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Steric Enhancement of Imidazole Basicity in *cis*-Urocanic Acid Derivatives: Models for the Action of Chymotrypsin

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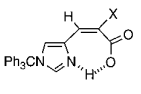
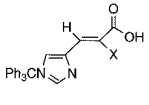
To test the hypothesis that substrate-induced steric compression between His 57 and Asp 102 at the active site of chymotrypsin can increase the basicity of His 57, we have synthesized the *cis*- and *trans*-isomers of 2-bromo-3-(*N*-tritylimidazole)-2-propenoic acid and 2-chloro-3-(*N*-tritylimidazole)-2-propenoic acid and compared selected properties with those of *cis*- and *trans*-urocanic acids. The *cis*-isomers display low field ^{1}H NMR signals at 17 ppm in dimethylsulfoxide, similar to *cis*-urocanic acid; whereas the *trans*-isomers do not show strong hydrogen bonds. Increasing the size of the C2 substituent (H < Cl < Br) in the *cis*-isomers increases the pK_a of the imidazolium group from 6.78 for H to 7.81 and 9.10 for Cl and Br, respectively; whereas the pK_as of the *trans* isomers are all 6.0 ± 0.1. The results indicate that the *cis*-urocanic acid derivatives with large substituents at C2 act as proton sponges in water, and they support the concept that steric compression in the catalytic triad of chymotrypsin can increase the basicity of His 57.

A new concept for the mechanism of general acid–base catalysis in serine proteases such as chymotrypsin has recently been proposed (*1*). The mechanism explains the function of the low barrier hydrogen bond (LBHB) between His 57 and Asp 102 (*2*, *3*). Chemical, spectroscopic, and X-ray crystallographic evidence indicate that a substrate-induced conformational change introduces steric compression between N$^{\delta 1}$ of His 57 and the β-carboxyl group of Asp 102. Strain is relieved by proton transfer from Ser 195 to N$^{\varepsilon 2}$ of His 57 and LBHB formation between N$^{\delta 1}$ of His 57 and Asp 102. Relief of strain by the LBHB can occur only when His 57 is protonated, so that this mechanism increases the basicity of His 57 and its effectiveness as a base in abstracating a proton from Ser 195. To test the hypothesis that steric compression can increase the basicity of imidazole, we have synthesized derivatives **1–4** of *cis*- and *trans*-urocanic acid, with chloro or bromo substituents at C2 of the side chain, and studied their properties.



1 X = Br
2 X = Cl



3 X = Br
4 X = Cl

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EXPERIMENTAL

Synthetic Protocols

N-Tritylurocanic acid, dibutylammonium salt (**6**). Urocanic acid 5 (10.0 g, 72.4 mmol) and trityl chloride (44.4 g, 159.3 mmol, 2.2 eq) were dissolved in 250 ml of *N*,*N*-dimethylformamide (DMF). Et_3N (54.0 ml, 39.2 g, 388 mmol, 5.4 eq) was added and the reaction was stirred for 14 h at room temperature. Methanol (250 ml) was added and the reaction was stirred for 24 h at 65°C. The solution was cooled to room temperature, diluted with 150 ml of ethyl acetate, and washed with 4×125 ml of 10% citric acid. The organic layer was condensed to 80 ml of a brown oil. Dibutylamine (13.0 ml, 10.0 g, 77.1 mmol, 1.1 eq) and 50 ml of ethylacetate was added. A white precipitate formed over 24 h. The solid was isolated and washed repeatedly with hexanes until pure by TLC (R_f = 0.8, 70% hexane/ethyl acetate) and dried under vacuum to give 36.7 g (quantitative yield) of white solid **6.** ^1H NMR (300 MHz, $CDCl_3$) 9.17 (br. s, 1H), 7.44 (d, J = 1.0 Hz, 1H), 7.35–7.31 (m, 9H), 7.28 (half of alkene doublet: other half obscured by 9H multiplet, 0.5H), 7.15–7.12 (m, 6H), 6.91 (d, J = 1.0 Hz, 1H), 6.49 (d, J = 15.8 Hz, 1H), 2.80 (t, J = 7.4 Hz, 4H), 1.67 (quintet, J = 7.4 Hz, 4H), 1.32 (sextet, J = 7.4 Hz, 4H), 0.88 (t, J = 7.4 Hz, 6H). ^{13}C NMR (75.5 MHz, $CDCl_3$, missing 1 quaternary C) 146.9, 143.9, 142.1, 129.7, 128.7, 128.1, 127.9, 127.7, 127.2, 126.9, 81.9, 47.6, 20.2, 13.7. LSIMS calculated for $C_{33}N_3O_2H_{39}$ M = 509 not observed; Ph_3C = 243.1, $C_6N_2O_2H_5$ = 136.0, $H_2N + Bu_2$ = 130.2.

