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Synthesis of novel keto-ACE analogues as domain-selective angiotensin I-converting enzyme inhibitors

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Abstract—Novel analogues of the angiotensin I-converting enzyme (ACE) inhibitor keto-ACE were synthesized via a facile Horner–Emmons olefination of a phosphonoketone precursor with ethyl glyoxylate. Introduction of a bulky aromatic tryptophan at the P'_2 position of keto-ACE resulted in a significant increase in C-domain-selectivity.

Angiotensin-converting enzyme (ACE) inhibitors are widely used in the treatment of hypertension and myocardial infarction. ACE is comprised of two enzymatically active (N- and C-) domains with different physiological functions. The adverse drug effects associated with current generation ACE inhibitors are thought to arise from inadequate domain-selectivity.[1] The recent determination of the X-ray structure of ACE in complex with various inhibitors[2–4] has paved the way for the protein structure-based design of new more domain-selective inhibitors with improved pharmacological profiles. Ketomethylene derivatives have been used extensively in the design of protease inhibitors.[5,6] 5-*S*-5-Benzamido-4-oxo-6-phenylhexanoyl-L-proline (keto-ACE), the first of such compounds, was shown to be a good inhibitor of ACE with modest domain selectivity.[7] Various strategies and modifications to the synthesis of keto-ACE have been described.[8–10] These synthetic approaches have a number of limitations: a modified Dakin–West reaction,[9] which proceeds via a 5-oxazolone intermediate, is accompanied by racemization; the use of a Grignard reagent with 2-pyridyl thiolate generates the keto acetal intermediate in low yield;[8,10] and the synthesis of ketomethylene dipeptide analogues using diazomethane is unattractive.[11,12] Here, we report a simple and facile synthesis of keto-ACE and its analogues as well as their ACE inhibitory profiles.

In synthesizing new analogues of keto-ACE, we chose a general strategy outlined in Scheme 1. The starting materials, N-protected-L-phenylalanine methyl esters **1** and **2**, were prepared from L-phenylalanine in the presence of either benzoyl chloride or di-*tert*-butyl dicarbonate in 1,4 dioxane and 1 M NaOH followed by treatment with thionyl chloride and methanol to give the resulting esters in quantitative yield. The methyl esters **1** and **2** were converted into the β-keto phosphonates **3** and **4** by treatment with 8 equiv of lithiated dimethyl methyl phosphonate in anhydrous THF at −78 °C as described by Dézil et al.[13] (Scheme 1). The key intermediates, α,β-unsaturated keto derivatives **5** and **6**, were obtained in excellent yield as isomeric mixtures by the Horner–Emmons olefination of **3** and **4**, respectively, with 1 equiv of freshly prepared ethyl glyoxylate[14] in the presence of potassium carbonate and dry ethanol at ambient temperature. However, the ^{1}H NMR spectra of **5** and **6** showed that the above reaction conditions gave a substantial amount of the *cis*-isomer [ratio, 85:15 (*trans*:*cis*)]. Separation of this isomeric mixture was unnecessary as catalytic hydrogenation of the double bond over 10% Pd on activated carbon afforded the intermediate ketomethylene esters **7** and **8** in 80% and 92% yields, respectively (Scheme 1). These esters were hydrolysed in the presence of 0.5 N LiOH in THF–MeOH (8:2) at room temperature to give the desired acids **9** and **10** in 84% and 98% yields, respectively. Coupling of the free acids **9** and **10** with the corresponding O-protected amino acids was effected under typical peptide coupling conditions using EDC·HCl in the presence of HOBt and diisopropylethylamine as a base to provide the corresponding esters **11**–**16** in excellent

