5-(Halomethyl)-2-pyranones as Irreversible Inhibitors of α-Chymotrypsin

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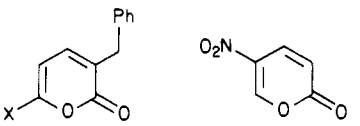
A series of 5-(halomethyl)-2-pyranones were prepared and assayed as potential mechanism-based inhibitors of chymotrypsin, in order to test whether substitution of a suitable electron-withdrawing group at position 5 in a 2-pyranone can activate the ring for catalytic hydrolysis, as well as form a mechanism-based inhibitor. 3-Benzyl-5-(chloromethyl)-2-pyranone (**3**) and 3-(1-naphthylmethyl)-5-(chloromethyl)-2-pyranone (**4**) rapidly and irreversibly inactivate chymotrypsin with high efficiency (I/E = 2.0 for **3** and 1.38 for **4**). However, evidence for their formation of an acyl enzyme is lacking, and thus they may simply be active-site alkylating agents. 6-Methyl substitution on **4**, or replacement of the chloromethyl of **3** with CF_3, prevents inactivation. Since 6-bromo substitution is capable of ring activation, but 5-bromo substitution (3-benzyl-5-bromo-2-pyranone (**11**)) is not, the 5-position of the pyrone does not appear to affect catalytic hydrolysis.

Recent reports of studies on 6-halo-2-pyranones (**1**) as mechanism-based inhibitors of serine proteases[1,2] prompted us to investigate the pyrone ring as the basis for other types of mechanism-based inhibitors.[3] Although the 6-halo-2-pyrones inactivate chymotrypsin, they all suffer from reactivation of the enzyme with time, a process which suggests, most likely, hydrolysis of an acyl serine (Ser-195) intermediate of chymotrypsin. Therefore, it appears that the 6-chloro group, which according to the original design was to form a reactive acyl chloride or ketene that was then to form a stable acyl link with the enzyme, does not fulfill this role.[1b] However, from the results of our initial structure–activity survey of 2-pyranones, we found that the pyrone ring needs further activation (such as by halogen substitution) for it to become reactive enough to acylate chymotrypsin; the 6-halo substitution does provide this activation.[3]

A Hückel molecular orbital calculation by Dines[4] indicated that positions 2, 4, and 6 of 2-pyranone are positively charged, while small negative charges exist on positions 3 and 5. Therefore, if electron-withdrawing groups (e.g., halogens) are required to activate the 2-pyranone ring toward reaction with nucleophiles (as would be required to form a serine acyl ester in chymotrypsin),[3] then it seemed reasonable that substitution on position 5 would have a greater effect than substitution on position 6. This hypothesis is supported by Dines' observation that 5-nitro-2-pyranone (**2**) reacts instantly with nucleophiles as weak as ether.[4] Thus, we imagined that by the proper selection of the 5-substituent on the 2-pyranone system, it might be possible to prepare mechanism-based inhibitors of chymotrypsin.

In this paper, we describe the synthesis of a series of pyrones substituted at position 5 with chloromethyl or trifluoromethyl groups. These groups are designed both to provide electronic activation of the pyrone system and, through an elimination reaction in the acyl enzyme, to provide a reactive Michael acceptor that might undergo further reaction with the enzyme. We have found that two of the 5-(chloromethyl)pyrones (**3** and **4**) are indeed capable of time-dependent, irreversible inhibition of chymotrypsin. The stoichiometry of this interaction, however, is complex and may not involve pyrone ring hydrolysis.



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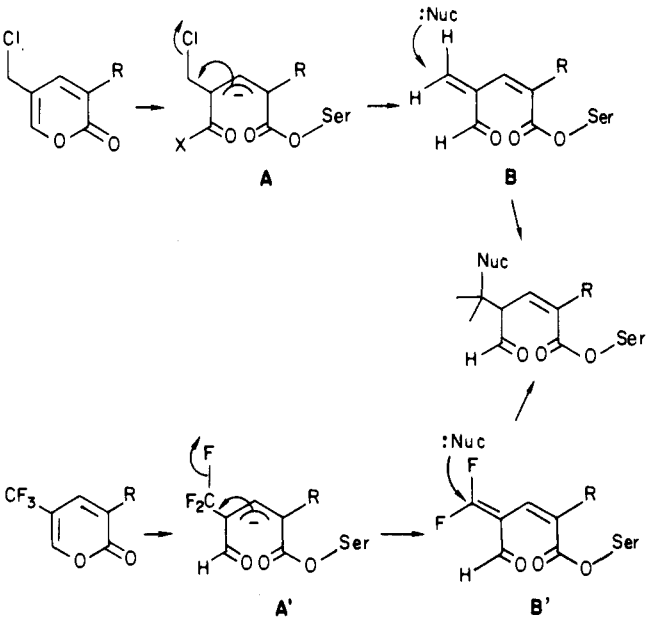
Scheme I



