Acylating Agents as Enzyme Inhibitors and Understanding Their Reactivity for Drug Design

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A series of bicyclic trans-fused γ-lactones and γ-lactams have been previously described for the inhibition of human neutrophil elastase and as possible development candidates. During the discovery program, it had been assumed that their acylating power was due in part to the inherent strain energy in the bicyclic structure that was released upon ring opening. This is now shown not to be the case, and in fact, these compounds are no more reactive than simple but analogous γ-lactams and γ-lactones. The strain energy is not released in the transition state for alkaline hydrolysis or alcoholysis because the reaction proceeds with rate-limiting formation of the tetrahedral intermediate. A reactivity index of k_{OH} is proposed as a simple guide to determine the usefulness of a potential inhibitor as an enzyme acylating agent.

Proteolytic enzymes are common targets for potential therapeutic application. In particular, their inhibition by mechanism-based and active-site-directed agents is a fruitful area of study.[1] In general, this involves the covalent modification of an active site residue that is not subsequently and readily regenerated. For example, many inhibitors are acylating,[2] phosphylating,[3] or sulfylating[4] agents of the active site serine residue of serine proteases. The mechanism of these processes involves, in the case of acylation, the displacement of a leaving group, Lg, from the acylating agent:

$$\text{EnzCH}_2\text{OH} + \text{RCOLg} \rightarrow \text{EnzCH}_2\text{O}-\text{COR} \quad (1)$$
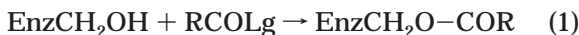
The structure of the acylenzyme is such that the regeneration of the enzyme by its further reaction with nucleophiles, such as water, is a relatively slow process, and this leads to effective inhibition.

Some of the other major requirements for successful inhibition are that the rate of the acylation process must be faster or at least competitive with the turnover of the natural substrate and the inhibitor itself must be relatively stable under in vivo conditions. The rate of acylation is controlled by two important factors: (i) the "chemical" reactivity of the inactivator and (ii) molecular recognition by the target enzyme.

Chemical reactivity is usually considered in terms of the electrophilic nature of the acyl center and the leaving group ability (nucleofugality) of the group displaced. In addition to these electronic effects, the intrinsic chemical reactivity of a compound may be modified by steric and strain effects. Increasing intrinsic chemical reactivity may lead to a faster rate of reaction with the target enzyme but may also lead to greater hydrolytic and metabolic instability.

Maximum activity gained by molecular recognition involves using the full catalytic apparatus of the enzyme, as is used for the natural substrate, and utilizing the favorable noncovalent interactions at the various subsites of the enzyme and inhibitor.[5] The most desirable inhibitor may be one where molecular recognition is optimized using favorable hydrophobic and other noncovalent interactions and where covalent modification of the enzyme, due to the inherent reactivity of the inhibitor, is minimized.

Improving the inhibition properties of a compound may therefore be achieved by modifying the structure to enhance either chemical reactivity or molecular recognition, or a combination of both. However, demonstrating the efficiency of the reaction when the inhibitor is modified is not straightforward. Modification of the inhibitor structure can affect the free energies of both the initial reactant state and the transition state, whereas the observed variation in rate constants for the various inhibitors reacting with the enzyme only reflects the *difference* in energies between these two states. Different substituents can affect the ease of bond-making and -breaking by classical electronic factors such as inductive, resonance, and steric effects. However, the free energy of activation of an enzyme-catalyzed reaction is also affected by the favorable binding energies between the protein and substrate substituents not directly involved with the reaction site. It is therefore important to separate these two effects before conclusions about the efficiency of inhibition can be made. We have suggested[6] that an "enzyme rate-enhancement factor" (EREF) can be evaluated by dividing the second-order rate constant for the enzyme-catalyzed reaction, k_{cat}/K_m, by that for hydrolysis of the same substrate catalyzed by hydroxide ion, k_{OH}.

