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THE SYNTHESIS OF POTENT MACROCYCLIC RENIN INHIBITORS

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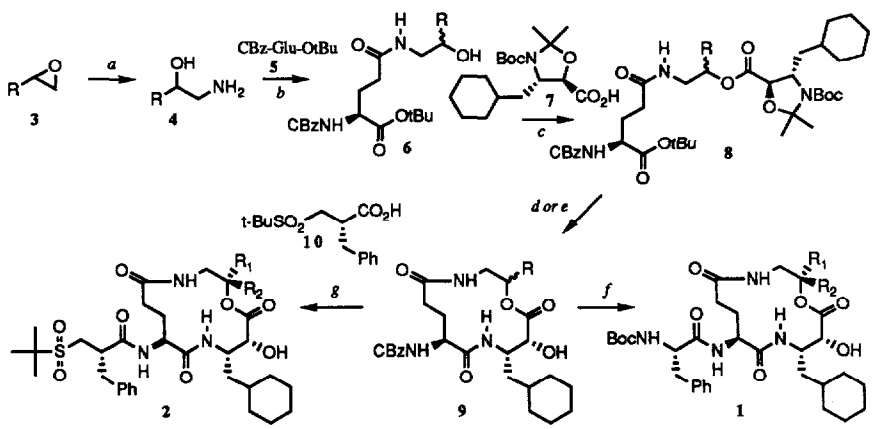
Abstract: *An efficient synthesis of a novel class of potent macrocyclic renin inhibitors exemplified by compounds 1 and 2, which involves the macrocyclization of 8 to 9 as the key step, is described. The macrocyclic design of renin inhibitors 1 and 2 disclosed here incorporates (2R,3S)-3-amino-4-cyclohexyl-2-hydroxybutanoic acid (norACHPA) as the transition-state isostere. Determination of stereochemistry of the substituent R (morpholinomethyl) at P_2' position of the more potent diastereomer of the 13- membered cyclic renin inhibitors 1, 2, and 9 is presented.*

The renin-angiotensin system (RAS), a proteolytic cascade, has been implicated in several forms of hypertension.[1] Renin, synthesized by the kidneys, is a highly specific aspartyl proteinase with only one known naturally occurring substrate, angiotensinogen.[2] Renin catalyzes the first and rate-limiting step of the RAS by cleaving the substrate angiotensinogen to produce the decapeptide angiotensin I in humans. Angiotensin I is further hydrolyzed by angiotensin-converting enzyme (ACE) to a biologically active octapeptide angiotensin II (AII), a potent vasoconstrictor that leads to the elevation of blood pressure. Because of the high substrate specificity of renin, its inhibitors could have pharmacological advantages over antihypertensive drugs with less specific modes of action such as ACE inhibitors.[3] Although many potent renin inhibitors have been reported, majority of these being peptidic in nature suffer from problems such as poor oral absorption, short duration of action, proteolytic instability and rapid biliary excretion.[4] The development of cyclic renin inhibitors to combat these problems has attracted considerable attention recently.[5] Some of the more promising macrocyclic renin inhibitors have recently been reported.[6] Advantages offered by the macrocyclic design of renin inhibitors include stabilization against cleavage by proteolytic enzymes and potential for increased binding interactions with active sites of the enzyme to achieve high potency due to their restricted conformations. We herein report on the synthesis of a novel class of highly potent macrocyclic renin inhibitors incorporating (2R,3S)-3-amino-4-cyclohexyl-2-hydroxybutanoic acid[7] (*nor*ACHPA) as the transition-state isostere.

The synthesis of the macrocycles **1** involves coupling of the appropriate aminoalcohol **4** with N-Cbz-L-glutamic acid-α-*t*-butyl ester **5** followed by acylation with Boc-*nor*ACHPA acetonide **7**[6,7] and macrocyclization of the precursors **8** as illustrated in scheme **1**. The aminoalcohol **4** was obtained by ring opening of the epoxide **3** with LiN_3 in DMF followed by hydrogenation of the azide using $Pd(OH)_2$ as a catalyst. Coupling of **4** with Cbz-GLu-OtBu **5** using ethyl(dimethylamino)propylcarbodiimide (EDC) and hydroxybenzotriazole (HOBt) afforded glutamine derivative **6** as an inseparable mixture of diastereomers (for R = Et, *n*-Bu, *n*-hexyl, morpholinomethyl) in 75-80% yield. The intermediate **6** was acylated with *nor*ACHPA derivative **7** utilizing EDC/DMAP to produce the macrocyclization precursor **8** in 56-90% yield. While the diastereomeric mixtures of **8a** (R = Et), **8b** (R = n-

Bu), and **8c** (R = n-hexyl) were inseparable, the diastereomers of **8d** (R = morpholinomethyl) were separated by flash column chromatography and/or MPLC in 37% yield for each diastereomer.