Results and Discussion

Compounds **3**, **4**, and **5** were initially examined as mechanism-based inhibitors of chymotrypsin. The mildly electron withdrawing 5-chloromethyl group (σ_m = 0.11[5]) is also intended both to activate the 2-pyranone ring and to provide for an elimination of chloride ion from the enolate **A** which results from acyl transfer to form the acyl enzyme; this elimination would generate a reactive Michael acceptor, **B** (Scheme I).[6] Since the 5-chloromethyl group may be insufficiently electron withdrawing to activate the pyrone ring toward reaction with the active-site serine in chymotrypsin, the 5-CF_3 group (σ_m = 0.43[5]) was also used in pyrones **6**, **7**, and **8**. These pyrones could undergo an

(1) (a) Westkaemper, R. B.; Abeles, R. H. *Biochemistry* **1983**, *22*, 3256. (b) Gelb, M. H.; Abeles, R. H. *Biochemistry* **1984**, *23*, 6596. (c) Ringe, D.; Seaton, A.; Gelb, M. H.; Aeles, R. H. *Biochemistry* **1985**, *24*, 64.
(2) Harper, J. W.; Hemmi, K.; Powers, J. C. *J. Am. Chem. Soc.* **1983**, *105*, 6518.
(3) Boulanger, W. A.; Katzenellenbogen, J. A. *J. Med. Chem.*, in press.
(4) Dines, M. B. Ph.D. Dissertation, University of Illinois, Urbana, IL, 1968.
(5) Hansch, C.; Leo, A.; Unger, S. H.; Kim, K. K.; Nakaitani, D.; Lien, E. J. *J. Med. Chem.* **1973**, *16*, 71.
(6) For a review article on mechanism-based inhibitors, including many in which a reactive Michael acceptor is generated by an elimination reaction from an enolate, see Walsh, C. *Tetrahedron* **1982**, *38*, 871.

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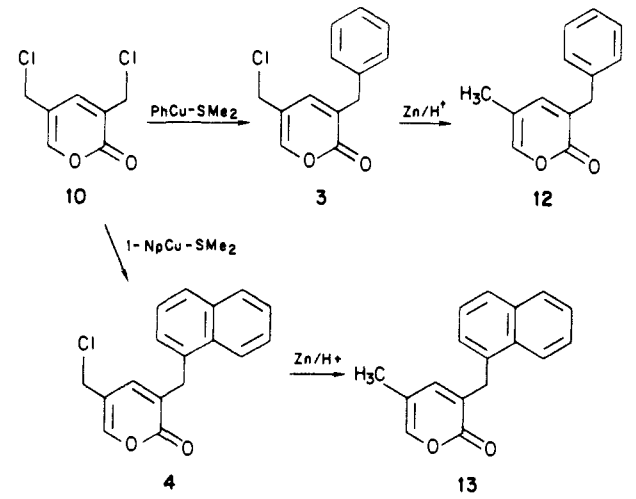
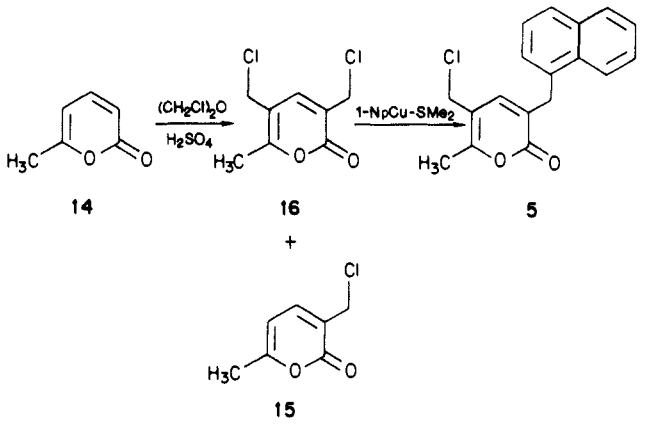
Scheme II



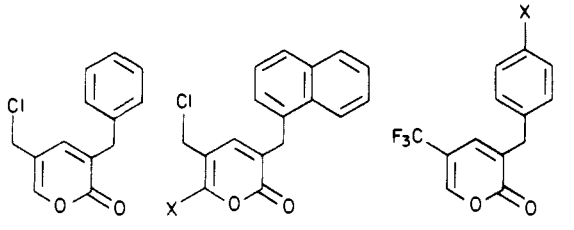
10 3 12 4 13

Scheme III



14 16 5 15

analogous elimination of fluoride ion from the enolate **A′**, generating the more highly reactive difluoromethylene system **B′** (Scheme I).[6]



3 4, (X = H) 5, (X = CH_3) 6, (X = H) 7, (X = OCH_3) 8, (X = OH)

