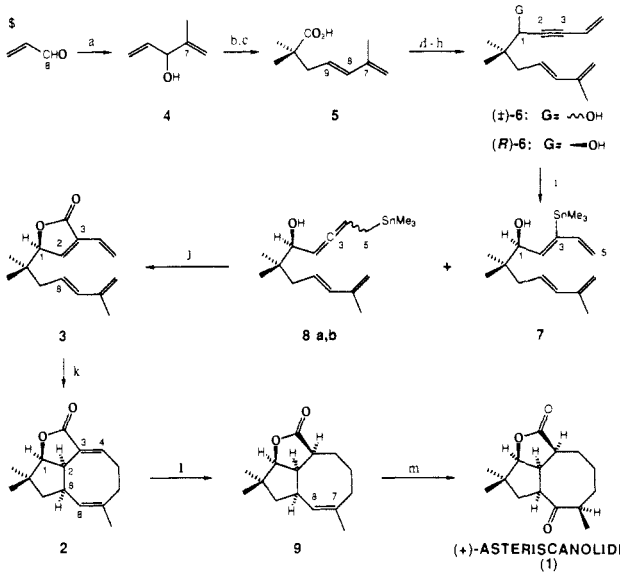
Scheme II[a]



[a] (a) Isopropenyl Grignard (57%); (b) isobutyric anhydride (99%); (c) LDA, -78 °C to 0 °C (69%); (d) LAH (93%); (e) DMSO, $(COCl)_2$, Et_3N (88%); (f) $LiC{\equiv}CCH{=}CH_2$ (88%); (g) DMSO, $(COCl)_2$, Et_3N (89%); (h) LAH/Darvon (97%); (i) Red-Al; Me_3SnCl (83%); (j) *n*-BuLi; CO_2 (56%); (k) $Ni(COD)_2$, Ph_3P, 90 °C (67%); (l) Red-Al, CuBr (74%); (m) BH_3·THF; PCC (48%).

of this methodology are under investigation and will be reported in due course.

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Supplementary Material Available: Experimental details for the asymmetric reduction and the nickel-catalyzed cycloaddition of **3** and spectral and physical data for **(*R*)-6**, **3**, and **2** (4 pages). Ordering information is given on any current masthead page.

Stereoelectronic Effects at Carboxylate: A Syn Oriented Model for the Histidine-Aspartate Couple in Enzymes

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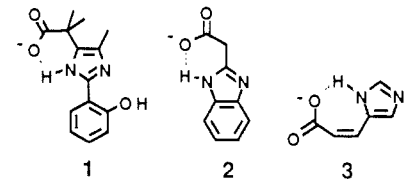
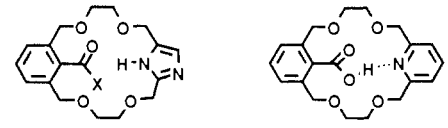
Features which recur in the active site of enzymes, unrelated by evolution, are particularly worthy of chemical modelling. It is essential, however, that these small organic molecules maintain the spatial relationships found in the enzymic system. Models of the serine proteases[1] (e.g., **1**[2a], **2**[2b], **3**[2c]) orient the anti lone pair of the carboxylate toward the imidazole, in contrast to the serine proteases,[3] malate and lactate dehydrogenase,[4] thermolysin,[5] and

Anti



Syn



4: X = O⁻
10: X = NH_2

5

DNAse I[6] which have all evolved to use His-Asp (Glu) couples with the N-3(H)-syn lone pair arrangement (Chart I).[7,8]

With respect to carboxylate orientation, Gandour has argued that there is a large stereoelectronic effect in operation whereby the carboxylate syn lone pair may be as much as 10^4 times more basic than the anti.[9,10] However, it is not known whether this will affect the pK_a of the His-Asp couple. In an elegant study, Craik has compared native trypsin with a mutant enzyme in which aspartate is replaced by neutral asparagine (D 102 N trypsin).[11] In this case, the carboxylate syn lone pair of Asp-102 increases the pK_a of His-57 by 1.5 units. In all anti imidazole-carboxylates the ΔpK_a, in aqueous medium,[12] is less than 1 pK_a unit. If the larger ΔpK_a seen in trypsin results from this syn orientation, then this could explain the preference seen for the N-3(H)-syn lone pair orientation in the enzymic His-Asp couple.

