β-Substituted β-Phenylpropionyl Chymotrypsins. Structural and Stereochemical Features in Stable Acyl Enzymes

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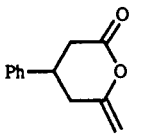
In order to develop effective alternate substrate inhibitors for serine proteases, we have prepared a series of β-substituted β-phenylpropionic acid esters related to some systems known to form stable acyl enzymes with α-chymotrypsin. Some of these compounds were prepared in enantiomerically pure form by asymmetric synthesis. Acyl enzyme species were generated from chymotrypsin by reaction with the active esters, and the progress of deacylation was monitored by the proflavin displacement assay. In some cases, it was possible to distinguish two different deacylation rates that correspond to the two enantiomers. β-Phenylpropionic acyl enzymes with β-substituents that are nonpolar were not especially stable, but a number of the polar derivatives and particularly the acylamino derivatives showed slow rates of deacylation (k_d less than 0.005 min^{-1}), with three systems showing deacylation enantioselectivities in the range of 500–1500. These results are consistent with a model in which additional stabilization of the acyl enzyme and enantioselectivity in the deacylation process derives from an additional hydrogen bond between the acyl enzyme species (as an acceptor) and the enzyme (as a donor). A number of active site residues that might be involved in this hydrogen bond are discussed.

Introduction

One effective strategy for inhibiting serine proteases[1] is to present them with substrates that undergo rapid acylation but are slow to deacylate. Although the inhibition is not permanent, the result is that during the lifetime of the acyl enzyme, the protease is effectively inhibited, as it is unable to act on other substrates.[2] This inhibition strategy, using alternate substrates, is effective to the extent to which the acyl enzyme intermediate is stable. Thus, the development of effective alternate substrate inhibitors requires an understanding of those features that result in the stabilization of the acyl enzyme species.

As will be presented in the Discussion section, there are a number of classes of serine protease inhibitors that are effective alternate substrates inhibitors.[3] In the course of our own investigation on the development of irreversible inhibitors of α-chymotrypsin using aryl-substituted halo enol lactone inactivators, we found that a bromo enol lactone and its protio analogue (1) were effective alternate substrate inhibitors.[4] A subsequent study using enantiomerically pure lactones uncovered a yet more potent reversible inhibitor, the 1-naphthyl α-substituted protio enol valerolactone **2**, and showed that the inhibitory properties of lactones **1** and **2** are caused primarily by the *R* enantiomer of each lactone.[5]



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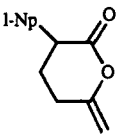

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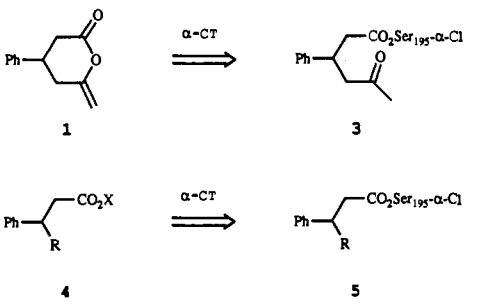
Scheme I



1 **3** **4** **5**

X = *p*-nitrophenyl (*p*NP) or *p*-cyanophenyl (*p*CP)

R = nonpolar: -H (**18**), -$CH_2CH(CH_3)_2$ (**21**), -$CH_2C(CH_3)=CH_2$ (**13**)

polar: -CH_2CN (**32**), -CH_2CO_2Me (**26**), -$OCOCH_3$ (**29**), -CH_2CHO (**38**), -CH_2CONH_2 (**24**), -CH_2CO_2H (**22**)

acylamino: -NHCHO (**45**), -$NHCOCH_3$ (**17**), -$NHCOCF_3$ (**46**), -$NHCOCH_2CH_3$ (**41**), -$NHCOC_6H_5$ (**42**)

The acyl enzymes formed from the *R* enantiomers of **1** and **2** are very stable, showing deacylation rates (k_d) of 0.0029 and 0.000 92 min^{-1}, respectively. Considering the fact that good chymotrypsin substrates such as Bz–Tyr–OEt and Bz–Phe–OEt have deacylation rates of 7900 and 5500 min^{-1}, respectively,[6] these are poor substrates (i.e., effective alternative substrates) indeed. A representation of the acyl enzyme **3**, formed from the β-phenyl lactone **1**, is shown in Scheme I.

In order to maximize the inhibitory potency of this type of alternate substrate inhibitor, we undertook an investigation of structural factors responsible for the stability of the acyl enzyme **3**. Thus, the present study was undertaken in order to determine the effect of the β substituent of **3** on the stability of the acyl enzyme. This was accomplished by replacement of the methyl ketone substituent on the acyl enzyme **3** with a variety of other substituents bearing nonpolar, polar, and acylamino moieties (**5**). The acyl enzymes were prepared from the acyclic *p*-nitrophenyl (*p*NP) or *p*-cyanophenyl (pCP) ester precursors **4**. Although most of the acyl enzyme precursors **4** were used as racemic mixtures, the deacylation enan-

(1) For general references on the medical importance of protease inhibitors see: (a) Rich, D. H. In *Comprehensive Medicinal Chemistry. The Rational Design, Mechanistic Study and Therapeutic Applications of Chemical Compounds*; Hansch, C., Sammes, P. G., Taylor, J. B., Eds.; Pergamon: New York, 1990. (b) *Design of Enzyme Inhibitors as Drugs*; Sandler, M., Smith, H. J., Eds.; Oxford University: Oxford, UK, 1989. (c) *Proteases. Potential Role in Health and Disease*; Hörl, W. H., Heidland, A., Eds.; Plenum: New York, 1984.

(2) See, for example: (a) Krantz, A.; Spencer, R. W.; Tam, T. F.; Liak, T. J.; Copp, L. J.; Thomas, E. M.; Rafferty, S. P. *J. Med. Chem.* **1990**, *33*, 464–479. (b) Tanizawa, K.; McLaren, A. B.; Lawson, W. B.; Kanaoka, Y. *Chem. Pharm. Bull.* **1986**, *34*(2), 913–916. (c) Miyano, M.; Deason, J. R.; Nakao, A.; Stealey, M. A.; Villamil, C. I.; Sohn, D. D.; Mueller, R. A. *J. Med. Chem.* **1988**, *31*, 1052–1061.

(3) Fisher, G. *Nat. Prod. Rep.* **1988**, 465–495.

(4) Sofia, M. J.; Katzenellenbogen, J. A. *J. Med. Chem.* **1986**, *29*, 230–238.

(5) Baek, D.-J.; Reed, P. E.; Daniels, S. B.; Katzenellenbogen, J. A. *Biochemistry* **1990**, *29*, 4305–4311.

(6) Fersht, A. *Enzyme Structure and Mechanism*; Freeman: New York, 1985; p 206.

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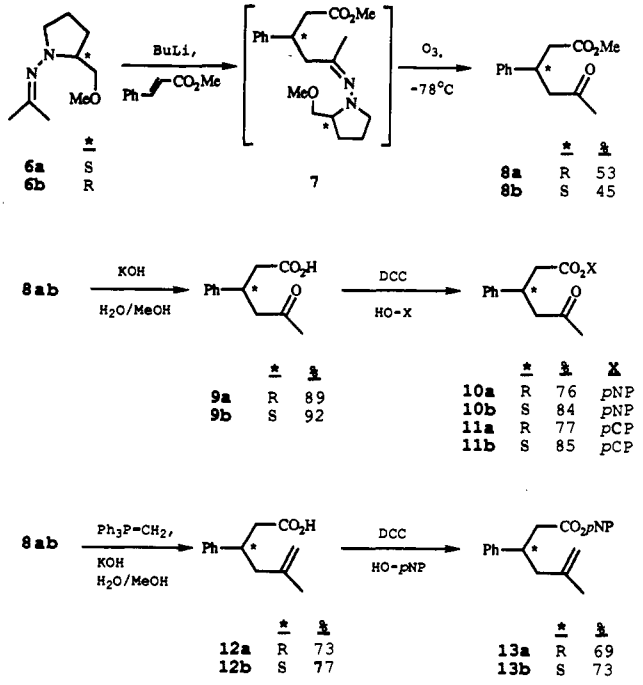
Scheme II



tioselectivities were generally high enough so that two deacylation rates, corresponding to the deacylation of each enantiomer, could be measured for each racemic acyl enzyme precursor. We found a number of β-substituted β-phenylpropionic acid systems that have comparably slow and in some cases higher enantioselective deacylation rates than those of the two systems we studied earlier.

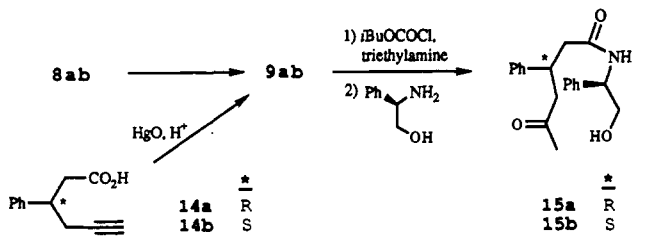
Results

Chemical Synthesis. The structures of the β-substituted phenylpropanoic esters that were prepared and studied in this investigation and their classification are shown below in Scheme I.

Enantiomerically Pure Esters. One enantiomerically pure ester was prepared for each type of β-substituent: nonpolar, polar, and acylamino. An asymmetric synthesis was used to prepare both enantiomers of the polar methylcarbonyl esters **10** and **11** and the nonpolar alkenoic ester **13** (Scheme II). Enders has prepared the keto ester **8a** with >99% ee (estimated by chiral shift reagent NMR) by use of the chiral auxiliary (*S*)-1-amino-2-(methoxymethyl)pyrrolidine (SAMP).[7] SAMP has been shown to react with a very high degree of asymmetric induction via its lithiated hydrazone derivative, a chiral enolate synthon.[8] By the use of methods developed by Enders, SAMP and RAMP (the *R* antipode of SAMP) were used to prepare **8a** and **8b**, as illustrated in Scheme II.

The SAMP/RAMP hydrazones of acetone, **6ab**, were prepared according to the literature procedure.[9] The resulting anion[8a] was reacted with methyl cinnamate to form the hydrazone alkylation adduct **7**. The physical properties of the adduct **7** were such that it was difficult to purify this diastereomeric intermediate by chromatographic methods. Instead, the crude adduct was subjected to conditions for oxidative removal of the chiral auxiliary to give, after chromatography, the desired keto ester **8**. SAMP removal by ozonolysis was found to give superior yields of **8** than an alternate method of SAMP removal employing methyl iodide in pentane/$HCl_{(aq)}$. Ester hydrolysis in aqueous KOH furnished the keto acid enantiomers **9ab** that were condensed with *p*-nitrophenol to give the esters **10ab** and with *p*-cyanophenol to give the esters **11ab**.

Scheme III



The alkenoic esters **13ab** were obtained by olefination of the keto esters **8ab** followed by hydrolysis to give the alkenoic acids **12ab**. Esterification of **12ab** with *p*-nitrophenol provided the desired alkenoic esters **13ab**.

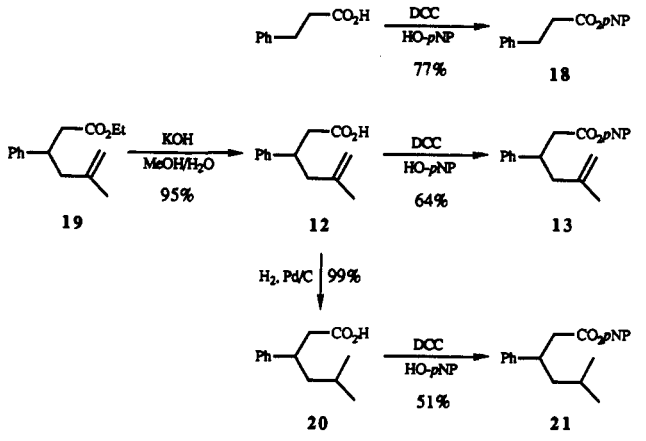
In order to accurately determine the enantiomeric purity of the esters obtained by this route, diastereomeric (*R*)-phenylglycinol amide derivatives of each enantiomer of the acid **9** were prepared (Scheme III). Helmchen[10] has shown that such diastereomeric amides show high separation factors in liquid chromatography. The amides **15ab** have a separation factor, α, of 2.76, and HPLC analysis of **15a** and **15b** show de's of >99% and >93%, respectively, showing that the esters **10**, **11**, and **13** are of high enantiomeric purity.

The assignment of an *R* configuration to the SAMP-derived keto ester **8a** was based on analogy to other products derived from lithiated SAMP-hydrazone conjugate additions.[7] This assignment is consistent with the configurational assignment of the diastereomeric phenylglycinol amides **15ab** obtained by using Helmchen's model for predicting the chromatographic elution order of phenylglycinol amides.[11] Definitive evidence for this assignment, outlined in Scheme III, was obtained by conversion of the acetylenic acid **14**, the precursor of the lactone **1**, to the (*R*)-phenylglycinol amide **15** via the keto acid **9**, obtained by hydration of **14** with mercuric oxide. The configuration of the acid enantiomer **14a** has been established unambiguously by an X-ray structure of its corresponding (*R*)-phenylglycinol amide.[12] The amide diastereomer **15a**, obtained from the enol lactone precursor **14a**, coeluted with **15a** obtained from the SAMP-derived **8a**. The *S* enantiomers **8b** and **14b** were likewise correlated. Thus, the esters **10a**, **11a**, and **13a** have configurations equivalent to the more potent *R* enantiomer of the enol lactone inhibitor **1**.

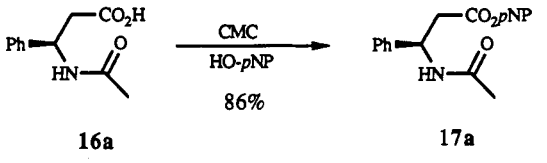
The *R* enantiomer of the acetylamino *p*-nitrophenyl ester, **17a**, was prepared by resolution of the cinchonidine salt of the acetylamino acid **16** by multiple recrystallizations from ethanol. The progress of resolution was monitored by ^1H NMR analysis of the acetylamino methyl group of each of the diastereomers, which gave well separated signals at ca. 2 ppm. In this way, enantiomerically pure (*R*)-acetylamino acid **16a** (ee = 97%) was recovered

(7) Enders. D.; Papadopoulas, K. *Tetrahedron Lett.* **1983**, *24*(*45*), 4967–4970.
(8) (a) Enders, D. In *Asymmetric Synthesis*; Morrison, J. D., Ed.; Academic Press: New York, 1984; Vol. 3; pp 275–339. (b) Enders, D.; Kipphardt, H.; Fey, P. In *Organic Synthesis*; Vedejs, E., Ed.; Wiley: New York, 1987; Vol. 65; pp 183–201.
(9) Enders, D.; Eiechenaur, H.; Pieter, R. *Chem. Ber.* **1979**, *112*, 3703–3714.
(10) Helmchen, G.; Nill, G.; Flockerzi, D.; Yourseff, M. S. K. *Angew. Chem., Int. Ed. Engl.* **1979**, *18*, 63–65.
(11) Helmchen, G.; Ott, R.; Sauber, K. *Tetrahedron Lett.* **1972**, *37*, 3873–3878.
(12) Baek, D.-J.; Daniels, S. B.; Reed, P. E.; Katzenellenbogen, J. A. *J. Org. Chem.* **1989**, *29*, 4305–4311.

Scheme IV



from the salt after eight recrystallizations. The configurational assignment of **16a** is based upon comparison of optical rotation to the literature value.[13] Conversion of the acid to the *p*-nitrophenyl ester 17a was effected by the water soluble diimide 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (CMC).

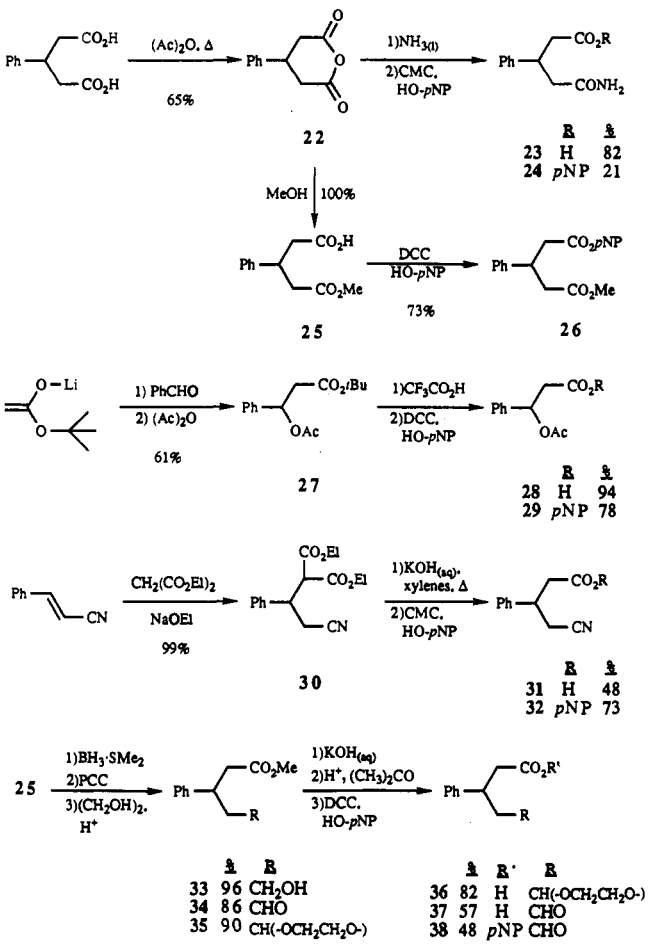


Nonpolar Esters. The synthesis of racemic *p*-nitrophenyl esters bearing nonpolar β-substituents is shown in Scheme IV. For comparative purposes, the unsubstituted ester **18** was prepared from hydrocinnamic acid and *p*-nitrophenol by using the coupling agent dicyclohexyl carbodiimide (DCC). The alkenoic ester **19**, prepared by the addition of methallyl zinc bromide to diethyl benzalmalonate followed by decarboxylation of the diester addition product according to the method of Stella et al.,[14] was saponified with aqueous KOH to give the isobutenyl-substituted acid **12** and then hydrogenated over a palladium catalyst to give the isobutyl-substituted acid **20**. Both acids were subjected to DCC-mediated coupling with *p*-nitrophenol, providing the isobutenyl ester **13** and the isobutyl ester **21**.