Synthesis. By use of the aryl–copper–dimethyl sulfide complex prepared from the corresponding Grignard reagent and CuBr–SMe_2 in THF at 0 °C, one chloride of 3,5-bis(chloromethyl)-2-pyrone (**10**)[7] could be selectively displaced to give **3** or **4**. The regiochemistry of this alkylation was proved by reduction of the remaining chloromethyl group with zinc in aqueous acetic acid to give putative methylpyrones **12** and **13** (Scheme II); only the signal arising from the chloromethylene group should show a large change in chemical shift in the ^1H NMR upon reduction to the methyl group. The respective benzylic and chloromethyl methylene signals could thus be assigned in the ^1H NMR's of **3** and **4**. In addition, this assignment was confirmed by a shift reagent experiment, using Aldrich

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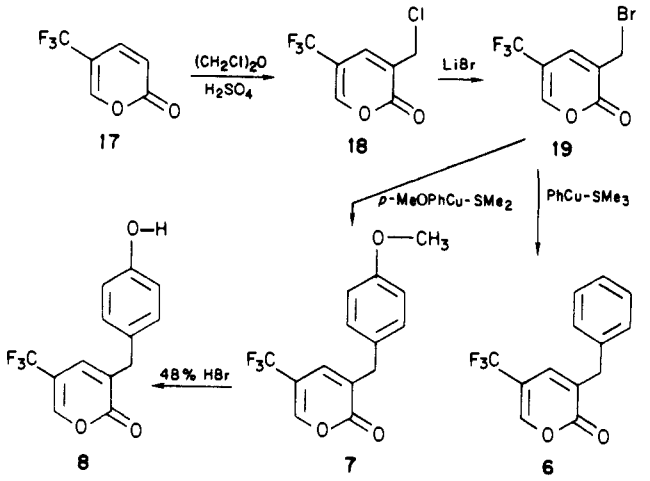
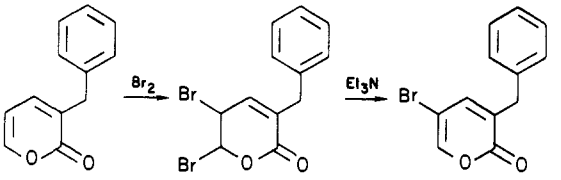
Scheme IV



17 18 19 8 7 6

Scheme V



20 21 11

Resolve-Al; with both sets of pyrones, the methylene assigned to the benzylic position shifted the most downfield, indicating that they are relatively closer to the pyrone carbonyl.

The 6-methylpyrone **5** was prepared in a manner similar to that of **3** and **4** (Scheme III); 6-methyl-2-pyranone[8] (**14**) was chloromethylated with H_2SO_4 and bis(chloromethyl) ether to give a mixture of **15** (47%) and **16** (27%). Reaction of **16** with 1-naphthylcopper–SMe_2 gives **5** (25%).

The 5-(trifluoromethyl)pyrones were accessed through **17**[9] (Scheme IV). Chloromethylation of **17** in concentrated H_2SO_4 using bis(chloromethyl) ether at 75 °C gives **18** in 45% yield. The chloride of **18** proved unreactive toward the organocopper reagent; however, after conversion to the bromide **19**, reaction with PhCu–SMe_2 in THF at 35 °C gives **6** (23%), while reaction with *p*-anisylcopper–SMe_2 gives **7** (66%). By heating at 100 °C in 48% HBr for 3 h, the methyl ether of **7** could be cleaved, giving **8** (65%).

Bromination of **20** in CCl_4 with excess bromine leads to dibromide, **21** (Scheme V). This could be debrominated with Et_3N in a one-pot synthesis to give **11** (75%, based on **20**).

Assays of Time-Dependent Inactivation and Competitive Inhibition of Chymotrypsin. Description of Assay Methods. Time-dependent inactivation of chymotrypsin was assayed by incubating the enzyme with the pyrone and periodically diluting aliquots into an assay mixture containing a chromogenic substrate (see Experimental Section). Various concentrations were used to determine that a maximum inactivation rate was being observed. Competitive inhibition was determined in the usual manner,[10] using short time periods for activity assay, to minimize the effect of the time-dependent inactivation.

(8) (a) Sokorina, L. P.; Zakharin, L. I. *Izv. Akad. Nauk SSSR Ser. Khim.* **1964**, *1*, 73. (b) Schirmann, J. P.; Dreux, J. *Bull. Soc. Chim. Fr.* **1967**, *10*, 3896.

(9) Bailey, T. D. (Reilly Tar and Chemical Corp.) U.S. Patent 4 230 864. Oct 28, 1980.

(10) Seigel, I. H. *Enzyme Kinetics*; Wiley-Interscience: New York, 1975.