Ethyl N-tritylurocanate (**7**). **6** (2.0 g, 3.93 mmol, 1 eq) was dissolved in 100 ml of $CHCl_3$. 2,4,6-Trichlorobenzoyl chloride (1.9 ml, 2.92 g, 12.0 mmol, 3 eq) and Et_3N (1.2 ml, 0.87 g, 8.6 mmol, 2.2 eq) were added and the reaction was stirred for 10 min. Ethanol (1.0 ml, 0.80 g, 17.4 mmol, 4.4 eq) and 4-dimethylaminopyridine (1.27 g, 10.4 mmol, 2.6 eq) were added and the reaction was stirred for 12 h. The $CHCl_3$ was reduced to about 10 ml *in vacuo* and the crude reaction mixture was chromatographed (SiO_2, 70% hexane/ethyl acetate) to give, after removal of solvent, 1.08 g (67% yield) of white solid. ^1H NMR (300 MHz, $CDCl_3$) 7.51 (d, J = 15.6 Hz, 1H), 7.47 (s, 1H), 7.37–7.32 (m, 9H), 7.16–7.11 (m, 6H), 6.53 (d, J = 15.6 Hz, 1 H), 4.21 (q, J = 7.4 Hz, 2H), 1.29 (t, J = 7.4 Hz, 3H). ^{13}C NMR (75.5 MHz, $CDCl_3$) 167.4, 141.9, 140.3, 137.2, 136.1, 129.7, 128.3–128.2 (large signal, 2 trityl C's), 123.8, 116.3, 75.7, 60.1, 14.3. LRMS calculated for $C_{27}N_2O_2H_{24}$ M = 408; found M = 408.

Ethyl 2,3-dibromo-3-(*N-tritylimidazole*) *propionate* (**8**). **7** (425 mg, 1.0 mmol) was dissolved in 30 ml CCl_4 and cooled to 0°C in an ice bath. Bromine (Br_2, 70 μl, 217 mg, 1.4 mmol, 1.3 eq) was added and the reaction was stirred for 1 h at 0°C. It was then warmed to room temperature and stirred for 1 h. The solvent was removed *in vacuo* to give 600 μl of pale yellow solid (quantitative yield). ^1H NMR (200 MHz, $CDCl_3$) 7.62 (br s, 1H), 7.40–7.34 (m, 9H), 7.16–7.11 (m, 6H), 6.92 (d, J = 1.5 Hz, 1H), 5.38 (d, J = 11.5 Hz, 1H), 5.14 (d, J = 11.5 Hz, 1H), 4.32 (q, J = 6.8 Hz, 2H), 1.34 (t, J = 6.8 Hz, 3H). LSIMS and HRMS and LRMS: dibromide decomposed rather than fragmenting in all cases. MS of bromoalkenes for which this compound is a precursor are provided.

Ethyl 2,3-dichloro-3-(*N-tritylimidazole*) *propionate* (**11, 12**). To condense Cl_2, a 15″ syringe needle was attached to a lecture bottle and inserted into an NMR tube in a dry ice/CH_3CN bath. The chlorine gas was bubbled into the tube until 100 μl of gas had been collected. The NMR tube was then rapidly inverted into 5 ml of precooled $CHCl_3$. Meanwhile, **7** (42 mg, 0.10 mmol) was dissolved in $CHCl_3$ and cooled to −45°C in a dry ice/CH_3CN bath. A stock $Cl_2/CHCl_3$ solution (480 μl, 1.1 eq) was added and the reaction was stirred for 2 h at −45°C. It was warmed to room temperature and solvent was removed *in vacuo.* A TLC (70% hexane/EtOAc) of the crude reaction product revealed two spots. Chromatography (SiO_2, 70% hexane/EtOAc) gave 8 mg of pure material ($R_f = 0.7$), 14 mg of pure material ($R_f = 0.6$), and 10 mg of a mixture of the two compounds (65% combined yield). The two products were assigned as the *syn-* and *anti*-diastereomers on the basis of elimination experiments in refluxing $CHCl_3$. The higher R_f material gave a mixture of products while the lower R_f material gave only the *trans*-urocanate derivative. Thus, the products result from *anti-* and *syn*-addition of Cl_2, respectively. In general, the diastereomeric mixture was carried on without separation. *anti*-Addition product: ^{1}H NMR (200 MHz, $CDCl_3$) 7.45 (d, $J = 1.5$ Hz, 1H), 7.37–7.32 (m, 9H), 7.15–7.10 (m, 6H), 6.91 (d, $J = 1.5$ Hz, 1H), 5.20 (d, $J = 10.3$ Hz, 1H), 4.89 (d, $J = 10.3$ Hz, 1H), 4.31 (q, $J = 6.8$ Hz, 2H), 1.37 (t, $J = 6.8$ Hz, 3H). *Syn*-addition product: ^{1}H NMR (200 MHz, $CDCl_3$) 7.40 (d, $J = 1.5$ Hz, 1H), 7.38–7.31 (m, 9H), 7.15–7.08 (m, 6H), 6.92 (br. s), 5.45 (d, $J = 6.5$ Hz, 1H), 5.00 (d, $J = 6.5$ Hz, 1H), 4.18 (q, $J = 7.5$ Hz, 2H), 1.24 (t, $J = 7.5$ Hz, 3H). Mixture of *syn-* and *anti*-dichlorides: LSIMS calculated for $C_{27}N_2O_2Cl_2H_{24}$ M = 478; found M + 1 = 479.1.