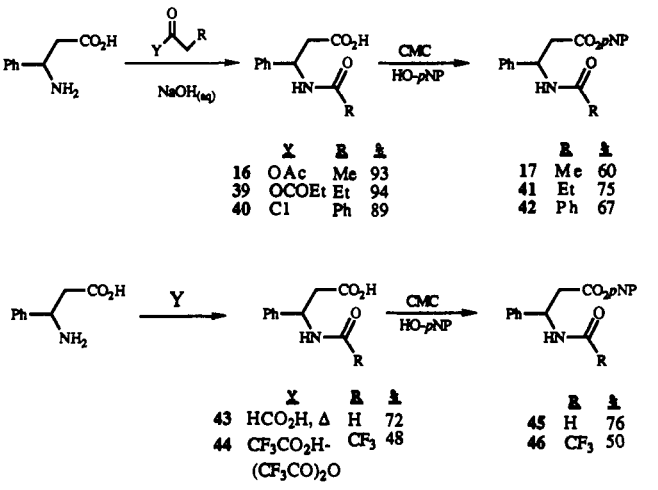
Polar Esters. The synthesis of racemic *p*-nitrophenyl esters bearing polar β-substituents is shown in Scheme V. 3-Phenylglutaric acid was dehydrated in refluxing acetic anhydride to give 3-phenylglutaric anhydride (**22**). The carboxamide ester **24** was prepared by aminolysis of the anhydride to give the acid **23**, followed by esterification using the diimide coupling reagent CMC. The low yield of the esterification step was caused by a major side reaction that formed β-phenylglutarimide. The carboxymethyl derivative **26** was prepared similarly, by methanolysis of the anhydride **22** to give the half-ester **25**, followed by esterification using DCC.

The acetyloxy ester derivative **29** was prepared by trapping the adduct formed from the addition of the lithium enolate of *tert*-butyl acetate to benzaldehyde with acetic anhydride to give the ester **27**. The *tert*-butyl ester was then cleaved with trifluoroacetic acid and replaced with a *p*-nitrophenyl ester by the use of DCC.

Scheme V



Scheme VI



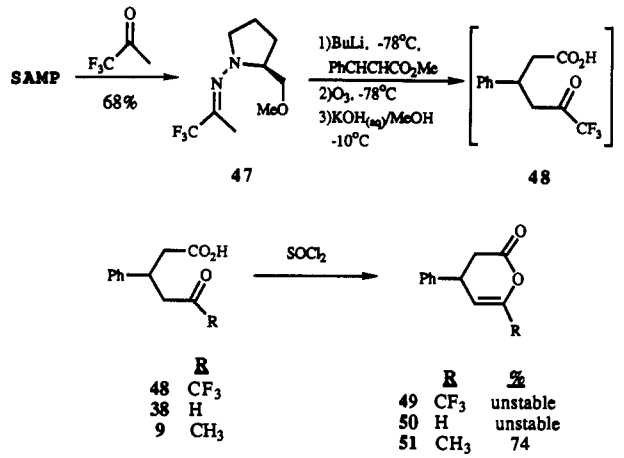
The cyano ester derivative **32** was prepared by CMC-mediated coupling of *p*-nitrophenol to the cyano acid **31**, which in turn was prepared by the addition of sodium diethyl malonate to cinnamonitrile followed by saponification and decarboxylation.

The carboxaldehyde derivative **38** was unstable and could only be isolated as an impure mixture, containing >80% product by NMR, that was stored below 0 °C in an acetonitrile solution. The carboxylic acid group of the half-ester **25** underwent reduction with borane·methyl sulfide complex followed by oxidation with pyridinium chlorochromate (PCC) to give the aldehyde **34** that could be stored in a freezer for several weeks without decom-

(13) Cohen, S. G.; Weinstein, S. Y. *J. Am. Chem. Soc.* **1964**, *86*, 725–728.

(14) Stella, L.; Raynier, B.; Surzur, J. M. *Tetrahedron* **1981**, *37*(*16*), 2843–2854.

Scheme VII



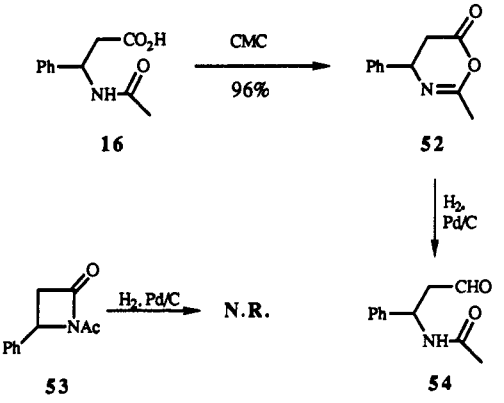
position. Hydrolysis of the methyl ester of **34** could only be achieved after protection of the aldehyde in the form of its dioxolane acetal **35**. Methyl ester hydrolysis then proceeded with aqueous KOH and was followed by deprotection to furnish the acid **37**, which gave the unstable ester **38** upon condensation with *p*-nitrophenol.

β-Acylamino-Substituted Esters. The synthesis of racemic *p*-nitrophenyl esters bearing acylamino β-substituents is shown in Scheme VI. The acetylamino, propionylamino, and benzoylamino acids **16**, **39**, and **40** were prepared by acylation of 3-amino-3-phenylpropanoic acid under Shotten-Baumann conditions with use of acetic anhydride, propanoic anhydride, and benzoyl chloride, respectively. Esterification with CMC proceeded to give the corresponding *p*-nitrophenyl esters **17**, **41**, and **42**. The formylamino acid **43** and the (trifluoroacetyl)amino acid **44** were formed by treatment of 3-amino-3-phenylpropanoic acid with hot formic acid and trifluoroacetic anhydride/trifluoroacetic acid, respectively. The target esters **45** and **46** were then obtained by CMC-mediated coupling of **43** and **44** with *p*-nitrophenol.

Cyclic Acyl Enzyme Precursors. Several attempts were made to prepare novel acyl enzymes from cyclic, dehydrated substrates rather than acyclic ester substrates. Most of these compounds, however, turned out to be too unstable for use as α-chymotrypsin substrates. An exception is the cyclic anhydride **22** (Scheme V), which acylated chymotrypsin readily in spite of its rapid rate of hydrolysis in buffer (0.4 min^{-1}), and so could be used to form an acyl enzyme possessing a carboxylate functional group.

The trifluoromethyl enol lactone **49** (Scheme VII) could potentially give the trifluoromethyl analogue of the methylcarbonyl acyl enzyme **3**. We attempted to prepare this lactone from its unstable keto acid precursor **48**, prepared from a SAMP hydrazone in the same way used to prepare the methylcarbonyl acid **9**. The trifluoromethyl SAMP hydrazone **47** would not form from SAMP and trifluoroacetone by using standard procedures, but could be formed in 68% yield when the condensation reaction was carried out at 90 °C in a sealed vial. Quenching experiments with D_2O showed that the hydrazone could be quantitatively lithiated with butyllithium at −78 °C. The intermediates leading to the formation of the acid **48**, and even the acid **48** itself, decomposed readily, so these intermediates, characterized by NMR and GCMS, were carried on immediately to the desired trifluoromethyl lactone **49**. Unfortunately, the same chemical instability was also observed in the enol lactone **49**, so it was not suitable for use as an enzyme substrate. The protio lactone analogue **50**,

Scheme VIII



prepared from the carboxaldehyde acid **37**, was also not isolable in pure form, although the methyl lactone analogue **51**, prepared from the keto acid **9**, proved to be stable.

The cyclic oxazinone **52** (Scheme VIII) is a more attractive precursor to an acetylamino-substituted acyl enzyme than the acyclic *p*-nitrophenyl ester **17**. The oxazinone could be prepared as a clear colorless oil by warming the acid **16** in the presence of CMC, but it decomposed readily to an insoluble white solid upon standing for several hours at room temperature. The decomposition is apparently due to an aldol-type self-condensation characteristic of oxazinones and oxazolinones with carbon–nitrogen double bonds not stabilized by conjugation.[15] The instability of the oxazinone **52**, along with its extremely rapid rate of hydrolysis in phosphate buffer (>0.5 min^{-1}), precluded its use as an enzyme substrate.

Dehydrative cyclization of the (acetylamino)propionic acid **16** could in principle produce 1-acetyl-4-phenylazetidin-2-one (**53**) instead of the isomeric oxazinone **52**. Although infrared and NMR data are insufficient to accurately distinguish between these isomers,[16] azetidin-2-ones are resistant to catalytic hydrogenation, while oxazin-6-ones undergo hydrogenation to yield 3-acylamino aldehydes.[16,17] Compound **52** produced the aldehyde **54** when hydrogenated over palladium on carbon, supporting our structural assignment.

Biochemical Studies

The interaction between a serine protease and its substrate is described by eq 1, where E = enzyme, S = substrate, E·S = Michaelis complex, E∼S = acyl enzyme intermediate, and P = product. The reaction is characterized by a binding constant K_S, an acylation rate constant k_a, and a deacylation rate constant k_d.

$$E + S \overset{K_S}{\rightleftharpoons} E{\cdot}S \xrightarrow{k_a} E{\sim}S \xrightarrow{k_d} E + P \qquad (1)$$

Determination of the Acylation Rate Constants for the Keto Esters 11ab. The methyl ketone bearing acyl enzyme **3** can be prepared from either the lactone **1** or the keto esters **10** or **11**. The acylation rate constants k_a and K_S for the cyclic lactone **1** are known.[4] In order to compare acyl enzyme formation from the cyclic lactone substrate **1** with acyl enzyme formation from an acyclic ester substrate **10** or **11**, we obtained the acylation rate constants for one of the ester substrates. The *p*-nitrophenyl esters **10ab** proved to be too reactive to follow the acylation

(15) Drey, C. N. C.; Ridge, R. J. *J. Chem. Soc., Perkin Trans.* 1 **1979**, 378–380.
(16) Carter, H. E. *Org. React.* **1946**, *3*, 198–239.
(17) Drey, C. N. C.; Ridge, R. J. *J. Chem. Soc., Chem. Commun.* **1975**, 948–949.

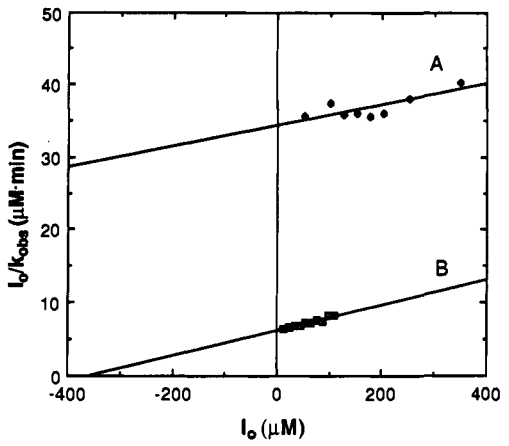


Figure 1. Competitive substrate assay of α-chymotrypsin with the *R* and *S* enantiomers of the acyclic methylcarbonyl *p*-cyanophenyl esters **11a** (A) and 11b (B). Enzyme acylation was monitored continuously in the presence of the chromogenic substrate succinyl-L-Ala-L-Ala-L-Pro-L-Phe *p*NA. A first-order decrease in chromogenic substrate turnover, k_{obs}, was measured for each initial ester concentration, I_o, used. A plot of I_o versus I_o/k_{obs}, shown here, gives a straight line with a slope of $1/k_a$ and an *x*-intercept of $-K_S(1 + S_o/K_M)$.

Table I. Comparison of the Formation of the Acyl Enzyme **3** from the Precursors 1 and 11

acyl enzyme precursor	k_a, min^{-1}	K_S, μM	k_a/K_S, M^{-1} s^{-1} × 10^{-3}
(*R*)-1[a]	35 ± 10	51 ± 14	12
(*S*)-1[a]	17 ± 2	8 ± 1	38
11a (*R*)	73 ± 23	630 ± 200	2
11b (*S*)	58 ± 6	91 ± 9	11

[a] Data for the lactone 1 was reproduced from ref 4.

transient (burst) without the use of stopped-flow methods, so we studied the less reactive *p*-cyanophenyl esters **11ab**. These esters could be used together with the chromogenic substrate succinyl-L-alanyl-L-alanyl-L-prolyl-L-phenylalanine *p*-nitroanilide in a competitive substrate assay,[18] where the competitively reduced burst rates led to more conveniently measured progress curves. The results are plotted in Figure 1, and the values obtained for the binding and acylation constants K_S and k_a are listed in Table I along with the values reported for the lactone 1.[4] The acylation rates for the esters **11ab** were somewhat faster than that for the lactones (*R*)- and (*S*)-1, but the binding was weaker. Thus, the overall second-order rate constant for acylation (k_a/K_S) were the same within a reasonably small factor, for both systems.

Determination of the Deacylation Rate Constants (k_d). Proflavin Displacement Assays. The stabilities of the acyl enzymes derived from the *p*-nitrophenyl substrates were determined by the proflavin displacement assay.[19] A representative progress curve is shown in Figure 2C. Initially, the enzyme–dye spectrum is destroyed during the rapid acylation phase. Subsequently, a slow first-order return of absorbance intensity back to its equilibrium value occurs at a rate equal to k_d.

When this assay is carried out with a racemic substrate that forms two epimeric acyl enzymes deacylating at different rates, a biphasic progress curve is observed (Figure 2A). If the two deacylation rates differ appreciably, both the fast deacylation rate (k_d(fast), Figure 2C) and the slower deacylation rate (k_d(slow), Figure 2B) can be

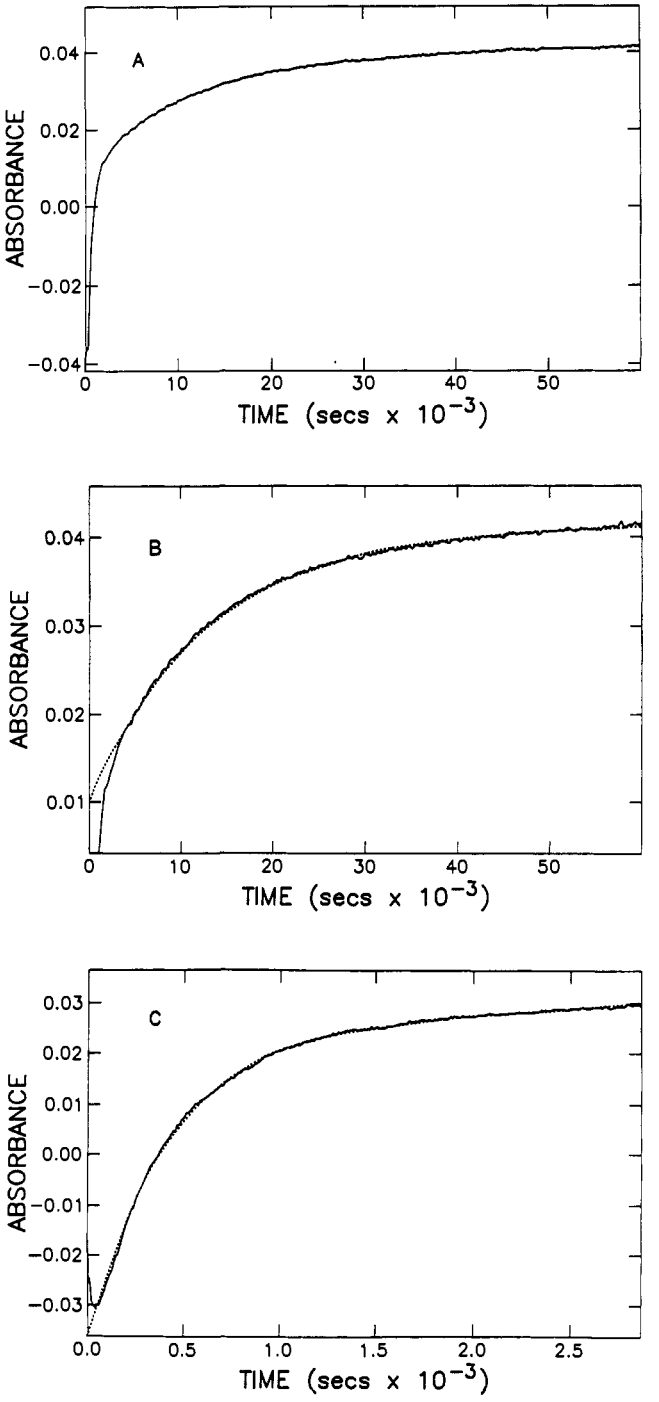
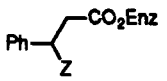


Figure 2. Proflavin displacement assay of the racemic carboxamide *p*-nitrophenyl ester **24**. The ester (90 μM) was added to a solution of α-chymotrypsin (90 μM) and proflavin (9 μM) dissolved in 1 mL phosphate buffer (0.1 M, pH 7.2).[19] Panel A: A time-based measurement at 466 nm gives a biphasic progress curve, characterized by a large first-order rate constant, k_d(fast), resulting from deacylation of the least stable acyl enzyme epimer, and a small first order rate constant, k_d(slow), resulting from deacylation of the more stable acyl enzyme epimer. The approximate deacylation enantioselectivity, k_d(fast)/k_d(slow), is 31 for this substrate. Panel B: *Y*-Scale expansion of Figure 2 panel A showing the deacylation of the more stable acyl enzyme species formed from the *R* enantiomer of the carboxamide ester **24**. The dotted line shows the progress curve calculated with the rate constant k_d(slow). Panel C: *Y*-Scale and *x*-scale expansion of Figure 2 panel A showing the deacylation of the less stable acyl enzyme species formed from the *S* enantiomer of the carboxamide ester **24**. The dotted line shows the progress curve calculated with the rate constant k_d(fast).