Human neutrophil elastase (HNE) is a serine enzyme that is one of the most destructive proteolytic enzymes, being able to catalyze the hydrolysis of the components of connective tissue. HNE is released in response to inflammatory stimuli and has a major role in protein digestion following phagocytosis. It has been implicated in the development of diseases such as emphysema, cystic fibrosis, and rheumatoid arthritis, and there have

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been numerous studies attempting to find small-molecule inhibitors of HNE.[7] The enzyme belongs to the trypsin class, and the structure of HNE has been determined by X-ray crystallography,[8] but many structural and inhibition studies have been conducted with the related, but more readily available, porcine pancreatic elastase (PPE).[9]

The majority of elastase inhibitors are similar to other serine protease inhibitors. They are, for example, peptidyl fluoroketones[10] or ketones attached to a strongly electron-withdrawing group, such as 2-benzoxazole, with suitable elements of molecular recognition.[11] An alternative strategy has been to use acylating agents as inhibitors that generate an acyl enzyme that does not turn over or rearranges to a more stable adduct or even generates a second electrophilic "trap" that subsequently reacts with a nucleophilic group on the enzyme. For example, bicyclic [3.3.0] systems containing a γ-lactone or γ-lactam that is trans-fused to the other five-membered ring inhibit HNE by acylating the nucleophilic hydroxy group of serine-195 in the active site of the enzyme.[12] The potency of these compounds has previously been attributed in part to the ring strain present in the trans-fused ring system, which was assumed to facilitate the rate of ring opening.[12a] This explanation follows the widespread belief that the ring strain present in the four-membered ring of β-lactam antibiotics is responsible for their ability to rapidly acylate bacterial transpeptidase enzymes. Although an intuitively appealing idea, we have shown that for these antibiotics this is not correct and that the energy released upon opening this strained ring does *not* contribute to a lowering of the activation energy for acylation.[13] We herein report a similar study on the reactivity of the formally strained trans-fused bicyclic [3.3.0] lactones and lactams.

Results and Discussion

The potency of the 5,5-trans-fused bicyclic pyrrolidine lactams **1** and lactones **2** ("*trans*-γ-lactones" and "*trans*-γ-lactams") as elastase inhibitors is attributed to a
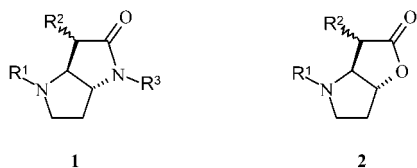

combination of conformational ring strain and molecular fit in the active site of the enzyme.[12a]

These 5,5-trans-fused ring systems are highly strained structures; for example, there is approximately 6−7 kcal mol^{-1} difference in strain energy between the cis and trans bicycloalkane analogues.[14] A similar energy difference in the lactams **1** and lactones **2** could lead to a rate enhancement of up to 10^5 for the trans-fused systems if this energy is released in the transition state.