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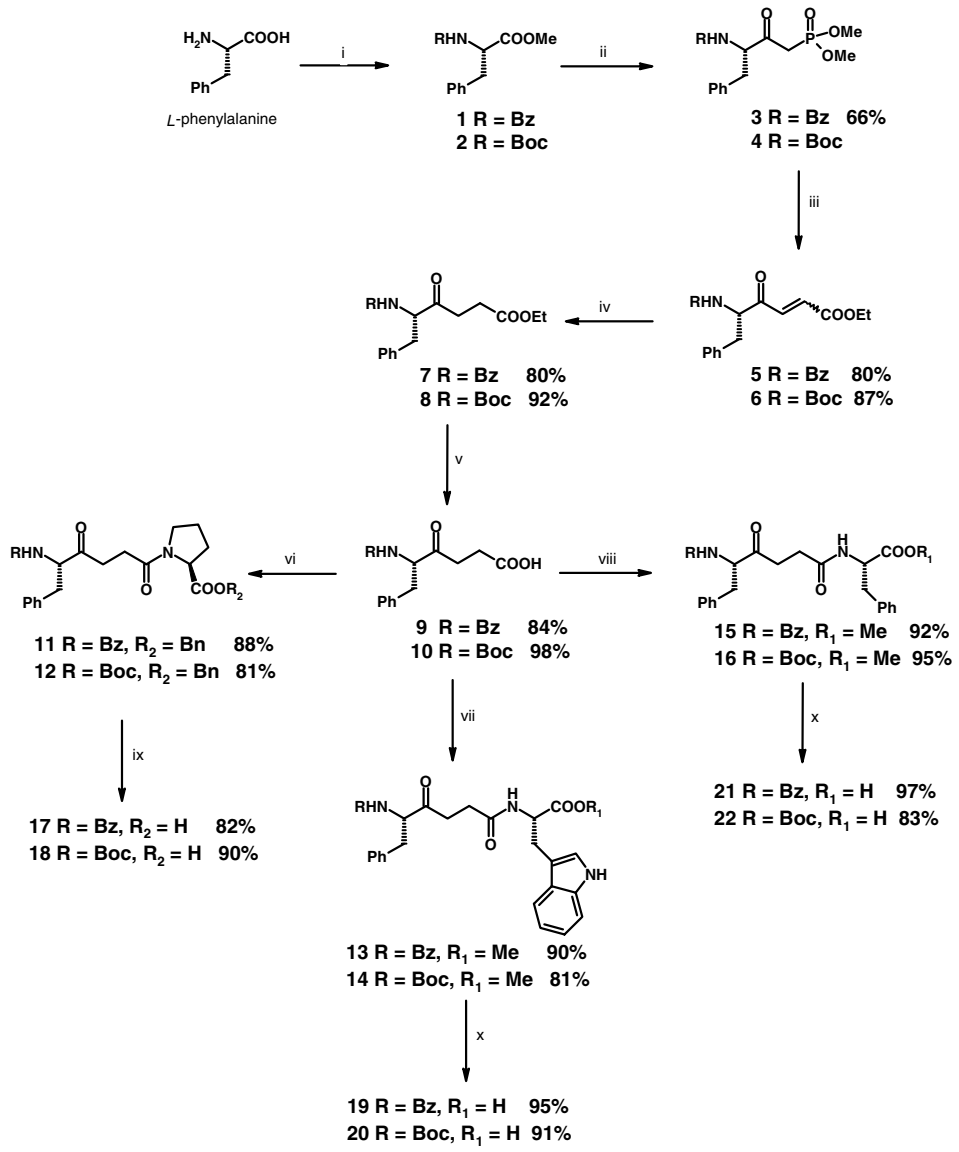


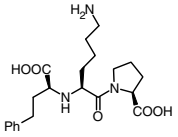
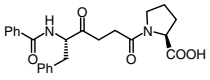
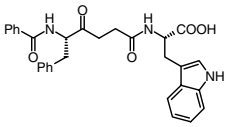
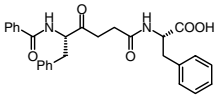
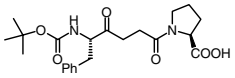
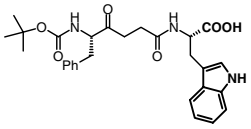
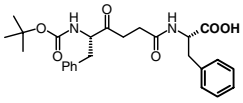
Scheme 1. Reagents and conditions: (i) a—RX, 1,4-dioxane, 1 M NaOH, 0 °C, 30 min; b—$SOCl_2$, dry MeOH, 0 °C to rt, 24 h; (ii) $(CH_3O)_2P(O)CH_3$, ^{n}BuLi, THF, −78 °C; (iii) EtOOCCHO, K_2CO_3, EtOH, 25 °C; (iv) H_2, Pd/C, EtOAc, rt, 2 h; (v) 0.5 N LiOH, THF–MeOH (8:2 ratio), rt, 3 h; (vi) L-proline benzyl ester hydrochloride, EDC·HCl, HOBt, ^{i}Pr$_2$NEt (2.0 equiv), DMF/CH_2Cl_2, rt, 48 h; (vii) L-tryptophan methyl ester,[15,16] EDC·HCl, HOBt, ^{i}Pr$_2$NEt (1.0 equiv), DMF/CH_2Cl_2, rt, 48 h; (viii) L-phenylalanine methyl ester hydrochloride,[17] EDC·HCl, HOBt, ^{i}Pr$_2$NEt (2.0 equiv), DMF/CH_2Cl_2, rt, 48 h; (ix) H_2, Pd/C, EtOAc–MeOH (3:1 ratio), rt, 24 h; and (x) 0.5 N LiOH, THF–MeOH (8:2 ratio), rt, 2 h.

