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The 3-(3-Pyridine)propionyl Anchor Group for Protease-Catalyzed Resolutions: *p*-Toluenesulfinamide and Sterically Hindered Secondary Alcohols

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Abstract: Compared to an acetyl acyl group, the 3-(3-pyridine)propionyl group increases substrate binding to many proteases and substrate solubility in water, thereby increasing the rates of protease-catalyzed reactions. For example, proteases reacted up to six hundred-fold faster with the 3-(3-pyridine)propionyl ester of 1-phenylethanol than with the corresponding acetate ester. In addition, the 3-(3-pyridine)propionyl group enables a simple, mild acid extraction to separate the remaining starting material and product. To demonstrate the synthetic usefulness of this strategy, we resolved multi-gram quantities of (*R*)- and (*S*)-*p*-toluenesulfinamide with α-chymotrypsin and gram quantities of (*R*)- and (*S*)-2,2-dimethylcyclopentanol with subtilisin Carlsberg. The 3-(3-pyridyl)propionyl group was better for these resolutions than the corresponding acetate or dihydrocinnamate because it decreased the reaction time due to increased reactivity, decreased the reaction volume due to increased substrate solubility and enabled purification without chromatography. Molecular modeling suggests the enantioselectivity of α-chymotrypsin toward (*R*)-*p*-toluenesulfinamide is high ($E = 52$) because of a favorable hydrophobic interaction between the *p*-tolyl group of the fast-reacting (*R*)-enantiomer and leaving group pocket. The enantioselectivity of subtilisin Carlsberg toward (*S*)-2,2-dimethylcyclopentanol is high ($E = 43$) because the large substituent (the 2,2-dimethyl quaternary carbon) of the slow-reacting (*R*)-enantiomer cannot fit in the S_1' leaving group pocket.

Keywords: biocatalysis; chiral auxiliary; enantioselective; protease; resolution

Introduction

Lipases and proteases are both useful enantioselective catalysts for resolutions,[1–3] but their preferred substrates differ significantly. Lipases favor insoluble substrates and bind them in deep hydrophobic pockets[4] in a folded conformation.[5] Proteases, on the other hand, require water-soluble substrates and bind them in a shallow active site in an extended conformation.[6] For example, during resolution of esters of secondary alcohols, lipases bind both substituents in hydrophobic pockets within the active site, but proteases bind only one substituent leaving the other substituent in the solvent. The shallow active site allows proteases to accept more sterically hindered substrates[7,8] than lipases and to accept polar substrates since one substituent can remain in water.[9]

While the hydrophobic nature of insoluble substrates binds them to the active site of lipases, proteases contain a specificity pocket to bind substrates.[10,11] For example, subtilisins and chymotrypsin favor esters and amides of amino acids containing hydrophobic side chains such as phenylalanine esters.[10–12] The dihydrocinnamoyl group mimics phenylalanine so dihydrocinnamoyl esters are often good substrates for proteases. α-Chymotrypsin binds dihydrocinnamoyl esters as tightly as *N*-acetylphenylalanine esters and they react at least one hundred-fold faster than *N*-acetylglycine esters.[10] We previously used the dihydrocinnamoyl group to extend subtilisin E to a new class of substrates, *N*-acyl arylsulfinamides, to make enantiopure sulfinamides.[7b] Subtilisin E did not catalyze the hydrolysis of *N*-acetyl arylsulfinamides, but it did catalyze the hydrolysis of *N*-dihydrocinnamoyl arylsulfinamides. In this paper, we introduce another analogue of phenylalanine – the 3-(3-pyridine)propionyl group. This group not only anchors the substrate in the active site of the protease, but also increases the water solubility of the ester as compared to the dihydrocinnamoyl group and, be-

cause it contains a basic nitrogen atom, enables separation of product and remaining starting material by mild acid extraction. We report efficient resolutions of *N*-3-(3-pyridine)propionyl-*p*-toluenesulfinamide (**1a**) and two sterically hindered secondary alcohol esters, 2,2-dimethylcyclopentyl 3-(3-pyridine)propionate (**3a**) and 1-(2-mesityl)ethyl 3-(3-pyridine)propionate (**4a**).

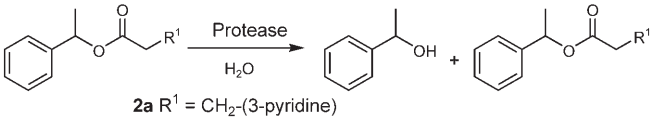
Results and Discussion

Carbodiimide-promoted coupling of 1-phenethyl alcohol with 3-(3-pyridine)propionic acid yielded 1-phenethyl 3-(3-pyridine)propionate (**2a**). Compound **2a** was stable in buffered solutions (pH 7.2) for >5 days, like the corresponding acetate **2b**. 1-Phenethyl 3-(3-pyridine)propionate (**2a**) reacted with six commonly used proteases up to 600-fold faster than the acetate **2b** (Table 1). Subtilisin BL (EC 3.4.21.62), *Aspergillus melleus* protease (EC 3.4.21.63), subtilisin Carlsberg (EC 3.4.21.62) and *Streptomyces griseus* (EC 3.4.21.80) protease-catalyzed hydrolyses proceeded 15- to 70-fold faster, α-chymotrypsin (EC 3.4.21.21) from bovine pancreas reacted 600-fold faster, while *Aspergillus oryzae* (EC 3.4.21.63) reacted at the same rate as acetate **2b**.