In the above structure **1** the R^1 group is thought to be important for molecular recognition with the enzyme but is unlikely to affect chemical reactivity, i.e., the rate of ring opening of the γ-lactam. The R^2 group has been shown through X-ray studies with PPE to dock into the S$_1$ specificity pocket[12b,c] but could also affect the chemical reactivity of the acylating agent because the rate of
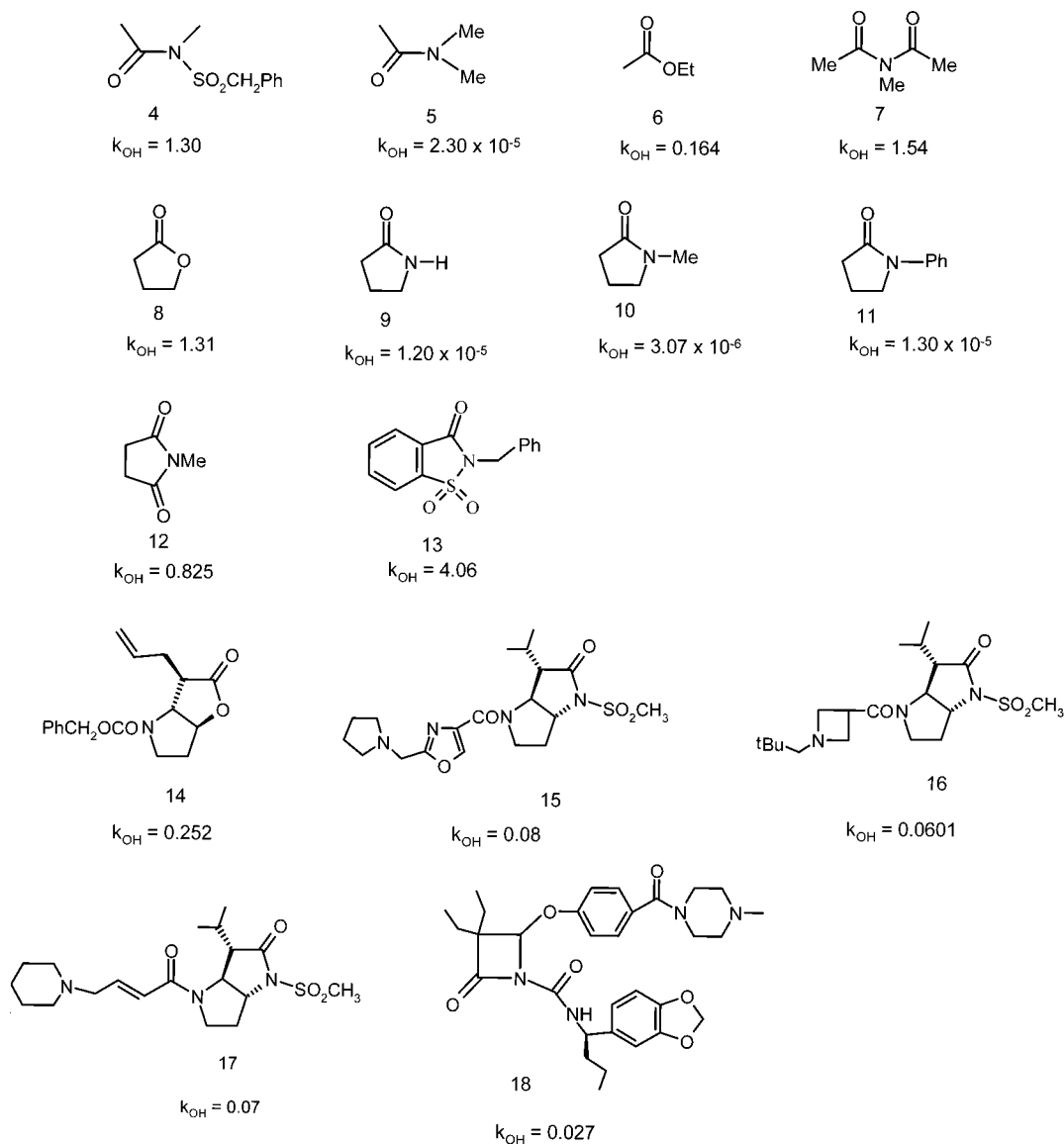
nucleophilic attack generally increases if R^2 is an electron-withdrawing group. The reaction rate usually increases when R^3 is an electron-withdrawing group so that the leaving group is weakly basic (i.e., amines with low pK_a's), a common example of which is an *N*-acylsulfonamide (R^3 = SO$_2$R). Although the presence of a sulfonyl center does present an alternative electrophilic site for nucleophilic attack, it has again been shown in X-ray studies of **1** with PPE that the carbonyl center acylates the serine.[12b,c] Further, in general, acyl transfer reactions occur much more readily than analogous sulfonyl transfers.[15] For example, the rate of alkaline hydrolysis of acyl derivatives is often about 10^3-fold faster than the equivalent sulfonyl derivative. For *N*-acylsulfonamides, **3**, which incorporate both centers in one molecule, nucleophiles preferentially attack the carbonyl group to displace the sulfonamide anion:[16]

$$R_1SO_2NHCOR_2 \xrightarrow{\text{NuH}} R_1SO_2NH_2 + R_2CONu \quad (2)$$
$$\mathbf{3}$$
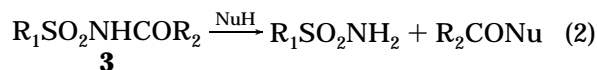
An additional factor favoring acyl attack in this case is the strong electron-withdrawing character of the sulfonamide group, with the sulfonyl group stabilizing the adjacent lone pair on nitrogen by a polarization effect rather than conjugative d−pπ bonding.[17] This activates the carbonyl group toward nucleophilic attack, and because sulfonamides are stronger acids than amides by about 5 pK units, sulfonamide anions are usually better leaving groups than amide anions. Only in highly strained and activated sulfonamides such as *N*-acyl-β-sultams do these compounds undergo hydrolysis with S−N fission and displacement of the amide group.[18]