We wish to report the synthesis and pK_a determinations of **4**, the first syn oriented model of the enzymic His-Asp couple. Crucial to the design of our system was the X-ray structure of pyrido-crown ether **5**,[13] synthesized by Cram,[14] in which the benzoic acid moiety engages in a syn hydrogen bond with the pyridine nitrogen. A serviceable route to **4** involved the reaction of 1-benzylimidazole with formalin to produce 2,5-bis(hydroxymethyl)imidazole **6** (Scheme I).[15-17] Conversion to the bis(chloromethyl)imidazole hydrochloride and reaction with a large excess of ethylene glycol produced diol **7**, which was debenzylated

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(2) (a) Rogers, G. A.; Bruice, T. C. *J. Am. Chem. Soc.* **1974**, *96*, 2473. (b) Komiyama, M.; Bender, M. L. *Bioorg. Chem.* **1977**, *6*, 13. (c) Roberts, J. D.; Yu, C.; Flanagan, C.; Birdseye, T. R. *J. Am. Chem. Soc.* **1982**, *104*, 3945. (d) Bruice, T. C.; Sturtevant, J. M. *J. Am. Chem. Soc.* **1959**, *81*, 2860.

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(16) All new compounds gave correct elemental analysis and/or high resolution mass spectral data and had spectroscopic properties which were in accord with the assigned structure. Compound **4** analyzed best as a hydrate. Anal. Calcd for $C_{18}H_{22}O_6N_2 \cdot 1.5H_2O$: C, 55.52; H, 6.47; N, 7.19. Found: C, 55.17; H, 6.10; N, 7.36.

(17) (a) We have since developed an improved synthesis of 2,4(5)-bis(benzyloxymethyl)imidazole and **8** (ref 17b). (b) Zimmerman, S. C.; Cramer, K. D.; Galan, A. A., manuscript in preparation.

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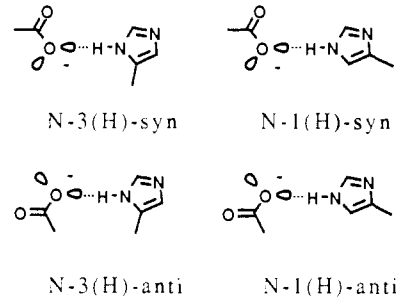
Table I. Equilibrium Acidities of Imidazolium Carboxylic Acids and Related Compounds

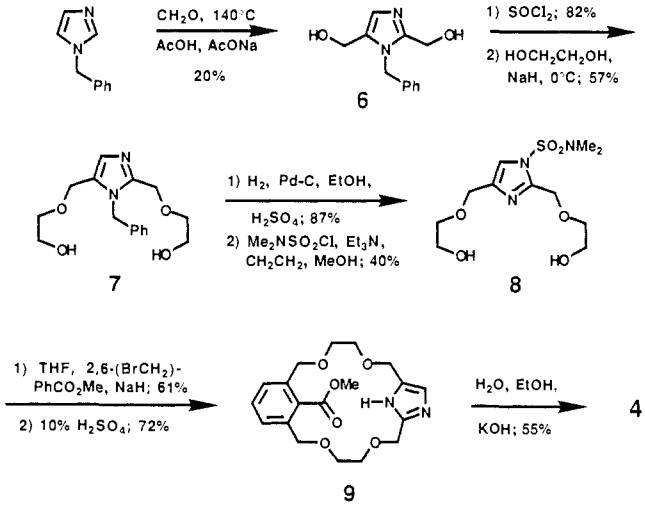
compd	$pK_{a_{COOH}}$	$pK_{a_{Im}}$	model compd	$pK_{a_{Im}}$	$\Delta pK_{a_{Im}}$	ref
4-(4-imidazole)butyric acid	4.26	7.62	methyl 4-(4-imidazole)butyrate	7.3	0.3	2d
N_α-acetyl histidine		7.07	N_α-acetyl histidine methylamide	6.45	0.62	8
1	3.18	6.93	**1**-methyl ester	6.17	0.76	2a
2		6.1	benzimidazole	5.43	0.7	2b
3	3.3	7.0	*trans*-**3**	6.1	0.9	2c
4	3.8	7.50	**11**	6.00	1.50	this work[a]
4	3.8	7.50	**10**	6.15	1.35	this work
trypsin		6.8	D 102 N trypsin	5.3	1.5	11

[a] pK_a values were determined from the ^1H NMR titration data by using the procedures described by Markley (ref 20). Duplicate runs gave values which agreed within 1%.

Chart I



Scheme I



and then converted to **8** with dimethylsulfamoyl chloride.[18] Cyclization of **8** with methyl 2,6-bis(bromomethyl)benzoate[19] afforded the corresponding macrocyclic ester, and subsequent desulfamylation produced **9** as a crystalline dihydrate whose structure was confirmed by X-ray analysis.[17b] Finally, ester hydrolysis produced **4**. For comparison ester **9** was converted into the corresponding amide **10** by reaction with sodium amide ($NaNH_2$, NH_3, reflux; 52%).