(18) Main, A. R. In *Essays in Toxicology*; Hays, W. J., Ed.; Academic Press: New York, 1973; Vol. 4; pp 59–105.
(19) Reference 4, method A.

Table II. k_d's of Modified Acyl Enzymes



precursor	k_d(fast), min^{-1}	k_d(slow), min^{-1}	k_d(fast)/k_d(slow)
nonpolar			
(1) H (18)	15[a]	15[a]	–
(2) $CH_2C(CH_3)=CH_2$ (13)	0.162[b] 0.172 (S)	0.162[b] 0.067 (R)	3[c]
(3) $CH_2CH(CH_3)_2$ (21)	0.096[b]	0.096[b]	–
polar			
(4) CH_2CN (32)	9	0.035	257
(5) CH_2CO_2Me (26)	2.34	0.048	49
(6) OAc (29)	0.067	0.01	7
(7) CH_2COCH_3 (10)	0.15	0.0036	42
(1)	0.17[d] (S)	0.0029[d] (R)	59[c]
(8) CH_2CHO (38)	0.9	0.005	180
(9) CH_2CONH_2 (24)	0.154	0.005	31
(10) $CH_2CO_2^-$ (22)	0.204[b]	0.204[b]	–
acylamino			
(11) NHCHO (45)	3.58	0.039	92
(12) NHCOMe (17)	1.57	0.02 0.0023 (R)	785
(13) NHCOEt (41)	4.35	0.0029	1500
(14) NHCOPh (42)	30	0.025	1200
(15) $NHCOCF_3$ (46)	0.0063[b]	0.0063[b]	–

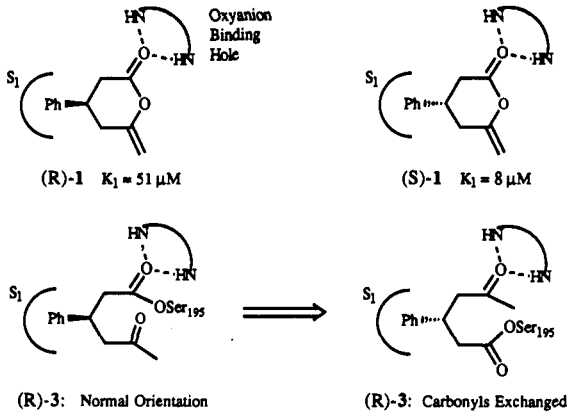
[a] This acyl enzyme is achiral and therefore shows only one k_d. [b] Only one deacylation rate could be measured for this acyl enzyme. [c] This value represents $k_d(S)/k_d(R)$. [d] Data reproduced from ref 4.

measured, and the values obtained can be used as estimates of the deacylation rates of the rapidly deacylating enantiomer and the slowly deacylating enantiomer. The accuracy of these estimates can be very good when the deacylation enantioselectivity is high. The deacylation rates thus obtained for the acyl enzymes derived from the racemic enzyme substrates prepared for this study are presented in Table II. The ratio k_d(fast)/k_d(slow) provides an approximate measure of deacylation enantioselectivity. Data obtained from homochiral substrates are also indicated in Table II.

Discussion

The acyl enzymes formed from alternate substrate inhibitors of serine protease can be stabilized in a number of ways. The acyl carbonyl can be stabilized by inductive or resonance effects of the inhibitors in acyl enzymes formed from azapeptides,[20] certain stabilized anhydrides such as isatoic anhydride,[21] and benzoxazinones.[2a] Additionally, acyl enzyme hydrolysis is often decelerated by factors that hinder the approach of water to the acyl carbonyl. This may be steric repulsion with acyl enzymes formed from pivaloyl esters[2c] and 5-substituted benzoxazinones,[22] and electrostatic interactions between the inhibitor and the enzyme as with 3-benzyl-6-chloropyrone, that generates a carboxylate functional group that hydrogen bonds to His-57, preventing access of water to the acyl carbonyl.[23] In other stable acyl enzymes, the acyl carbonyl twists out of its normal orientation where it is activated by hydrogen bonding in the oxyanion binding hole. The causes of this acyl enzyme twist have been ascribed to both electrostatic interactions (with porcine pancreatic elastase and a guanidino-substituted isocoumarin),[24] and hydrophobic interactions (with indoleacryloyl chymotrypsin).[25]

Scheme IX



Although an X-ray structure of the acyl chymotrypsins we have prepared in this study is unavailable, certain assumptions about its structure can be made. The protio enol lactone 1 resembles the aromatic amino acids found at the P_1 site of preferred chymotrypsin substrates. Therefore, in the methylcarbonyl acyl enzyme 3, the β-phenyl group resides in the primary specificity pocket (the S_1 subsite), as is the case with the aromatic group of indoleacryloyl chymotrypsin. The focus of this study is to determine the effect of the β-alkyl substituent on the stability of the acyl enzyme intermediate 3. This moiety could force the inhibitor to twist the acyl carbonyl out of alignment in the oxyanion binding hole, due to geometric constraints or to hydrophobic or electrostatic interactions. Alternately, the β-substituent could simply block the access of water to the acyl carbonyl by steric or electronic interactions.

Recent studies have shown that the *R* enantiomers of 1 and 2 are responsible for forming stable acyl enzymes, but it is the *S* enantiomers that bind more readily to chymotrypsin.[5] One interesting possibility, shown in Scheme IX, is that the subsite interactions of the conformationally liberated (i.e., noncyclic) acyl enzyme derived from the less-well-bound *R* enantiomers might readjust in such a way that they resemble the more favorable binding mode of the *S* enantiomer by exchanging the remote carbonyl of the β-substituent with the acyl carbonyl in the oxyanion binding hole. If this "carbonyl exchange mechanism" were operative, the remote carbonyl could bind more tightly to the oxyanion binding hole by converting to its hydrated form in order to resemble the tetrahedral intermediate this binding region is designed to stabilize.[26]

The Use of Racemic Ester Precursors to Study Acyl Enzyme Stability. The acyl enzymes we have

(20) Gupton, F. B.; Carroll, D. L.; Tuhy, P. M.; Kam, C.-M.; Powers, J. C. *J. Biol. Chem.* **1984**, *259*(7), 4279–4287.
(21) Moorman, A. R.; Abeles, R. H. *J. Am. Chem. Soc.* **1982**, *104*, 6785–6786.
(22) Krantz, A.; Spencer, R. W.; Tam, T. F.; Thomas, E.; Copp, L. J. *J. Med. Chem.* **1987**, *30*, 591–597.
(23) Ringe, D.; Mottonen, J. M.; Gelb, M. H.; Abeles, R. H. *Biochemistry* **1986**, *25*, 5633–5638.
(24) Powers, J. C.; Oleksyszyn, J.; Narasimhan, S. L.; Kam, C.-M.; Radhakrishnan, R.; Meyer, E. F. *Biochemistry* **1990**, *29*, 3108–3118.
(25) Henderson, R. *J. Mol. Biol.* **1970**, *54*, 341–354.
(26) In unpublished modeling studies (Bemis, G. W.; Katzenellenbogen, J. A., University of Illinois, unpublished) we have found that there is a low energy pathway to the carbonyl exchanged structure for the acyl enzyme derived from (*R*)-1 but not for (*S*)-2. Although we have no direct evidence for such a carbonyl exchange process, it is energetically feasible and its enantioselectivity matches the pattern of enantioselectivity for the deacylation rate we have observed for (*R*)- and (*S*)-1.

studied were almost all prepared from active ester precursors, most of which were racemic compounds. These substrates form acyl enzymes readily from both enantiomers, as shown by the rate constants obtained for the acylation of chymotrypsin by the keto methyl esters **11a** and **11b** (Table I).

Studies on the enantiomerically pure substrates provide good evidence that the most stable acyl enzyme is derived from the *R* enantiomer of the substrate (Table II). The *R* acyl enzyme epimer is the most stable epimer for the nonpolar isobutenyl-substituted acyl enzyme (entry 2), the polar keto methyl substituted acyl enzyme (entry 7), and the acetylamino-substituted acyl enzyme (entry 12). Thus it is likely that α-chymotrypsin shows the same enantioselectivity toward the structurally similar racemic analogues of the enantiomerically pure substrates.

The rate constants k_d(fast) and k_d(slow) obtained from the racemic substrates seem to give reasonable approximations of the rate constants for each individual enantiomer, $k_d(S)$ and $k_d(R)$, when the deacylation enantioselectivity is high enough to give distinctly biphasic progress curves for deacylation. This is seen by a comparison of the deacylation rates measured for the methylcarbonyl acyl enzyme (entry 7) and the acetylamino acyl enzyme (entry 12) in their racemic and enantiomerically pure forms. The approximations obtained from the racemic structures tend to underestimate $k_d(S)$ and overestimate $k_d(R)$, but these errors are small considering that the range of deacylation rates measured encompasses many orders of magnitude.

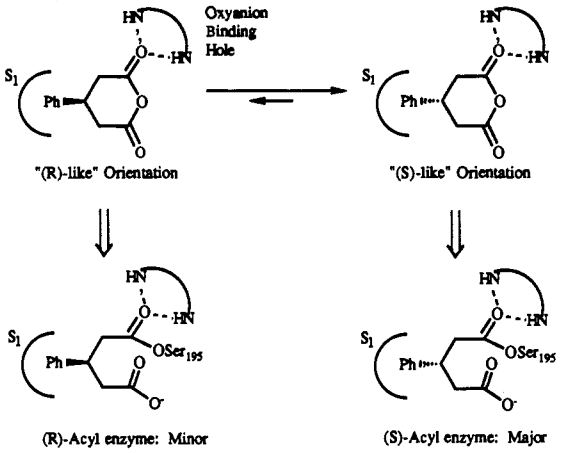
Structure–Activity Relationships between the β-Substituent and the Stability of the Acyl Enzyme Intermediate. For the most part, the acyl enzymes that are most stable also show the greatest deacylation enantioselectivities (Table II). This phenomenon has also been observed to occur with α- and β-aryl-substituted protio enol valerolactone chymotrypsin substrates.[5]

(a) Nonpolar Acyl Enzymes. The unsubstituted hydrocinnamoyl acyl enzyme is turned over very rapidly (entry 1), but the isobutyl- and isobutenyl-substituted acyl enzymes (entries 2 and 3) are about 100 times more stable. The deacylation enantioselectivity for these substrates is very small, so that only one composite deacylation rate is observed. The enhanced stability of entries 2 and 3 may arise from a steric or hydrophobic interaction that twists the acyl carbonyl out of its binding site in the oxyanion binding hole, or one in which the alkyl moiety blocks the access of water to the acyl carbonyl. However, these effects can account for only about half of the stabilizing effect of certain of the polar β-substituents.

(b) Polar Acyl Enzymes. Deacylation rates measured for the acyl enzymes bearing polar β-substituents show that electronic factors can impart even greater stabilization to the acyl enzyme. In general, the trends observed in the stability of the acyl enzymes (nitrile, ester < ketone, aldehyde, carboxamide) roughly parallel the ability of the remote heteroatom of the β-substituent to form a hydrogen bond with the enzyme, suggesting that such an interaction may be involved in stabilizing the acyl enzyme.

The carboxylate derivative, however, does not show a stable acyl enzyme (entry 10). These results appear surprising considering that it contains the most effective hydrogen bond acceptor in this class and because a structurally related carboxylate-containing acyl chymotrypsin forms a very stable acyl enzyme.[23] The data obtained for the carboxylate derivative (entry 10) also appears anomalous for a polar substrate because only one deacylation rate was observed. One must note, however, that the acyl enzyme involved is unique in that it was

Scheme X



prepared from a cyclic precursor, 3-phenylglutaric anhydride **22**, that is an achiral meso compound. Consequently, the chirality of the acyl enzyme formed from the anhydride will depend on which anhydride carbonyl is acylated by Ser-193, (i.e., the relative orientation of the anhydride in the Michaelis complex shown in Scheme X). Since chymotrypsin prefers to bind to the *S* enantiomer of the isosteric lactone 1 (Table I),[5] it is reasonable to expect that the anhydride will bind mostly in the "*S*-like" orientation shown in Scheme X. As a result, the (*S*)-acyl enzyme will form predominantly, so only the more rapid deacylation rate corresponding to this less stable acyl enzyme will be observed.

(c) Acylamino-Substituted Acyl Enzymes. As a class, the acyl enzymes bearing acylamino β-substituents give the most stable (*R*)-acyl enzymes. Due to amide resonance, these substituents are also the most effective hydrogen bond acceptors. The stability of the acetylamino derivative (entry 12) exceeds that of the parent keto methyl compound (entry 7). Acyl enzyme stability decreases as the acylamino group becomes larger (entries 13 and 14), indicating that the acylamino group binds to the enzyme in a region that is sensitive to the size of the terminal functional group, with the optimal size being that of an acetylamino or the isosteric keto methyl group. The formylamino acyl enzyme (entry 11) is smaller than the acetylamino derivative (entry 12) but deacylates more readily. The results obtained for the (trifluoroacetyl)amino derivative (entry 15) are hard to interpret because only one deacylation rate was observed. Greater than 80% of enzyme activity was recovered after a period of several half-lives at the measured k_d, indicating that the measured k_d represents the most stable acyl enzyme. The measured k_d could represent a composite of both $k_d(S)$ and $k_d(R)$, or the (*S*)-acyl enzyme could deacylate so rapidly that k_d(fast) could not be observed.

Proposals for the Structure of the Stable (*R*)-Acyl Enzymes. In the absence of crystallographic data, kinetic or computer modeling data must be used to propose a structure for the stable acyl enzymes prepared in this study. Although one must be careful when proposing a common structure based on data obtained from different acyl enzymes, because each acyl enzyme could adopt a very different structure,[27] very minor perturbations were made in the structure of the acyl enzymes used in this study, and the results are consistent enough to indicate that these acyl enzymes share many common structural features.

(27) Bode, W.; Meyer, E.; Powers, J. C. *Biochemistry* **1989**, *28*(5), 1951–1963.

Two factors appear to be primarily responsible for the stabilization of the acyl enzymes we have studied. First, steric or van der Waals interactions between the enzyme and the β-substituent impart a large measure of stability to the acyl enzyme intermediate. This is seen in the 100-fold difference in the stabilities of the hydrocinnamoyl acyl enzyme (entry 1) compared to the nonpolar isobutenyl-substituted (*R*)-acyl enzyme (entry 2). It is not clear whether the β-substituent causes the inhibitor to adopt a conformation that is unfavorable for hydrolysis, by pulling the acyl carbonyl out of the oxyanion binding hole, or whether it is blocking the access of water to the acyl carbonyl. The latter hypothesis conflicts with ongoing molecular dynamics calculations being carried out on the methylcarbonyl acyl enzyme **3** that show that low energy conformations of **3** have an extended inhibitor conformation, placing the β-substituent (the methylcarbonyl group in the case studied) away from the vicinity of the acyl carbonyl.[28]

The second major factor that stabilizes these acyl enzymes appears to be a hydrogen bond between the enzyme and the carbonyl oxygen of the β-substituent. The enhanced stability of the polar and acylamino acyl enzymes shows that electrostatic interactions are important for stabilization, and the general trend among acyl enzymes bearing functional groups on their β-substituent is that acyl enzyme stability roughly parallels the ability of the functional group to act as a hydrogen bond acceptor. Several residues on chymotrypsin could act as hydrogen bond donors to the remote carbonyl of the inhibitor, with the most likely being the backbone amides of Met-192 or Gly-216, or the hydroxyl of Ser-218.[28] The site of this hydrogen bond is sensitive to the size of the β-substituent and appears to be optimal for β-substituents terminating in acetyl groups such as the methylcarbonyl derivative (entry 7) and the acetylamino derivative (entry 12).