To compare the relative reactivity of the strained *trans*-γ-lactams **1** and *trans*-γ-lactones **2**, it is necessary to have the relevant data for analogous unstrained derivatives. Hence, we required the second-order rate constants for the alkaline hydrolysis of analogous acyclic and monocyclic esters and amides (Scheme 1). The alkaline hydrolysis in water of the acyclic *N*-acylsulfonamide, **4**, occurs by *N*-acyl fission as a result of hydroxide ion attack on the carbonyl group followed by displacement of the sulfonamide anion. This is demonstrated by product analysis of UV and ^{1}H NMR spectra, ESIMS evidence, and titration of the acetate anion produced. The second-order rate constant for the alkaline hydrolysis of k_{OH} of **4** is 1.30 dm^3 mol^{-1} s^{-1} at 30 °C (Scheme 1), showing the high reactivity of these amide derivatives and the good leaving group ability of the sulfonamide anion. This activated amide shows a 10^5 greater reactivity than "normal" amides, e.g., **5**, and is similar to that for imides, e.g., **7** and **12**, in its susceptibility to attack by hydroxide ion and acyl transfer potential.

The second-order rate constants for the alkaline hydrolysis of other various acylating compounds are also given in Scheme 1. Cyclization of acyl centers into five-membered rings does not generally have a large effect upon reactivity. γ-Lactones, e.g., γ-butyrolactone **8**, are about 10-fold more reactive than acyclic "*trans*" esters, e.g., **6**, an effect which has been well documented.[19] However, no such effect is observed between the relative reactivities of amides, e.g., **5**, and γ-lactams, e.g., **9−11**. As already mentioned, making the carbonyl group of

Scheme 1. Second-Order Rate Constants ($M^{-1} s^{-1}$) for the Alkaline Hydrolysis of Various Acyl Centers in Water at 30 °C

4
k_{OH} = 1.30

5
k_{OH} = 2.30 x 10^{-5}

6
k_{OH} = 0.164

7
k_{OH} = 1.54

8
k_{OH} = 1.31

9
k_{OH} = 1.20 x 10^{-5}

10
k_{OH} = 3.07 x 10^{-6}

11
k_{OH} = 1.30 x 10^{-5}

12
k_{OH} = 0.825

13
k_{OH} = 4.06

14
k_{OH} = 0.252

15
k_{OH} = 0.08

16
k_{OH} = 0.0601

17
k_{OH} = 0.07

18
k_{OH} = 0.027

amides more electrophilic and reducing the basicity of the leaving group amine so that it may be expelled as its anion, as in imides and *N*-acylsulfonamides, dramatically increase reactivity so that these derivatives are 10^5–10^6 more reactive than simple amides; e.g., compare the reactivities of **7** and **4** with that of **5**. Interestingly,. the five-membered cyclic imide **12** shows a reactivity similar to that of the acyclic imide **7**, as does the cyclic *N*-acylsulfonamide, *N*-benzylsaccharin (**13**), compared with the acyclic analogue **4**.

The reactivities, as expressed by the second-order rate constants for alkaline hydrolysis, of the bicyclic *trans-γ*-lactam and *trans-γ*-lactone inhibitors of elastase are also given in Scheme 1. The k_{OH} value for the *trans-γ*-lactone **14** of 0.252 dm^3 mol^{-1} s^{-1} is a similar order of magnitude to that of *γ*-butyrolactone, **8**, which has a k_{OH} of 1.31 dm^3 mol^{-1} s^{-1}, but counterintuitively, the "strained" bicyclic *trans-γ*-lactone is actually 3-fold *less* reactive than the simple *γ*-lactone. There is no rate enhancement due to ring strain. This suggests that the release of ring strain does not lower the activation energy, which is probably because of the rate-limiting

formation of the tetrahedral intermediate so that the ring is still intact in the transition state.