Ethyl 2-bromo-3-(*N-tritylimidazole*)*-2-propenate* (**9**). E_2 reaction conditions: **8** (115 mg, 0.20 mmol) was dissolved in 5.0 ml CH_2Cl_2 and cooled to −78°C in a dry ice/acetone bath. DBU (61 μl, 103 mg 0.68 mmol, 3.3 eq) was added and the reaction was stirred at −78°C for 10 min, until TLC (70% hexane/ethyl acetate) indicated no starting material remained. The reaction was warmed to room temperature and solvent was removed *in vacuo* to give 187 mg of brown oily solid which was a 20:1 mixture of isomers, as determined by NMR. Chromatography (SiO_2, 70% hexane/EtOAc) gave 75 mg of white solid (76% yield). ^{1}H NMR (300 MHz, $CDCl_3$) 7.55 (br. s, 1H), 7.41 (d, $J = 1.1$ Hz, 1H), 7.35–7.33 (m, 9H-trityl, ^{1}H-alkene), 7.15–7.12 (m, 6H), 4.16 (q, $J = 7.2$ Hz, 2H), 1.20 (t, $J = 7.2$ Hz, 3H). ^{13}C NMR (75.5 MHz, $CDCl_3$) 164.0, 142.0, 138.9, 135.6, 134.8, 129.7, 128.2, 124.6, 107.5, 75.7, 61.9, 13.9. HRMS calculated for $C_{27}N_2O_2BRH_{23}$ M = 486.094, 488.092; found M = 486.094, 488.090.

Ethyl 2-bromo-3-(*N-tritylimidazole*)*-2-propenate* (**10**). β-Elimination reaction conditions: **8** (100 mg, 0.18 mmol) was dissolved in 2.5 ml $CHCl_3$. Et_3N (25 μl, 18.2 mg. 0.18 mmol, 1 eq) was added and the reaction was refluxed for 14 h. The solvent was removed *in vacuo* to give 100 mg of brown solid. Chromatography (SiO_2, 70% hexane/EtOAc) gave 25 mg of *cis* bromoalkene **9** and 32 mg of *trans* bromoalkene **10** (65% yield). ^{1}H NMR (300 MHz, $CDCl_3$) 8.28 (s, 1H), 7.90 (d, $J = 0.9$ Hz, 1H), 7.49 (d, $J = 0.9$ Hz, 1H), 7.34–7.30 (m, 9H), 7.15–7.10 (m, 6H), 4.26 (q, $J = 7.3$ Hz, 2H), 1.30 (t, $J = 7.3$ Hz, 3H). ^{13}C NMR (75.5 MHz, $CDCl_3$) 163.3, 141.8, 139.3, 135.8, 135.4, 129.5, 128.2, 128.0, 125.6, 110.1, 76.0, 62.3, 14.1. HRMS calculated for

$C_{27}N_2O_2BrH_{23}$ M − 486.094, 488.090; found M − 486.094, 488.090. X-Ray crystal structure was also obtained.