Table I. Competitive Inhibition and Inactivation Constants for the Substituted 2-Pyranones with Chymotrypsin[a]

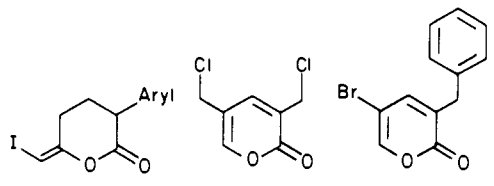
compd no.	substituent 3	4	5	6	K_i, μM	k_{inact}, min^{-1}	I/E
3	CH_2Ph		CH_2Cl		412	0.030	2.00
4	CH_2(1-Np)		CH_2Cl		[b]	0.085	1.38
5	CH_2(1-Np)		CH_2Cl	CH_3	[b]	NI	NI
10	CH_2Cl		CH_2Cl		[b]	NI	NI
6	CH_2Ph		CF_3		470	NI	NI
7	CH_2(4-MeOPh)		CF_3		893	NI	NI
8	CH_2(4-HO-Ph)		CF_3		1610	NI	NI
11	CH_2Ph		Br		687	NI	NI

[a] The competitive inhibition constant (K_i) was determined by standard methods using either *N*-acetyltyrosine *p*-nitrophenyl ester or *p*-nitroanilide. The inactivation rate constant (k_{inact} was determined by exposing chymotrypsin to different concentrations of the pyrone and periodically diluting aliquots into an assay medium containing one of the chromogenic substrates above. The inactivation efficiency (I/E) was determined by incubating different ratios of pyrone and chymotrypsin for 18 h and then assaying the surviving enzyme activity, relative to an untreated control. NI indicates no inactivation. These assays were performed according to protocols described previously.[11] [b] Competitive inhibition is too weak to measure.

The inactivation efficiency (I/E), which corresponds to the inactivator-to-enzyme stoichiometry required to achieve complete inhibition after prolonged incubation (i.e., turnovers per inactivation), was determined by the ultimate activity assay method described previously.[11] The inactivation and inhibition data are summarized in Table I.

5-(Chloromethyl)pyrones (3, 4, 5, and 10). The 5-(chloromethyl)pyrones **3** and **4** are potent inactivators of chymotrypsin; at high concentrations, they each consumed chymotrypsin in an irreversible, time-dependent manner, with half-lives of 23.3 min for **3** and 11.8 min for **4** (cf. Table I). This inactivation shows substrate protection, indicating that it is active-site directed and it follows first-order kinetics, if solutions of **3** and **4** are prepared in Me_2SO; freshly prepared solutions of **3** and **4** in acetonitrile also give first-order inactivation kinetics, but acetonitrile solutions that had been stored appear to accumulate an impurity that causes deviations from first-order kinetics.

As in the 6-halo-2-pyranone series described earlier,[3] substitution on position 3 of the pyrone (presumed to be analogous to the hydrophobic binding group of normal substrate) affects binding and inactivation. In the 5-chloromethyl series, however, 3-(1-naphthyl)methyl substitution results in 2-fold increase in inactivation rate relative to 3-benzyl (Table I); this substitution had just the opposite effect in the 6-halopyranones.[3] The fact that orientation in the active site by the binding group of the pyrone is important in the 5-(chloromethyl)pyrones is demonstrated by the lack of inactivation by **10**. The additional methyl group in pyrone **5** also blocks inactivation. Neither of these compounds is a substrate for chymotrypsin, as no spectroscopic changes could be observed upon prolonged incubation with enzyme.



9 **10** **11**

The inactivation efficiencies of pyrone **3** (I/E = 2.0) and **4** (I/E = 1.38) are very high (cf. Table I and Figure 1). Because very few turnovers are required for complete inactivation, it is difficult to establish (by spectroscopic means or product isolation) whether these pyrones are acting as substrates for chymotrypsin. The shape of the ultimate activity assay curves for **3** and **4** (Figure 1) differ significantly from those of other compounds known to act as mechanism-based inactivators of chymotrypsin, such as the 6-iodomethylene-tetrahydro-2-pyranones, **9**, we have worked with earlier.[11] These enol lactones appear to inactivate chymotrypsin by a two-step acylation–alkylation sequence, and they give ultimate activity assays that are descending straight lines (with some concave nature, if spontaneous hydrolysis consumes a significant quantity of lactone prior to complete inactivation).