In order to determine the basicity of the imidazole moiety, titrations were carried out in D_2O and monitored by ^1H NMR.[20] As seen in Figure 1, the imidazole C–H and three of the observable benzylic methylene resonances of **4** show two distinct inflection points. The relative magnitude of the shifts allows definitive assignment of the lower pK_a value (ca. 3.8, corrected[21]) to be that of the carboxylic acid and the higher value (pK_a = 7.50) to be that of the imidazole group. Similar titrations of model compounds **9**, **10**, and 2,4(5)-bis(methoxymethyl)imidazole (**11**) exhibited single inflection points.

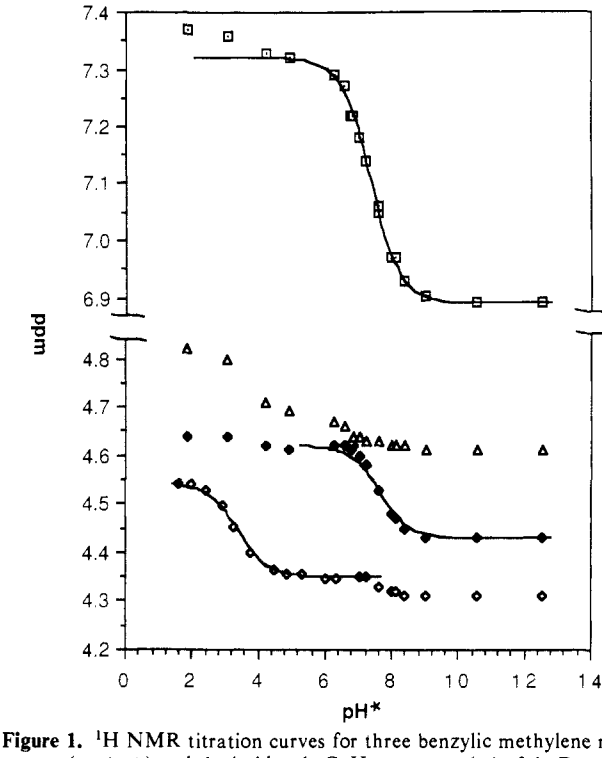


Figure 1. ^1H NMR titration curves for three benzylic methylene resonances (△, ♦, ◇) and the imidazole C–H resonance (□) of **4**. Data was obtained at 500 MHz in D_2O at a constant ionic strength of 0.5 M (KCl). Referencing (0.00 ppm) was to a solution of TSP in D_2O contained in a coaxial insert. Solid lines represent the theoretical curve obtained from a modified Hill equation (ref 20) with n = 1.

Strikingly, the pK_a increase in imidazole **4** relative to model **11** is 1.50, substantially higher than the increase seen in anti models (e.g., **1**, **2**, and **3**) and identical with the trypsin value of ΔpK_a = 1.5 (Table I). A more appropriate comparison is with amide **10** where ΔpK_a = 1.35. The increase in basicity of **4** over that of macrocyclic ester **9** is 1.07 pK_a units, and this smaller increase probably reflects a change in the microenvironment of the imidazole, as the conformation of ester **9** is distinctly nonplanar.[17b]

These data demonstrate that a carboxylate group can cause a larger increase in the pK_a of a proximate imidazole than observed in previous model systems. The higher ΔpK_a values seen in **4** and trypsin can be attributed to the greater stabilization of an imidazolium ion by a syn carboxylate. In terms of previously proposed models, this stabilization is through a stronger electrostatic interaction and/or a stronger hydrogen bond resulting from the greater basicity of the syn lone pair as proposed by Gandour.[9] In those enzymes where histidine functions as a base, evolutionary selection may have favored the slightly higher pK_a attending the N-3(H)-syn lone pair orientation in the His–Asp couple.[22] This argument will be strengthened if many more enzymes are found

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to contain a syn oriented His-Asp couple,[23] and if additional anti and syn models are found to have ΔpK_a values of less than and greater than about 1 pK_a unit, respectively.

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Note Added in Proof. We thank Professor R. S. Brown for informing us of an anti model, structurally similar to **1**, which gives $\Delta pK_a = 1.2$.[24] This model will be discussed in detail in a future paper.

(23) A preference for a different orientation might be expected when the histidine residue is acting solely as a general acid. However, thermolysin is the only enzyme where the His-Asp couple is proposed to function in this manner, and it also uses the N-3(H)-syn lone pair combination (ref 5).

(24) Skorey, K. I.; Somayaji, V.; Brown, R. S. *J. Am. Chem. Soc.*, in press.