The hydrolysis of the *trans-γ*-lactams, which are *N*-acylsulfonamides, **15**–**17**, could occur with C–N or S–N fission depending on whether nucleophilic attack occurs at the acyl or sulfonyl center, respectively. Ring opening with C–N fission occurs to generate a sulfonamide, as indicated by spectrophotometric titration of the latter, giving a pK_a of 10.85 ± 0.05 for this leaving group. The k_{OH} values for the *trans-γ*-lactams **15**–**17** of 0.07 ± 0.01 dm^3 mol^{-1} s^{-1} can be compared with those of the simple monocyclic *γ*-lactams **9**–**11**. The increased reactivity of ca. 10^4 of the *trans-γ*-lactams over the unsubstituted monocyclic *γ*-lactams can, however, be attributed to the good leaving sulfonamide group rather than any strain effect. The k_{OH} value for the simple monocyclic *N*-acylsulfonamide, benzylsaccharin, **13**, is actually 60-fold greater than those of the analogous *trans-γ*-lactams, **15**–**17**, which in turn are even 20-fold less than that for the acyclic *N*-sulfonamide **4**! This probably arises from the steric retardation brought about by the isopropyl group α to the carbonyl carbon,
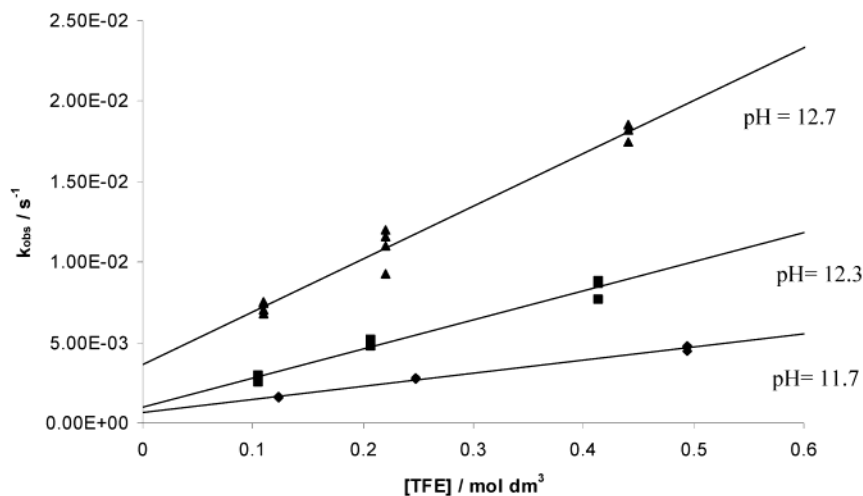
Figure 1. Observed pseudo-first-order rate constants, k_{obs}, for the reaction of the *trans-γ*-lactam **16** in aqueous solutions of trifluoroethanol as a function of alcohol concentration at the pH indicated at 30 °C and $I = 1.0$ M (KCl).

Scheme 2
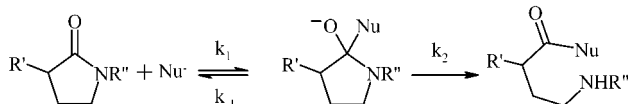
so again, ring strain makes no contribution to the reactivity of **15**.

For comparison, the recently reported[20] inhibitor of elastase, the *β*-lactam derivative **18**, has a second-order rate constant for alkaline hydrolysis, k_{OH}, of 0.027 M^{-1} s^{-1}, which is of the same order as those for the bicyclic *trans-γ*-lactams (Scheme 1).

The two important factors of the acylating power of amides and lactams are the activation of the C=O group toward nucleophilic attack and the ability of the amine leaving group to facilitate C−N bond fission. The alkaline hydrolysis of amides/lactams generally proceeds through the formation of a tetrahedral intermediate, the formation or breakdown of which may be rate-limiting (Scheme 2). The similar reactivity shown by the thermodynamically strained trans-fused *γ*-lactones and *γ*-lactams compared to that of unstrained acyclic analogues indicates that the rate-limiting step is the formation of the tetrahedral intermediate. In the transition state, no C−N bond fission has occurred and the strained ring is still present; hence, the thermodynamic strain energy is not released kinetically.

The rate of the reaction shown in Scheme 2 is

$$v = \frac{k_1 k_2 [\text{Nu}][\text{S}]}{k_{-1} + k_2} \quad (3)$$

The rate of formation k_1 of the tetrahedral intermediate is the rate-limiting step when $k_2 \gg k_{-1}$; i.e., the rate of breakdown of the intermediate is fastest in the forward direction. Further evidence for rate-limiting formation of the tetrahedral intermediate is provided from studies of the alcoholysis of the *trans-γ*-lactams.

