

22), 401 ($M^+ - CO$, 43), 373 ($M^+ - 2CO$, 54), 345 ($M^+ - 3CO$, 72), 317 ($M^+ - 4CO$, 58).

Anal. Calcd. for $C_{12}H_{18}N_2S_2O_4CrNi$: C, 33.59; H, 4.19; N, 6.53. Found: C, 33.21; H, 4.02; N, 6.32.

Preparation of $[Ni(SCH_2CH_2N(CH_3)CH_2CH_2N(CH_3)CH_2CH_2S)]Mo(CO)_4$ (III). The same procedure was taken as described in the preparation of II. The yield was 0.05 g (30%). mp. 185°C. 1H NMR (DMSO- d_6): δ 3.9-1.9 (m, 12H, CH_2), 2.5 (s, 6H, CH_3); IR (on KBr pellet; cm^{-1}) 3008 (w), 2917 (m), 2000 (s), 1870 (s), 1810 (s), 1450 (w), 1260 (w), 1060 (w), 684 (w), 642 (w); mass spectrum, m/e (relative intensity) 473 (M^+ , 17), 445 ($M^+ - CO$, 38), 417 ($M^+ - 2CO$, 54), 389 ($M^+ - 3CO$, 64), 361 ($M^+ - 4CO$, 58).

Anal. Calcd. for $C_{12}H_{18}N_2S_2O_4MoNi$: C, 30.47; H, 3.81; N, 5.92. Found: C, 30.02; H, 3.62; N, 5.72.

Preparation of $[Ni(SCH_2CH_2N(CH_3)CH_2CH_2N(CH_3)CH_2CH_2S)]W(CO)_4$ (IV). The same procedure was taken as described in the preparation of II. The yield was 0.08 g (36%). mp. 187°C. 1H NMR (DMSO- d_6): δ 4.0-2.15 (m, 12H, CH_2), 2.75 (s, 6H, CH_3); IR (on KBr pellet; cm^{-1}) 3012 (w), 2910 (m), 1990 (s), 1850 (sh), 1830 (m), 1790 (m), 1460 (m), 1440 (sh), 1430 (sh), 1280 (w), 1260 (w), 1190 (w), 1150 (w), 1060 (w), 1030 (w), 770 (w), 670 (w), 620 (m); mass spectrum, m/e (relative intensity) 561 (M^+ , 18), 533 ($M^+ - CO$, 38), 505 ($M^+ - 2CO$, 58), 577 ($M^+ - 3CO$, 62), 547 ($M^+ - 4CO$, 42).

Anal. Calcd. for $C_{12}H_{18}N_2S_2O_4WNi$: C, 25.69; H, 3.21; N, 5.00. Found: C, 25.19; H, 3.08; N, 4.83.

Preparation of $[Ni(SCH_2CH_2N(CH_3)CH_2CH_2N(CH_3)CH_2CH_2S)]ZrCp_2$ (V). To a stirred methylene chloride solution (4 ml) of I (0.2 g, 0.8 mmol) was added $Cp_2Zr(CO)_2$ (0.222 g, 0.8 mmol) dissolved in CH_2Cl_2 (2 ml) dropwise. The reaction was continued for 20 hr and the hexane (3 ml) was added. The resulting orange solid was filtered. Recrystallization from methylene dichloride/hexane gave yellow crystals. The yield was 0.23 g (60%). mp. 192°C. 1H NMR (DMSO- d_6): δ 6.4 (s, 5H, Cp), 3.0-1.9 (m, 12H, CH_2), 2.75 (s, 6H, CH_3); IR (on KBr pellet; cm^{-1}) 3014 (w), 2922 (m), 1620 (w), 1460 (m), 1430 (w), 1260 (w), 1230 (w), 1200 (w), 1060 (m), 1040 (w), 1020 (w), 1010 (w), 950 (w), 920 (w), 810 (w), 770 (m), 750 (m); mass spectrum, m/e (relative intensity) 486 (M^+ , 38), 421 ($M^+ - C_5H_5$, 58).

Anal. Calcd. for $C_{18}H_{28}N_2S_2NiZr$: C, 44.45; H, 5.76; N, 5.76. Found: C, 44.07; H, 5.89; N, 5.38.

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A Three-Dimensional Active Site Model of Carboxypeptidase A

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Carboxypeptidase A (CPA, EC 3.4.17.1) is a much studied prototypic zinc-containing peptidase¹ which preferentially cleaves off the C-terminal amino acid residue having a hydrophobic side chain with L-stereochemistry. Although the X-ray crystallographic structural information of CPA is available², the development of empirical, yet functional active site model which portrays the substrate as well as stereospecificity of the enzyme is highly desirable. Most of the proposed models are two dimensional and thus inadequate for explaining the stereospecificity of the enzymic action. We herein propose a simple three dimensional model of the active site of CPA, which ameliorate the existing models. This three dimensional model enables one to visualize not only the observed stereochemical course of the enzymic reaction³ but also explains the stereoselective binding of inhibitors.