Stereoelectronic Effects and the Active Site of the Serine Proteases

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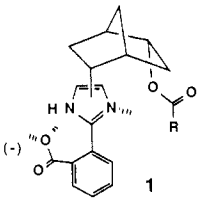
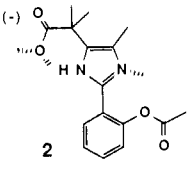
Modelling the active site of the serine proteases is a popular undertaking, and a number of systems have been developed to show how binding forces can lead to large rate enhancements.[1] In cases where both the carboxyl and imidazole groups of the catalytic triad (Chart I) are incorporated into the model, some questions arise concerning stereoelectronic effects at the carboxyl oxygen. Specifically, in structures such as **1**[2] or **2**[3] the less basic anti lone



1



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pair of the carboxylate is directed toward the imidazole nucleus. Gandour[4] has pointed out that carboxylates at the sites of the serine proteases (and other enzymes) feature the more basic syn lone pairs directed toward the substrate (Chart II). We recently introduced a series of compounds in which carboxyl groups can be oriented with respect to other structural elements in the same molecule.[5] Here we apply these advantages to elaborate new models for the serine proteases.

Chart I

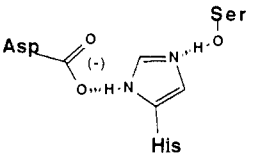
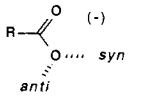


Chart II



Scheme I

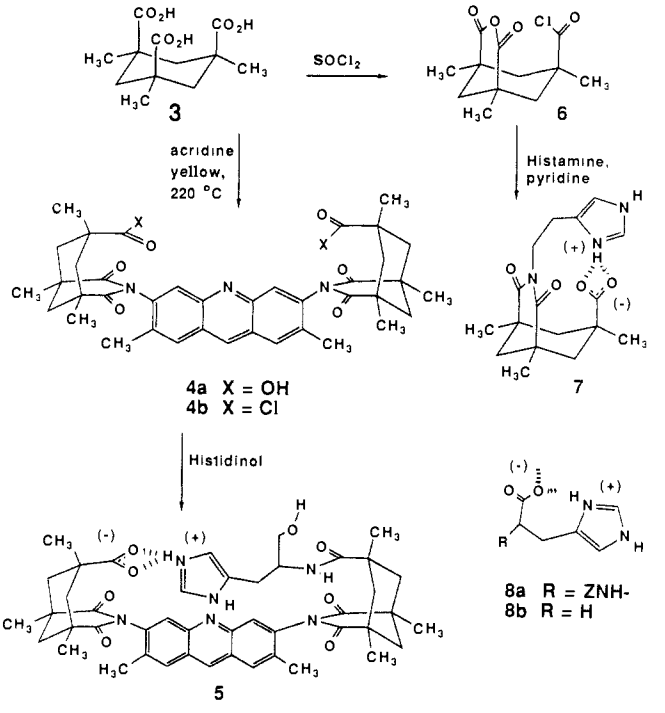


Table I. Acid Dissociation Constants

entry	structure	pK_a COOH	pK_a $NH^{(+)}$	solvent
1	**5**	4.7	7.2	$EtOH/H_2O$
2	**8a**(Z-His)	4.0	6.9	$EtOH/H_2O$
3	**7**	5.7	7.3	$EtOH/H_2O$
4	**9**	6.0		$EtOH/H_2O$
5	**7**	4.8	7.7	H_2O
6	10	5.0		H_2O
7	**11**	3.3	7	H_2O
8	**2**	3.2	7.05	H_2O
9	**8b**	3.8	7.4	H_2O

The systems are rapidly and efficiently assembled from the condensation of Kemp's triacid[6] **3** with appropriate amines. By using acridine yellow in triglyme (Scheme I) a diacid structure (**4a**) is obtained. The carboxyl OH groups converge on a molecular cleft in which a distance of ca. 8 Å separates opposing oxygen atoms.[5] The corresponding diacid chloride[7] **4b** was used to acylate histidinol. Hydrolysis of the intermediate gave th emonofunctionalized derivative[8] **5**. The second structure **7** is

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(8) All new compounds were characterized by high resolution spectroscopy and/or elemental analyses. For **5** mp > 300 °C; **7** mp = 270d (microanalysis invariably indicated the presence of solvent molecules); **10** mp = 272-275 °C. Titrations were performed as described in the following: Gordon, W. E. *J. Phys. Chem.* **1979**, *83*, 1365-1377. Gordon, W. E. *Anal. Chem.* **1987**, *54*, 1595-1601.

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