The 3-(3-pyridine)propionate **2a** also reacted 3- to 40-fold faster than the dihydrocinnamate **2c** for these six proteases (Table 1), likely due to the increased solubility of **2a** over **2c**. Subtilisin BL, *Aspergillus melleus* protease, *Aspergillus oryzae* protease and *Streptomyces griseus* protease-catalyzed hydrolyses proceeded 10- to 23-fold faster, subtilisin Carlsberg reacted 40-fold faster, while α-chymotrypsin from bovine pancreas reacted only 3-fold faster. Although dihydrocinnamate **2c** mimics phenylalanine, five of the six proteases reacted no faster with **2c** than with acetate **2b**, likely because of the lower water solubility of **2c** as compared to acetate **2b**. The exception was α-chymotrypsin, which reacted 200-fold faster with dihydrocinnamate **2c** than with acetate **2b**. This exception is consistent with the strong preference of α-chymotrypsin for phenylalanine as acyl group.[13]

A different leaving group – *p*-toluenesulfinamide – showed a similar trend. Both *N*-3-(3-pyridine)propionyl-*p*-toluenesulfinamide (**1a**) and *N*-dihydrocinnamoyl-*p*-toluenesulfinamide (**1c**) were good substrates for α-chymotrypsin and subtilisin BPN′, while *N*-acetyl-*p*-toluenesulfinamide (**1b**) did not react (Table 2).[14] α-Chymotrypsin and subtilisin BPN′ gave 21–42% conversion for **1a** and 19–39% conversion for **1c** after 3 h, while acetate **1b** showed no reaction after 24 h. In this case, both **1a** and **1c** showed similar activity in contrast to **2a** and **2c** above where the 3-(3-pyridine)propionate **2a** reacted 3 to 40-fold faster. A possible reason for this similarity is that compound **1c** already dissolves in water at a concentration near its K_M value (solubility *ca.* 5 mM in 10% DMF) and therefore the higher solubility of **1a** (solubility *ca.* 50 mM in 10% DMF) does not significantly increase the fraction of protease containing bound

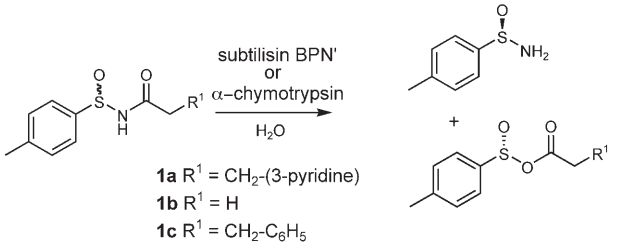
Table 1. Specific activity of proteases toward secondary alcohol esters **2a**, **2b** and **2c**.



Protease	Prot. conc. [mg/mL][a]	Specific activity[b] 2a	2b	2c
Bacillus lentus subtilisin[c]	2.4	10	0.6	0.7
Aspergillus melleus[c]	4.4	4.6	0.3	0.3
Aspergillus oryzae[c]	5.1	1.0	1.0	0.1
subtilisin Carlsberg[d]	9.0	20	0.5	0.5
α-chymotrypsin[d]	8.6	19	0.03	5.4
Streptomyces griseus[d]	6.6	7	0.1	0.3

[a] Protein concentration was determined by the method of Bradford using bovine serum albumin (BSA) as standard.[43]
[b] Specific activity is calculated in μmol/min mg^{-1} protein.
[c] Altus Biologics (Cambridge, USA).
[d] Sigma–Aldrich (St. Louis, USA).

Table 2. Reactivity and enantioselectivity of α-chymotrypsin and subtilisin BPN′ towards **1a**, **1b** and **1c.**



Protease	1a %c[a]	*E*[b]	1b %c	*E*	1c %c	*E*
α-chymotrypsin[c]	42[e]	52	n.r.[f]	n.d.[g]	39[e]	56
subtilisin BPN′[d]	21	125	n.r.	n.d.	19	75

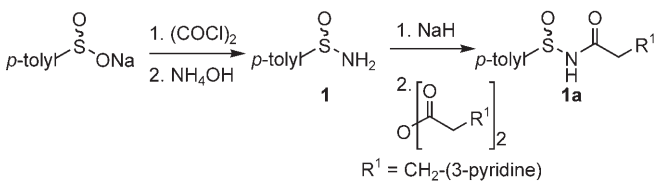
[a] % Conversion: amount of sulfinamide formed after 24 h except where noted.
[b] Enantiomeric ratio: the enantiomeric ratio *E* measures the relative rate of hydrolysis of the fast-reacting enantiomer as compared to the slow-reacting enantiomer as defined by Sih.[44]
[c] Sigma–Aldrich (St. Louis, USA).
[d] For protein expression and purification details see ref.[9]
[e] Reaction for 3 h.
[f] No reaction.
[g] Not determined.

substrate. Nevertheless, compound **1a** is still preferred because its higher solubility decreases the reaction volume and simplifies separation of product and remaining starting material (see below).

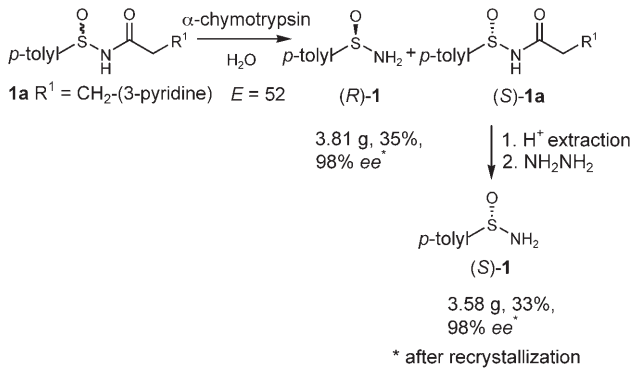
The 3-(3-pyridine)propionyl derivatives showed similar or better enantioselectivity than the dihydrocinnamoyl derivatives (Table 2). α-Chymotrypsin showed an enantioselectivity of 52 favoring the (*R*)-enantiomer in the hydrolysis of *N*-3-(3-pyridine)propionyl **1a** versus 56 for *N*-dihydrocinnamoyl **1c**. Subtilisin BPN′ showed an enantioselectivity of 125 favoring the (*R*)-enantiomer in the hydrolysis of *N*-3-(3-pyridine)propionyl **1a** *versus* 75 for *N*-dihydrocinnamoyl **1c**. In previous work subtilisin E showed an enantioselectivity > 150 favoring the (*R*)-enantiomer for *N*-dihydrocinnamoyl **1c**.[7b,15] Subtilisin BPN′ or subtilisin E are the best proteases for preparative-scale resolution of **1a**. However, preparation of subtilisin BPN′ or subtilisin E requires a fermentation,[16] and many organic chemistry laboratories lack access to fermentation facilities. For this reason, we use the commercially available α-chymotrypsin as the large-scale example in this work. The enantioselectivity of commercial α-chymotrypsin toward sulfinamides varied with different samples from $E = 21$ to 63 for **1a** and $E = 18$ to 87 for **1c**. If one has access to fermentation facilities, we recommend using subtilisin BPN′ or E as both show consistently high enantioselectivity.

