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A NEW POTENT INHIBITOR FOR ANGIOTENSIN CONVERTING ENZYME: (*R,S*)-CAPTOPRIL-F_3

Iwao Ojima* and Fabian A. Jameison
Department of Chemistry, State University of New York at Stony Brook, Stony Brook, New York 11794

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Abstract: *Trifluoromethyl analogs of captopril, i.e (R,S)- and (S,S)-captopril-f_3, are synthesized and submitted to in vitro assay for inhibition of angiotensin converting enzyme (ACE). It is found that (R,S)-captopril-f_3 is substantially more potent than captopril (captopril-f_3: $IC_{50} = 2.9 \times 10^{-10}$ M). Stereoelectronic and conformational effects attributed to trifluoromethyl incorporation serve to explain the enhanced inhibitory activity.*

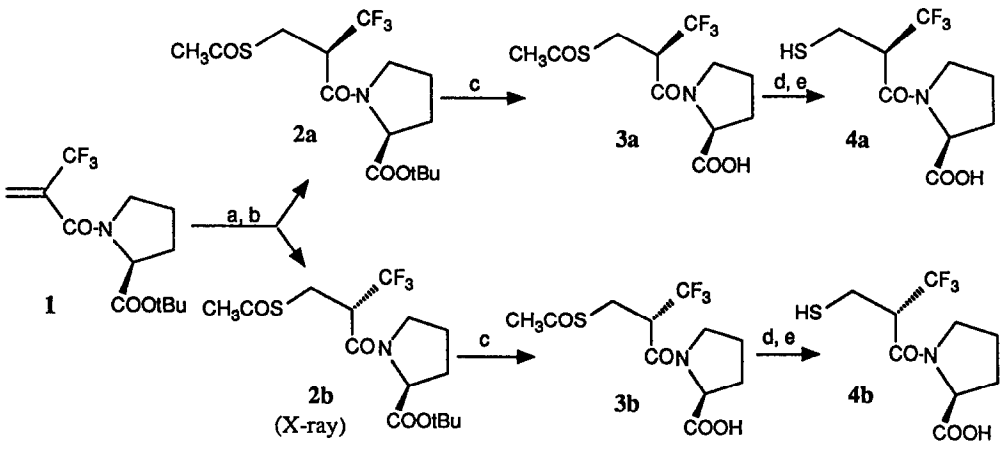
Since the discovery of the early generation angiotensin converting enzyme (ACE) inhibitors captopril[1] and Enalaprilat,[2,3] other inhibitors displaying potencies greater than or equal to the aforementioned compounds have been prepared.[4-10] However, much attention has not been directed toward the investigation of fluorinated analogs of ACE inhibitors.[7] It has been demonstrated that fluorine or trifluoromethyl incorporation into biologically active molecules imparts unique physiochemical properties including increased lipophilicity and enhanced drug transport and delivery.[12] We describe here the discovery of a new and highly potent ACE inhibitor, (*R,S*)-captopril-f_3 (**4a**), which clearly demonstrates such effects of trifluoromethyl group.[11]

During the course of our study on the asymmetric Michael-type addition of sulfur and nitrogen nucleophiles to 2-trifluoromethylacrylic acid derivatives,[13] it became apparent that this method could successfully be applied to the synthesis of novel trifluoromethyl analogs of captopril. Coupling of (*S*)-proline *tert*-butyl ester with 2-trifluoromethylacryloyl chloride derived from α-trifluoromethylacrylic acid,[14] gave the requisite Michael acceptor, 2-trifluoromethylacryloyl-(*S*)-proline *tert*-butyl ester (**1**) in a good yield.

Conjugate addition of thiolacetic acid to **1** afforded a 1:2 (*RS/SS*) diastereomeric mixture of adducts, **2a** (*R,S*) and **2b** (*S,S*), which were separated by medium pressure liquid chromatography[16] on silica gel (Scheme 1). The stereochemistry of **2a** and **2b** was assigned on the basis of the X-ray crystal structure of the (*S,S*)-isomer, **2b** (Figure 1). Deblocking of the *tert*-butyl ester by conventional trifluoroacetic acid-anisole, though successful, was inexplicably plagued by poor yields. The application of a procedure employing iodotrimethylsilane as an agent for the facile cleavage of *tert*-butyl esters,[16,17] gave mono-acids **3a** and **3b** in good to excellent yields. Removal of the thioacetyl group with methanolic-ammonia, followed by treatment with sodium borohydride-^{i}PrOH[18] resulted in the formation of *(R,S)*- and *(S,S)*-captopril-f_3, **4a** and **4b**, respectively.[19]

These compounds were compared to (*S,S*)-captopril with regard to their ACE inhibitory activities. For the enzyme inhibitory assay, the tripeptide, [3-(2-furyl)acryloyl]-Phe-Gly-Gly, was used as the substrate.[20] The enzyme (ACE) from rabbit in a buffered bovine serum base, was obtained commercially (Sigma) and reconstituted with 1 mL of water. Aliquots (100 μL) were added to assay solutions containing inhibitor at different concentrations.