Ethyl 2-chloro-3-(N-tritylimidazole)-2-propenate (**13, 14**). A diastereomeric mixture of dichlorides **11** and **12** (370 mg, 0.77 mmol) was dissolved in 20 ml CH_2Cl_2 and cooled to −78°C in a dry ice/acetone bath. DBU (231 μl, 235 mg, 1.55 mmol, 2.0 eq) was added and the reaction was stirred for 2 h at −78°C. The reaction was warmed to room temperature and solvent was removed *in vacuo.* Chromatography (70% hexane/EtOAc) gave 65 mg (2% yield) of **13** and 200 mg (59% yield) of **14.** (**13**) ^{1}H NMR (300 MHz, $CDCl_3$) 7.74 (dd, J = 1.5, 0.8 Hz, 1H), 7.44 (d, J = 1.5 Hz, 1H), 7.37 − 7.32 (m, 9H), 7.25 (d, J = 0.8 Hz, 1H), 7.17 − 7.13 (m, 6H), 4.17 (q, J = 7.0 Hz, 2H), 1.20 (t, J = 7.0 Hz, 3H). ^{13}C NMR (75.5 MHz, $CDCl_3$) 163.2, 142.0, 138.8, 134.5, 132.8, 129.8, 129.3, 128.4, 125.4, 118.7, 75.8, 61.7, 14.0. (**14**) ^{1}H NMR (300 MHz, $CDCl_3$) 8.00 (s, 1H), 7.76 (d, J = 1.5 Hz, 1H), 7.53 (d, J = 1.5 Hz, 1H), 7.37–7.32 (m, 9H), 7.17–7.14 (m, 6H), 4.28 (q, J = 7.0 Hz, 2H), 1.32 (t, J = 7.0 Hz, 3H). ^{13}C NMR (75.5 MHz, $CDCl_3$) 163.0, 141.7, 139.3, 135.0, 131.8, 129.5, 128.0, 127.9, 125.8, 119.3, 75.9, 62.0, 14.1. HRMS of *cis*/*trans* mixture: Calculated for $C_{27}N_2O_2ClH_{23}$ M = 442.1448; Found 442.1433.

Ester Hydrolysis

Esters of the four *cis*- and *trans*-urocanates were all synthesized by same procedure, one example of which is given here.

2-Bromo-3-(N-tritylimidazole)-2-propenoic acid (**1**). **9** (20 mg, 0.04 mmol) was dissolved in 1.4 ml THF. LiOH · H_2O (5 mg, 0.12 mmol, 2.9 eq) was dissolved in 600 μl of H_2O and added to the THF solution. The reaction was stirred for 2 days at room temperature until no more starting material was present (TLC, 70% hexane/EtOAc). The THF was removed *in vacuo* and the aqueous solution was acidified to pH 7.0 using 3 N HCl. White precipitate formed and was filtered and washed with water. Drying for 24 h on a drying pistol with P_2O_5 (toluene) gave 18 mg (95% yield) of product. ^{1}H NMR (500 MHz, d_6-DMSO) 16.82 (s, 1H, COOH), 7.77 (s, 1H), 7.64 (s, 1H), 7.37 (m, 10H), 7.09 (m, 6H). ^{13}C NMR (125.8 MHz, d_6-DMSO, 1C overlapped) 173.6, 152.4, 149.2, 140.1 (large signal, trityl), 139.4 (large signal, trityl), 139.2 (large signal, trityl), 136.2, 117.2, 90.2, 86.7. HRMS calculated for $C_{25}N_2O_2BrH_{19}$ M = 459.3492 not observed: M-CO_2 = 414.0698 (calc 414.0687, M-Br-Co_2 = 334.1470 (calc 334.1471), Trityl group = 243.1121 (calc 243.1171).

(**2**) ^{1}H NMR (300 MHz, d_6-DMSO) 17.2 (s, 1H, COOH), 7.60 (s, 1H), 7.37 (m, 10H), 7.07 (m, 6H). ^{13}C NMR (125.8 MHz, D_2O) 161.6, 141.2, 138.2, 132.2, 129.1, 128.4, 128.3, 127.5, 125.8, 79.1, 75.9.

(**3**) ^{1}H NMR (500 MHz, d_6-DMSO) 7.85 (s, 1H, COOH), 7.71 (s, 1H), 7.48 (s, 1H), 7.41 (m, 10H), 7.10 (m, 6H).

(**4**) ^{1}H NMR (200 MHz, D_2O) 7.50 (br. s, 2H), 7.40 (s, 1H), 6.95 (m, 15H). ^{1}H NMR (500 MHz, d_6-DMSO) 7.50 (s, 1H), 7.42 (m, 10H), 7.12 (m, 6H). (C_2H and COOH exchanged out—not detected). ^{13}C NMR (125.8 MHz, d_6-DMSO) 166.0, 150.0, 147.8, 147.8, 143.1, 137.7, 137.5, 136.5, 134.3, 132.8, 83.7.

Evaluation of pK_as. The imidazolium pK_as of *trans*-urocanic acid and **1–4** were evaluated from potentiometric titration data obtained at 25°C by use of an iterative

computer program written by W. W. Cleland. For **1,** subtraction of pH increases caused by NaOH addition to H_2O was required. For **2–5,** this subtraction was unnecessary as the pK_a-values were below the region where NaOH interfered.