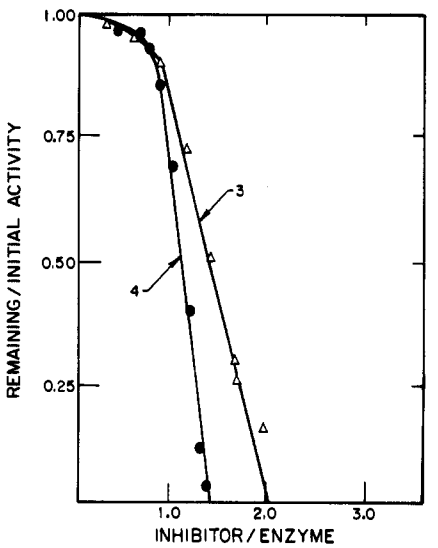


Figure 1. Stoichiometry of chymotrypsin inactivation by the 5-(chloromethyl)-2-pyrones **3** and **4**. Various ratios of pyrone **3** (Δ) and **4** (●) were incubated with chymotrypsin. After 18 h, remaining activity was measured by dilution into *N*-benzoyltyrosine ethyl ester. The plot is constructed from the activity remaining relative to an untreated control vs. the molar ratio of pyrone to chymotrypsin. For details, see Experimental Section.

By contrast, with the 5-(chloromethyl)pyrones **3** and **4**, 1 equiv of inhibitor has relatively little effect, but beyond this, enzyme activity is lost very rapidly, so the ultimate activity plots are clearly convex (cf. Figure 1). This behavior would be consistent with the capture of two enzyme nucleophiles of differing reactivity, the more reactive of which is not critical to catalytic activity, the less reactive being highly critical.

Still, if this were the case, the nonintegral number of equivalents required for total inactivation of chymotrypsin by pyrone **4** is curious, and while it might be rationalized by suggesting that the reaction at the less reactive (critical) site blocks reaction at the more reactive (noncritical) site, such speculations would be difficult to verify without performing chemical and structural analyses on inactivated

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forms of the enzyme. It is known, however, that the inactivation stoichiometry is not skewed by pyrone hydrolysis, as both **3** and **4** were shown to be stable for 1 week at pH 7.5, 25 °C, in 0.1 M phosphate buffer.

In an effort to identify the active site nucleophile(s) that might be involved in chymotrypsin inactivation by the 5-(chloromethyl)pyrones **3** and **4**, methionine-192 *S*-oxide chymotrypsin was prepared.[12] This methionine residue is situated at the lip of the active site of chymotrypsin[13] and is known to react with other active-site-directed alkylating agents.[14] It is not, however, involved in the catalytic activity of chymotrypsin and may be modified by oxidation to the sulfoxide[12] or methylation to the sulfonium salt[15] with only minor effect on the catalytic activity of the enzyme. However, when methionine-192 *S*-oxide chymotrypsin was exposed to the 5-(chloromethyl)pyrones **3** and **4**, inactivation proceeded in a manner identical with that observed with native chymotrypsin.

A clue to the site of action of these pyrones comes from a study with mercaptoethanol. When chymotrypsin was treated with mercaptoethanol, which cleaves an exposed disulfide bridge near the active site,[16] the enzyme retains catalytic activity but is no longer inactivated by the (chloromethyl)pyrones **3** and **4**. Although the pyrones are slowly attacked by mercaptoethanol, the half-life of this reaction under the assay conditions was on the order of 12 h, and therefore is too slow to affect the assay. Further investigation of the suggested interaction between the 5-chloromethyl pyrones and this disulfide link would obviously be interesting.

5-(Trifluoromethyl)pyrones 6, 7, and 8 and 5-Bromopyrone 11. The 5-(trifluoromethyl)pyrones **6**, **7**, and **8** did not inactivate chymotrypsin; they were only weak competitive inhibitors (Table I) and showed no activity as substrates. (Trifluoromethyl)pyrone **6** proved to be remarkably lipophilic and consequently poorly soluble in buffer, even with Me_2SO as a cosolvent. Initially, the poor binding of **6** was attributed to its low solubility, and **7** and **8** were prepared in an effort to offset this effect induced by the CF_3 group by decreasing the hydrophobicity of the 3-substituent. However, binding decreased **6** > **7** > **8** (see Table I).

In the 6-substituted-2-pyranones series studied earlier,[3] we found that CF_3 substitution on position 6 did not activate the ring for catalytic hydrolysis, while halogen substitution did. In order to demonstrate that the lack of substrate activity of **6**, **7**, and **8** is not due to choice of substituent (or low solubility), but insensitivity of position 5 for ring activation, 5-bromopyrone **11** was assayed; no substrate activity or inactivation was observed. This suggests that the original thesis of ring activation for catalytic hydrolysis may not be correct.

Conclusion

It was our original intention in this study to develop a mechanism-based inactivator of chymotrypsin by substituting position 5 of 2-pyranone with a group that would both provide activation of the ring toward hydrolysis (and thus promote acylation of the active site serine) and undergo an elimination of the resulting enolate, to form a reactive Michael acceptor function. The 5-(chloromethyl)-2-pyrones **3** and **4** proved to be rapid and efficient irreversible inactivators of chymotrypsin. However, the stoichiometry of their inactivation is complex, and there is no direct evidence that they are inactivating via the acyl serine intermediate, as opposed to a direct alkylating reaction. The 5-(trifluoromethyl)-2-pyrones **6**, **7**, and **8**, as well as the 5-bromo-2-pyrone (**11**), proved to be inactive, suggesting that substitution at position 5 in a 2-pyrone does not provide the desired enhancement of acylating activity needed to develop a mechanism-based chymotrypsin inhibitor. Still, the high inactivation efficiency of the 5-(chloromethyl)-2-pyranones is intriguing.