To prepare substrate **1a**, we treated *p*-toluenesulfinic acid with oxalyl chloride, followed by ammonolysis to give the *p*-toluenesulfinamide in 86% yield (Scheme 1).[17] Acylation using a mixed anhydride prepared from isobutyl chloroformate and 3-(3-pyridine)propionic acid gave < 10% yield,[17] so we used the symmetrical anhydride of 3-(3-pyridine)propionic acid. This symmetrical anhydride was prepared by $KMnO_4$ oxidation[18] of 3-(3-pyridine)propanol and coupling of the resulting acid using carbodiimide.[17] Thus, treating *p*-toluenesulfinamide with NaH, followed by addition of the symmetrical anhydride of 3-(3-pyridine)propionic acid yielded the crude **1a**. Trituration with hexanes/ethyl acetate removed unreacted *p*-toluenesulfinamide **1** and gave **1a** in 73% yield. This synthetic strategy is simple, inexpensive and does not require low temperatures, special equipment or chromatography.

We resolved **1a** (20.2 g, 70 mmol) with α-chymotrypsin (12 g) to give (*R*)-**1** (4.48 g, 41% yield; the



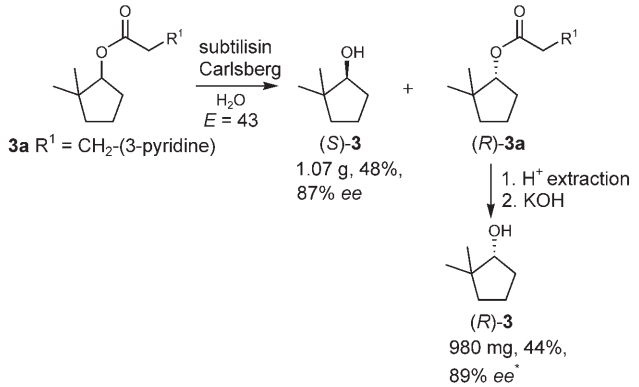
Scheme 1. Synthesis of racemic *N*-3-(3-pyridine)propionyl-*p*-toluenesulfinamide (**1a**).



Scheme 2. α-Chymotrypsin-catalyzed resolution of **1a**.

maximum yield is 50% in a resolution) with 87% *ee* at 51% conversion after 4 d (Scheme 2). The remaining starting material was conveniently separated from the product *via* acid extraction (0.1 N HCl). Treating (*S*)-**1a** with hydrazine hydrate[19] gave (*S*)-**1** (4.29 g, 40% yield) with 92% *ee*. Recrystallization from hexanes/ethyl acetate gave (*R*)-**1** (3.81 g, 35% yield) with 98% *ee* and (*S*)-**1** (3.58 g, 33% yield) with 98% *ee*. The enantioselectivity ($E = 47$) was slightly lower than with small-scale reactions.

To demonstrate the usefulness of the 3-(3-pyridine)-propionyl group for resolving hindered secondary alcohols, we resolved 2,2-dimethylcyclopentanol (**3**) using subtilisin Carlsberg. 2,2-Dimethylcyclopentanol (**3**) can be prepared *via* asymmetric reduction using chiral organoborane reagents, but it requires long reaction times and low temperatures, and shows variable selectivity.[20–22] Subtilisin Carlsberg previously showed high enantioselectivity with a structurally related substrate, *cis*,*cis*-6-(2,2-dimethylpropamido)-spiro[4.4]nonan-1-ol.[18] Subtilisin Carlsberg also showed good enantioselectivity toward 2,2-dimethylcyclopentyl 3-(3-pyridine)propionoate (**3a**) ($E = 43$). Thus, we resolved **3a** (4.95 g, 20 mmol) with subtilisin Carlsberg (4.5 g) to give (*S*)-**3** (1.07 g, 48% yield) with 87% *ee* at 51% conversion (Scheme 3). The

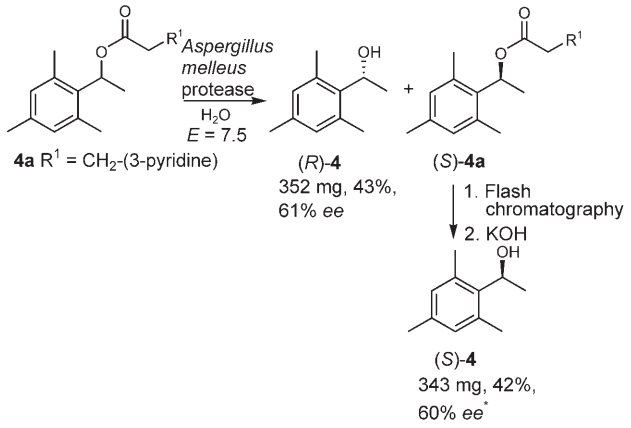


Scheme 3. Subtilisin Carlsberg-catalyzed resolution of **3a**.