RESULTS AND DISCUSSION

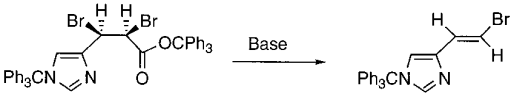
Synthesis of cis- and trans-2-halo-3-(N-tritylimidazole)-2-propenoic acids **1–4.** The synthesis of compounds **1–4** is shown in Scheme 1. *N*-Triphenylmethyl (trityl) protection of commercially available *trans*-urocanic acid **5** following the procedure described by Mutter and Hersperger gave **6** in quantitative yield (*4*).[1] Yamaguchi esterification *via* formation of the mixed anhydride using 2,4,6-trichlorobenzoyl chloride gave ethyl ester **7** in 70% yield (*5*).[2] Bromination of **7** afforded **8** (100%). Elimination of HBr at low temperature (−78°C) with DBU afforded an 80% yield of bromoalkenes **9** and **10,** with **9** formed as the major product (>20:1).[3] At higher temperatures (refluxing $CHCl_3$) with a weaker base (Et_3N) (*6*), the β-elimination product **10** was the major product while E_2 elimination to give **9** as the minor product (3:2; 65% combined yield). Esters **9** and **10** were separated and independently subjected to basic hydrolysis to give products **1** and **3** (95 and 100%; Scheme 1c).

The chlorides were obtained from **7** in a similar fashion (Scheme 1b). Chlorination of 7 gave a mixture of *syn*- and *anti*-addition products **11** and **12** (65%). Reaction of this mixture with DBU at −78°C gave a 1:3 mixture of **13** and **14** in 80% yield. E_2 elimination of HCl from **11** produced **13,** while E_2 elimination from **12** and β-elimination from **11** both produced **14.** Esters **13** and **14** were separated and hydrolyzed to afford **2** and **4** (both 80%; Scheme 1c).

Low field signals in the ^1H *NMR spectra of* **1–4.** The ^1H NMR spectra of **1–4** were obtained in DMSO-d^4. The chemical shift of the carboxylic acid proton in the spectra of both the chloro- and the bromo-substituted derivatives of *cis*-urocanic acid is about 17 ppm (**1,** 16.8 ppm; **2,** 17.2 ppm in DMSO). Low field signals have been used as a diagnostic of LBHBs (*7*), and such a signal was originally reported

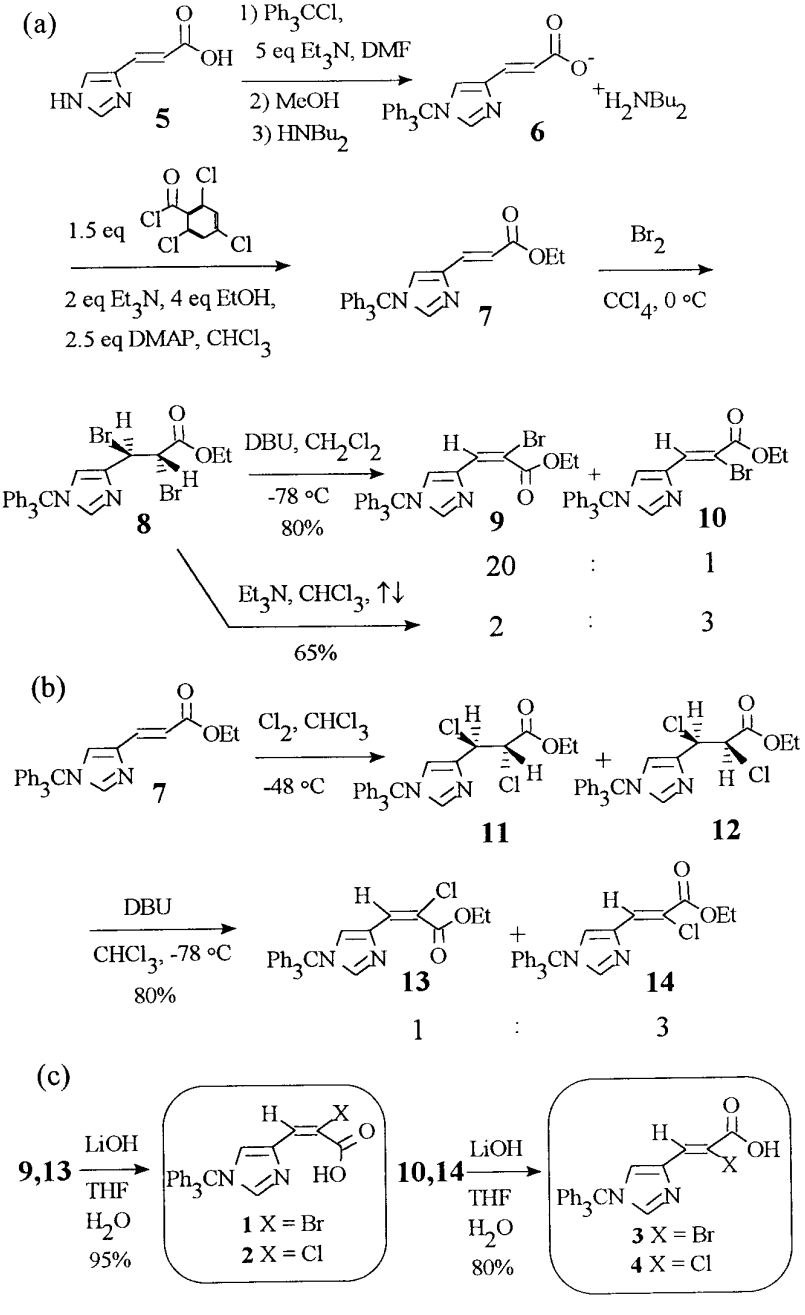
[1] *N*-Trityl protection was required as bromination of dimethyl-protected **3** occurred exclusively on the imidazole ring.