Scheme 1. Synthesis of Captopril-f_3



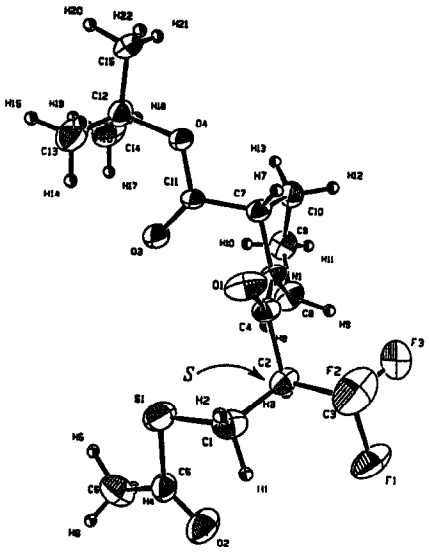
(a). CH_3COSH, THF, r.t., 20h; (b). MPLC separation, **2a/2b** = 1/2, 70% total (2 steps);
(c) Iodotrimethylsilane, $CHCl_3$, r. t. 85-92%; (d). methanolic-ammonia, r.t.; (e). $NaBH_4$, 2-propanol, 78-82% (2 steps) .

Table 1. ACE Inhibitory Activity of captopril-f_3[a]

ACE Inhibitor	IC_{50}[b]
(R,S)-captopril-f_3 (**4a**)	2.9×10^{-10} M
(S,S)-captopril-f_3 (**4b**)	4.8×10^{-7} M
(S,S)-captopril[c]	3.6×10^{-9} M

[a]Inhibitors were assayed for rabbit ACE activity by using a commercial assay kit from Sigma using [3-(2-furyl)acryloyl]-Phe-Gly-Gly as the substrate.[20]
[b]Error in IC_{50} values is approximately ± 20%. The enzyme concentration was kept at 8.3% (v/v).
[c]The reported IC_{50} values for (S,S)- and (R,S)-captopril are 2.3×10^{-8} M and 2.4×10^{-6} M, respectively.[1b]

Figure 1. X-ray crystal structure of **2b**



As Table 1 shows, (*R,S*)-captopril-f_3 (**4a**) is a highly potent inhibitor of ACE (IC_{50} 10^{-10}M level), whereas its diastereomer (*S,S*)-captopril-f_3 (**4b**) is much less active (IC_{50} 10^{-7}M level). This result is in accord with the reported fact that (*S,S*)-captopril is more potent than its (*R,S*)-diastereomer by a factor of 100.[1b] It should be noted that the trifluoromethyl analog **4a** is at least one order of magnitude *more active than (S,S)-captopril.* It is reasonable to assume that the improved potency of the fluorinated analogs is due to the hydrophobicity and the stereoelectronic effect of trifluoromethyl group. Namely, the replacement of methyl group by trifluoromethyl group at the C-2 position of 3-mercaptopropanoyl moiety may well contribute to the increase in attractive interaction with the hydrophobic binding subsite of ACE. Also, the stereospecific introduction of (*2R*)-CF_3 may cause stronger restriction of rotation about the 3-mercaptopropanoyl-proline amide bond than that of (*2S*)-CH_3 because of the stereoelectronic effect of trifluoromethyl group. This would fix the inhibitor in the favorable conformation such that strong binding with the active site is achieved without sacrificing energy for conformational change.[1b-d] Molecular mechanics energy calculations of (*S,S*)-captopril and (*R,S*)-captopril-f_3 showed the latter being more stable by 1.3 kcal/mol, and the calculated energy difference between (*R,S*)- and (*S,S*)-captopril is 6.48 kcal/mol and (*S,S*)- and (*R,S*)-captopril-f_3 8.33 kcal/mol, respectively.[21] It should be noted that these calculated energy differences are consistent with the observed differences in the *in vitro* ACE inhibitory activities of these compounds.