yields. Keto-ACE **17** and compound **18** were obtained in 82% and 90% yields, respectively, by catalytic hydrogenation (10% Pd/C) of the benzyl ester group of compounds **11** and **12**.

The methyl ester derivatives **13**–**16** were saponified with aqueous 0.5 N LiOH and a mixture of THF–MeOH at room temperature to afford the desired compounds **19**–**22** in quantitative yields. We also found that compound **17** could easily be obtained through standard removal of the Boc group from compound **12** by treatment with 0.5 M triflic acid in the presence of dichloromethane. Benzoylation of the free amine with an excess of benzoyl chloride in dry pyridine followed by hydrogenolysis produced compound **17** in 90% yield. All new compounds gave ^{1}H and ^{13}C NMR, MS, and purity consistent with their structures.

ACE inhibitory activities of compounds were determined as previously described[7] with some modifications. Recombinant truncated forms of the C- and N-domains were used in the enzyme assays. tACEΔ36NJ is a testis ACE construct lacking the first 36 residues and truncated at Ser^{625} that is identical to the C domain of somatic ACE.[18] Enzyme (4 μg/ml) was preincubated with inhibitor at concentrations ranging from 0 to 500 μM. Residual enzyme activity was determined from enzyme–inhibitor solution using Z-Phe-His-Leu at two substrate concentrations. The enzyme assay was adapted so it could be carried out in a 96-well plate and the fluorescence was measured at Ex = 360 nm; Em = 486 nm on a Varian Cary Eclipse plate reader. All assays were conducted in triplicate. Inhibition curves were plotted using GraphPad Prism 4.01 and K_i values were determined from Dixon plots of $1/V$ versus $[I]$.

Table 1. ACE inhibitory activity profile[19]

Compound	Structure	K_i C domain	K_i N domain	K_i N/C selectivity
Lisinopril		51.0 nM	131.5 nM	2.6
17		1.8 μM	45.2 μM	25
19		0.8 μM	195.7 μM	245
21		23.7 μM	84.3 μM	3.6
18		143.5 μM	33.6 μM	0.2
20		381.3 μM	13.3 μM	0.04
22		313.0 μM	43.0 μM	0.1

The inhibition constants of compounds **17**–**22** were determined for the hydrolysis of the peptide substrate Z-Phe-His-Leu by the two separate N- and C-domains of ACE (Table 1). All the compounds displayed K_i values in the micromolar range as compared with the nanomolar K_i value of lisinopril (Table 1).

The activity of either ACE N- or C-domain was inhibited by the tryptophan derivative **19** (195.7 and 0.8 μM, respectively) and its counterpart with a P_2 Boc group **20** (13.3 and 381.3 μM, respectively). Thus, **19** was more C domain-selective, whereas **20** was more potent against the N domain. In the N domain the S2 Phe 391 is replaced by a more polar Tyr369 which reduces the size of the binding pocket decreasing the distance from the Tyr side chain to the Boc group of compound **20** and favouring hydrogen bonding with a lone electron pair on the oxygen of the Boc group. Therefore, the N domain S_2 pocket may tolerate a non-aromatic group more readily than an aromatic group and as a result this renders compound **20** more N-selective. The increased C-selectivity of **19** is likely due to the hydrophobic interactions between the P_2' tryptophan of **19** and Val379 and Val380 of the C domain active site which are replaced by polar residues (Ser and Thr, respectively) in the N domain. Moreover, the C-selectivity of this compound (245-fold) was significantly greater than that determined for keto-ACE **17** (25-fold) and also reported previously by Deddish et al.[7]

In summary, we have developed an efficient and versatile approach for synthesizing selective ACE inhibitors where the introduction of different fragments can be used to optimize the interactions between the inhibitor and the active site residues of the enzyme.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.06.003.

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