Experimental Section

General Procedures. Proton magnetic resonance spectra were obtained on a Varian EM-390, XL-200, or HR-220 spectrometer. Low-resolution mass spectra were run on a Varian MAT CH-5, and high-resolution mass spectra were run on a Varian MAT 731 mass spectrometer, both by electron impact at 70 eV. Elemental analyses were performed by the microanalytical service of the University of Illinois. Infrared spectra were run on either a Beckman IR-12 or Nicolet 7199 FT IR spectrometer. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Flash chromatography[17] was performed on a 25-mm-diameter column of silica gel, with adsorbant heights that varied (30–60 cm) depending on sample size.

Chemical Synthesis. 3-Benzyl-5-(chloromethyl)-2-pyranone (3). The Grignard from bromobenzene (1.712 g, 10.9 mmol) and magnesium (0.2643 g, 10.9 mmol) was formed in THF (20 mL) in the usual manner. This was cooled to −10 °C, and CuBr–SMe_2 (2.24 g, 10.9 mmol) was sifted in under a positive pressure of nitrogen. The mixture was stirred at 0 °C for 10 min; then **10**[3] (1.997 g, 10.35 mmol) in 10 mL of THF was slowly added. The mixture was rapidly brought to 50 °C and stirred at this temperature for 4.5 h. After cooling, ether was added, and the mixture was washed with saturated NaCl (pH 3). After drying over magnesium sulfate, the solvent was removed in vacuo. The residue was flash-column chromatographed, using 20–40% ethyl acetate in pentane. After a fore-run of biphenyl, came putatively a bis-substituted pyrone (0.2309 g, 8.1%). Following this came **3** (0.2414 g, 10%), giving crystals: mp 87 °C; NMR ($CDCl_3$) 7.3 (1 H, s, CH), 7.2 (5 H, s, Ph), 6.9 (1 H, s, CH), 4.1 (2 H, s, CH_2Cl), 3.7 (2 H, s, CH_2Ph). Anal. ($C_{13}H_{11}ClO_2$) C, H.

3-(1-Naphthylmethyl)-5-(chloromethyl)-2-pyranone (4). This compound was prepared by a reaction sequence similar to that used for compound **3**, using 1-chloronaphthalene to prepare the Grignard reagent. Eluting after binaphthyl was putatively a bis-substituted pyrone (6.7%), followed by **4** (9.2%): NMR ($CDCl_3$) 7.6–7.9 (3 H, m, Np), 7.20–7.55 (5 H, m, Np, CH), 6.66 (1 H, m, CH), 4.18 (2 H, s, CH_2Np), 3.95 (2 H, s, CH_2Cl). Anal. ($C_{17}H_{13}ClO_2 \cdot 0.5H_2O$) C, H.

3-Benzyl-5-methyl-2-pyranone (12). Following the method of Shusherina,[7] powdered zinc (0.85 g, 13 mmol) was added to a solution of **3** (0.3015 g, 1.29 mmol) in aqueous acetic acid (17 mL, 88:12 HOAc/H_2O). To this mixture was added 10 mg of silver acetate, and the resulting mixture was refluxed for 22 h. The solvent was then evaporated under reduced pressure and the resulting residue triturated with ether. The ethereal solution was filtered and concentrated in vacuo, then flash-column chromatographed, using 5–20% ethyl acetate in cyclohexane on silica gel. After a small amount of impurity, **12** (0.1338 g, 51.8%) eluted, giving an oil: NMR ($CDCl_3$) 7.3 (5 H, s, Ph), 7.1 (1 H, s, CH), 6.75 (1 H, s, CH), 3.75 (2 H, s, CH_2Ph), 1.9 (3 H, s, Me). Anal. ($C_{13}H_{12}O_2$) C, H.

3-(1-Naphthylmethyl)-5-methyl-2-pyranone (13). This compound was prepared from **4**, by a reaction sequence similar to that used to prepare compound **12**. Compound **13** was obtained (29%) as an oil after chromatography using 20% petroleum ether in methylene chloride: NMR ($CDCl_3$) 7.6–7.95 (3 H, m, Np),

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(17) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

7.25–7.50 (4 H, m, Np), 7.05 (1 H, m, CH), 6.40 (1 H, m, CH), 4.15 (2 H, s, CH_2Np), 1.70 (3 H, s, Me). Anal. ($C_{17}H_{14}O_2 \cdot 0.5H_2O$) C, H.