Alcoholysis of the *trans-γ*-Lactam 16. The alcoholysis of the *trans-γ*-lactams is a model reaction for the acylation step of the serine enzyme catalyzed reaction. The second-order rate constants, k_{RO^-}, for the reaction of the *γ*-lactam **16** with various alcohols were determined by using aqueous solutions of the alcohol,
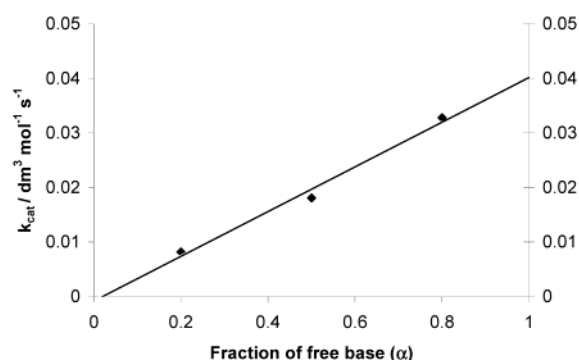
Figure 2. Plot of the catalytic rate constants, k_{cat}, as a function of the fraction of free base, α, in the alcoholysis of the *trans-γ*-lactam **16** in water at 30 °C and $I = 1.0$ M (KCl).

which acted as both buffer and nucleophile. The pseudo-first-order rate constants (k_{obs}) were measured at varying alcohol concentrations at a fixed pH and ionic strength (Figure 1) and are given, in principle, by

$$k_{obs} = k_0[\text{H}_2\text{O}] + k_H[\text{H}^+] + k_{OH}[\text{OH}^-] + k_{RO^-}[\text{RO}^-] + k_{ROH}[\text{ROH}]$$

$$= k_0[\text{H}_2\text{O}] + k_H[\text{H}^+] + k_{OH}[\text{OH}^-] + \{\alpha k_{RO^-} + (1 - \alpha)k_{ROH}\}[\text{ROH}]_{total} \quad (4)$$

where α is the fraction of free base (RO$^-$) present in the buffer.

The slope, k_{cat}, of a plot of k_{obs} against total alcohol concentration, [ROH]$_{total}$, is the sum of the rate constants for the alcoholysis of the *trans-γ*-lactam by the alkoxide ion and the free alcohol, while the intercept is the sum of all the pH-independent, acid-catalyzed, and base-catalyzed hydrolysis reactions. Values of k_{cat} were obtained at different pH's, and when they are plotted against the fraction α of free base (Figure 2), the results show that alcoholysis occurs only through the alkoxide ion because the intercept at α = 0 is indistinguishable from zero. The intercept at α = 1.0 gives the second-order rate constant for the alcoholysis reaction with alkoxide ion acting as the nucleophile, the mechanism of which is also likely to follow that outlined in Scheme 2, with Nu$^-$ = RO$^-$.

Table 1. Second-Order Rate Constants, k_{RO^-}, for the Reaction of Alkoxide Ions with the *trans*-γ-Lactam **16** in Water at 30 °C and $I = 1.0$ M (KCl)

alcohol	pK_a	k_{RO^-}, dm^3 mol^{-1} s^{-1}
trifluoroethanol, CF$_3$CH$_2$OH	12.43	0.0410
hexafluoroisopropanol, (CF$_3$)$_2$CHOH	9.30	0.00102

The rate constants for the reaction of alkoxide ions with the *trans*-γ-lactam, **16**, are given in Table 1. As expected, the rate constants increase with increasing basicity of the alkoxide ion. With only two alcohols, it is not possible to construct a reliable Brønsted plot. Nonetheless, the Brønsted β_{nuc} value can be estimated to be 0.5, which is compatible with rate-limiting formation of the tetrahedral intermediate (Scheme 2) so that for alcoholysis there is also no significant release of ring strain in the transition state.

Inactivation of elastase by the relatively planar trans-fused γ-lactams is a result of attack of the active site serine-195 hydroxyl group on the carbonyl group followed by ring opening to generate an acyl enzyme. Once the enzyme is acylated with the ring-opened *trans*-γ-lactam, X-ray studies show a nonplanar configuration;[12b,c] the pyrrolidine ring of the acylating agent displaces the catalytic histidine from its normal position, which is likely to be a factor in preventing ring closure and regeneration of the enzyme and the *trans*-γ-lactam. In addition, the ring closure reaction is not favored by the reintroduction of strain into the bicyclic system.

k_{OH} as a Therapeutically Useful Index. The magnitude of the second-order rate constant, k_{OH}, for the alkaline hydrolysis of potential enzyme inhibitors is a crude, but useful, indicator to aid judgments about their ability to be effective and therapeutically useful acylating agents. This reactivity index is a guide to both acylating power and hydrolytic and metabolic stability and should prove to be a powerful tool in drug design. *A brief survey[21] of acylating agents of enzymes that are therapeutically useful indicates that k_{OH} for these compounds often falls in the range 0.01–1.0 M^{-1} s^{-1}.*