[2] Esterifications using dimethyl sulfate or methyl iodide were unsuccessful, presumably due to the low solubility of **4** in appropriate solvents. The trityl ester was not suitable because exposure of the derived dibromide to base caused cleavage of the trityl group followed by loss of CO_2:



[3] X-ray crystal structures of **7, 8** and **12** were obtained to verify product assignments.

[4] In organic solvents such as $CDCl_3$ or d_6-DMSO, the pK_a of the carboxylic acid will be elevated relative to the aqueous pK_a, while the pK_as of positively charged acids such as imidazolium ions will be similar to their values in water. Thus, a low aqueous pK_a for the carboxylic acid should create a system which has been more highly optimized for LBHB formation. For further explanation, see reference [8] (and references cited therein).



SCHEME 1. (a) The synthesis of **1** and **3.** (b) The synthesis of **2** and **4.** (c) The last step (**1–4**).

as present in *cis*-urocanic acid (17.4 ppm) (*2*). The corresponding proton in *trans*-urocanic acid derivative **3** is at 7.9 ppm. Inasmuch as 7.9 ppm is very close to the chemical shift of the acidic proton in 1-methylimidazolium *p*-toluenesulfonate in $CDCl_3$ (7 ppm) (*8*), **3** exists as the zwitterion. Accordingly, **3** served as a control, in which very little (if any) strong hydrogen bonding occurs. In **1** and **2,** the hydrogen bond between imidazole and the carboxylic acid is expected to be stronger, and the 9 ppm difference (**1** and **2** vs **3**) can be attributed to the difference between a strongly hydrogen bonded ion-pair and a zwitterionic structure.

The halogen substituents C2 of **1–4** are acid strengthening and can therefore be expected to alter the strength of the hydrogen bond between the imidazole and carboxylic acid groups of **1** and **2.** The hydrogen bond is not optimal in the case of *cis*-urocanic acid (17.4 ppm in DMSO), perhaps because the pK_a of the carboxylic acid group is 3, whereas the optimal pK_a may be somewhat lower (*8*). We thought that the chloro- or bromo-substituents on C2 might lower the pK_a enough to strengthen the hydrogen bond; however, the chemical shift values did not indicate a stronger bond. The plot of chemical shift for the hydrogen bond against aqueous pK_a for analogous 1-methylimidazole/carboxylic acid complexes showed a maximum at $pK_a = 2.1$, with a positive slope at low pK_as and a negative slope at higher pK_as (*8*). *cis*-Urocanic acid falls on the negative slope of this plot. If the chloro- and bromo-substituents shift the pK_a lower than 2.1, the values for **1** and **2** may fall on the positive slope of the plot and coincidentally display proton chemical shifts similar to *cis*-urocanic acid. The pK_as of fumarate (3.02) and 2-bromofumarate (1.7) and 2-chlorofumarate (1.7) indicate that halogen substituents on C2 significantly increase the acidity of unsaturated acids (*9*), perhaps too much to optimize the hydrogen bonds in **1** and **2.** In addition, it is not clear that the structures of these molecules allow the formation of linear LBHBs.

Basicities of the imidazole rings in cis- and trans-urocanic acid derivatives. In the catalytic triad of the chymotrypsin active site, it has been proposed that His 57 abstracts a proton from Ser 195. Inasmuch as histidine is not normally basic enough ($pK_a = 6$) to be optimally effective in deprotonating serine, the pK_a of which is >13 (*10*), an LBHB between His 57 and Asp 102 is thought to elevate the pK_a of His 57 (*1*). With this in mind, we obtained the imidazolium-pK_as of our model compounds.