The results obtained in this study do not support an acyl enzyme structure in which the acyl carbonyl exchanges with the remote carbonyl in the oxyanion binding hole. If this mechanism were operative, one would expect the remote carbonyls that hydrate most readily, like the aldehyde, formylamino, and (trifluoroacetyl)amino acyl enzymes, to form more stable acyl enzymes than their acetyl analogues. Instead, the acetylamino and propionylamino derivatives, which are among the least likely to become hydrated, form very stable acyl enzymes.

Conclusion

This study was undertaken in order to determine the stabilizing effect of the β-substitutent of acyl chymotrypsins formed from nonpolar, polar, and acylamino β-substituted β-phenylpropionates. A series of β-substituted β-phenylpropanoic ester substrates of α-chymotrypsin were prepared and the deacylation rates of the acyl chymotrypsins derived from them were measured. Interpretation of the structure–activity relationships seen in this study suggested that the β-substituent imparts stabilization both by steric effects and by forming a hydrogen bond with the enzyme. These results will be useful in designing more potent alternate substrate inhibitors of serine proteases.

Experimental Section

A. Chemical Synthesis. General. Reaction progress was monitored by analytical thin-layer chromatography, performed with 0.25-mm silica gel glass-backed plates with F-254 indicator (Merck), or gas chromatography, performed with a Hewlett-Packard 5790A GC, using a Hewlett-Packard Ultra 1 fused silica capillary column. Visualization of TLC was done by UV light, iodine vapor, or phosphomolybdic acid stain. Flash chromatography[29] was performed by using 15 cm of Woelm 32–63-μm silica gel packing. Column diameter and eluent are indicated parenthetically. All reactions using nonaqueous reagents were run under a dry nitrogen atmosphere with magnetic stirring.

Proton magnetic resonance (^{1}H NMR) spectra were recorded on a Varian XL-200 (200 MHz), a General Electric QE-300 (300 MHz), a Nicolet NT-360 (360 MHz), or a General Electric GN-500 (500 MHz) spectrometer. Field strength is indicated parenthetically. Tetramethylsilane was used as an internal standard and data are presented in the form: δ value of signal (peak multiplicity, integrated number of protons, coupling constant, assignment). Carbon magnetic resonance (^{13}C NMR) were recorded on a General Electric QE-300 (75 MHz) spectrometer. Tetramethylsilane was used as an internal standard and data are presented in the form: δ value of signal (assignment). Fluorine magnetic resonance spectra (^{19}F NMR) were recorded on a Nicolet NT-360 (90 MHz) spectrometer. Fluorotrichloromethane was used as an internal standard. Mass spectral data, unless otherwise indicated, were obtained by using electron-impact ionization on a Varian MAT CH-5 spectrometer (low-resolution data) or a Varian MAT 731 spectrometer (high-resolution data) at 70 eV and are reported in the form: *m/z* (intensity relative to base peak = 100). Fast atom bombardment (FAB) mass spectral data were obtained on a ZAB-SE mass spectrometer. Infrared spectral data were recorded on an IBM IR-32 FTIR spectrophotometer and presented as cm^{-1} for important diagnostic bands. Ultraviolet spectral data were recorded on a Newlett-Packard 8451A diode array single beam spectrophotometer and are reported in the form: λ_{max} (ε). HPLC was performed with a Varian 5060 analytical HPLC. Peak areas were measured by using a Hewlett-Packard 3390A integrator–plotter. Elemental analyses were performed by the Microanalytical Service Laboratory of the University of Illinois School of Chemical Sciences.

Solvents and reagents used were purchased as analytical reagent grade, and the following were further purified by distillation from the indicated drying agent: methylene chloride (P_2O_5), oxalyl chloride (P_2O_5), dimethyl sulfoxide (CaH_2), chloroform (P_2O_5), triethylamine (CaH_2), acetonitrile (CaH_2), and tetrahydrofuran (sodium benzophenone ketyl). Methyl cinnamate was distilled prior to use. Trifluoroacetone, (*S*)-1-amino-2-(methoxymethyl)pyrrolidine (SAMP), (*R*)-1-amino-2-(methoxymethyl)-pyrrolidine (RAMP), (*R*)-(−)-2-phenylglycinol, and 3-amino-3-phenylpropanoic acid were purchased from Aldrich Chemical Co. (−)-Cinchonidine was purchased from Sigma Chemical Co.

(*R*)-Methyl 5-Oxo-3-phenylhexanoate (8a). This procedure follows general methods published by Enders.[7,9] A solution of the hydrazone **6a** (850 mg, 5.0 mmol) dissolved in 5 mL THF was metalated at 0 °C with *n*-butyllithium according to the literature procedure.[8a] The resulting solution was cooled to −78 °C and a solution of methyl cinnamate (4.04 mL dissolved in 2.5 mL of THF, 5.5 mmol) was added dropwise over a 15-min period. Stirring was continued for 6 h at −78 °C, followed by 1 h at −64 °C; 1 h at −46 to −20 °C; and 0.5 h at −10 to 0 °C. The reaction mixture was then poured into a saturated ammonium chloride solution and extracted with ether. The combined ether extracts were then dried (Na_2SO_4) and concentrated to give the crude adduct **7** (1.58 g, 95%), which was used without further purification.

In order to prevent exposure to the carcinogenic SAMP nitrosamine byproduct formed during removal of the chiral auxillary, all manipulations described below were done in a fume hood while wearing gloves and all glassware was cleaned in an HBr/HOAc bath[30] after use. A portion of the hydrazone adduct **7** (1.00 g, 3.01 mmol) was dissolved in 10 mL methylene chloride and cooled to −78 °C under O_2. Ozone was then bubbled through the solution for 15 min, during which time the solution color changed from orange to clear green. The reaction was warmed to room tem-

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(30) Eisenbrand, V. G.; Preussmann, R. *Arzneim. Forsch.* **1970**, *10*, 1513–1517.

perature under O_2 and concentrated to an orange oil (1.21 g). The white solid, **8a** (338 mg, 51%), was isolated by flash chromatography (45 mm, 1:1 ether/hexane): mp 47 °C; NMR (300 MHz, $CDCl_3$) δ 7.29 (m, 2), 7.21 (m, 3), 3.68 (qn, 1, J = 7 Hz), 3.59 (s, 3, OCH_3), 2.83 (d of AB qt, 2, J_1 = 7 Hz, $\Delta\delta$ = 0.04, J_2 = 17 Hz), 2.65 (d of AB qt, 2, J_1 = 8 Hz, $\Delta\delta$ = 0.07, J_2 = 15 Hz, CH_2COCH_3), 2.06 (s, 3, CH_3CO); mass spectrum, m/z 220 (M^+, 26), 189 (34), 160 (100), 147 (42), 145 (46), 131 (31); IR ($CHCl_3$) 3025, 2401, 1724, 1437, 1363, 1020 cm^{-1}. Anal. ($C_{13}H_{16}O_3$) C, H.

(*S*)-Methyl 5-Oxo-3-phenylhexanoate (8b). This compound was prepared from **6b** (1.70 g, 10 mmol) by the same procedure used for **8a**. The NMR spectrum of the product (1.00 g, 45%) is identical with **8a**, mp 47 °C. Anal. ($C_{13}H_{16}O_3$) C, H.

(*R*)-5-Oxo-3-phenylhexanoic Acid (9a). Method A. The ester **8a** (205 mg, 0.93 mmol) was dissolved in a solution, prepared from 4 mL of 5% KOH and 8 mL methanol, and stirred for 45 min. The reaction solution was then poured into 50 mL of 10% sodium bicarbonate and washed with ether. The aqueous phase was then acidified with HCl and extracted with ethyl acetate. The combined ethyl acetate extracts were washed (saturated NH_4Cl), dried ($MgSO_4$), and concentrated to a white solid (170 mg, 89%). Recrystallization from ether provided analytically pure product: mp 93–94 °C; NMR (300 MHz, $CDCl_3$) δ 7.27 (m, 5, C_6H_5), 3.68 (m, 1), 2.82 (m, 2, $CH_2CO_2CH_3$), 2.69 (d of AB qt, 2, J_1 = 8 Hz, $\Delta\delta$ = 0.07, J_2 = 15 Hz), 2.06 (s, 3); mass spectrum, m/z 206 (M^+, 13), 188 (23), 160 (100), 147 (20), 145 (38), 107 (14); IR (KBr) 3104, 1730, 1683, 1492, 1451, 1398, 1149, 1078 cm^{-1}. Anal. ($C_{12}H_{14}O_3$) C, H.

Method B. The acid **14a** (28 mg, 149 μmol) was combined with HgO (5.2 mg, 24 μmol), concentrated H_2SO_4 (50 μL), and water (0.75 mL). The mixture was stirred and heated to 70 °C for 1 h, then poured into 20 mL 20% sodium bicarbonate, and washed with ether. The aqueous phase was then acidified with HCl and extracted with ethyl acetate. The combined ethyl acetate extracts were washed (saturated NH_4Cl), dried ($MgSO_4$), and concentrated to a white solid (22 mg, 77%). The product, >95% pure by GC, gave MS and NMR spectra identical with the product prepared by method A. Exact mass calcd for $C_{12}H_{14}O_3$ m/e 206.0943, obsd 206.0949.

(*S*)-5-Oxo-3-phenylhexanoic Acid (9b). This compound was prepared from **8b** (500 mg, 2.27 mmol) by method A. The NMR spectrum of the product, 430 mg of light brown crystals (92%), was identical with **9a**. Recrystallization from ether provided analytically pure material. Anal. ($C_{12}H_{14}O_3$) C, H.

(*R*/*S*)-5-Oxo-3-phenylhexanoic Acid (9). This compound was prepared from racemic **14** (40 mg, 213 μmol) by method B, yielding a white solid (30 mg, 68%). The product, >99% pure by GC, gave MS and NMR spectra identical with **54a**. Exact mass calcd for $C_{12}H_{14}O_3$ m/e 206.0943, obsd 206.0949.

(3*R*,1′*R*)-*N*-(2-Hydroxy-1-phenethyl)-5-oxo-3-phenylhexanamide (15a). This procedure was adapted from a similar procedure.[12] The acid **9a** (22 mg, 108 μmol) was dissolved in 1 mL of a 0.235 M anydrous solution of triethylamine in THF. After the mixture was cooled to −10 °C, isobutyl chloroformate (13.6 μL, 108 μmol) was added and the solution was stirred for 15 min. After the solution was warmed to 0 °C, (*R*)-phenylglycinol (14.6 mg, 108 μmol) was added, and the reaction mixture was stirred for 40 min. After warming to room temperature, the reaction mixture was filtered through a transfer pipet containing a 10-mm bed of silica gel and concentrated to a white solid (25.1 mg, 71%). The de of the product was shown by HPLC to be >99%: mp 121–124 °C; NMR (300 MHz, $CDCl_3$) δ 7.28 (m, 3), 7.14 (d, 2, J = 7.0 Hz), 5.94 (d, 1, J = 6.0 Hz), 4.93 (m, 1), 3.69 (m, 3), 2.92 (d, 1, J = 7.5 Hz), 2.82 (d, 1, J = 7.0 Hz), 2.59 (d, 1, J = 7.0 Hz), 2.52 (d, 1, J = 7.5 Hz), 2.08 (s, 3), 0.90 (s, 1); mass spectrum, m/z 325 (M^+, 13), 295 (18), 294 (36), 264 (16), 189 (17), 131 (25), 106 (100); IR (KBr) 3318, 3021, 1710, 1639, 1530, 1399, 753 cm^{-1}. Exact mass calcd for $C_{20}H_{23}NO_3$ m/e 325.1678, obsd m/e 325.1674.

(3*S*,1′*R*)-*N*-(2-Hydroxy-1-phenethyl)-5-oxo-3-phenylhexanamide (15b). This compound was prepared from **9b** according to the procedure described above for **15a**. The product (18 mg, 51%) was shown to have >93% de by HPLC: mp 107–110 °C; NMR (300 MHz, $CDCl_3$) δ 7.25 (m, 3), 7.03 (m, 2), 6.02 (d, 1, J = 7.2 Hz), 4.95 (m, 1), 3.78 (d, 2, J = 5.4 Hz), 3.67 (qn, 1, J = 7.2 Hz), 2.88 (m, 2), 2.56 (m, 2), 2.07 (s, 3), 0.91 (s, 1). Exact mass calcd for $C_{20}H_{23}NO_3$ m/e 325.1678, obsd m/e 325.1684.

(3*R*)-*p*-Nitrophenyl 5-Oxo-3-phenylhexanoate (10a). The acid **9a** (111 mg, 0.54 mmol) was added to mixture of chloroform (2 mL) and *N*,*N*-dicyclohexylcarbodiimide (11 mg, 0.54 mmol) and allowed to stir for 5 min. The mixture was then cooled to 0 °C, *p*-nitrophenol (90 mg, 0.65 mmol) was added, and the cooling bath was removed. Stirring was continued for 1 h, after which the reaction mixture was filtered and concentrated. The mixture was then purified by flash chromatography (30 mm, 1% ether/chloroform) to give the cream colored solid **10a** (134 mg, 76%): mp 82–83 °C; NMR (300 MHz, $CDCl_3$) δ 8.20 (d, 2, J = 9 Hz), 7.30 (m, 5), 7.02 (d, 2, J = 9 Hz), 3.81 (qn, 1, J = 7 Hz), 3.04 (dd, 1, J_1 = 7 Hz, J_2 = 15 Hz), 2.91 (d, 2, J = 7 Hz), 2.86 (dd, 1, J_1 = 9 Hz), J_2 = 15 Hz), 2.12 (s, 3); mass spectrum, m/z 190 (13), 189 ($M^+ - O(C_6H_4)NO_2$, 100), 132 (7), 131 (78); IR (KBr) 1745, 1712, 1520, 1342, 1200, 1123, 923, 868, 854 cm^{-1}; UV (20% acetonitrile/0.1 M phosphate buffer, pH 7.2) 210 (11 490), 274 (7745). Anal. ($C_{18}H_{17}NO_5$) C, H, N.

(3*S*)-*p*-Nitrophenyl 5-Oxo-3-phenylhexanoate (10b). This compound was prepared from **9b** (206 mg, 1.0 mmol) by the same method used to prepare the *p*-nitrophenyl ester **10a**. The product (275 mg, 84%) was identical with **10a** by NMR analysis, mp 82–83 °C. Anal. ($C_{18}H_{17}NO_5$) C, H, N.

(3*R*)-*p*-Cyanophenyl 5-Oxo-3-phenylhexanoate (11a). This compound was prepared from **9a** (50 mg, 0.24 mmol) and *p*-cyanophenol (35 mg, 0.29 mmol) by the same method used to prepare the *p*-nitrophenyl ester **10a**. The product (57 mg, 77%) was isolated by flash chromatography (12 mm, $CHCl_3$): mp 86–87 °C; NMR (300 MHz, $CDCl_3$) δ 7.61 (d, 2, J = 9 Hz), 7.29 (m, 5), 6.97 (d, 2, 9 Hz), 3.80 (qn, 1, J = 8 Hz), 3.02 (dd, 1, J_1 = 6.5 Hz, J_2 = 15 Hz), 2.90 (d, 2, J = 8 Hz), 2.84 (dd, 1, J_1 = 8.5 Hz, J_2 = 15 Hz) 2.12, (s, 3); mass spectrum, m/z 190 (14), 189 ($M^+ - O(C_6H_4)CN$, 100), 132 (9), 131 (91), 43 (76); IR (KBr) 2905, 2225, 1756, 1701, 1602, 1498, 1208, 1169, 1126 cm^{-1}; UV (10% DMSO/0.1 M phosphate buffer, pH 7.2) 243 (6300). Anal. ($C_{19}H_{17}NO_3$) C, H, N.

(3*S*)-*p*-Cyanophenyl 5-Oxo-3-phenylhexanoate (11b). This compound was prepared from **9b** by the same procedure described above for **11a** and at the same scale. The white solid obtained (63 mg, 85%) was identical with **11a** by NMR. Anal. ($C_{19}H_{17}NO_3$) C, H, N.

***p*-Nitrophenyl 3-Phenylpropanoate (18).** This compound was prepared from hydrocinnamic acid (300 mg, 2.0 mmol), *N*,*N*-dicyclohexylcarbodiimide (412 mg, 2.0 mmol), and *p*-nitrophenol (334 mg, 2.4 mmol) by the same method used to prepare the *p*-nitrophenyl ester **10a**. Flash chromatography (30 mm, $CHCl_3$) provided the white solid product (418 mg, 77%): mp 99 °C; NMR ($CDCl_3$ δ 8.25 (d, 2, J = 11 Hz), 7.27 (m, 5), 7.18 (d, 2, J = 10 Hz), 3.10 (m, 2), 2.96 (m, 2); mass spectrum, m/z 271 (M^+, 5), 133 (86), 105 (100), 91 (41); IR ($CHCl_3$) 3015, 2401, 1763, 1595, 1527, 1348, 1226, 1122 cm^{-1}; UV (20% acetonitrile/0.1 M phosphate buffer, pH 7.2) 275 (5730). Anal. ($C_{15}H_{13}NO_4$) C, H, N.