There is usually a minimum and a maximum chemical reactivity, indicated by k_{OH}, required of inhibitors that act by covalent modification of enzymes. There is an obvious upper limit to this rate constant for hydrolytic stability. For example, a k_{OH} of 10^2 M^{-1} s^{-1} would give a half-life of only 2 h at pH 8. Attempts to increase the acylating power of an inhibitor by modifying its chemical structure face several opposing factors. The stepwise mechanism of acylation means that either formation, k_1, or breakdown, k_2, of the tetrahedral intermediate is rate-limiting (Scheme 2). The rate of a reaction occurring with rate-limiting breakdown can go faster by incorporating a better leaving group. However, there is a limit to the improvement because when $k_2 \gg k_{-1}$ (Scheme 2), the rate of formation of the intermediate, the k_1 step, becomes rate-limiting and making k_2 bigger will have no effect upon the rate. Modifying the structure of the acylating agent to increase k_1 also has limits because eventually this rate constant could become so large that a noncovalent step (diffusion control) could become rate-limiting. Furthermore, as already mentioned, increasing acylation rates by structural modification will almost invariably increase the rate of hydrolysis so that the inhibitor may be converted to an inactive product before it reaches its target enzyme. Of course, structural modification to increase molecular recognition and the rate of acylation of the target enzyme without a concomitant increase in hydrolytic lability can overcome these limitations.

Similarly, structural modification can decrease susceptibility to metabolism while maintaining the rate of acylation of the enzyme. This strategy proved to be successful in the design of more metabolically robust *trans*-γ-lactams by swapping an *n*-propyl for an isopropyl group (as in **15−17**),[12c] which affords increased stability to *trans*-γ-lactam ring opening in blood and plasma.

There is usually also a minimum and maximum chemical reactivity required for an inhibitor to react with the target enzyme, for example, as an effective acylating agent, and a k_{OH} of 0.01 to 1.0 M^{-1} s^{-1} is also a useful guide to this. The second-order rate constant for the rate of inactivation of an enzyme, k_{inact}, is related to the rate constant for hydrolysis of the inhibitor, k_{OH}, through the enzyme rate enhancement factor (EREF)[3,6] given by the ratio $(k_{inact})/k_{OH}$. These ratios are indicators of the enzyme's ability to use its catalytic apparatus to increase the rate of a reaction. EREF values[3] are commonly around 10^6, and so if k_{OH} is significantly less than 0.01 M^{-1} s^{-1}, the enzyme is unlikely to be able to catalyze fast acylation. For example, an enzyme is unlikely to be able to catalyze its acylation by an inhibitor with a k_{OH} value of 10^{-4} M^{-1}s^{-1} with k_i of more than 10^2 M^{-1} s^{-1}. If the enzyme concentration is 0.1 μM, this would give a rate constant of 10^{-5} s^{-1} and a half-life for acylation of 20 h, and the agent is therefore unlikely to be effective. Conversely, if k_{OH} is very high, then it is likely that selectivity between the inhibitor and different enzymes will be reduced.

Experimental Section

Kinetics. Standard UV spectroscopy was carried out on a Cary 1E UV−visible spectrophotometer (Varian, Australia) equipped with a 12-compartment cell block. The instrument was used in double-beam mode, allowing six reaction cells to be followed in a single run. The cell block was thermostated using a Peltier system. Stopped-flow experiments used an SX.18 MV Spectrakinetic monochromator (Applied Photophysics, Leatherhead, England) equipped with an absorbance photomultiplier. The reagent syringes were thermostated with a Grant thermostated water circulator. pH-stat experiments were performed on an ABU 91 autoburet (Radiometer, Copenhagen, Denmark) controlled by a VIT 90 video titrator. The SAM 90 sample station incorporated a machined aluminum E2000 sample block rotor thermostated by a MGW Lauda M3 water circulator. The pH was measured by a pHG200-8 glass pH electrode and a REF200 "red rod" reference electrode (Radiometer). Temperature was monitored by a T101 temperature sensor.

pH measurements were made with either a ϕ40 (Beckman, Fullerton, CA) or 3020 (Jenway, Dunmow, England) pH meter. Electrodes were semimicro Ag/AgCl and calomel (Russel (Fife, Scotland) and Beckman, respectively). A calibration of the pH meter was carried out at 30 °C using pH 7.00 ± 0.01, pH 4.01 ± 0.02, or pH 10.00 ± 0.02 calibration buffers.

