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Peptidomimetic Inhibitors of Renin Incorporating Topographically Modified Isosteres Spanning the $P_1(\rightarrow P_3)$-P_1' Sites

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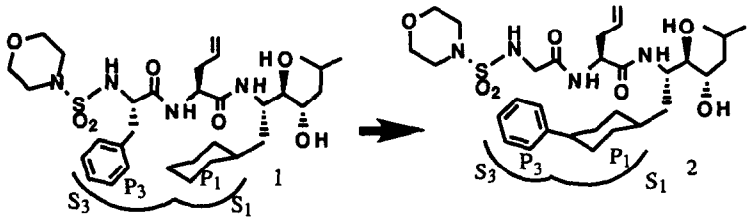
Abstract: Dihydroxyethylene isostere- modified dipeptides incorporating topographically modified $P_1(\rightarrow P_3)$ side chains were investigated as structurally novel renin inhibitors; the binding affinity of selected compounds exhibited near nanomolar binding affinity.

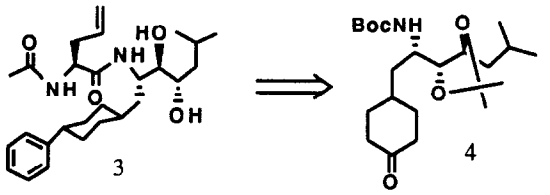
Blockade of a proteolytic step leading to the pressor agent angiotensin II, in the renin- angiotensin system (RAS), has been shown to be an effective means of controlling hypertension as previously demonstrated by the success of angiotensin converting enzyme (ACE) inhibitors. Hence, inhibition of the aspartyl proteinase renin, the rate- limiting enzyme in the cascade leading to angiotensin II, may prove to be an effective means of controlling hypertension.[1,2] However, limited oral bioavailability and short duration of action have typically compromised peptide-like renin inhibitors.[2]

Since the initial work by Boger and coworkers,[3] who increased potency of statine based renin inhibitors by replacing the P_1 isobutyl sidechain with a cyclohexylmethyl group, the latter group has been the standard P_1 sidechain in a variety of transition state based renin inhibitors. Recently, cyclic pseudopeptidyl inhibitors of the aspartyl protease pepsin have been described[4] in which the sidechains of P_1 and P_3 are covalently linked taking advantage of a continuous binding pocket. We decided to exploit the observation[5] that the cyclohexylmethyl group of P_1 and the phenyl group of P_3 also occupy a contiguous hydrophobic pocket in the enzyme as shown in **1**. In contrast to the work of Rich, we chose to append the

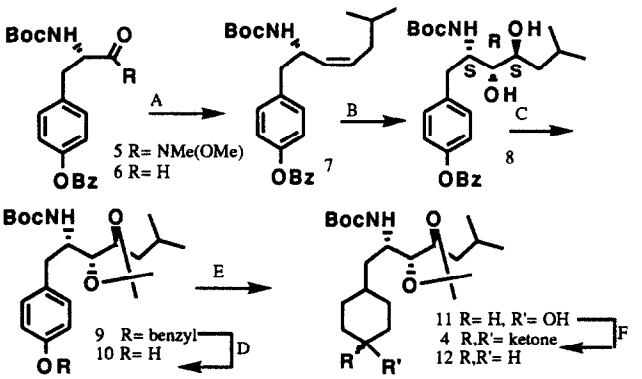


P_3 side chain directly to the P_1 side chain and eliminate the P_3 side chain linkage to peptide backbone providing a structure such as **2**. The P_3-P_1 spatial relationship (inhibitor enzyme complex) was explored by appending aromatic and alkyl groups to the cyclohexylmethyl group in P_1. Conceptually, by extending the P_1 sidechain to the S_3 binding pocket, truncation of the peptide backbone as in **3** might be possible, allowing potential improvement in bioavailability and duration of action of such inhibitors. Herein we report

the synthesis and binding affinity of renin inhibitors incorporating these novel transition state analogues through the versatile intermediate **4**.[11]



A flexible convergent synthesis of a variety of transition state analogues with alkyl and aryl groups appended from the 4-position of the cyclohexylmethyl group was desired. Specifically, the 4-ketocyclohexyl derivative **4** was the targeted intermediate to the desired appended analogues by either Grignard addition into or Wittig olefination of the 4- keto group. The synthesis of key intermediate **4** began from commercially available N-Boc-O-benzyl tyrosine which was converted via its mixed anhydride[6A] to the N,O- dimethylamide **5**.[6B] Reduction with lithium aluminum hydride provided the aldehyde **6** which reacted