product and remaining starting material were conveniently separated *via* acid extraction (0.1 N HCl) of the remaining starting material. Subsequent hydrolysis of the remaining starting material gave (*R*)-**3** (980 mg, 44% yield) with 89% *ee* (Scheme 3). Lipases from *Candida rugosa* (CRL, EC 3.1.1.3) and *Pseudomonas cepacia* (PCL, EC 3.1.1.3) did not catalyze the hydrolysis of **3a**; however, lipase B from *Candida antarctica* (CALB, EC 3.1.1.3) catalyzed the enantioselective hydrolysis of **3a** ($E=108$) to give the (*R*)-enantiomer. This discovery provides an enantiocomplementary strategy for synthesizing **3**. Although kinetic resolution yields both enantiomers, enantiocomplementary enzymes can simplify the next synthetic steps by providing the necessary enantiomer without further manipulation.[8]

We also resolved 1-(2-mesityl)ethanol (**4**), a useful chiral inductor for cycloaddition reactions,[23] with moderate enantioselectivity on a milligram scale using the 3-(3-pyridine)propionyl group. Lipases react slowly with esters of **4** because of steric hindrance of the *ortho*-methyl substituents with active site residues[24] and the acetate and dihydrocinnamate derivatives react only slowly with proteases (<1% conversion in 24 h). In contrast, all proteases tested catalyzed the hydrolysis of **4a** and *Aspergillus melleus* protease showed the highest enantioselectivity ($E=7.5$). We resolved **4a** (1.48 g, 5 mmol) with *Aspergillus melleus* protease (ca. 1.5 g) to give (*R*)-**4** (352 mg, 43% yield) with 61% *ee* at 49% conversion (Scheme 4). Acid extraction did not cleanly separate product and remaining starting material due to the poor solubility of the hydrophobic ester in acidic solutions, so we used chromatography to separate the product and remaining starting material. Subsequent hydrolysis of the remaining starting material gave (*S*)-**4** (343 mg, 42% yield) with 60% *ee*.

Molecular modeling showed that α-chymotrypsin binds the sulfinamide moiety of the fast-reacting (*R*)-enantiomer of **1a** similarly to subtilisin E, but binds the slow-reacting (*S*)-enantiomer differently.[7b] Modeling the first tetrahedral intermediate for the hydrolysis of **1a** with α-chymotrypsin gave one catalytically productive conformation for each enantiomer (Figure 1). The two other plausible conformations lacked catalytically essential hydrogen bonds or encountered severe steric clashes with the protein.[25] Both productive conformations bind the 3-(3-pyridine)propionyl group in the hydrophobic acyl pocket (specificity pocket)[26] of α-chymotrypsin, based on its similarity to phenylalanine.[13] The productive conformation of (*R*)-**1a** has the *p*-tolyl group in the hydrophobic leaving group pocket (*n*-site)[26] and the hydrophilic sulfoxide oxygen in the solvent. The *p*-tolyl group fits tightly in the leaving group pocket: [C_{ips}–S_γ (Cys42) distance = 3.79 Å, C_{ortho}–S_γ (Cys42) distance = 3.79 Å, C_{ortho}–$C_{\delta 2}$ (His57) distance = 3.67 Å, C_{ortho}–C_δ (Phe41) distance = 4.42 Å, C_{meta}–C_α (Cys58) distance = 3.92 Å].[27] This tight fit suggests a favorable hydrophobic interaction – tendency of non-polar compounds to transfer from an aqueous phase to an organic phase – between the *p*-tolyl group and leaving group pocket residues.[28] This favorable hydrophobic interaction may account for the faster reaction of this enantiomer. In contrast, for the slow-reacting enantiomer (*S*)-**1a**, steric interactions between the active site and the sulfinamide moiety prevent it from binding in this pocket so it only sits on top of this region: Met192 ($S_{sulfinamide}$–S_δ distance = 3.41 Å, $S_{sulfinamide}$–C_ε distance = 3.60 Å and C_{ipso}–C_ε distance = 3.65 Å). This productive conformation differs from the productive conformation of slow enantiomer (*S*)-**1c** bound to subtilisin E.[7b] With subtilisin E, the substituents at stereocenter are exchanged, which put the sulfoxide oxygen in the S_1' pocket and the *p*-tolyl group in the solvent. With α-chymotrypsin, the entire sulfinamide moiety of (*S*)-**1a** remains outside of the active site and exposed to the solvent and this lack of hydrophobic interaction may account for the slower reaction of this enantiomer.



Scheme 4. *Aspergillus melleus* protease-catalyzed resolution of **4a**.

The enantiopreference of subtilisin toward (*S*)-2,2-dimethylcyclopentanol [(*S*)-**3**] agrees with predictive models for subtilisin.[9,29] The enantiopreference of subtilisin toward secondary alcohol esters depends both on relative substituent polarity[9] and relative substituent size.[29] Subtilisin favors the (*R*)-enantiomer of secondary alcohols when the large substituent is approximately the size of phenyl because the phenyl-sized substituent makes a favorable hydrophobic interaction with the S_1' leaving-group pocket of subtilisin, as described above. Subtilisin favors the (*S*)-enantiomer of secondary alcohols when the large substituent is polar (hydrophilic) or too large to bind in the S_1' leaving-group pocket. For (*S*)-2,2-dimethylcyclopentanol [(*S*)-**3**], the large substituent is the 2,2-dimethyl quaternary carbon, which is too large for the