3-(Chloromethyl)-6-methyl-2-pyranone (15) and 3,5-Bis(chloromethyl)-6-methyl-2-pyranone (16). A mixture of 14[8] (5.25 g, 4.77 mmol), concentrated sulfuric acid (7 mL), and crude chloromethyl methyl ether (10 mL, 0.13 mol) was heated at 45 °C for 3.5 h under a reflux condenser. The mixture was added to ice water then extracted with ether. The extracts were washed with 10% sodium carbonate and saturated NaCl, then dried over magnesium sulfate. The solvent was removed in vacuo, and the residue was flash-column chromatographed with methylene chloride on silica gel to give (eluting first) **16** (26.5%): mp 64–65 °C; NMR ($CDCl_3$) 7.45 (1 H, s, CH), 4.36 (2 H, s, CH_2), 4.34 (2 H, s, CH_2), 2.30 (3 H, s, Me). Anal. ($C_8H_8Cl_2O_2$) C, H.

Eluting next is **15** (46.7%) as an oil: NMR ($CDCl_3$) 7.30 (1 H, d, J = 5 Hz, CH), 6.2 (1 H, d, J = 5 Hz), 4.38 (2 H, s, CH_2), 2.35 (3 H, s, Me). Anal. ($C_7H_7ClO_2$) C, H.

3-(1-Naphthylmethyl)-5-(chloromethyl)-6-methyl-2-pyranone (5). This compound was prepared by using 1-naphthylcopper–SMe_2 and **16**,[3] except that it was chromatographed with methylene chloride. Compound **5** was obtained (25.2%): NMR ($CDCl_3$) 7.6–7.9 (3 H, m, Np), 7.20–7.55 (4 H, m, Np), 6.4 (1 H, s, CH), 4.15 (2 H, s, CH_2Np), 4.0 (2 H, s, CH_2Cl), 2.2 (3 H, s, Me). Anal. ($C_{18}H_{15}ClO_2$) C, H.

3-(Chloromethyl)-5-(trifluoromethyl)-2-pyranone (18). A mixture of 17[9] (5.0 g, 30.5 mmol), bis(chloromethyl) ether (19.82 g, 150 mmol), and concentrated sulfuric acid (15 mL) was heated at 85 °C for 7 h. The reaction was then diluted with ice and extracted with methylene chloride. The organic layer was washed with 10% sodium carbonate and dried over magnesium sulfate. The solvent was removed in vacuo and the residue taken up in 10% EtOAc/hexane. This solution was filtered through silica gel, the solvent removed in vacuo, and the residue vacuum distilled at 60–70 °C/0.1 mm to give **18** (3.24 g, 50.1%): NMR ($CDCl_3$) 7.9 (1 H, s, CH), 7.75 (1 H, s, CH), 4.45 (2 H, s, CH_2). Anal. ($C_7H_4ClF_3O_2 \cdot 0.2kH_2O$) C, H, F. This appears to be a water–azeotropic mixture.

3-(Bromomethyl)-5-(trifluoromethyl)-2-pyranone (19). Under nitrogen, anhydrous lithium bromide (29.7 g, 0.342 mmol) and **18** (2.9 g, 13.67 mmol) were dissolved in 150 mL of anhydrous acetone. An exothermic reaction ensued upon addition of the lithium bromide. The reaction was 95% complete after 5 min; the mixture was briefly brought to reflux, then cooled for 1 h; the mixture was not 99% bromide by GLC (3% OV-17 on CSW, 1/2 in. × 12 ft; 175 °C). The solvent was removed in vacuo and the residue triturated with ether. After washing with water and drying over magnesium sulfate, the solvent was removed to give **19** (3.05 g, 86.8%), with only a trace of **18**: NMR ($CDCl_3$) 7.9 (1 H, m, CH), 7.55 (1 H, d, J = 3 Hz, CH), 4.3 (2 H, s, CH_2). Anal. ($C_7H_4F_3BrO_2 \cdot 0.5H_2O$) C, H.

3-Benzyl-5-(trifluoromethyl)-2-pyranone (6). This compound was prepared, using PhCu–SMe_2 and **19**, by the sequence used to prepare **3**. The crude **6** was recrystallized from pentane at −40 °C to give crystals (0.336 g, 22.6%): mp 56–57 °C; NMR ($CDCl_3$) 7.75 (1 H, s, CH), 7.2–7.4 (5 H, m, Ph), 6.9 (1 H, s, CH), 3.75 (2 H, s, CH_2). Anal. ($C_{13}H_9F_3O_2$) C, H, F.