Consequently, it is concluded that trifluoromethyl incorporation has led to the substantial increase in ACE inhibitory potency (*in vitro*). Its *in vivo* and clinical properties are as yet unknown. The development of other trifluoromethyl-containing compounds and their use as potential ACE inhibitors is actively underway.

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11. A patent claimed the synthesis of a *diastereomeric* mixture of captopril-f_3 (**4**), but no ACE inhibitory activity was reported: Ondetti, M.A. *U.S. Patent* 4,154,935 (1977). Accordingly, the acquisition of enantiomerically as well as diastereomerically pure isomers was essential for the finding of a large difference (1,000 times) between (*R,S*)- and (*S,S*)-captopril-f_3 in their ACE inhibitory activities in this work.
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15. **2a**: colorless oil; ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9H), 1.9-2.3 (m, 4H), 2.35 (s, 3H), 3.2-3.42 (m, 2H), 3.42-3.8 (m, 3H), 4.36-4.44 (dd, 1H, J=3.8, 3.4 Hz); ^{19}F NMR ($CDCl_3$): δ -67.5 (d, J = 7.7 Hz); IR (neat): 1738, 1698, 1635, 1441 cm^{-1}; $[\alpha]_D^{22}$ -158.3° (c 2.77, $CHCl_3$). Anal. Calcd for $C_{15}H_{22}F_3NO_4S$: C, 48.78; H, 5.96. Found: C, 49.00; H, 5.97.
2b: yellow needles; mp 93-93.5°C; ^{1}H NMR ($CDCl_3$) δ 1.48 (s, 9H), 1.9-2.25 (m, 4H), 2.37 (s, 3H), 3.24-3.44 (m, 2H), 3.5-3.72 (m, 3H), 4.35-4.45 (dd, 1H, J= 3.5, 3.6 Hz); ^{19}F NMR ($CDCl_3$) δ -67.7 (d, J = 7.7 Hz); IR (nujol): 1738, 1698, 1653, 1441 cm^{-1}; $[\alpha]_D^{22}$ +48.3° (c 1.74, $CHCl_3$). Anal. Calcd for $C_{15}H_{22}F_3NO_4S$: C, 48.78; H, 5.96. Found: C, 48.86; H, 6.01.
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18. Iodotrimethylsilane presumably produces species capable of oxidizing the *in situ* generated mercapto-acid (**4**) to the corresponding disulfide. Treatment with sodium borohydride in 2-propanol cleaves the disulfide linkage to liberate the desired mercapto-acid (**4**).
19. **4a**: colorless oil; ^{1}H NMR (MeOH-d_4) δ 2.0-2.4 (m, 4H), 2.8-3.05 (m, 2H), 3.64-4.0 (m, 3H), 4.84-4.52 (dd, 1H, J= 3.7, 2.8 Hz), 4.9 (s, 2H); ^{19}F NMR (MeOH-d_4): δ -65.55 (d, CF_3, J= 7.5 Hz); IR (neat): 2566, 1731, 1651, 1436 cm^{-1}; $[\alpha]_D^{22}$ -69.6° (c 0.23, MeOH). Anal. Calcd for $C_9H_{12}F_3NO_3S$: C, 39.85; H, 4.43. Found: C, 39.67; H, 4.23.
4b: yellow solid; mp 72-74°C; ^{1}H NMR (MeOH-d_4): δ 1.9-2.4 (m, 4H), 3.0-3.2 (m, 2H), 3.7-3.8 (m, 1H), 3.8-4.0 (m, 2H), 4.4-4.5 (dd, 1H, J=3.5, 3.3 Hz), 4.9 (s, 2H); IR (nujol) 2962, 1731, 1651, 1436, 1157, 880, 760 cm^{-1}; $[\alpha]_D^{22}$ -12.4° (c 1.29, MeOH). Anal. Calcd for $C_9H_{12}F_3NO_3S.0.7\ H_2O$: C, 38.02; H, 4.78. Found: C, 38.15; H, 4.34.
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21. The calculation were performed with a program, SYBIL, 5.0, Tripos Associates, Inc., St. Louis, Missouri. The calculated relative energies for the diastereomers of captopril and captopril-f_3 are as follow: (*S,S*)-captopril, +1.30 kcal/mol; (*R,S*)-captopril, +7.78 kcal/mol; (*R,S*)-captopril-f_3 (**4a**), 0.00 kcal/mol; (*S,S,*)-captopril-f_3, +8.33 kcal/mol.