The pK_as of the imidazolium ring protons of compounds **1–4** and *trans*-urocanic acid were determined by potentiometric titration with the results shown in Table 1. In the *trans*-urocanic acid series, the results show that the bromo- and chloro-substituents have essentially no effect on the pK_a-values, all of which are 6.0 ± 0.1 (**3, 4,** and **5**). Therefore, the inductive effects of the chloro- and bromo-substituents in **3** and **4** are not base-weakening in this system. The presence of the trityl group also has no significant effect on the pK_a values in the *trans* series (**3** and **4** vs **5**).

Interesting results were obtained from the substituted *cis*-urocanic acids. The pK_a for *cis*-urocanic acid is 0.85 higher than that for the *trans*-urocanic acid **5,** presumably because of the proximity of the imidazole and carboxyl groups. The pK_a of chloro-substituted **2** is 1 unit higher and that of bromo-substituted **1** is nearly 2.5 units higher than that of *cis*-urocanic acid. The effect of bromine at C2 represents a 3.2 kcal/mol difference in the ionization free energy between **1** and *cis*-urocanic

TABLE 1
Imidazole Ring pK_as of *cis*- and *trans*-Urocanic Acids and Halogenated Derivatives

Compound	pK_a	Compound	pK_a
cis-Urocanic acid	6.78[a]	*trans*-Urocanic acid (**5**)	5.92[b]
cis-2-Chloro-3-(*N*-tritylimidazole)-propenoic acid (**2**)	7.81	*trans*-2-Chloro-3-(*N*-tritylimidazole)-propenoic acid (**4**)	6.02
cis-2-Bromo-3-(*N*-tritylimidazole)-propenoic acid (**1**)	9.10	*trans*-2-Bromo-3-(*N*-tritylimidazole)-propenoic acid (**3**)	5.99

[a] Value from Ref. 11.
[b] Ref. 11 value = 5.89 ± 0.03.

acid and a 4.3 kcal/mol difference between **1** and **3.** That the molecules have different solvation energies might account for part of the observed change in pK_a, but it would not be expected to account for most of the difference.

The substituent-induced pK_a elevations in **1** and **2** (relative to *cis*-urocanic acid) are not caused by an electronic effect. Inductive electron withdrawal by halogens would lower the basicity of the imidazole ring, but pK_a-elevation is observed. Furthermore, any electronic effect would be observed in the *trans*-isomers, all of which display essentially the same imidazole pK_a.

It appears that increased basicity in **1** and **2** relative to *cis*-urocanic acid is brought about by the steric requirements of Cl and Br as substituents at carbon-2. The atomic radii of chlorine (99 pm) and bromine (114 pm) are significantly larger than that of hydrogen (32 pm). The halogens occupy considerably more space than hydrogen, and this must force **1** and **2** to be in slightly different conformations than that of *cis*-urocanic acid. Bromine is the larger substituent and causes the greater increase in basicity. A nearer proximity of the carboxylic acid and the imidazole ring, which shortens the internal hydrogen bond, is the change that would most obviously account for the increased basicity of the imidazole ring with increased substituent size.

Steric compression and low-barrier hydrogen bonding in chymotrypsin. The LBHB-facilitated general base catalysis mechanism recently postulated for the action of chymotrypsin in cleaving peptide bonds is illustrated in Fig. 1 (*1*, *16*). The mechanism was put forward to explain how His 57 can be basic enough to be an optimally effective Bronsted acid–base catalyst in the acylation of chymotrypsin. An optimal catalyst would be basic enough to abstract the proton from Ser 195 in the transition state for tetrahedral adduct formation, and its conjugate acid would be acidic enough to protonate the leaving group, the *N*-terminal amino group of a peptide in the case of chymotrypsin. Thus, an ideal catalytic group would display a pK_a value between that of the 3-OH group of serine (about 13) and the *N*-terminal ammonium group of a peptide (about 9). His 57 in peptidyl trifluoromethylketone adducts of chymotrypsin displays pK_a values between 10.6 and 12.1, depending on the structure of the peptidyl moiety (*1*, *16*). These values are in the range for effective catalysis of acylation. Peptidyl trifluoromethylketone adducts of chymotrypsin are