ESIMS experiments were carried out on a VG Quattro SQ II (Micromass, Altrincham, England), and NMR experiments were carried out on a 400 MHz instrument (Bruker, Germany).

AnalaR grade reagents and deionized water were used throughout. Sodium hydroxide solutions were titrated prior to use against a 1.00 M ± 0.1% hydrochloric acid volumetric reagent (D. H. Scientific, Huddersfield, England) using phenolphthalein as an indicator. For solution pH values of ≥3 and

≤11, the pH was controlled by the use of ≤0.2 M buffer solutions of formate (pK_a = 3.75), ethanoate (pK_a = 4.72), MES (pK_a = 6.1), MOPS (pK_a = 7.2), TAPS (pK_a = 8.4), CAPSO (pK_a = 9.6), and CAPS (pK_a = 10.4). For general pH work, buffers were prepared by partial neutralization of solutions of their sodium salts to the required pH. For the alcoholysis reactions, buffers were prepared by the addition of 0.25, 0.50, or 0.75 aliquots of 1 M NaOH to solutions of the alcohol. In all experiments, temperatures were maintained at 30 °C and ionic strength was maintained at 1.0 M with Analar grade KCl unless otherwise stated. Reaction concentrations were generally within the range ≥2 × 10^{-5} and ≤2 × 10^{-4} M to ensure pseudo-first-order conditions.

Hydroxide ion concentrations were calculated using pK_w-(H_2O) = 13.883 at 30 °C.

Reactions studied by UV spectrophotometry were usually commenced by injections of acetonitrile or dioxan stock solutions of the substrate (5–50 μL) into the cells containing preincubated buffer (2.5 mL). Final reaction cells contained ≤5% acetonitrile or dioxan, v/v. The pH of the reaction cells was measured before and after each kinetic run at 30 °C; kinetic runs experiencing a change of greater than 0.05 units were rejected. Reactant disappearance or product appearance was followed by monitoring absorbance change maxima for individual compounds. The solubility of compounds was ensured by working within the linear range of absorbance in corresponding Beer–Lambert plots. If required, greater than 1% MeCN v/v was used to aid solubility. Pseudo-first-order rate constants from exponential plots of absorbance against time or gradients of initial slopes were obtained using the Enzfitter package (Elsevier Biosoft, Cambridge, England) or the CaryBio software (Varian). pH–rate profiles were modeled with theoretical equations using the Scientist program (version 2.02, Micromath Software, Ltd.).

Reactions studied by stopped-flow UV spectrophotometry used stock solutions prepared at twice the standard UV concentration in 1 M KCl. Hydroxide solutions, buffer solutions, or solutions of nucleophilic reagents were prepared at twice the required concentration. The substrate solution and the reaction mixture were placed in separate syringes and thermostated at 30 °C before pneumatic injection into the reaction cell. Where applicable, the pH of solutions was measured prior to use. If greater than 1% acetonitrile v/v was required for solubility, then the organic solvent concentration of all solutions used was fixed at the required reaction cell amount. The photomultplier voltage was set to a maximum on deionized water, and absorbance wavelengths common to the standard UV experiments were used. Pseudo-first-order rate constants from exponential plots of absorbance against time were obtained using the supplied fitting software (Applied Photophysics).

For reactions studied by pH-stat, standardized NaOH was delivered to a stirred sample solution (10 mL) held within the thermostated sample station. All reactions were performed under nitrogen to prevent CO_2 absorption. Data were exported to a Windows PC via an RS232 interface and the terminal program (Microsoft Corp.). Conversion into an appropriate format was by means of an Excel (Microsoft Corp.) macro, and results were fitted to first-order equations via the Enzfitter program (Elsevier Software). The titrant used was 0.01–0.1 M NaOH standardized prior to use by means of phenolphthalein titration against 1.00 M HCl (volumetric reagent, D. H. Scientific). Reactions were performed in 1 M KCl and 5% MeCN v/v with a pH set point of 6–7. Concentrations of sample were in the range 1–2 mM with expected titrant-added volumes of up to 1.0 mL.

Materials. The trans-fused bicyclic γ-lactones and γ-lactams were synthesized as previously described.[12] Other compounds were purchased from Aldrich or synthesized using standard literature procedures.

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