The cyclization precursors **8a-d** were cyclized by two different methods of macrocyclization. In method A,[8] the intermediate **8** was deprotected by treatment with TFA, dried over P_2O_5/KOH, neutralized with N-methylmorpholine (NMM) and then subjected to macrocyclization conditions (EDC, HOBt, THF, 0.001M) under high dilution to afford the macrocycle **9** in (30-70%) yield. In method B,[9] **8** was treated with TFA, dried over P_2O_5/KOH and a THF solution of the resultant deprotected aminoacid was added via a syringe pump over 20 h to a mixture of EDC/DMAP-HCl/DMAP in refluxing ethanol-free $CHCl_3$ to produce **9** in 55-65% yield. Although diastereomers of **9a** (R = Et) and **9c** (R = n-hexyl) were obtained as inseparable mixtures, the diastereomers of **9b** (R = n-Bu) and **9d** (R = morpholinomethyl) were isolated as pure compounds. The N-Cbz group of **9** was removed by hydrogenation and the resultant macrocyclic amine upon coupling with N-Boc-phenylalanine (BocPheOH) or (2R)-t-butylsulfonylmethyl-3-phenylpropionic acid **10**[10] using EDC/HOBt, after purification, afforded the pure diastereomers of macrocyclic renin inhibitors **1** and **2** respectively.[11]



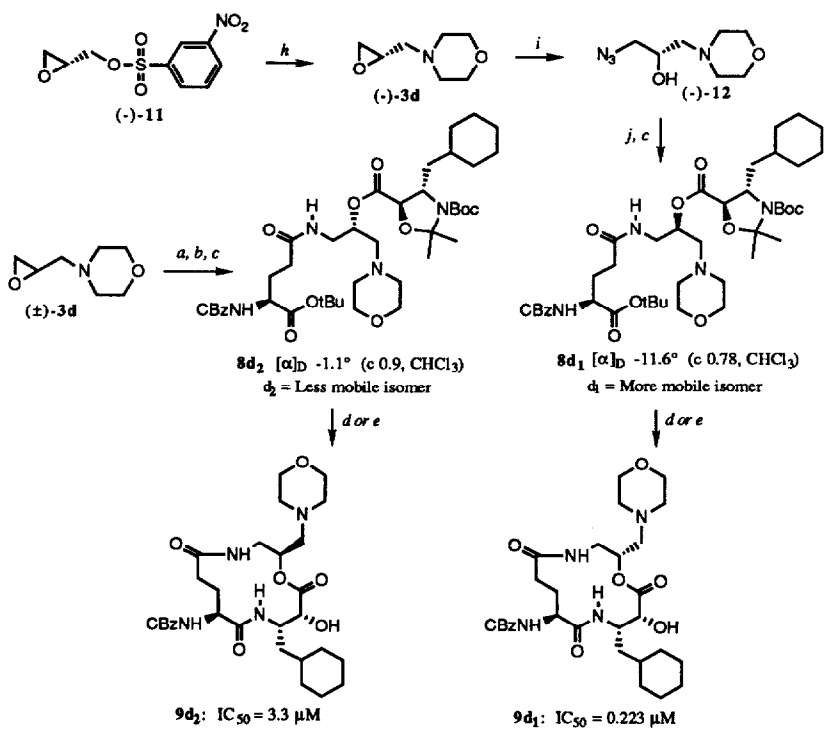
Scheme 1: (a) i. LiN_3, DMF; ii. H_2, $Pd(OH)_2$, MeOH; 68-78% for 2 steps; (b) **5**, EDC, HOBt, CH_2Cl_2; 75-80%; (c) **7**, EDC, DMAP, CH_2Cl_2; 56-90%; (d) i. TFA, CH_2Cl_2; ii. EDC, HOBt, NMM, THF, 0.001M, 90 h; 34-70% for 2 steps; (e) i. TFA, CH_2Cl_2; ii. EDC, DMAP-HCl, DMAP, $CHCl_3$, THF, reflux; (55-65%) for 2 steps; (f) i. H_2, Pd-C, EtOAc, THF, MeOH; ii. BocPheOH, EDC, HOBt, NMM, CH_2Cl_2; 45-75% for 2 steps; (g) i. H_2, Pd-C, EtOAc, THF, MeOH; ii. **10**, EDC, HOBt, NMM, CH_2Cl_2; 30-40% for 2 steps.