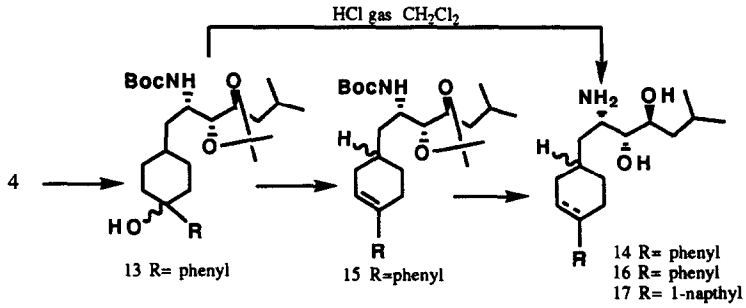


A) isoamyltriphenylphosphonium bromide 2.05 equiv.,THF, KHMDS 2.1 equiv., toluene, -50 °C to RT 86% three steps; B) OsO_4 0.02equiv., NMO 2.0 equiv., acetone/water(5:1), 40%; C) CH_2Cl_2/dimethoxypropane(1:1), PPTS reflux, 100%; D) 20%Pd/C 10 weight percent, methanol, 55psi H_2 ,98%; E) 10%Ru/C 20 weight percent, ethanol, 50 °C, 1500psi H_2, 88%; F) 2.5M Jones reagent, acetone, -10 °C, 59%.

with the ylide derived from isoamyltriphenylphosphonium bromide to give exclusively the cis- olefin **7**.[11] Hydroxylation of the olefin proceeded smoothly to give approximately a 2:1 mixture of diastereomers which were readily separable by flash column chromatography.[7,8] The major isomer **8**[11] was carried forward and was shown to be the desired S,R,S- isomer (*vide infra*).The diol was protected in quantitative yield as its acetonide **9**[11] employing dimethoxypropane/PPTS in refluxing dichloromethane. Hydrogenolysis of the benzyl protecting group over Pd/C provided the phenol **10**; reduction of the aromatic ring using 10% Ru/C in ethanol at 50 °C and 1500 psi hydrogen gave a mixture of cis and trans cyclohexanols **11**[11] in 90% yield

for these two steps. Alternatively, reduction of **10** with PtO_2 resulted in a significant quantity of the hydrogenolysis product **12**. Although **12** was an undesired by-product, it was useful for establishing the stereochemistry of the major diol isomer **8**. Conversion of the known 2S,3R,4S-2-[(*tert*-butyloxycarbonyl)amino]-1-cyclohexyl-3,4,-dihydroxy-6-methylheptane[8] to its acetonide provided **12**, independently demonstrating that the major diastereomer from the hydroxylation reaction had the desired 2S,3R,4S- stereochemistry shown in **8**.[8] Jones oxidation of **11** provided the 4-ketocyclohexyl intermediate **4** in 17% overall yield from the commercially available protected amino acid.

Reaction of **4** with organolithium reagents was found to be superior to the corresponding Grignard reagent, which lead predominantly to enolization of the ketone or protected amine resulting in precipitation during the reaction and recovery of starting material. Treatment of **4** in THF with commercially available phenyllithium provided a mixture of alcohols **13**. These alcohols could be treated with hydrogen chloride in dichloromethane to provide the styryl derivative **14**, a dihydroxyethylene isostere suitable for further elaboration to a renin inhibitor, directly in 60% yield from the starting ketone **4**. Alternatively, the crude

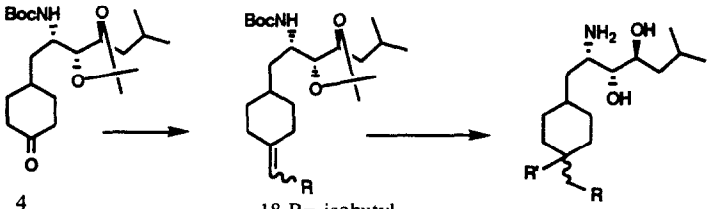


mixture of alcohols was eliminated to the olefin **15** using a catalytic amount of p-TSA in dichloromethane for 4h at room temperature. The olefin **15** was reduced with 20% Pd/C at one atmosphere of hydrogen to give a ~1:1 mixture of cis\trans- cyclohexane derivatives which were inseparable by thin layer chromatography; deprotection with hydrogen chloride in dichloromethane provided the desired amine **16**[11] in 53% yield from ketone **4**. The conversion of **4** to **16** was carried out without purification of intermediates since chromatography of the alcohol or olefin derivatives resulted in substantial material losses. In a like manner transmetalation of 1-bromonapthalene followed by addition into ketone **4** gave the 1-naphthyl derivative, **17**, as a 1:1 mixture of isomers in 38% yield from the ketone **4**.