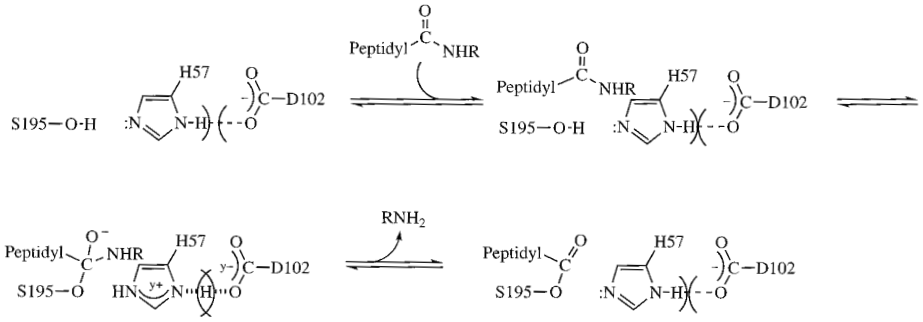


FIG. 1. The mechanism of LBHB-facilitated general base catalysis postulated for the acylation of chymotrypsin. In this mechanism, remote binding interactions between the peptidyl moiety and the active center are postulated to induce a conformational change in the enzyme that results in steric compression between His 57-N$^{\delta 1}$ and Asp 102-O$^{\gamma}$. Protonation of His 57-Ne2 relieves the strain by allowing LBHB-formation between His-57-N$^{\delta 1}$ and Asp 102-O$^{\gamma}$, whose acidities can be matched only when His 57 is in its conjugate acid form. Thus, the potential for LBHB-formation increases the basicity of His 57 in the Michaelis complex, where there is compression, but not in the free enzyme, where there is no steric compression. The deacylation mechanism should be in many respects analogous to acylation, with water reacting in place of the leaving group for acylation.

regarded as close analogs of the transition state. Therefore, His 57 appears to display nearly ideal acid–base properties for its function in catalysis.

It has been postulated that LBHB-formation between His 57 and Asp 102 facilitates general base catalysis by His 57 through stabilization of tetrahedral intermediates such as that shown in Fig. 1. The mechanism shown accounts for this effect as follows. The binding of a substrate induces a conformational change in the enzyme that leads to steric compression between His 57 and Asp 102. The compression between His 57-N$^{\delta 1}$ and Asp 102-O$^{\gamma}$ would be relieved by LBHB-formation because an LBHB is intrinsically short and would relieve the strain. However, LBHB-formation is possible only when His 57 is protonated on N$^{\varepsilon 2}$. The strain of compression between His 57 and Asp 102 therefore potentiates the protonation of His 57-N$^{\varepsilon 2}$; that is, it increases the basicity of His 57. The action of His 57 as a base is directed toward Ser 195 and facilitates its nucleophilic addition to the peptide carbonyl group. The resulting tetrahedral intermediate is stabilized by all of the binding interactions between it and the active site, in addition to the stabilization provided by the LBHB. The LBHB stabilizes the complex of the tetrahedral intermediate with the active site, but the stabilization is not so great as to prevent His 57 from protonating the leaving group, as shown by its pK_a in the peptidyl trifluoromethylketone adducts (*1*, *16*), which are structurally similar to the tetrahedral intermediate.

LBHB-formation can be observed in free chymotrypsin at low pHs (*2*, *17*, *18*), but the LBHB does not lead to high basicity for His 57 in resting chymotrypsin. This is because increased basicity is brought about by compression between His-N$^{\delta 1}$ and Asp 102-O$^{\gamma}$, which occurs only in the Michaelis complex when a specific substrate is bound (*1*). Thus, by the mechanism in Fig. 1, the basicity of His 57 is

increased only when a specific substrate is bound at the active site. Correlations of the basicity of His 57 and the strength of the LBHB with the structures of peptidyl trifluoromethylketones and substrates indicate that the best substrates induce the formation of the strongest LBHBs (*16*).

Conclusion. An efficient synthesis of bromo- and chloro-substituted derivatives of *cis*- and *trans*-urocanic acids has enabled the discovery that small steric changes can have dramatic effects on the basicity of the imidazole ring in the *cis*- but not the *trans*-isomers of urocanic acid derivatives. The results discussed above indicate that the halo substituents of **1** and **2** cause a change in geometrical structure, which makes the internally hydrogen-bonded proton much more difficult to remove relative to that in *trans*-urocanic acid. The simplest explanation is that the internal hydrogen bond is shortened and thereby strengthened. Shortening and strengthening of hydrogen bonds has been suggested to explain certain aspects of enzymatic mechanisms (*1*, *2*, *12–15*). Alternatively, the steric compression brought about by the halo substituents may increase the probability of internal hydrogen bonding, which stabilizes the protonated imidazole ring and increases the value of its pK_a. In either case, the steric effect increases the basicity of the imidazole ring in **1, 2,** and *cis*-urocanate relative to the corresponding *trans*-isomers. Compression in the 2-halo-*cis*-urocanic acids may serve as a chemical counterpart and model for compression between the imidazole ring of His 57 and the carboxylic acid group of Asp 102 of chymotrypsin, which has been postulated to strengthen the LBHB of the incipient tetrahedral intermediate at the transition state for acylation of Ser 195 (*1*, *16*). A similar stabilization of the transition state for deacylation of the acyl-chymotrypsin intermediate may also make a contribution to catalysis.

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