. L.; Rempel, C. A.; Stein, H. H.; Cohen, J. *J. Chem. Soc., Chem. Commun.* **1990**, 666. (i) Sham, H. L.; Bolis, G.; Stein, H. H.; Fesik, S. W.; Marcotte, P. A.; Plattner, J. J.; Rempel, C. A.; Greer, J. Renin Inhibitors. *J. Med. Chem.* **1988**, *31*, 284.

6 Wester, R. T.; Holt, W. F.; Murphy,W. R. *Abstracts,199th ACS National meeting in Boston, Division of Medicinal Chemistry* **April**, **1990** abstract 128.

7 Boden, E. P.; Keck, G. E. *J. Org. Chem.* **1985**, *50*, 2394.

8 Trost, B. M.; Curran, D. P. *Tetrahedron Lett.* **1981**, *21*, 1287.

9 The human plasma renin assay was conducted at pH 7.4 using DMF as co-solvent according to protocol described in: Boger, J.; Payne, L. S.; Perlow, D. S.; Lohr, N. S.; Poe, M.; Blaine, E. H.; Ulm, E. H.; Schorn, T. W.; LaMont, B. I.; Lin, T.-Y.; Kawai, M.; Rich, D. H.; Veber, D. F. *J. Med. Chem.* **1985**, *28*, 1779.

10 Nefkens, G. H. L.; Zwaneburg, B. *Tetrahedron* **1983**, *39*, 2995.

11 Miyano, S.; Lu, L. D.-L.; Viti, S. M.; Sharpless, K. B. *J. Org. Chem.* **1983**, *48*, 3608.

12 Protocol for oral administration of renin inhibitors in conscious sodium deficient rhesus monkeys: Williams, P. D.; Perlow, D. S.; Payne, L. S.; Holloway, M. K.; Siegl, P. K. S.; Schorn, T. W.; Lynch, R. J.; Doyle, J. J.; Straus, J. F.; Vlasuk, G. P.; Hoogsteen, K.; Springer, J. P.; Bush, B. L.; Halgren, T. A.; Richards, A. D.; Jay, K.; Veber, D. F. *J. Med. Chem.*, **1991**, 34, 887.

(Received in USA 22 July 1993; accepted 31 August 1993)