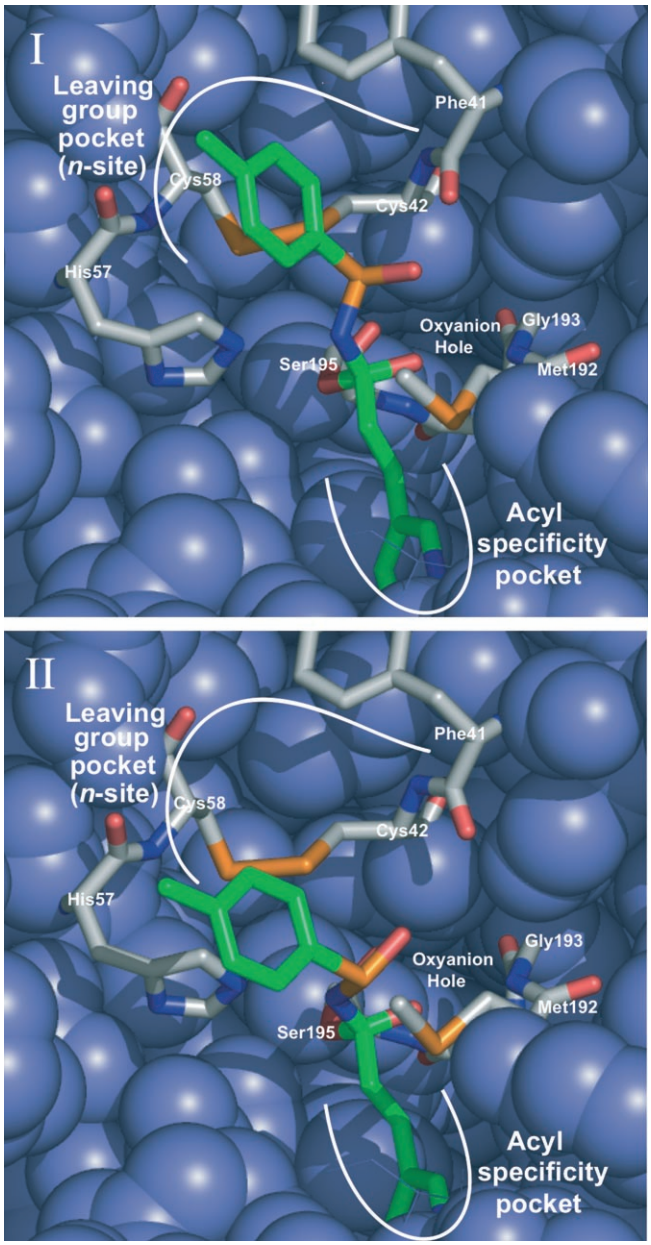


Figure 1. Catalytically productive tetrahedral intermediates for the α-chymotrypsin-catalyzed hydrolysis of (*R*)-**1a** (I) and (*S*)-**1a** (II) as identified by molecular modeling. The important active site and substrate atoms (sticks) are colored as follows: green (substrate carbon), grey (enzyme carbon), red (oxygen), blue (nitrogen) and orange (sulfur). Surrounding atoms (space fill) of α-chymotrypsin are shown in blue. For clarity, all hydrogen atoms and water molecules are hidden and Ser219 is hidden to expose the acyl group pocket. Both I and II maintain all catalytically essential hydrogen bonds and the 3-(3-pyridine)methyl moiety of the 3-(3-pyridine)propionate group binds in the S_1 pocket, as expected based on its similarity to phenylalanine.[13,26] In the fast-reacting enantiomer, (*R*)-**1a** (I), the *p*-tolyl group binds in the hydrophobic leaving group pocket and sulfoxide oxygen is exposed to solvent water. In the slow-reacting enantiomer, (*S*)-**1a** (II), the sulfoxide oxygen is forced out of the hydrophobic leaving group pocket because of steric interaction between the sulfinamide moiety and active site residues. This interaction places both the *p*-tolyl group and sulfoxide oxygen in solvent where this is no favorable hydrophobic interaction with leaving group pocket residues. The non-productive conformations of (*R*)-**1** and (*S*)-**1** (not shown) encountered severe steric clash with the active site residues and lacked catalytically essential hydrogen bonds.

S_1' pocket. This poor fit destabilizes reaction of the (*R*)-enantiomer and thus, the smaller methylene group binds in the S_1' pocket, favoring reaction of the (*S*)-enantiomer.

Sulfinamides, such as *p*-toluenesulfinamide, are useful chiral auxiliaries for synthesis of amines.[30] When condensed with an aldehyde or ketone to give the sulfinimine, the *N*-sulfinyl group directs nucleophilic addition across the C=N bond. This addition yields the *N*-alkylsulfinamide, which upon hydrolysis of the S–N link yields an amine. Enantioselective syntheses using *p*-toluenesulfinimine include preparations of amines,[31] α- and β-amino acids,[30] aziridines[32] and aminophosphonic acids.[33]

This enzyme-catalyzed route to enantiopure *p*-toluenesulfinamide **1** using the 3-(3-pyridine)propionyl group compares well with current chemical routes to sulfinamides. One chemical route to enantiopure *p*-toluenesulfinamide starts with menthyl *p*-toluenesulfinate (Andersen's reagent) prepared from menthol.[34] The natural (−)-enantiomer of menthol is readily available, but the unnatural (+)-enantiomer of menthol is *ca.* six times more expensive. The other synthetic route to enantiopure *p*-toluenesulfinamide is a double displacement strategy using a chiral auxiliary derived from indanol.[35] This route includes complicated steps requiring low temperatures and moisture- and air-sensitive reagents. Our enzymatic resolution is simple, convenient, avoids the use of costly auxiliaries and allows facile separation of product and remaining starting material.

Proteases are also useful for resolving sterically hindered alcohols, such as 2,2-dimethylcyclopentanol (**3**) and 1-(2-mesityl)ethanol (**4**), that cannot be easily prepared through chemical routes and react slowly with lipases. Proteases show higher reactivity toward these large alcohols when 3-(3-pyridine)propionic acid is used as acyl group and the enantioselectivity can be high when there is a large difference in the relative size[29] or polarity[9] of the alcohol substituents.