(*R*/*S*)-5-Methyl-3-phenylhex-5-enoic Acid (12). Ethyl 5-methyl-3-phenylhex-5-enoate (**19**) (686 mg, 2.96 mmol), prepared by the addition of methallylzinc bromide to diethyl benzalmalonate followed by decarboethoxylation of the diester addition product in $DMSO/NaCl_{(aq)}$ according to the method of Stella et al.,[14] was dissolved in a solution prepared from 60 mL of methanol and 20 mL of 5% aqueous potassium hydroxide and stirred 16 h under a nitrogen atmosphere. After removal of methanol in vacuo, the reaction solution was diluted with 25 mL of 5% sodium bicarbonate, washed with three portions of ether, and acidified to pH 3 with concentrated hydrochloric acid. The solution was then extracted four times with ethyl acetate and the combined ethyl acetate extracts were dried ($MgSO_4$) and concentrated to give the amber oil **12** (572 mg, 95%): ^1H NMR (300 MHz, $CDCl_3$) δ 7.27 (m, 2, o-aromatic), 7.19 (m, 3, *m*,*p*-aromatic), 4.71 (s, 1, =C*H*H), 4.63 (s, 1, =CH*H*), 3.30 (m, 1, PhC*H*RR′), 2.70 (dd, 1, J_1 = 6.0 Hz, J_2 = 15.8 Hz, RC*H*HC(CH_3)=CH_2), 2.55 (dd, 1, J_1 = 8.7 Hz, J_2 = 15.8 Hz, RCH*H*C(CH_3)=CH_2), 2.33 (d, 2, J = 7.7 Hz, RCH_2CO_2H), 1.68 (s, 3, CH_3); mass spectrum m/z 204 (M^+, 3), 189 (8), 149 (10), 144 (15), 107 (100), 79 (25), 76 (20); IR (neat) 3073, 3031, 2972, 2934, 1707 (C=O), 1647 (C=C), 893 cm^{-1}. Anal. ($C_{13}H_{10}O_2$) C, H.

(*R*)-5-Methyl-3-phenylhex-5-enoic Acid (12a). *n*-Butyllithium (0.252 mL of a 1.26 M THF solution, 0.315 mmol) was

added over a 5-min period to a cooled (−10 °C) mixture of methyltriphenylphosphonium bromide (114 mg, 0.315 mmol) and THF (1.5 mL). The resulting solution was stirred for 45 min at room temperature. After cooling to −78 °C, a solution of the keto ester **8a** (66 mg, 0.300 mmol) dissolved in 1 mL THF was added over a 10-min period, and the reaction mixture was then stirred for 1 h at −78 °C and warmed to room temperature. Ether (5 mL) was added and the reaction mixture was filtered (using 5 mL ether to wash over), concentrated, and purified by flash chromatography (10% ethyl acetate/hexane) to isolate the alkenoic ester intermediate methyl 5-methyl-3-phenylhex-5-enoate (50 mg, R_f = 0.37 in 10% ethyl acetate/hexane).

The alkenoic ester was saponified by dissolution into a mixture of 6 mL of methanol and 2 mL of 5% aqueous KOH. After stirring for 11 h, the reaction solution was poured into 5% aqueous HCl/ethyl acetate, and the aqueous phase was extracted twice with ethyl acetate. The combined ethyl acetate phases were washed (saturated NH_4Cl), dried ($MgSO_4$), and concentrated to a clear colorless oil that was pure by GC and coeluted with **12** (49 mg, 73%). Exact mass calcd for $C_{13}H_{11}O_2$ *m/e* 204.1150, obsd *m/e* 204.1150.

(*S*)-5-Methyl-3-phenylhex-5-enoic Acid (12b). This compound was prepared from the keto ester **8b** (99 mg, 0.450 mmol) with methyltriphenylphosphonium bromide (169 mg, 0.472 mmol) and *n*-butyllithium (0.375 mL of a 1.26 M THF solution, 0.472 mmol) by the same procedure used to prepare **12a**. Saponification of the intermediate alkenoic ester in 9.5 mL of methanol/3 mL of 5% aqueous KOH provided a clear colorless oil (71 mg, 77%) that coeluted with **12a** on GC. Anal. ($C_{13}H_{10}O_2$) C, H.

***p*-Nitrophenyl 5-Methyl-3-phenylhex-5-enoate (13).** A solution of the hexenoic acid **12** (166 mg, 0.814 mmol) and DCC (168 mg, 0.814 mmol) dissolved in 4 mL of methylene chloride was stirred at room temperature for 2 min (a white precipitate formed) and then cooled to 0 °C. *p*-Nitrophenol (136 mg, 0.977 mmol) was added, and the now homogeneous solution was stirred for 5 min at 0 °C and 2 h at room temperature, during which time a second precipitate formed. The mixture was then filtered, concentrated, and subjected to flash chromatography (30 mm, 75% methylene chloride/hexane) to isolate the white solid **13** (169 mg, 64%): mp 68–69 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.18 (d, 2, *J* = 9.0 Hz, arom CH ortho to NO_2), 7.30 (m, 5, Ph), 6.94 (d, 2, *J* = 9.0 Hz, arom CH ortho to OCO), 4.80 (s, 1, =C*H*H), 4.73 (s, 1, =CH*H*), 3.42 (m, 1, PhC*H*RR′), 2.98 (dd, 1, J_1 = 5.6 Hz, J_2 = 15.2 Hz, C*H*HCO$_2$R), 2.79 (dd, 1, J_1 = 9.7 Hz, J_2 = 15.2 Hz, CH*H*CO$_2$R), 2.44 (d, 2, *J* = 7.6 Hz, CH_2C(CH$_3$)=CH_2), 1.75 (s, 1, CH_3); mass spectrum, *m/z* 325 (M^+, 0.4), 228 (21), 187 (51), 145 (35), 91 (36), 83 (100), 55 (83); IR (neat) 3081, 2932, 1763 (C=O), 1593, 1524 (NO_2), 1491, 1347 (NO_2), 1206 (CO), 1117 (CO) cm^{-1}. Anal. ($C_{19}H_{19}NO_4$) C, H, N.

(*R*)-*p*-Nitrophenyl 5-Methyl-3-phenylhex-5-enoate (13a). This compound was prepared according to the procedure described above for racemic **13**, with use of the acid **12a** (41 mg, 0.201 mmol), DCC (42 mg, 0.201 mmol), *p*-nitrophenol (33 mg, 0.241 mmol), and methylene chloride (2 mL), mp 79–80 °C. Anal. ($C_{19}H_{19}NO_4$) C, H, N.

(*S*)-*p*-Nitrophenyl 5-Methyl-3-phenylhex-5-enoate (13b). This compound was prepared according to the procedure described above for racemic **13**, with use of the acid **12b** (49 mg, 0.240 mmol), DCC (49 mg, 0.240 mmol), *p*-nitrophenol (40 mg, 0.288 mmol), and methylene chloride (2 mL), mp 80–81 °C. Anal. ($C_{19}H_{19}NO_4$) C, H, N.

5-Methyl-3-phenylhexanoic Acid (20). To a solution of the alkenoic acid **12** (204 mg, 1.00 mmol) dissolved in 6 mL absolute ethanol was added 18 mg of 5% palladium on carbon. The mixture was stirred under a hydrogen atmosphere for 20 h, filtered through a short bed of silica gel, and concentrated to the white solid **20** (205 mg, 99%), which contained <5% (by ^{1}H NMR) of the double bond isomerized starting material 5-methyl-3-phenylhex-4-enoic acid as an impurity: mp 52–55 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29 (m, 2, o-aromatic), 7.20 (m, 3, *m,p*-aromatic), 3.17 (m, 1, PhC*H*RR′), 2.60 (dd, 1, J_1 = 1.0 Hz, J_2 = 7.5 Hz, RC*H*HCO$_2$H), 2.57 (dd, 1, J_1 = 2.3 Hz, J_2 = 7.5 Hz, CH*H*CO$_2$H), 1.38–1.65 (m, 2, RCHCH_2CH), 1.30 (m, 1, C*H*(CH$_3$)$_2$), 0.87 (d, 3, *J* = 6.3 Hz, RCH_3), 0.82 (d, 3, *J* = 6.4 Hz, RCH_3); mass spectrum, *m/z* 206 (M^+, 29), 150 (34), 146 (36), 107 (89), 105 (37), 104 (59), 91 (100); IR (neat) 3029, 2957, 2870, 1707 (C=O), 1452, 1410, 1279 cm^{-1}. Exact mass calcd for $C_{13}H_{18}O_2$ *m/e* 206.1308, obsd *m/e* 206.1310.

***p*-Nitrophenyl 5-Methyl-3-phenylhexanoate (21).** A solution of the hexanoic acid **20** (80 mg, 0.388 mmol) and DCC (80 mg, 0.388 mmol) dissolved in 4 mL of methylene chloride was stirred at room temperature for 2 min (a white precipitate formed) and then cooled to 0 °C. *p*-Nitrophenol (65 mg, 0.466 mmol) was added and the now homogeneous solution was stirred for 5 min at 0 °C and 1 h at room temperature, during which time a second precipitate formed. The mixture was then filtered, concentrated, and subjected to flash chromatography (20 mm, 60% methylene chloride/hexane) to isolate the white solid **21** (65 mg, 51%): mp 60–61 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.18 (d, 2, *J* = 9.1 Hz, arom CH ortho to NO_2), 7.31 (m, 5, Ph), 6.94 (d, 2, *J* = 9.1 Hz, arom CH ortho to OCO), 3.30 (m, 1, PhC*H*RR′), 2.76–2.93 (m, 2, R′CH_2CO$_2$R), 1.71 (m, 1, RC*H*HCH(CH$_3$)$_2$), 1.51 (m, 1, R′CH*H*CH(CH$_3$)$_2$), 0.92 (d, 3, *J* = 6.4 Hz, CH_3), 0.87 (d, 3, *J* = 6.5 Hz, CH_3); mass spectrum, *m/z* 327 (M^+, 2), 189 (23), 147 (65), 105 (28), 91 (100); IR (neat) 2957, 1765 (C=O), 1524 (NO_2), 1346 (NO_2), 1207 (C–O), 1102 (C–O) cm^{-1}. Anal. ($C_{19}H_{21}NO_4$) C, H, N.

3-Phenylglutaric Anhydride (22). To 50 mL of acetic anhydride was added 10.00 g (48 mmol) of 3-phenylglutaric acid and the mixture was heated to reflux temperature. After 1 h, the acetic anhydride was removed by shortpath distillation, and the product was distilled under reduced pressure (180–190 °C, 0.5 Torr) to yield 6.6 g of white solid. Improved yields are obtained if this distillation is carried out as quickly as possible while using a heating flame to prevent solidification of the product before it reaches the receiver. Cold-finger sublimation (80 °C, 0.5 Torr) gave 5.96 g (65%) pure white crystals of **22**: mp 104–105 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.39 (m, 3), 7.21 (m, 2), 3.41 (m, 1), 3.12 (dd, 2, J_1 = 1.3 Hz, J_2 = 13 Hz), 2.87 (dd, 2, J_1 = 1.3 Hz, J_2 = 11 Hz); mass spectrum, *m/z* 190 (M^+, 10), 104 (100), 78 (6); IR ($CHCl_3$) 1812, 1761, 1064, 947 cm^{-1}. Anal. ($C_{11}H_{10}O_3$) C, H.

4-(Aminocarbonyl)-3-phenylbutanoic Acid (23). A solution of 3-phenylglutaric anhydride **22** (300 mg, 1.58 mmol) dissolved in 3 mL of THF was added to 15 mL of $NH_{3(l)}$ and allowed to stir overnight. The reaction mixture was then concentrated and triturated twice with 10-mL portions of ethyl acetate. The solids remaining after trituration were acidified at 0 °C with 2 mL of 5% HCl, filtered, washed on the filter paper with ice cold water, and dried to give a white powder (269 mg, 82%): mp 163–164 °C; ^{1}H NMR (300 MHz, DMSO) δ 7.15–7.28 (m, 5, arom CH's), 6.71 (br s, 2, NH_2), 3.43 (m, 1, PhCHRR′), 2.61 (dd, 1, J_1 = 15.6 Hz, J_2 = 5.8 Hz, RC*H*HCO$_2$H), 2.48 (dd, 1, J_1 = 15.6 Hz, J_2 = 9.2 Hz, RCH*H*CO$_2$H), 2.35 (m, 2, RCH$_2$ON); mass spectrum, *m/z* 207 (M^+, 24), 189 (M − H_2O, 19), 162 (48), 161 (100), 118 (68), 103 (50), 78 (54), 77 (62). Anal. ($C_{11}H_{13}NO_3$) C, H, N.

***p*-Nitrophenyl 4-(Aminocarbonyl)-3-phenylbutanoate (24).** A mixture of the acid **23** (104 mg, 0.502 mmol), *p*-nitrophenol (105 mg, 0.754 mmol), 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (213 mg, 0.502 mmol), and 4 mL of methylene chloride was stirred overnight and then treated with 100 mL of ethyl acetate/30 mL of water. The ethyl acetate phase was washed (brine), dried ($MgSO_4$), concentrated, and purified by flash chromatography (30 mm, 90% ethyl acetate/hexane) to give a light yellow solid (35 mg, 21%): mp 125–126 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.19 (d, 2, *J* = 9.1 Hz, arom CH's ortho to NO_2), 7.28–7.39 (m, 5, C_6H_5), 7.01 (d, 2, *J* = 9.1 Hz, arom CH's ortho to OCO), 5.41 (br s, $CONH_2$), 3.80 (m, 1, PhCHRR′), 3.15 (dd, 1, J_1 = 15.4 Hz, J_2 = 6.1 Hz, RC*H*HCO$_2$R′), 2.94 (dd, 1, J_1 = 15.4 Hz, J_2 = 8.8 Hz, RCH*H*CO$_2$R′), 2.67 (m, 2, RCH_2CONH$_2$); mass spectrum, *m/z* 189 (M − HOC$_6$H$_4$NO$_2$, 85), 139 (76), 131 (66), 104 (100), 65 (98); IR ($CHCl_3$) 3526 (amide), 3407 (amide), 1761 (ester C=O), 1686, 1593, 1527 (NO_2), 1348 (NO_2), 1208, 1125 cm^{-1}. Anal. ($C_{17}H_{16}N_2O_5$) C, H, N.

4-Carbomethoxy-3-phenylbutanoic Acid (25). The anhydride **22** (4.0 g, 2.10 mmol) was dissolved in 350 mL of anhydrous methanol, heated to reflux, and allowed to stir for 6 days. The reaction time is highly dependent on the reaction scale; reaction progress was monitored by ^{1}H NMR analysis of concentrated aliquots. Removal of methanol under vacuum afforded the half-ester **25** in quantitative yield (4.67 g), mp 94–95 °C: ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.24 (m, 5), 3.61 (m, 1), 3.60 (s, 3), 2.71 (m, 4); mass spectrum, *m/z* 222 (M^+, 5), 204 (7), 191 (15), 176 (100),

162 (46), 118 (67); IR ($CHCl_3$) 3180, 1732, 1718, 1438, 1272, 1150 cm^{-1}. Anal. ($C_{12}H_{14}O_4$) C, H.

p-Nitrophenyl 4-Carbomethoxy-3-phenylbutanoate (26). A solution of the acid **25** (100 mg, 0.450 mmol) and DCC (93 mg, 0.450 mmol) dissolved in 4 mL of methylene chloride was stirred at room temperature for 2 min (a white precipitate formed), and then cooled to 0 °C. *p*-Nitrophenol (75 mg, 0.541 mmol) was added and the now homogeneous solution was stirred for 5 min at 0 °C and 6 h at room temperature, during which time a second precipitate formed. The mixture was then filtered, concentrated, and subjected to flash chromatography (1% $Et_2O/CHCl_3$) to isolate the white solid ester **26** (112 mg, 73%): mp 108 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 8.20 (d, 2, J = 9.1 Hz, arom CH's ortho to NO_2), 7.25–7.38 (m, 5, Ph), 7.00 (d, 2, J = 9.1 Hz, arom CH's ortho to NO_2), 7.25–7.38 (m, 5, Ph), 7.00 (d, 2, J = 9.1 Hz, arom CH's ortho to OCO), 3.78 (m, 1, PhCHRR'), 3.64 (s, 3, CH_3OCO), 3.08 (dd, 1, J_1 = 15.4 Hz, J_2 = 6.3 Hz, RC*H*$HCO_2(C_6H_4)NO_2$), 2.92 (dd, 1, J_1 = 15.4 Hz, J_2 = 9.0 Hz, RCH*H*$CO_2(C_6H_4)NO_2$), 2.77 (d, 2, J = 7.5 Hz, RCH_2CO_2Me); mass spectrum, m/z 312 (M – CH_3OH, 4), 205 (M–O(C_6H_4)NO_2, 100), 173 (43), 131 (85), 121 (98), 117 (26), 104 (23); IR ($CHCl_3$) 1761 (arom ester C=O), 1734 (methyl ester C=O), 1528 (NO_2), 1348 (NO_2), 1208, 1121 cm^{-1}. Anal. ($C_{18}H_{17}NO_6$) C, H, N.