Reaction of **4** with the ylide derived from isoamyltriphenylphosphonium bromide provides the olefin **18**[11] in 89% yield. Deprotection with hydrogen chloride gas in dichloromethane provides the tertiary alkylchloride **19** which was dehydrohalogenated with palladium on carbon giving the desired aminodiol **20**[11] in 82% yield (Scheme 3). Treatment of **4** with the ylide derived from benzyltriphenylphosphonium chloride gave the corresponding olefin in 60% yield; this compound was hydrogenated then deprotected, as previously described, providing the aminodiol **21**.[11] Thus, transition state analogues with a variety of

aromatic, alkyl or benzylic substituents appended to the 4- position of the cyclohexylmethyl group can be easily prepared.

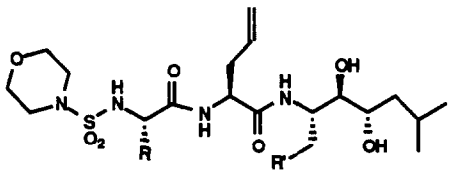
These transition state isosteres were then coupled to Boc- allyl glycine using standard carbodiimide chemistry. The Boc- protecting group was removed followed by coupling to N-(4-morpholinosulfonyl) glycine[9] providing the fully elaborated renin inhibitors.

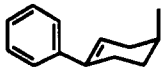
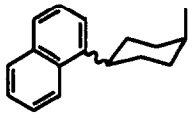


These inhibitors were tested against monkey plasma renin according to a previously described method.[10] The binding affinities were compared to two standard renin inhibitors **22**[11] and **23**[11] to determine if the concept of tethering a group from the cyclohexylmethyl P_1 moiety into the S_3 binding pocket was feasible. The first tethered compound synthesized contained a phenyl group appended to the cyclohexyl ring providing, **24**[11], a compound with 60.5 nM binding affinity. This binding affinity is similar to the surprisingly good affinity of the P_3 glycine analogue, **23**, at 82 nM and approximately 300 fold less active than the P_3 phenylalanine parent compound **22**. Interestingly, the incorporation of an olefin into the cyclohexyl-phenyl sidechain afforded **25**[11]; a compound with a three fold increase in binding affinity over **24**. The presence of this olefin obviously changes the conformation of the cyclohexane ring resulting in a slightly modified spatial orientation of the phenyl tether. This highlights the sensitivity of this approach to minor structural modifications. In fact, replacing the phenyl appendage in **25** with the benzyl appendage found in **26**[11] resulted in a decreased binding affinity of 202 nM. The incorporation of the an alkyl appendage as in **27**[11] resulted in a drastically lowered binding affinity. Fortunately, the activity was restored when the 1-naphthyl appendage was employed resulting in **28**[11] whose binding affinity is 11 nM, a 7.5 fold increase over the P_3 glycine standard **23**. However, compound **28** is still less potent than the parent P_3 phenyl derivative, **22**, suggesting that the appendages employed here do not optimally fill the hydrophobic space normally occupied by the P_3 phenylalanine moiety. The preliminary results described here support the concept that appending sidechains complimentary to contiguous binding pockets may lead to increased binding affinity when an appropriate appendage is employed.

The key intermediate **4** is a versatile and readily available starting material for the synthesis of novel transition-state mimics of P_1-P_1' dipeptides that append sidechains to neighboring binding pockets. The ability to extend from the transition state isostere directly to relatively distant binding pockets without involving the peptide backbone is seen as a promising strategy toward designing smaller substrate- based

enzyme inhibitors that may eventually overcome the problems associated with more peptide- like enzyme inhibitors.[2] The binding affinities of other renin inhibitors incorporating such topographically modified transition state mimics will be reported elsewhere.



No.	R	R'	Binding affinity IC_{50} (nM)
22	Benzyl	Cyclohexyl	0.2
23	H	Cyclohexyl	82
24	H		60.5
25	H		18.5
26	H		202
27	H		29% at 10^{-6}
28	H		11

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11. Analytical data for these compounds includes IR, MS, ^{1}H NMR, and C,H, N analysis.