The 3-(3-pyridine)propionyl group maintains reactivity and enantioselectivity with subtilisin and α-chymotrypsin by mimicking phenylalanine and anchoring substrate to the active site. The pyridine moiety allows mild acid extraction to separate product and

remaining starting material and thus avoid chromatographic separation. Researchers have also tested other acyl groups that simplify separation of product and starting material. For example, Fukui and coworkers[36,37] used the succinyl monoester acyl group in a lipase-catalyzed resolution of menthol. While the succinate group allowed base extraction to separate product and remaining starting material, the charged succinate esters were poor substrates for lipases, which favor non-polar, insoluble substrates.[2] We previously tested the monomethyl ester succinyl acyl group for a subtilisin E-catalyzed resolution of *p*-toluenesulfinamide, but it showed lower enantioselectivity ($E=52$) than the dihydrocinnamoyl derivative ($E=>150$).[7b]

Conclusions

The 3-(3-pyridine)propionyl group increases substrate binding to proteases, increases substrate solubility in aqueous solutions, and provides a facile route for separation of remaining starting material and product. This is a cost-effective route for large-scale preparation of the useful chiral auxiliary, *p*-toluenesulfinamide **1**, and sterically hindered secondary alcohols that are poor substrates for other hydrolases.

Experimental Section

General Remarks

^{1}H and ^{13}C NMR spectra were obtained as $CDCl_3$ solutions at 300 MHz and 75 MHz, respectively. Chemical shifts are expressed in ppm (δ) and are referenced to tetramethylsilane or solvent signal. Coupling constants are reported in Hertz (Hz). GC analyses were performed on a 25 m × 0.25 mm Chrompack CP-Chiralsil-Dex CB column (Varian Inc., Palo Alto, USA) with He as carrier gas using one of the two following temperature programs: **A** 17.5 psi, 50 °C, 5 °C min^{-1}, 150 °C held for 5 min, 2.5 °C min^{-1}; 175 °C held for 5 min, 5 °C min^{-1}, 200 °C held for 30 min; **B** 17.5 psi, 100 °C, held for 15 min, 25 °C min^{-1}; 200 °C held for 21 min). HPLC analyses were performed on a 4.6×250 mm Daicel Chiralcel OD column (Chiral Technologies, Exton, USA) and monitored at 254 nm. Subtilisin Carlsberg was purchased from Sigma–Aldrich (St. Louis, USA) and α-chymotrypsin was purchased from Sigma–Aldrich (St. Louis, USA) or Amresco (Solon, USA). Other hydrolases were a generous gift from Altus Biologics (Cambridge, USA). All reagents, buffers, starting materials and anhydrous solvents were purchased from Sigma–Aldrich (Milwaukee, USA) and used without purification. All air- and moisture-sensitive reactions were performed under argon. Substrates **1b**, **1c**, **2c** and **4c** were available from previous studies.[7b,9]

Racemic Toluenesulfinamide (±)-1

Oxalyl chloride (67.2 g, 530 mmol) was added drop-wise to *p*-toluenesulfinic acid sodium salt (89.0 g, 500 mmol) in toluene at 0 °C.[17] After 1 h at room temperature, the reaction mixture was added to a biphasic mixture of NH_4OH (500 mL) and EtOAc (500 mL) at 0 °C and then stirred at room temperature. After 1 h, the reaction mixture was diluted with EtOAc (500 mL) and the two layers were separated. The aqueous layer was extracted with EtOAc (2×500 mL). The combined EtOAc layers were washed with saturated NaCl (500 mL) and dried over Na_2SO_4. The aqueous layer was then extracted with CH_2Cl_2 (2×500 mL). The combined CH_2Cl_2 layers were washed with saturated NaCl (500 mL) and dried over Na_2SO_4. The combined organic layers were concentrated under vacuum to give a white powder; yield: 66.7 g (86 %); mp 115–117 °C (lit.[17] 117–118 °C); ^{1}H NMR: $\delta=2.42$ (s, 3H, PhCH_3), 4.30 (br s, 2H, NH_2), 7.30 (d, $J=$ 8.1, 2H, phenyl), 7.62 (d, $J=8.1$, 2H, phenyl); ^{13}C NMR: $\delta=21.7$ (PhCH_3), 125.6, 129.7, 141.5, 143.6 (phenyl).

The enantiomers were separated using HPLC [Chiralcel OD-H column, 85:15 hexanes/EtOH, 0.75 mL/min, 254 nm; (*R*)-**1**, $t_R=9.8$ min; (*S*)-**1**, $t_R=11.1$ min].

3-(3-Pyridine)propionic Acid

Solid $KMnO_4$ (256 g, 1.62 mol) was added in portions over 30 min to a solution of 3-(3-pyridine)propanol (200 g, 1.46 mol) in 3 N H_2SO_4 (1.5 L) at 0 °C.[18] The reaction mixture was stirred at room temperature. After 24 h, the reaction mixture was adjusted to pH 6 by addition of solid KOH. Insoluble MnO_2 was pelleted by centrifugation and the clear solution was decanted. Water was removed under vacuum to afford 3-(3-pyridine)propionic acid;[38] yield: 153 g (70 %); ^{1}H NMR: $\delta=2.72$ [t, $J=7.2$, 2H, C(O)CH_2], 3.03 (t, $J=7.5$, 2H, CH_2Ph), 7.34 (m, 1H, pyridyl), 7.68 (m, 1H, pyridyl), 8.52 (m, 2H, pyridyl).

3-(3-Pyridine)propionic Acid Anhydride

N-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (25.2 g, 132 mmol) was added to a solution of 3-(3-pyridine)propionic acid (40.0 g, 265 mmol) in CH_2Cl_2 (750 mL) and NEt_3 (37 mL) at 0 °C and stirred at room temperature.[17] After 24 h, the reaction was washed with ice-cold saturated $NaHCO_3$ (3×500 mL), dried over $MgSO_4$ and concentrated under vacuum to give a pale yellow oil; yield: 34.1 g (91 %); ^{1}H NMR: $\delta=2.72$ [t, $J=7.2$, 2H, C(O)CH_2], 3.03 (t, $J=7.5$, 2H, CH_2Ph), 7.24 (m, 1H, pyridyl), 7.58 (m, 1H, pyridyl), 8.50 (m, 2H, pyridyl).