***tert*-Butyl 3-(Acetyloxy)-3-phenylpropanoate (27).** To a cooled (0 °C) solution of butyllithium (3.50 mL of a 1.43 M solution in hexane, 5.0 mmol) dissolved in 4.0 mL of hexane was added diisopropylamine (0.70 mL, 5.0 mmol) over a 5-min period and the resulting solution was stirred for 5 min at 0 °C, then cooled to –78 °C. *tert*-Butyl acetate (0.68 mL, 5.0 mmol) was then added over 15 min, followed 30 min later by the addition of THF (0.250 mL) and benzaldehyde (0.51 mL, 5.0 mmol) over a 1-min period. The mixture was then allowed to warm to room temperature over a 2-h period and concentrated to an oil. THF (10 mL), acetic anhydride (1.1 mL, 11.6 mmol), and (dimethylamino)pyridine (10 mg) were then added, and the solution was heated to reflux and stirred for 8 h.

The reaction mixture was dissolved in 150 mL of ethyl acetate and washed successively with $NH_4Cl_{(satd)}$, water, three portions of 5% $NaHSO_{3(aq)}$, water, three portions of $NaHCO_{3(aq)}$, and $NaCl_{(satd)}$. The organic phase was dried ($MgSO_4$) and concentrated to an oil which provided the clear colorless oil **27** (804 mg, 61%) after isolation by flash chromatography (60 mm, 11% EtOAc/hexane): ^1H NMR (300 MHz, $CDCl_3$) δ 7.29–7.36 (m, 5, C_6H_5), 6.13 (dd, 1, J_1 = 9.0 Hz, J_2 = 5.4 Hz, PhCHRR'), 2.88 (dd, 1, J_1 = 15.4 Hz, J_2 = 9.0 Hz, RC*H*HCO_2R'), 2.67 (dd, 1, J_1 = 15.4 Hz, J_2 = 5.4 Hz, RCH*H*CO_2R'), 2.05 (s, 3, CH_3COR), 1.40 (s, 9, $(CH_3)_3COCO$); mass spectrum, m/z 208 (M – $(CH_3)C{=}CH_2$, 26), 166 (14, 165 (87), 147 (26), 131 (40), 105 (55), 57 (94), 43 (100); IR (neat) 2978, 1744 (br, C=O's), 1369, 1289, 1232, 1151, 1024 cm^{-1}. Anal. ($C_{15}H_{20}O_4$) C, H.

3-(Acetyloxy)-3-phenylpropanoic Acid (28). A solution of the ester **27** (319 mg, 1.21 mmol) dissolved in 4 mL of methylene chloride was cooled to 0 °C, and 4 mL of precooled trifluoroacetic acid was added. After stirring under nitrogen for 4 h, the solution was concentrated to a cream-colored solid. Recrystallization from EtOAC/hexane provided the pure acid **28** (237 mg, 94%): mp 100–101 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.35 (m, 5, C_6H_5), 6.16 (dd, 1, J_1 = 9.1 Hz, J_2 = 4.9 Hz, PhCHRR'), 3.03 (dd, 1, J_1 = 16.4 Hz, J_2 = 9.1 Hz, RC*H*HCO_2H), 2.80 (dd, 1, J_1 = 16.4 Hz, J_2 = 4.9 Hz, RCH*H*CO_2H), 2.07 (s, 3, CH_3COR); mass spectrum, m/z 208 (M^+, 2), 165 (49), 107 (44), 105 (57), 77 (33), 43 (100); IR (melt) 2955, 1740 (acid C=O), 1716 (ester C=O), 1374, 1239, 1028 cm^{-1}. Anal. ($C_{11}H_{12}O_4$) C, H.

p-Nitrophenyl 3-(Acetyloxy)-3-phenylpropanoate (29). A mixture of the acid **28** (172 mg, 0.827 mmol), *p*-nitrophenol (138 mg, 0.992 mmol), and methylene chloride (4 mL) was cooled to 0 °C, and DCC (170 mg, 0.827 mmol) was added. The reaction mixture was stirred for 1 h at 0 °C, stirred for 5 h at room temperature, filtered, and concentrated to a solid. The white solid **29** (211 mg, 78%) was isolated by flash chromatography (33 mm, 1% $Et_2O/CHCl_3$): mp 94–95 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 8.26 (d, 2, J = 9.1 Hz, arom CH ortho to NO_2), 7.36–7.45 (m, 5, Ph), 7.20 (d, 2, J = 9.1 Hz, arom CH ortho to OCO), 6.29 (dd, 1, J_1 = 8.8 Hz, J_2 = 5.2 Hz, PhCHRR'), 3.25 (dd, 1, J_1 = 15.6 Hz, J_2 = 8.8 Hz, RC*H*HCO_2R'), 3.07 (dd, 1, J_1 = 15.6 Hz, J_2 = 5.2 Hz, RCH*H*CO_2R'), 2.11 (s, 3, CH_3COR); mass spectrum, m/z 191 (M – O(C_6H_4)NO_2, 13), 131 (74), 107 (19), 77 (11), 43 (100); IR (melt) 1763 (C=O), 1750 (C=O), 1527 (NO_2), 1348 (NO_2), 1240, 1207, 1129 cm^{-1}. Anal. ($C_{17}H_{15}NO_6$) C, H, N.

Ethyl 2-Carbethoxy-4-cyano-3-phenylpropanoate (30). Elemental sodium, 80 mg (3.5 mmol), was dissolved in 10 mL of anhydrous methanol, and diethyl malonate (3.20 g, 20.0 mmol) was then added. Cinnamonitrile (1.29 g, 10.0 mmol) was added dropwise over a 2-min period, and the reaction mixture was stirred for 1 h at room temperature and 5 h at reflux temperature. HCl (5%, 50 mL) was then added, and the reaction solution was extracted with three portions of ethyl acetate. The organic extracts were combined and washed three times with water, dried ($MgSO_4$), and concentrated under vacuum. Excess diethyl malonate was removed by Kugelrohr distillation (110 °C/2 Torr), and the remaining dark amber oil was purified by flash chromatography (50 mm, 1:4 ethyl acetate/hexane) to give 2.86 g (99%) of the clear colorless oil **30**: ^1H NMR (200 MHz, $CDCl_3$) δ 7.30 (s, 5), 4.23 (q, 2, J = 8 Hz), 3.92 (q, 2, J = 8 Hz), 3.73 (m, 1), 2.89 (m, 2), 1.28 (t, 3, J = 8 Hz), 1.00 (t, 3, J = 8 Hz); mass spectrum, m/z 289 (M^+, 1), 261 (6), 189 (100), 29 (30); FAB MS, 290 (M + 1); IR ($CHCl_3$) 2980, 2243, 1730, 1318, 1173, 1017 cm^{-1}. Anal. ($C_{16}H_{19}NO_4$) C, H, N.

4-Cyano-3-phenylpropanoic Acid (31). To a solution, prepared from 50 mL of methanol and 30 mL of 10% KOH, was added 1.16 g (4.00 mmol) of the diester **30**. After the solution was stirred for 1 h, the methanol was removed under vacuum and 50 mL of water was added. The solution was then acidified to pH 1 with concentrated HCl and extracted three times with ethyl acetate. The organic phase was dried ($MgSO_4$) and concentrated under vacuum to yield the diacid intermediate, which was subjected to decarboxylation conditions (refluxing *m*-xylene, 1.5 h) without further purification. The xylene solution was then extracted three times with 5% $NaHCO_3$, and the combined aqueous extracts were acidified to pH 1 with concentrated HCl. Extraction three times with ethyl acetate, followed by drying ($MgSO_4$) and concentration of the combined organic extracts under vacuum, yielded 0.361 g (48%) of the white solid **31**: mp 64–69 °C; NMR (200 MHz, $CDCl_3$) δ 7.30 (m, 5), 3.50 (m, 1), 2.89 (dd, 2, J_1 = 1 Hz, J_2 = 7 Hz), 2.76 (d, 2, J = 7 Hz); mass spectrum, m/z 189 (M^+, 12), 162 (24), 134 (43), 107 (100), 77 (72); IR ($CHCl_3$) 3090, 2255, 1712, 1422, 1205 cm^{-1}. Anal. ($C_{11}H_{11}NO_2$) C, H, N.

p-Nitrophenyl 4-Cyano-3-phenylbutanoate (32). A solution of the acid **31** (100 mg, 0.529 mmol) and DCC (109 mg, 0.529 mmol) dissolved in 4 mL of methylene chloride was stirred at room temperature for 2 min (a white precipitate formed) and then cooled to 0 °C. *p*-Nitrophenol (88 mg, 0.635 mmol) was added, and the now homogeneous solution was stirred for 5 min at 0 °C and 1 h at room temperature, during which time a second precipitate formed. The mixture was then filtered, concentrated, and subjected to flash chromatography (1% $Et_2O/CHCl_3$) to isolate the ester **32** as a semisolid (119 mg, 73%): ^1H NMR (300 MHz, $CDCl_3$) δ 8.23 (d, 2, J = 9.0 Hz, arom CH's ortho to NO_2), 7.29–7.46 (m, 5, Ph), 7.08 (d, 2, J = 9.0 Hz, arom CH's ortho to OCO), 3.65 (m, 1, PhCHRR'), 3.07–3.25 (m, 2, RCH_2CO_2R'), 2.83 (m, 2, RCH_2CN); mass spectrum, m/z 172 (M – O(C_6H_4)NO_2, 100), 144 (25), 131 (31), 130 (86), 103 (24); IR (neat) 3082, 2928, 2247 (CN), 1761 (C=O), 1524 (NO_2), 1348 (NO_2), 1206, 1127 cm^{-1}. Exact mass calcd for $C_{17}H_{14}N_2O$ m/e 310.0954, obsd m/e 310.0953.

Methyl 5-Hydroxy-3-phenylpentanoate (33). To a solution of the acid **25** (2.22 g, 10.0 mmol) dissolved in 6 mL of THF was added 24 mL of diethyl ether. Borane/dimethyl sulfide (5.24 mL of a 2 M solution in THF, 10.5 mmol) was then added dropwise with stirring over a 30-min period (after dropwise addition ceased to produce gas evolution, the last 1 mL was added at reflux temperature). After 1.5 h at reflux (50 °C heating bath oil), during which time a white precipitate formed, the reaction mixture was cooled and concentrated under reduced pressure to give a cloudy oil. CH_2Cl_2 (100 mL) and 50 mL of water was added, and after separation the organic phase was washed three times with saturated $NaHCO_3$, washed once with saturated NaCl, dried ($MgSO_4$), and concentrated under vacuum to give 1.99 g (96%) of cloudly colorless oil: ^1H NMR (200 MHz, $CDCl_3$) δ 7.21 (m, 5), 3.60 (s, 3), 3.50 (t, 2, J = 3 Hz), 3.30 (m, 1), 2.65 (d, 2, J = 2.5 Hz); mass spectrum, m/z 208 (M^+, 12), 190 (26), 176 (42), 148 (28), 130 (100), 117 (79), 105 (74), 104 (74), 91 (80); IR ($CHCl_3$) 3520, 2960, 1728, 1263, 1160, 1080 cm^{-1}. Anal. ($C_{12}H_{16}O_3$) C, H.

Methyl 5-Oxo-3-phenylpentanoate (34). To a stirring suspension of pyridinium chlorochromate (431 mg, 2.00 mmol) and 10 mL of CH_2Cl_2 (distilled from P_2O_5) and added dropwise the alcohol **33** (412 mg, 1.98 mmol), dissolved in 5 mL of CH_2Cl_2, over the course of 10 min. After the mixture was heated to reflux, with stirring, for 25 min, 50 mL 1:1 ether/CH_2Cl_2 was added. Filtration through a bed of Fluorosil (5 g), followed by concentration of the filtrate under vacuum gave a clear, light green oil that was used without further purification (312 mg, 86%). Analytically pure material was obtained by distillation to a clear colorless oil (150 °C, 0.1 Torr): ^{1}H NMR (200 MHz, $CDCl_3$) δ 9.67 (s, 1), 7.24 (m, 5), 3.74 (m, 1), 3.61 (s, 3), 2.83 (d, 2, J = 1.5 Hz), 2.68 (d, 2, J = 7 Hz); mass spectrum, m/z 206 (M^+, 9), 178 (21), 175 (17), 146 (35), 117 (21), 105 (78), 74 (50); IR ($CHCl_3$) 3000, 2950, 2822, 2722, 1734, 1718, 1255, 1152 cm^{-1}. Anal. ($C_{12}H_{16}O_3$) C, H.

Methyl 4-(2,5-Dioxolanyl)-3-phenylbutanoate (35). To 100 mL of benzene was added the aldehyde **35** (1.63 g, 7.91 mmol), ethylene glycol (2.46 g, 39.55 mmol), and toluenesulfonic acid (23 mg, 0.12 mmol). The mixture was brought to reflux and water was removed azeotropicly by use of a Dean-Stark trap. After 5.5 h, 50 mL of 50% $NaHCO_3$ was added, and the phases were separated. The organic phase was washed twice with saturated $NaHCO_3$, washed once with water, dried ($MgSO_4$), and concentrated under vacuum to an oil (1.78 g, 90%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.23 (m, 5), 4.62 (dd, 1, J_1 = 1 Hz, J_2 = 2 Hz), 3.94 (m, 2), 3.78 (, 2), 3.57 (s, 3), 3.39 (m, 1), 2.70 (dq, 2, J_1 = 7 Hz, J_2 = 14 Hz), 2.0 (m, 2); mass spectrum, m/z 250 (M^+, 1), 219 (3), 188 (5), 172 (9), 104 (44), 73 (100), 45 (52); IR ($CHCl_3$) 3005, 2995, 2890, 1729, 1440, 1138 cm^{-1}. Anal. ($C_{14}H_{18}O_4$) C, H.

4-(2,5-Dioxolanyl)-3-phenylbutanoic Acid (36). The acid **35** (1.69 g, 6.75 mmol) was dissolved in 60 mL of methanol. Then 40 mL of 5% KOH was added, and the solution was stirred for 2 h under N_2. Removal of methanol under vacuum was followed by acidification with 6 N HCl to pH 1 and extraction three times with ethyl acetate. The organic extracts were dried ($MgSO_4$) and concentrated under vacuum to an oil that was dissolved in ether and reconcentrated under vacuum to a white solid (1.31 g, 82%). Analytically pure material was obtained by recrystallization from ether/hexane: NMR (200 MHz, $CDCl_3$) δ 7.24 (m, 5), 4.63 (dd, 1, J_1 = 1 Hz, J_2 = 2 Hz), 3.92 (m, 2), 3.78 (m, 2), 3.38 (m, 1), 2.71 (dq, 2, J_1 = 7 Hz, J_2 = 14 Hz), 2.02 (m, 2); mass spectrum, m/z 236 (M^+, 2), 176 (11), 158 (22), 104 (27), 73 (100), 45 (34); IR ($CHCl_3$) 3520, 3090, 2960, 2890, 1715, 1408, 1136 cm^{-1}. Anal. ($C_{13}H_{16}O_4$) C, H.

5-Oxo-3-phenylpentanoic Acid (37). To 200 mL of acetone was added the dioxolane **36** (1.08 g, 4.59 mmol), toluenesulfonic acid (100 mg, 0.53 mmol), and 3 mL of water. The resulting solution was heated to reflux for 48 h, then concentrated under vacuum to an amber oil. Purification by flash chromatography (40 mm, 9:0.6:0.1 methylene chloride/methanol/acetic acid), followed by bulb-to-bulb distillation (230 °C/0.1 Torr), gave the white solid **37** (0.505 g, 57%): mp 83–85 °C; ^{1}H NMR (200 MHz, acetone-d_6) δ 9.67 (s, 1), 7.33 (m, 5), 3.73 (m, 1), 2.83 (m, 2), 2.79 (t, 2, J = 8 Hz); mass spectrum, m/z 192 (M^+, 2), 147 (6), 118 (52), 104 (90), 91 (54), 77 (100). Anal. ($C_{11}H_{12}O_3$) C, H.

***p*-Nitrophenyl 5-Oxo-3-phenylpentanoate (38).** To a mixture of the acid **37** (90 mg, 0.470 mmol), DCC (97 mg, 0.470 mmol), and *p*-nitrophenol (78 mg, 0.563 mmol) was added cold (0 °C) methylene chloride (3 mL), and the resulting mixture was stirred for 30 min at 0 °C and 30 min at room temperature. The reaction mixture was then filtered and subjected to flash chromatography (23 mm, 3% ether/chloroform) to isolate the carboxaldehyde ester **38** (71 mg, 48%, R_f = 0.31 in 3% ether/chloroform). This unstable product, >80% pure by ^{1}H NMR, was used without further purification: ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.77 (s, 1, CHO), 8.24 (d, 2, J = 9.5 Hz, arom CH's ortho to NO_2), 7.31 (m, 5, Ph), 7.04 (d, 2, J = 9.5 Hz, arom CH's ortho to OCO), 4.07 (m, 1, PhCHRR'), 2.96 (d, 2, J = 8.0 Hz, CH_2CO), 2.78 (m, 2, CH_2CO); mass spectrum, m/z 313 (M^+, 6), 174 (M − $HOC_6H_4NO_2$, 20), 146 (34), 131 (100), 103 (25).