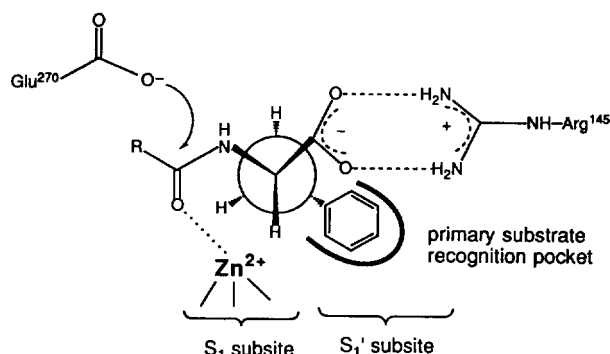


Figure 1. Three dimensional schematic representation of the active site of CPA, which is occupied by *N*-acetyl-L-Phe to form a Michaelis complex.

Furthermore, it provides bioorganic chemists with the essential stereochemical informations needed for an inhibitor design.

Three major binding sites and one catalytic site have been identified in the active site of CPA. The active site Zn^{2+} which is coordinated to the backbone amino acid residues of His-69, Glu-72, and His-196 is essential for the catalytic activity, serving to activate the scissile peptide bond of the substrate through ligation for the nucleophilic attack by the catalytic center. Other principal binding sites are Arg-145 and the substrate recognition pocket.⁴ The recognition pocket which has a narrow mouth and a wide interior is shaped complementary to an aromatic ring, and accommodates the aromatic side chain of P_1' residue of the substrate.⁵ The Arg-145 is involved in forming a salt link with the terminal carboxylate of the substrate. The X-ray structure of the enzyme revealed that the hydrophilic guanidinium moiety of Arg-145 and the carboxylate group of Glu-270 are located at the surface of the enzyme molecule, and the recognition pocket is invaginated from the surface deep into the core.⁴ The Zn^{2+} which is found also deep in the active site crevice is positioned in a transoid relationship to the pocket within the active site cleft.⁴

When a substrate binds to the active site, the scissile carbonyl carbon of the substrate would be positioned in the vicinity of the carboxylate of Glu-270. There then ensues a nucleophilic attack by the carboxylate of Glu-270 at the activated carbonyl carbon with the resultant formation of an anhydride intermediate. The latter decomposes rapidly to products with regeneration of the enzyme (anhydride pathway).⁶ In an alternative mechanism (general base mechanism), the carboxylate serves as a general base, activating a nearby water molecule which in turn attacks at the peptide carbonyl.⁷

Figure 1 shows the proposed three-dimensional representation of the CPA active site which is occupied by a substrate having L-configuration. In this representation, the recognition pocket is depicted with a bold line to indicate that it is invaginated into the core of the enzyme molecule. The Zn^{2+} which is positioned deep inside the molecule is also shown with bold letters. The Newman's projection is used in showing the bound substrate as it better reflects the spatial relationship of its functionalities involved in the binding, and thus also the topology of the active site which accommodates

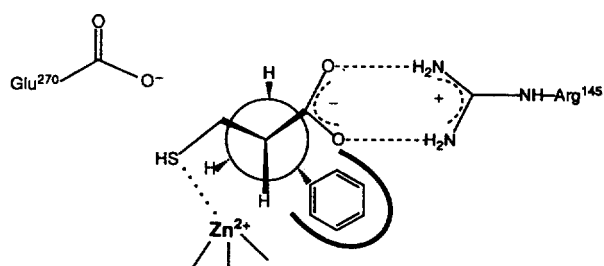


Figure 2. Three dimensional schematic representation of the active site of CPA, which is occupied by (*S*)-BMPA, a substrate analog inhibitor.

the substrate.