3-[(4-Methoxyphenyl)methyl]-5-(trifluoromethyl)-2-pyranone (7). This compound was prepared, using *p*-anisyl-Cu–SMe_3 and **19**, by the sequence used to prepare **3**. The chromatographed material (50% hexane/methylene chloride on silica gel) was recrystallized from methylene chloride/hexane to give **7** (66%) as fine, white needles: mp 67–68 °C; NMR ($CDCl_3$) 7.7 (1 H, s, CH), 7.1 (2 H, d, J = 8 Hz, Ph), 6.9 (1 H, s, CH), 6.82 (2 H, d, J = 8 Hz), 3.75 (3 H, s, OMe), 3.70 (2 H, s, CH_2). Anal. ($C_{14}H_{11}F_3O_2$) C, H, F.

3-[(4-Hydroxyphenyl)methyl]-5-(trifluoromethyl)-2-pyranone (8). A suspension of **7** (0.262 g, 0.992 mmol) in 48% HBr (10 mL) was heated to 100 °C in a flask open to air for 3 h. The mixture was then cooled and extracted with methylene chloride. The extracts were treated with Norit then dried over magnesium sulfate, and the solvent was removed in vacuo. White crystals of **8** could be obtained from the residue by crystallizing from methylene chloride/hexane at −40 °C (0.163 g, 65.5%): mp 109 °C (softens at 94 °C). upon recrystallization, crystals were obtained: mp 115–116 °C; NMR (acetone-d_6) 6.8–8.2 (1 H, br, s, OH), 8.15 (1 H, s, CH), 7.32 (1 H, m, CH), 7.1 (2 H, d, J = 8 Hz, Ph), 6.75 (2 H, d, J = 8 Hz, Ph). Anal. ($C_{13}H_9F_3O_2 \cdot H_2O$) C, H, F.

3-Benzyl-5,6-dibromo-5,6-dihydro-2-pyranone (21). To a solution of **20**[1a] (0.3221 g, 1.73 mmol) in carbon tetrachloride (10 mL) was added bromine (11.96 g, 74.7 mmol); this was refluxed for 1 h, after which the excess bromine and solvent were removed in vacuo. The residue was flash-column chromatographed with 10% pentane in methylene chloride to give **21** (0.5441 g, 91.7%) as an oil which slowly lost HBr: NMR ($CDCl_3$) 7.0–7.3 (5 H, m, Ph), 6.7 (1 H, d, J = 1.5 Hz, =CH), 6.50 (1 H, dd, J = 6 Hz, 1.5 Hz, CHBr), 4.9 (1 H, d, J = 6 Hz, CHBrO); UV λ_{max} (CH_3CN) = 230 nm, e = 7070. Anal. ($C_{12}H_{10}Br_2O_2$) C: calcd, 41.65; found, 42.40. H: calcd, 2.91; found, 3.00.

3-Benzyl-5-bromo-2-pyranone (11). To a solution of **20** (0.4587 g, 2.46 mmol) in 20 mL of CCl_4 was added bromine (1.18 g, 7.4 mmol), and the mixture was refluxed for 1 h, after which the excess bromine and solvent were removed in vacuo. The crude residue was taken up in THF (25 mL), and triethylamine (2.18 g, 21.5 mmol) was added. The mixture was refluxed for 2 h, then the solvent and excess triethylamine were removed in vacuo. The residue was taken up in ether; this solution was washed with dilute HCl and saturated NaCl and dried over magnesium sulfate. The solvent was removed in vacuo and the residue flash-column chromatographed on silica gel with methylene chloride. This gave **11** (0.2994 g, 75.3% based on **20**): mp 125–126 °C; NMR ($CDCl_3$) 7.4 (1 H, d, J = 2.5 Hz, CH), 7.1–7.3 (5 H, m, Ph), 6.85 (1 H, m, CH), 3.72 (2 H, s, CH_2). Anal. ($C_{12}H_9BrO_2$) C, H, Br.

Biological Methods. α-Chymotrypsin was obtained from Worthington Biochemicals and *N*-benzoyltryrosine ethyl ester from Sigma. Inactivation assays and the determination of competitive inhibition constants were performed as described in our previous paper.[3] Inactivation by **3** and **4** in the presence of mercaptoethanol is also the same as described, except the chymotrypsin solution was made 100 mM in mercaptoethanol immediately prior to addition of inhibitor.

Ultimate Activity Assays of 3 and 4.[11] To 1.00 mL of a 2.69 μM solution of α-chymotrypsin in 0.1 M potassium phosphate buffer, pH 7.5, at 25 °C, was added, in separate experiments, 0–60 μL (in 5-μL increments) of a freshly prepared solution of inhibitor, 0.15 mM in acetonitrile. After 18 h, a 50-μL aliquot was removed from each and the activity of the enzyme determined by adding this aliquot to 0.95 mL of a 50 mM solution of benzoyl tyrosine ethyl ester in 15% acetonitrile/buffer and following the rate of hydrolysis at 256 nm. The percent activity was found by dividing the slope of the progress curve of each by that of the blank (0 μL).

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