(*R*/*S*)-3-(Acetylamino)-3-phenylpropanoic Acid (16). This procedure was adapted from Bodanski and Bodanski.[31] To a solution of 3-amino-3-phenylpropanoic acid (1.98 g, 12.0 mmol) dissolved in 12.60 mL of 1 N NaOH at 0 °C was added 2.52 mL of 1 N NaOH followed by 0.24 mL (2.52 mmol) acetic anhydride, and the mixture was stirred for 10 min. The sequence of 2.52 mL of 1 N NaOH addition, 0.24 mL of acetic anhydride addition, and 10 min stir at 0 °C was repeated four more times while maintaining an alkaline pH during the stirring period by addition of 1 N NaOH when necessary. The reaction mixture was then acidified to Congo red with concentrated HCl, allowed to stand for 30 min in an ice bath, and filtered. The precipitate was washed with ice-cold water and dried in a vacuum desiccator over $Mg(ClO_4)_2$, giving the white crystalline **16** (2.32 g, 93%): mp 169–170 °C; NMR (200 MHz, acetone-d_6) δ 7.33 (m, 5), 5.39 (t, 1, J = 6 Hz), 2.81 (dd, 2, J_1 = 5 Hz, J_2 = 6 Hz), 1.89 (s, 3); mass spectrum, m/z 189 (1), 164 (100), 119 (33), 106 (80), 43 (49); FAB MS, 208 (M + 1); IR (KBr) 3328, 2497, 1970, 1699, 1599, 1267, 1084 cm^{-1}. Anal. ($C_{11}H_{13}NO_3$) C, H, N.

(*R*)-3-(Acetylamino)-3-phenylpropanoic Acid (16a). A mixture of the racemic amino acid **16** (1.00 g, 4.82 mmol) and (−)-cinchonidine (1.42 g, 4.82 mmol) was dissolved in 20 mL of boiling absolute ethanol, and the resulting solution was allowed to stand 1 day at room temperature and 1 day in the freezer. The 850 mg of white precipitate that formed was then harvested, and recrystallization was repeated seven more times with use of 15–5 mL of boiling ethanol until the extent of enrichment of the diastereomeric salt containing the *R* enantiomer of **16** was constant. Stereochemical purity was monitored by ^{1}H NMR analysis (500 MHz) of the methyl groups of the epimeric salts at ca. 2 ppm and showed >97% de after eight recrystallizations.

The recrystallization product was then dissolved in 2 mL of pH 2 HCl and allowed to stand for 2 days at 40 °C. The crystals that formed were isolated by filtration, washed on the filter paper with ice-cold 10% HCl and ice-cold water, and dried to yield crystalline **16a** (63 mg 6%). The mass spectrum of the product is identical with the racemic compound: mp 193 °C; $[\alpha]_D$ +98 (*c* 1.5, ethanol) (literature[13] $[\alpha]_D$ +103 (c 1.0, ethanol). Anal. ($C_{11}H_{13}NO_3$) C, H, N.

(*R*/*S*)-*p*-Nitrophenyl 3-(Acetylamino)-3-phenylpropanoate (17). A mixture of the acid **16** (60 mg, 0.290 mmol), 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (CMC, 123 mg, 0.290 mmol), and methylene chloride (3 mL) was stirred under nitrogen for 20 min, and *p*-nitrophenol (60 mg, 0.435 mmol) was then added. After stirring overnight (a precipitate formed), the mixture was combined with 75 mL of EtOAc/20 mL of water and separated. The organic phase was washed with brine, dried ($MgSO_4$), and concentrated to a white solid which was purified by flash chromatography (22 mm, 80% EtOAc/hexane) to provide the white solid **17** (57 mg, 60%), mp 170 °C: ^{1}H NMR (300 MHz, acetone-d_6) δ 8.29 (d, 2, J = 8.9 Hz, arom CH's ortho to NO_2), 7.10 (m, 1, NH), 7.27–7.40 (m, 7, Ph and arom CH's ortho to OCO), 5.56 (m, 1, PhCHRR'), 3.16 (d, 2, J = 7.6 Hz, RCH_2CO_2R'), 1.92 (s, 3, CH_3CONHR); mass spectrum, m/z 328 (M^+, 2), 285 (18), 148 (50), 131 (61), 106 (100), 104 (22), 43 (39); IR ($CHCl_3$) 3443 (NH), 1761 (ester C=O), 1676 (amide C=O), 1528 (NO_2), 1348 (NO_2), 1206, 1146 cm^{-1}. Anal. ($C_{17}H_{16}N_2O_5$) C, H, N.

(*R*)-*p*-Nitrophenyl 3-(Acetylamino)-3-phenylpropanoate (17a). This compound was prepared according to the procedure described above for racemic **17**, with use of the amino acid **16** (25 mg, 0.121 mmol), CMC (51 mg, 0.121 mmol), *p*-nitrophenol (25 mg, 0.181 mmol), and methylene chloride (1.5 mL). The product (34 mg, 86%) comigrated with racemic **17** on TLC (R_f = 0.40 in 90% ethyl acetate/hexane). Anal. ($C_{17}H_{16}N_2O_5$) C, H, N.

3-Phenyl-3-(propionylamino)propanoic Acid (39). To a cooled (0 °C) solution of 3-amino-3-phenylpropanoic acid (200 mg, 1.21 mmol) dissolved in 1.27 mL of 1 N NaOH (1.27 mmol) was added 255 μL of 1 N NaOH (0.255 mmol) followed by 33 μL (0.255 mmol) of propionic anhydride, and the mixture was stirred for 10 min. The sequence of 255 μL of 1 N NaOH addition, 33 μL of propionic anhydride addition, and 10-min stir at 0 °C was repeated four more times while maintaining an alkaline pH during the stirring period by addition of 1 N NaOH when necessary. The reaction mixture was then stirred for 30 min at 0 °C and 20 min at room temperature, acidified to Congo red with concentrated HCl, allowed to stand for 30 min in an ice bath, and filtered. The white precipitate was washed with ice cold water and dried in a

(31) Bodanski, M.; Bodanski, A. *The Practice of Peptide Synthesis*; Springer-Verlag: Berlin, 1984; pp 231–232.

vacuum desiccator to give the acid **39** (251 mg, 94%): mp 172–173 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.27 (d, 1, J = 8.3 Hz, NH), 7.21–7.30 (m, 5, Ph), 5.17 (m, 1, PhCHRR′), 2.64 (d, 2, J = 7.6 Hz, RCH_2CO$_2$H), 2.07 (qt, 2, J = 7.6 Hz, CH$_3$CH_2NHCOR), 0.95 (t, 3, J = 7.6 Hz, CH_3CH$_2$NHCOR); mass spectrum, m/z 221 (M$^+$, 3), 164 (100), 120 (33), 119 (30), 106 (79), 104 (38), 77 (29), 57 (36); IR (Nujol) 3355 (N—H), 1719 (acid C=O), 1626 (amide C=O), 1535, 1412, 1237, 1194 cm^{-1}. Anal. ($C_{12}H_{15}NO_3$) C, H, N.

3-Phenyl-3-(benzoylamino)propanoic Acid (40). To a cooled (0 °C) solution of the 3-amino-3-phenylpropanoic acid (200 mg, 1.21 mmol) dissolved in 0.727 mL of 2 N NaOH (1.46 mmol) was added 145 μL of 1 N NaOH (0.291 mmol) followed by 31 μL (0.255 mmol) of benzoyl chloride, and the mixture was stirred for 10 min. The sequence of 291 μL of 1 N NaOH addition, 31 μL of benzoyl chloride addition, and 10-min stir at 0 °C was repeated four more times while maintaining an alkaline pH during the stirring period by addition of 2 N NaOH when necessary. The reaction mixture was then stirred for 30 min at 0 °C and 20 min at room temperature, diluted with 1 mL of water, acidified to Congo red with concentrated HCl, allowed to stand for 30 min in an ice bath, and filtered. The white precipitate was washed with ice cold water and dried in a vacuum desiccator over $MgSO_4$ to give a white solid which was treated to insure removal of the by-product benzoic acid by combining with 2 mL of boiling CCl_4 and filtering (the solid was washed on the filter with three portions of 1 mL of boiling CCl_4). Recrystallization of the solid from boiling ethanol provided the pure acid **40** (290 mg, 89%): mp 194 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.90 (d, 1, J = 8.2 Hz, NH), 7.83 (d, 2, J = 7.7 Hz, arom CH's ortho to CONHR), 7.22–7.53 (m, 8, arom CH's), 5.43 (m, 1, PhCHRR′), 2.88 (dd, 1, J_1 = 15.7 Hz, J_2 = 9.0 Hz, RCHHCO$_2$H), 2.76 (dd, 1, J_1 = 15.7 Hz, J_2 = 6.1 Hz, RCHHCO$_2$H); mass spectrum, m/z 269 (M$^+$, 2), 164 (61), 105 (100), 77 (60); IR (Nujol) 3360 (N—H), 1701 (acid C=O), 1636 (amide C=O), 1316, 1088 cm^{-1}. Anal. ($C_{16}H_{15}NO_3$) C, H, N.

***p*-Nitrophenyl 3-(Propionylamino)-3-phenylpropanoate (41).** A mixture of the acid **39** (90 mg, 0.407 mmol), 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (173 mg, 0.407 mmol), and methylene chloride (4 mL) was stirred under nitrogen for 20 min, and then *p*-nitrophenol (85 mg, 0.610 mmol) was added). After stirring overnight (a precipitate formed), the mixture was combined with 75 mL of EtOAc/20 mL of water and separated. The organic phase was washed with brine, dried ($MgSO_4$), and concentrated to a white solid which was purified by flash chromatography (33 mm, 60% EtOAc/hexane) to provide the white solid **41** (105 mg, 75%): mp 156–157 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.22 (d, 2, J = 9.0 Hz, arom CH's ortho to NO_2), 7.37 (m, 5, Ph), 7.11 (d, 2, J = 9.0 Hz, arom CH's ortho to OCO), 6.17 (d, 1, J = 6.9 Hz, amide NH), 5.60 (m, 1, PhCHRR′), 3.26 (dd, 1, J_1 = 15.2 Hz, J_2 = 6.7 Hz, RCHHCO$_2$R′), 3.11 (dd, 1, J_1 = 15.2 Hz, J_2 = 6.3 Hz, RCHHCO$_2$R′), 2.27 (qt, 2, J = 7.5 Hz, NHCOCH_2CH$_3$), 1.17 (t, 3, J = 7.5 Hz, NHCOCH$_2$CH_3); mass spectrum, m/z 342 (M$^+$, 2), 285 (22), 204 (34), 162 (37), 131 (59), 106 (100); IR ($CHCl_3$) 3446 (NH), 1761 (ester C=O), 1674 (amide C=O), 1528 (NO_2), 1348 (NO_2), 1206, 1145 cm^{-1}. Anal. ($C_{18}H_{18}N_2O_5$) C, H, N.

***p*-Nitrophenyl 3-(Benzoylamino)-3-phenylpropanoate (42).** A mixture of the acid **40** (90 mg, 0.335 mmol), 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (142 mg, 0.335 mmol), and methylene chloride (4 mL) was stirred under nitrogen for 20 min, and *p*-nitrophenol (70 mg, 0.503 mmol) was then added. After stirring overnight, the mixture was combined with 75 mL of EtOAc/20 mL of water and separated. The organic phase was washed with brine, dried ($MgSO_4$), and concentrated to an oil which was purified by flash chromatography (33 mm, 65% ether/hexane) to provide the white solid **42** (87 mg, 67%): mp 147–149 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.22 (d, 2, J = 9.0 Hz, arom CH's ortho to NO_2), 7.80 (d, 1, J = 7.4 Hz, arom CH's ortho to CONH), 7.36–7.53 (m, 8, C$_6H_5$CHRR′ and arom CH's meta and para to CONH), 7.12 (d, 1, J = 9.0, arom CH's ortho to OCO), 7.03 (d, 1, J = 7.9, amide NH), 5.80 (m, 1, PhCHRR′), 3.39 (dd, 1, J_1 = 15.4 Hz, J_2 = 6.3 Hz, RCHHCO$_2$R′), 3.23 (dd, 1, J_1 = 15.4 Hz, J_2 = 6.1 Hz, RCHHCO$_2$R′); mass spectrum, m/z 390 (M$^+$, 3), 285 (68), 252 (79), 251 (100), 223 (67), 139 (75), 105 (45); IR ($CHCl_3$) 3451 (NH), 1761 (ester C=O), 1663 (amide C=O), 1528 (NO_2), 1348 (NO_2), 1206, 1127 cm^{-1}. Anal. ($C_{22}H_{18}N_2O_5$) C, H, N.

3-Phenyl-3-(formylamino)propanoic Acid (43). This procedure was adapted from Cohen and Weinstein.[13] 3-Amino-3-phenylpropanoic acid (2.0 g, 12.1 mmol) was dissolved in 3 mL of formic acid, heated to a gentle reflux for 3 h, and concentrated. This procedure was repeated two more times and the resulting oil was dissolved in 50 mL of water and extracted with three 50-mL portions of ethyl acetate. The combined ethyl acetate extracts were dried (Na_2SO_4), treated with charcoal, filtered, and concentrated to a cream-colored solid, 1.78 g. Recrystallization from 10 mL of boiling water gave light yellow crystals (1.69 g, 72%): mp 123–124 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.62 (d, 2, J = 8.5 Hz, amide NH), 8.01 (s, 1, NCHO), 7.21–7.36 (m, 5, arom CH's), 5.23 (m, 1, PhCHRR′), 2.68 (d, 2, J = 7.3 Hz, RCH_2CO$_2$H); mass spectrum, m/z 193 (M$^+$, 16), 164 (M − CHO, 65), 147 (m − HCO$_2$H, 96), 119 (48), 106 (83), 104 (100), 79 (87), 77 (91). Anal. ($C_{10}H_{11}NO_3$) C, H, N.

***p*-Nitrophenyl 3-Phenyl-3-(formylamino)propanoate (45).** This ester was prepared from the formylamino acid **43** (97 mg, 0.503 mg), by the same procedure used to prepare the carboxamide *p*-nitrophenyl ester **24**. Purification by flash chromatography (30 mm, 80% ethyl acetate/hexane) gave a yellow solid (120 mg, 76%): mp 95–96 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.23 (s, 1, NCHO), 8.20 (d, 2, J = 9.1 Hz, arom CH's ortho to NO_2), 7.32–7.43 (m, 5, C_6H_5), 7.11 (d, 2, J = 9.1 Hz, arom CH's ortho to OCO), 6.65 (d, 1, J = 8.1 Hz, NH), 5.66 (m, 1, PhCHRR′), 3.27 (dd, 1, J_1 = 15.5 Hz, J_2 = 6.9 Hz, RCHHCO$_2$R′), 3.13 (dd, 1, J_1 = 15.5 Hz, J_2 = 6.2 Hz, RCHHCO$_2$R′); mass spectrum, m/z 176 (M − OC$_6$H$_4$NO$_2$, 22), 175 (M − HOC$_6$H$_4$NO$_2$, 15), 77 (43), 65 (63); IR ($CHCl_3$) 3430 (amide), 2875, 1763 (ester C=O), 1690, 1593, 1528 (NO_2), 1491, 1348 (NO_2), 1134 cm^{-1}. Anal. ($C_{16}H_{14}N_2O_5$) C, H, N.

***p*-Nitrophenyl 3-[(Trifluoroacetyl)amino]-3-phenylpropanoate (46).** To a cooled (0 °C) solution of 3-amino-3-phenylpropanoic acid (250 mg, 1.52 mmol) dissolved in trifluoroacetic acid (9.0 mL) was added trifluoroacetic anhydride (257 μL, 1.81 mmol) over a 10-min period. The resulting solution was allowed to stir under nitrogen for 5 min at 0 °C and 30 min at room temperature. The solution was then concentrated, dissolved in ethyl acetate (60 mL), and washed successively with 5% $HCl_{(aq)}$ (three portions), $NH_4Cl_{(satd)}$, and $NaCl_{(satd)}$. The organic phase was dried ($MgSO_4$) and concentrated to a white solid that was recrystallized from ethyl acetate/hexane to yield 192 mg of the trifluoroacetylated amino acid **44**: ^{19}F NMR (90 MHz, CD_3OD) δ −75.55 (s).