As shown in Figure 1, when a substrate having a L-configuration binds the enzyme, there occurs the nucleophilic attack of the carboxylate at the activated carbonyl of the substrate with the formation of an anhydride intermediate.⁸ In the case of its epimer, however, the carbonyl carbon of the scissile bond would not be situated within a distance for the nucleophilic attack, even if it may bind: The carbonyl group and the hydrogen are reversed compared with those of the bound L-amino acid (Figure 1). The nucleophilic attack on the prochiral carbonyl is predicted to be taken place in the "si" fashion by this model. In agreement with the prediction and thus in supporting the proposed model, Mock and Zhang recently reported that the nucleophile must approach toward the "si" face of the peptide bond.⁹

Recently, we have reported that when racemic 2-benzyl-3-mercapto propanoic acid (BMPA), a potent substrate analog inhibitor of CPA reported by Ondetti *et al.*¹⁰ is resolved, only the (*S*)-isomer has the CPA inhibitory activity¹¹ with $K_i = 7.8 \times 10^{-9}$ M. This stereospecific inhibitory activity of (*S*)-BMPA against CPA may now be readily envisaged with the proposed model: When (*S*)-BMPA anchors the active site, its mercapto group may rest at a position which is within a ligating distance to the Zn^{2+} (Figure 2). On the other hand, (*R*)-BMPA would not be fit in the receptor site, thus to show no inhibitory activity. The high inhibitory potency of mercapto containing inhibitors is believed to be due to the strong binding affinity of the sulfhydryl group as a ligand¹² to the Zn^{2+} .

The change of conformational energy of *N*-acetyl-L-Phe with respect to the dihedral angle (ϕ) of $C(Ph)-C_\beta-C_\alpha-CO_2H$ was calculated by the PM3 method¹³, and is shown in Figure 3. The energies of gauche conformers having $\phi = 67^\circ$ and 299° are only 0.6 and 0.7 kcal/mol higher, respectively, compared with that of the *trans*-conformer, indicating that the depicted gauche conformer of the bound substrate (Figure 1) is energetically quite feasible. Examples of such conformer are also found in the X-ray structures of ligands complexed with CPA.¹⁴ For example, the corresponding dihedral angle for the bound Gly-L-Tyr¹⁴⁴ is shown to be 86° , and that of Cbz-Ala-Ala^(O)Phe to be 59° ^{14b}. In the case of (*S*)-BMPA, the gauche conformer (Figure 2) having $\phi = 72^\circ$ is only 0.4 kcal/mol higher in energy compared with the ground state conformer, *i.e.*, the *trans*-BMPA having $\phi = 166^\circ$.

With the proposed representation of the active site the stereospecificity of the CPA enzymic reaction and inhibitor binding is now readily envisaged. It is a model of predictive

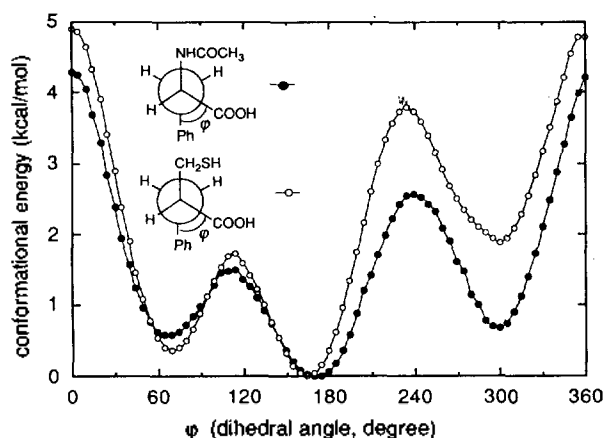


Figure 3. The conformational energy profiles of *N*-acetyl-L-Phe and BMPA calculated by the PM3 method (ϕ =dihedral angle of C(Ph)-C β -C α -C(CO₂H)).

value for the stereochemical output of the enzymic reaction, and furthermore it serves as a guiding ground for design of chiral inhibitors of not only CPA but also enzymes whose active sites are structurally similar to that of CPA.

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Synthesis of γ,γ,γ -Trifluoro- β -Hydroxy Ketones

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Trifluoromethylated compounds are the subject of renewed interest due to the unique properties of these compounds in the field of medicinal and agricultural chemistry.¹ The influence of the trifluoromethyl group in biologically active molecules is often associated with the increased lipophilicity that this substituent imparts. In addition, its electronegativity and relatively small size are also contributing factors.² Development of a general method for trifluoromethylated heterocycles and the related organofluorine compounds would be valuable for the syntheses of many trifluoromethylated target compounds. We wish to report here a very efficient method for the generation of β -trifluoromethyl β -hydroxy ketones.

Our method for the generation of trifluoromethylated heterocycles and the related organofluorine compounds is based on the nitrile oxide cycloaddition with 3,3,3-trifluoropropene³ followed by the reductive cleavage of corresponding cycloadducts⁴ (Eq. 1). Another cycloadditive approach using trifluoroacetonitrile oxide⁵ as a source of trifluoromethyl group provided 3-trifluoromethyl 2-isoxazoline cycloadducts in low yield in our hand, probably due to the volatility of trifluoroacetaldehyde oxime intermediate. Thus, we sought the source