Racemic *N*-3-(3-Pyridine)propionyl-*p*-toluenesulfinamide [(±)-1a]

Sodium hydride (60 % dispersion in oil; 12.0 g, 300 mmol) was added portion-wise over 15 min to a solution of *p*-tolu-

enesulfinamide (15.5 g, 100 mmol) in THF (750 mL) at 0 °C. The symmetric anhydride of 3-(3-pyridine)propionic acid (32.1 g, 113 mmol) was added drop-wise over 15 min at 0 °C and the reaction mixture was then stirred at room temperature for 3 h.[17] The reaction mixture was diluted with EtOAc (400 mL) and saturated $NaHCO_3$ (400 mL) was added slowly. The layers were separated and the aqueous layer was extracted with EtOAc (3×250 mL). The combined EtOAc layers were washed with saturated $NaHCO_3$ (500 mL) and dried over $MgSO_4$. The aqueous layer was extracted with CH_2Cl_2 (2×250 mL). The combined CH_2Cl_2 layers were washed with $NaHCO_3$ (250 mL) and dried over $MgSO_4$. The combined organic layers were concentrated under vacuum to give a pale yellow solid. Trituration with hexane/ethyl acetate gave a white powder; yield: 21.1 g (73%); mp 161–163 °C; ^{1}H NMR: δ=2.39 (s, 3H, $PhCH_3$), 2.72 [m, 2H, C(O)CH_2], 3.01 (t, J=7.2, 2H, CH_2Pyr), 4.78 (br s, 1H, N*H*), 7.25–7.48 (m, 3H, phenyl or pyridyl), 7.47 (m, 2H, phenyl or pyridyl), 7.68 (m, 1H, phenyl or pyridyl), 8.25 (m, 2H, pyridyl); ^{13}C NMR ([DMSO-d_6): δ=21.4 (Ph*C*H_3), 27.9 (*C*H_2Pyr), 36.9 [C(O)*C*H_2], 124.0, 125.4, 130.2, 136.4, 136.6, 140.9, 142.1, 147.9, 150.2 (phenyl or pyridyl), 173.8 (*C*=O); HR-MS: m/z=289.0989, calcd. for $C_{15}H_{17}N_2O_2S$ $[M+H]^+$: 289.1010.

The enantiomers were separated using HPLC [Chiralcel OD-H column, 85:15 hexanes/EtOH, 0.75 mL/min, 254 nm; (*R*)-**1a**, t_R=20.0 min; (*S*)-**1a**, t_R=22.5 min].

General Procedure for the Synthesis of Racemic Esters 2a–4a

N-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (7.5 mmol) was added portion-wise to a stirred solution of a secondary alcohol (5 mmol), 3-(3-pyridine)propionic acid (7.5 mmol), 4-(dimethylamino)-pyridine (0.5 mmol) and NEt_3 (7.5 mmol) in CH_2Cl_2 (25 mL) at 0 °C. The ice bath was removed and the reaction mixture was stirred at room temperature for 48 h. The reaction was quenched with the addition of saturated $NaHCO_3$ (25 mL). The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (2×25 mL). The combined organic layers were washed with saturated $NaHCO_3$ (2×25 mL) and dried over $MgSO_4$. The organic layer was concentrated under vacuum to give the crude ester. The relevant analytical data are given below:

Racemic 3-(3-Pyridine)propionic acid 1-phenyl-ethyl ester [(±)-2a]: Purification on silica gel (100% hexanes to 60:40 hexanes/acetone) gave a clear liquid; yield: 1.10 g (87%); ^{1}H NMR: δ=1.51 (d, J=6.6, 3H, CH_3), 2.70 [m, 2H, C(O)CH_2], 2.98 (t, J=7.5, 2H, CH_2Pyr), 5.88 (q, J=6.6, 1H, C*H*), 7.32–7.39 (m, 6H, phenyl), 7.54–7.58 (m, 1H, phenyl), 8.46–8.50 (m, 2H, pyridyl); ^{13}C NMR: δ=22.2 (*C*H_3), 28.1 (*C*H_2Pyr), 35.6 [C(O)*C*H_2], 72.7 (*C*H), 123.5, 126.1, 128.0, 128.6, 135.9, 136.1, 141.5, 147.7, 149.8 (phenyl or pyridyl), 171.6 (*C*=O); HR-MS: m/z=256.1336, calcd. for $C_{16}H_{18}NO_2$ $[M+H]^+$: 256.1337. The enantiomers were separated using GC [program **A**; (*S*)-**2a**, t_R=52.3 min; (*R*)-**2a**, t_R=52.5 min].

Racemic 3-(3-Pyridine)propionic acid 2,2-dimethylcyclopentanol [(±)-3a]: Purification on silica gel (100% hexanes to 75:25 hexanes/acetone) gave a clear liquid; yield: 1.05 g (86%); ^{1}H NMR: δ=0.90 (s, 3H, CH_3), 0.93 (s, 3H, CH_3), 1.43 (m, 2H, CH_2), 1.52 (m, 2H, CH_2), 1.67 (m, 2H, CH_2), 2.08 (m, 2H, CH_2), 4.73 (m, 1H, C*H*), 7.27 (m, 1H, phenyl), 7.61 (m, 1H, phenyl), 8.49 (m, 2H, phenyl); ^{13}C NMR: δ=20.6 (*C*H_2), 22.2 (*C*H_2), 26.6 (*C*H_2), 28.2 (*C*H_2Pyr), 30.5 (*C*H_2), 35.6 [C(O)*C*H_2], 38.0 (*C*H_2), 41.9 (*C*), 123.4, 135.9, 147.7, 149.8 (pyridyl), 172.1 (*C*=O); HR-MS: m/z=248.1650, calcd for $C_{15}H_{21}NO_2$ $[M+H]^+$: 248.1655. The enantiomers could not be separated using GC.