Results of the *in vitro* plasma renin assay[12] (*PRA*) of the cyclic inhibitors **1** and **2** summarized in Table I revealed that one of the diastereomers (d_1, the more mobile isomer on tlc) of BocPhe analogues **1a-d**, consistently displayed 5-25 fold higher potency[13] for renin inhibition than their epimers (d_2, the less mobile isomer on tlc). The incorporation of hydrophobic groups such as Et, n-Bu, n-hexyl and morpholinomethyl (CH_2Morph) in **1a-d** increased the renin inhibitory potency over that of the unsubstituted analogue **1e** (R_1 = H, R_2 = H, IC_{50} = 610 nM)[7a] by 10-25 fold. We attribute this higher potency to the binding of R (R_1/R_2) to S_2' subsite of enzyme, the binding pocket that accommodates the hydrophobic P_2' side chain of the angiotensinogen. The *PRA* demonstrate that replacement of BocPhe at the N-terminus of **1** with (2R)-t-butylsulfonylmethyl-3-phenylpropionic acid **10** affects a 5-8-fold enhancement in the potency of the macrocycles **2b and 2d.**

Table I: Human Plasma Renin Inhibition by Macrocycles **1** and **2**[12,13]

Compound	R_1	R_2	IC_{50}, nM
$1a_1$	Et	H	22.9
$1a_2$	H	Et	200
$1b_1$	n-Bu	H	24.8
$1b_2$	H	n-Bu	133.8
$1c_1$	n-Hexyl	H	48.85
$1c_2$	H	n-Hexyl	663.5
$1d_1$	CH_2Morph	H	55.4
$1d_2$	H	CH_2Morph	1300
$2b_1$	n-Bu	H	5.0
$2d_1$	CH_2Morph	H	6.9

The stereochemistry of the side chain (R = Et, n-Bu, n-hexyl) in **1a-c** was assigned by analogy to the stereochemistry of the chiral macrocycle $\mathbf{1d_1}$ (R_1 = CH_2Morph, R_2 = H), established by enantiospecific synthesis of the macrocyclic precursor $\mathbf{8d_1}$ to yield $\mathbf{1d_1}$, from the optically pure epoxide (2R)-(-)-glycidyl-3-nitrobenzenesulfonate **11**[14] (scheme **2**). Treatment of **11** with the potassium salt of morpholine yielded epoxide **3d** by displacement of 3-nitrobenzenesulfonate group of **11**.[14] Ring opening of the chiral epoxide **3d** with LiN_3 gave the optically active hydroxyazide **12**. Reduction of **12** followed by coupling of the resultant aminoalcohol with the glutamic acid derivative **5** and subsequent acylation with the Boc-*nor*ACHPA acetonide **7** as described in



Scheme 2: (h) Morpholine, KH, THF, 0°C-RT, (60%); (i) LiN_3, THF, (73%); (j) H_2, Pd $(OH)_2$, MeOH, (70%); (a-e) Refer to scheme **1**.

scheme **1**, yielded a single enantiomerically pure diastereomer **8d$_1$**, $[\alpha]_D$ -11.6° (c 0.78, $CHCl_3$). The chiral intermediate **8d$_1$** was found to display spectral data (^{1}H NMR, R_f, $[\alpha]_D$) identical to that of the more mobile diastereomer **8d$_1$** of the two diastereomers (**8d$_1$** and **8d$_2$**) obtained from the racemic epoxide **3d** by the synthetic route illustrated in scheme **1**. The deprotection of **8d$_1$** (TFA/CH_2Cl_2) and subsequent macrocyclization (method B) gave the most active isomer **9d$_1$** (IC_{50} = 223 nM) in the N-Cbz series of macrocycles **9a-d**. Removal of the Cbz group (H_2/Pd-C) of **9d$_1$** followed by coupling with **10** as described in scheme **1** yielded the cyclic inhibitor **2d$_1$** (IC_{50} = 6.9 nM).

In summary, we have described a highly convergent synthesis of enantiomerically pure macrocyclic renin inhibitors **1**, **2**, and **9**. The absolute configuration of the stereocenter bearing the P_2' substituent R (CH_2-Morph) in macrocyclic inhibitors **1**, **2** and **9** has been established as (S) in the more potent series of diastereomers (d_1-series). We have also shown that the incorporation of P_2' substituents in the P_2-P_1' linked 13-membered macrocyclic renin inhibitors of the glutamine series, incorporating *nor*ACHPA as the transition-state mimic of the tetrahedral intermediate for hydrolysis of the scissile bond, yield potent inhibitors of renin. Further modification of the macrocyclic design **1** by replacement of the BocPhe amino-terminus with (2R)-t-butylsulfonylmethyl-3-phenylpropionic acid **10** provides a potent class of macrocyclic renin inhibitors **2b$_1$** (IC_{50} = 5 nM) and **2d$_1$** (IC_{50} = 6.9 nM). The synthesis and design of the macrocyclic renin inhibitors **1** and **2** presented here offer possibilities for the development of new enzyme inhibitors of the aspartic proteinase class.

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