A portion of the acid (126 mg, 0.483 mmol) was added to a mixture of 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (205 mg, 0.483 mmol) and methylene chloride (3 mL). *p*-Nitrophenyl (60 mg, 0.435 mmol) was then added and the homogeneous solution was stirred for 5.5 h under nitrogen, and then concentrated to a solid which was purified by flash chromatography (20 mm, 25% ethyl acetate/hexane) to provide the white solid **46** (93 mg, 24%): mp 117–118 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.24 (d, 2, J = 9.1 Hz, arom CH's ortho to NO_2), 7.40 (m, 5, Ph), 7.23 (br d, 1, J = 7.9 Hz, NH), 7.11 (d, 2, J = 9.1 Hz, arom CH's ortho to OCO), 5.58 (m, 1, PhCHRR′), 3.28 (d of AB qt, 2, J_1 = 16.0 Hz, J_2 = 6.1 Hz, Δδ = 0.13, RCH$_2$CO$_2$R′); ^{19}F NMR (90 MHz, $CDCl_3$) −76.31 (s); mass spectrum, m/z 244 (M − O(C$_6$H$_4$)NO$_2$, 34), 202 (100), 131 (34), 79 (22); IR ($CHCl_3$) 3420 (N—H), 1763 (ester C=O), 1728 (amide C=O), 1527 (NO_2), 1348 (NO_2), 1266, 1165 cm^{-1}. Anal. ($C_{17}H_{13}F_3N_2O_5$) C, H, N, F.

(*S*)-[[1′-(Trifluoromethyl)ethylidene]amino]-2-(methoxymethyl)pyrrolidine (47). A mixture of trifluoroacetone (1.4 mL, 1.54 mmol) and SAMP (1.0 g, 7.69 mmol) was placed in a 3-mL reacti-vial, sealed, placed in a fume hood behind a blast shield, and heated to 90 °C with stirring for 1 h. The resulting oil was purified by flash chromatography (62 mm, 15% ethyl acetate/hexane) to give a clear colorless oil (1.17 g, 68%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.61 (m, 2, CH_2O), 3.52 (m, 1), 3.41 (m, 1), 3.40 (s, 3, OCH_3), 2.84 (m, 1), 2.01 (s, 3, CCH_3), 1.85–2.03 (m, 3), 1.73 (m, 1); ^{19}F NMR (90 MHz, $CDCl_3$) δ −70.73 (s); mass spectrum, m/z 224 (M$^+$, 3), 179 (M − CH_2OCH_3, 100), 155 (16), 69 (30), 43 (52); IR ($CHCl_3$) 3017, 2880, 1329, 1227, 1111 (C–F) cm^{-1}. Anal. ($C_9H_{15}F_3N_2O$) C, H, N, F.

4-Phenyl-6-methyl-3,4-dihydro-2-pyranone (51). The methylcarbonyl acid **9** (28 mg, 0.136 mmol) was dissolved in thionyl

chloride (1 mL) and stirred under nitrogen for 30 min. The reaction solution was then concentrated, and the resulting crude product was distilled (150 °C/0.8 Torr) to give a clear colorless oil (19 mg, 74%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.18–7.36 (m, 5, Ph), 5.17 (d, 1, J = 7.1 Hz, C=CH), 3.77 (m, 1, PhCHRR'), 2.93 (dd, J_1 = 14.4 Hz, J_2 = 7.4 Hz, $CHHCO_2R$), 2.69 (dd, J_1 = 14.4 Hz, J_2 = 9.3 Hz, $CHHCO_2R$), 2.01 (s, 3, CH_3); mass spectrum, m/z 188 (M^+, 27). 160 (48), 145 (87), 130 (100), 103 (42), 43 (91). Exact mass calcd for $C_{12}H_{12}O_2$ m/e 188.0837, obsd m/e 188.0850.

4,5-Dihydro-1,3-oxazin-6-one (52). To 3.5 mL of $CHCl_3$, freshly distilled from P_2O_5, was added the acid **16** (103 mg, 0.50 mmol) and 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (233 mg, 0.55 mmol), weighed under a nitrogen atmosphere. The mixture was heated to gentle reflux with stirring for 2.5 h, then allowed to stir at room temperature for 3 h. The reaction mixture becomes homogeneous upon heating and forms a heavy white precipitate after 0.50–2 h at reflux. Then 40 mL of ether was added, the reaction mixture was filtered, and the filtrate was washed briefly with H_2O, dried (Na_2SO_4), and concentrated to a clear colorless oil (91 mg, 96%). The product will decompose rapidly into a white solid unless kept in the freezer, where it will remain stable for several days: ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.32 (m, 5) 4.80 (m, 1), 2.92 (dd, 1, J_1 = 7 Hz, J_2 = 16 Hz), 2.55 (dd, 1, J_1 = 10 Hz, J_2 = 16 Hz), 2.20 (d, 3, J = 1.3 Hz); mass spectrum, m/z 189 (M^+, 21), 161 (14), 147 (17), 132 (10), 119 (16), 104 (55), 43 (100); IR ($CHCl_3$) 2996, 1783, 1704, 1380, 1117, 989 cm^{-1}. Anal. ($C_{11}H_{11}NO_2$) C, H, N.

3-(Acetylamino)-3-phenylpropanal (54). To a 5 mL of benzene was added 76 mg (0.40 mmol) of freshly prepared oxazinone **53** and 30 mg of 5% palladium on carbon. The mixture was stirred under a hydrogen atmosphere for 12 h, filtered, and concentrated. Purification by preparative scale thin-layer chromatography on silica gel with ethyl acetate development gave the oil **54** (47 mg, 61%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 9.79 (s, 1), 7.35 (m, 5), 6.10 (br s, 1), 5.51 (m, 1) 3.10 (m, 5), 2.00 (s, 3); mass spectrum, m/z 191 (M^+, 1), 163 (5), 148 (8), 106 (15), 86 (78), 84 (100), 47 (28); IR ($CHCl_3$) 3430, 3310, 2995, 2925, 2850, 2725, 1725, 1662, 1494, 1370, 1095 cm^{-1}. Exact mass calcd for $C_{11}H_{13}NO_2$ m/e 191.0946, obsd m/e 191.0941.

B. Biochemical Procedures. General. Kinetic assays were performed by using a Hewlett-Packard 8451A diode array spectrophotometer. α-Chymotrypsin (three times crystallized and free of autolysis products and low molecular weight contaminants) was obtained from Worthington Biochemical. *N*-Succinyl-L-alanyl-L-alanyl-L-prolyl-L-phenylalanine *p*-nitroanilide (Suc-L-Ala-L-Ala-L-Pro-L-Phe *p*NA) was obtained from Sigma Chemical Co. Dimethyl sulfoxide (DMSO) was obtained from Fisher Scientific.

Active enzyme concentrations were determined by active site titration with *N-trans*-cinnamoylimidazole,[32] or from published kinetic parameters for the substrate Suc-L-Ala-L-Ala-L-Pro-L-Phe *p*NA.[33] All kinetic studies were performed at 25 °C, and the concentrations given below represent final concentrations after all the incubation solutions are combined.

Proflavin Displacement Assays. This procedure follows that of Baek et al.[5] To a mixture of 100 μL of a 100 μM proflavin solution in pH 7.2 phosphate buffer (0.1 M) and 1.0 mL of a 100 μM α-chymotrypsin stock solution in phosphate buffer was added 10 μL of a 10 μM solution of the *p*-nitrophenyl ester substrate in DMSO at 25 °C. (The final concentrations of proflavin, enzyme, and substrate were 9 μM, 90 μM, and 90 μM, respectively.) The change in absorbance was then followed at 466 nm, and a semilogarithmic plot of absorbance versus time gave a straight line with a slope of k_d. Racemic substrates often gave a biphasic progress curve from which two deacylation rates could be obtained, k_d(fast) and k_d(slow).

Competitive Substrate Assays. Progress curves were obtained as follows. Into a 1.5-mL cuvet were combined 875 μL of 0.1 M phosphate buffer (pH 7.2), 25 μL of a 5.1 mM DMSO solution of MeO-Suc-L-Ala-L-Ala-L-Pro-L-Phe *p*NA (128 μM), 2–70 μL of a 5.00 μM DMSO stock solution of lactone **11a** or **11b** (final concentrations are 50–350 μM for **11a** and 10–110 μM for **11b**), and 5–73 μL of DMSO (to give, with lactone stock solution, a total DMSO volume of 100 μL). To this cuvet was added 25 μL of a buffered 1 μM α-chymotrypsin stock solution (25 nM), and the change in absorbance at 410 nM was recorded over a time intervals ranging from 0–32 to 0–300 seconds. A semilogarithmic plot of absorbance versus time gave a straight line with a slope of k_{obs}. Triplicate determinations of k_{obs} were made from three progress curves obtained at each initial ester concentration. Binding constants, K_S, and acylation rate constants, k_a, were obtained from graphs of I_o/k_{obs} versus I_o by use of eq 2, derived by Main.[18] In

$$I_o/k_{obs} = (1/k_a)I_o + (K_I/k_a)[1 + (S_o/K_M)] \quad (2)$$

eq 2, I_o represents the initial β-substituted substrate concentration, S_o represents the initial chromogenic substrate concentration, and K_M represents the Michaelis constant for the chromogenic substrate (50 μM for Suc-L-Ala-L-Ala-L-Pro-L-Phe *p*NA with α-chymotrpysin in the indicated buffer[4]). Error limits, representing the absolute standard deviations of k_a and K_I, were obtained from the absolute standard deviations of the slope and y intercept of the graph of I_o/k_{obs} versus I_o as described by Baek et al.[5]

Deacylation of the substrates **11a** and **11b** was noted during data collection. This resulted in a slow regeneration of free enzyme, causing a linear rate of chromogenic substrate turnover to be observed in the progress curves. This limiting rate was estimated for both substrates and subtracted from the data to obtain the true exponential approach to the steady state, k_{obs}.

Hydrolysis Rate Determinations. Hydrolysis in buffer at 25 °C was monitored spectrophotometrically or by HPLC. A semilogarithmic plot of analyte concentration versus time gave a straight line with a slope equal to the first order spontaneous hydrolysis rate, k_h.

k_h of the Methylcarbonyl *p*-Nitrophenyl Ester 10. Initially, a calibration curve relating absorbance at 400 nm to concentration of *p*-nitrophenol in 20% acetonitrile/buffer (0.1 M phosphate, pH 7.2) was constructed. The results showed that Beer's Law was obeyed in concentrations up to 100 μM (ε = 10 800). A 500 μM solution of **10** in buffer was incubated at 25 °C and absorbance at 400 nm was monitored continuously over the initial 20% of the reaction. A spontaneous hydrolysis rate (k_h) of 0.000 843 min^{-1} was determined from a semilogarithmic plot of *p*-nitrophenol concentration versus time.

k_h and k_c of 3-Phenylglutaric Anhydride (22). Anhydride hydrolysis was followed by HPLC. Analysis was made by direct injection of reaction solution aliquots onto a 4 mm × 30 cm Varian MCH-10 Micro Pak reverse-phase column, eluting with a 30:70 mixture of acetonitrile/water at a flow rate of 2.0 mL/min. The water eluent contained 0.05% trifluoroacetic acid. A Hewlett-Packard Model 3390A integrater was used to calculate peak areas from UV signals detected at 200 nM. A calibration curve showed a linear correlation between HPLC area and concentration of anhydride **22** over the range of anhydride concentrations used in this study. Anhydride concentration was monitored with 5-μL aliquots taken from a 30 mM solution of **22** dissolved in a 0.1 M phosphate buffer, pH 7.2, containing 10% acetonitrile. The retention times from the anhydride **22** and its hydrolysis product, 3-phenylglutaric acid, were 9.7 min and 2.6 min, respectively.

Using data covering the first three minutes of the reaction, obtained by combining data from several runs, we obtained a first-order rate constant, k_h, of 0.41 min^{-1}. First-order hydrolysis rates were also obtained from incubation solutions containing 10 nM α-chymotrypsin. In this case, the spontaneous hydrolysis rate, k_h, was subtracted from the hydrolysis rate obtained with enzyme to give a chymotrypsin-catalyzed hydrolysis rate, k_c, of 0.12 min^{-1}.

Acknowledgment. We are grateful for support of this research by a grant from the National Institutes of Health (Grant No. PHS 5RO1 DK27526). High-resolution mass spectra were obtained on instruments supported by grants from the National Institutes of Health (Grant No. RR 01575) and the National Science Foundation (Grant No PCM-8121494). Nuclear magnetic resonance spectra were obtained on instruments supported by grants from the National Institutes of Health (Grant No. NIH 1531957) and the National Science Foundation (Grant No. NSF

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CHE 85-14500). We thank Guy Bemis for helpful discussions.

Registry No. **6a**, 65651-52-7; **6b**, 72724-02-8; **7** (isomer 1), 131905-98-1; **7** (isomer 2), 89393-62-4; **7** (isomer 3), 131906-33-7; **7** (isomer 4), 131906-34-8; **8a**, 89393-74-8; **8b**, 108056-65-1; **9**, 122088-92-0; **9a**, 122020-03-5; **9b**, 108056-64-0; **10a**, 131905-99-2; **10b**, 131906-00-8; **11a**, 131906-01-9; **11b**, 131906-02-0; **12**, 131906-03-1; **12a**, 132015-37-3; **12b**, 132015-38-4; **13**, 131906-04-2; **13a**, 132046-17-4; **13b**, 132046-18-5; **14a**, 122088-88-4; **14b**, 122088-89-5; **15a**, 122020-04-6; **15b**, 122020-05-7; **16**, 91094-99-4; **16a**, 117020-31-2; **17**, 131906-05-3; **17a**, 132015-39-5; **18**, 17895-71-5; **19**, 131906-06-4; **20**, 131906-07-5; **21**, 131906-08-6; **22**, 4160-80-9; **23**, 131906-09-7; **24**, 131906-10-0; **25**, 132015-40-8; **26**, 131906-11-1; **27**, 131906-12-2; **28**, 132015-41-9; **29**, 131906-13-3; **30**, 131906-14-4; **31**, 131906-15-5; **32**, 131906-16-6; **33**, 131906-17-7; **34**, 132015-42-0; **35**, 131906-18-8; **36**, 131906-19-9; **37**, 131906-20-2; **38**, 131906-21-3; **39**, 131906-22-4; **40**, 132015-43-1; **41**, 131906-23-5; **42**, 131906-24-6; **43**, 126575-05-1; **44**, 64482-09-3; **45**, 131906-25-7; **46**, 64482-08-2; **47**, 131906-26-8; **48**, 131906-27-9; **49**, 131906-28-0; **50**, 131906-29-1; **51**, 131906-30-4; **52**, 131906-31-5; **54**, 131906-32-6; hydrocinnamic acid, 501-52-0; 3-phenylglutaric acid, 4165-96-2; *tert*-butyl acetate, 540-88-5; 3-amino-3-phenylpropanoic acid, 3646-50-2; α-chymotrypsin, 9004-07-3.

Biologically Active Taxol Analogues with Deleted A-Ring Side Chain Substituents and Variable C-2′ Configurations

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Taxol (1), a potent inhibitor of cell replication, enhances the assembly of tubulin into stable microtubules and promotes the formation of microtubule bundles in cells. In addition to its unique mechanism of action, taxol exhibits unusual promise as an antitumor agent, but its application in cancer chemotherapy is hampered by its limited availability. In order to better define the structure-activity profile of taxol for the design of more accessible drugs and to provide insight into the chemical features of the taxol–microtubule interaction, taxol analogues **3**–**8**, with deleted A-ring side chain substituents and both *R* and *S* C-2′ configurations, were synthesized from baccatin III (**2**) through esterification at the hindered 13-hydroxyl. Employing an improved hydroxyl protection strategy, lactate analogues **3** and **4** were prepared with reasonable efficiency owing to their simple side-chain structures, while *N*-benzoylisoserine analogues **7** and **8** were synthesized through esterification reactions whose rates were enhanced greatly by the participation of the amide functionality. Although less biologically active than taxol, analogues **5**–**7** were found to promote the polymerization of tubulin and to be cytotoxic; **5** and **6** were considerably more effective than **7**, whereas **3**, **4**, and **8** were least active. Interestingly, tubulin polymerization was sensitive to the C-2′ configuration only when the amide substituent was present in the side chain. This observation suggests that the 3′-amide substituent plays an important role in preorganizing the taxol side chain to bind to microtubules.

Introduction

Taxol[1] (**1**) is unique among antimitotic drugs in that it promotes the *assembly* of stable microtubules from tubulin under otherwise unfavorable conditions, i.e. low temperature, the absence of microtubule associated proteins or exogenous GTP, and the presence of Ca ions. The drug binds to microtubules, stabilizing them against depolymerization.[2,3] A presumed consequence of these effects is the significant antitumor activity exhibited by taxol, most notably, at this time, against drug-refractory human ovarian tumors.[4] Currently, the only impediment to the more widespread application of taxol in cancer chemotherapy is its extremely limited availability: taxol is isolated in exceptionally poor yield from the bark of the slow-growing Pacific yew (*Taxus brevifolia*; 1 kg of taxol/20 000 lb of bark from 2000–3000 yew trees),[5] which is indigenous to the ecologically threatened old-growth forests of the Pacific northwest.

Since the initial biological evaluation of taxol and baccatin III (**2**) that established the critical role played by the taxol A-ring side chain,[1] the insignificance of the identity of the acyl group on the 3′-amino substituent, and the importance of the 2′-hydroxyl have become apparent.[3,6] We have sought to define further the taxol structure–activity profile for the locus of the side chain with two

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