Racemic 3-(3-Pyridine)propionic acid 2-mesityl-ethyl ester [(±)-4a]: Purification on silica gel (100% hexanes to 60:40 hexanes/acetone) gave a clear liquid; yield: 1.27 g (86%); ^{1}H NMR: δ=1.53 (d, J=6.9, 3H, CH_3), 2.26 (s, 3H, *p*-CH_3), 2.40 (s, 6H, *o*-CH_3), 2.68 [m, 2H, C(O)CH_2], 2.97 (t, J=7.5, 2H, CH_2Pyr), 6.28 (q, J=6.9, 1H, C*H*), 6.83 (s, 2H, phenyl), 7.24–7.28 (m, 1H, pyridyl), 7.56–7.60 (m, 1H, pyridyl), 8.46–8.50 (m, 2H, pyridyl); ^{13}C NMR: δ=19.6 (*C*H_3), 20.5 (*C*H_3), 20.9 (*C*H_3), 28.1 (*C*H_2Pyr), 35.6 (C(O)*C*H_2), 69.9 (*C*H), 123.5, 130.1, 134.2, 135.9, 136.0, 136.1, 137.2, 147.7, 149.8 (phenyl or pyridyl), 171.7 (*C*=O); HR-MS: m/z=298.1813, calcd. for $C_{17}H_{24}NO_2$ $[M+H]^+$: The enantiomers could not be separated using GC.

Racemic Acetic Acid 1-Phenylethyl Ester [(±)-2b]

Acetyl chloride (15 mmol) was added drop-wise to a stirred solution of 1-phenethyl alcohol (10 mmol) and pyridine (15 mmol) in CH_2Cl_2 (50 mL) at 0 °C. The ice bath was removed and the mixture stirred until the reaction was complete by TLC. The reaction was quenched with the addition of saturated $NaHCO_3$ (25 mL). The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (2×25 mL). The combined organic layers were washed with 1 N HCl (2×25 mL), saturated $NaHCO_3$ (2×25 mL), saturated NaCl (25 mL) and dried over Na_2SO_4. The organic layer was concentrated under vacuum to give the crude ester. Purification on silica gel (100% hexanes to 95:5 hexanes/EtOAc) gave the **2b** as a clear liquid; yield: 1.80 g (73%); ^{1}H NMR: δ=1.55 (d, J=6.6, 3H, CH_3), 2.09 [m, 3H, C(O)CH_3], 5.90 (q, J=6.6, 1H, C*H*), 7.36–7.38 (m, 5H, phenyl); ^{13}C NMR: δ=21.4 (*C*H_3), 22.3 (*C*H_3), 72.4 (*C*H), 126.2, 128.0, 128.6, 141.8 (phenyl), 170.4 (*C*=O). The enantiomers were separated using GC [program **B**; (*S*)-**2b**, t_R=9.7 min; (*R*)-**2b**, t_R=11.9 min].

Racemic 2,2-Dimethylcyclopentanol [(±)-3]

$NaBH_4$ (3.42 g, 90 mmol) was added portion-wise to a stirred solution of 2,2-dimethylcyclopentanone (6.72 g, 60 mmol) in EtOH (100 mL) at 0 °C. The ice bath was removed and the reaction solution was stirred for 3 h at room temperature. The solution was cooled to 0 °C, quenched with 1 N HCl (50 mL) and extracted with EtOAc (3×50 mL). The combined organic layers were washed with saturated $NaHCO_3$ (50 mL), saturated NaCl (50 mL), dried over $MgSO_4$ and concentrated under vacuum to give a clear liquid; yield: 5.87 g (86%); ^{1}H NMR: δ=0.95 (s, 3H, CH_3),

0.97 (s, 3H, CH_3), 1.40 (m, 2H, CH_2), 1.57 (m, 2H, CH_2), 1.72 (m, 2H, CH_2), 2.03 (m, 2H, CH_2), 3.69 (t, $J=6.3$, 1H, CH); ^{13}C NMR: $\delta=19.9$ (CH_2), 21.3 (CH_2) 26.7 (CH_2), 32.7 (CH_2), 37.5 (CH_2), 42.1 [$C(CH_3)_2$], 81.4 (CHOH). The enantiomers were separated using GC [program **B**; (*R*)-**3**, $t_R=5.6$ min; (*S*)-**3**, $t_R=5.8$ min].

Small-Scale Protease-Catalyzed Hydrolysis of 2a and 3a

Bacillus lentus subtilisin (subtilisin BL), *Aspergillus melleus* protease, *Aspergillus oryzae* protease, subtilisin Carlsberg, α-chymotrypsin from bovine pancreas, *Streptomyces griseus* protease [10 mg/mL; solution in 50 mM BES buffer (pH 7.2, 450 μL)] and substrate (100 mM in CH_3CN, 50 μL) containing 50 mM *n*-decane as internal standard were mixed in a $^1/_2$ dram glass vial. The reaction mixture was shaken at 30°C for 6 h with **2a** and 24 h with **2b**, **2c**, **3a** and **4a**. For specific activity measurements the reaction was stopped at a conversion below 10%. The reaction was terminated with the addition of CH_2Cl_2 (500 μL). The phases were separated by centrifugation, and the organic layers were collected. The aqueous phase was extracted with CH_2Cl_2 (2×500 μL) and the combined organics were evaporated under a stream of air. The residue was diluted with EtOAc (150 μL) and analyzed by GC. The enantiomers were separated using the conditions described above.

α-Chymotrypsin-Catalyzed Resolution of (±)-1a

α-Chymotrypsin (12 g) was added to a solution of BES buffer (3.15 L, 1 mM, pH 7.2) and 100 mM KCl and stirred for 15 min to ensure complete dissolution. Substrate **1a** (20.2 g, 70 mmol) was dissolved in dimethylformamide (350 mL) and added drop-wise to the enzyme solution. The rate of hydrolysis was monitored by pH stat, which maintained the pH at 7.2 by automatic titration with 1 N NaOH. At *ca.* 50% conversion (4 d), the reaction was terminated by extraction of remaining starting material and product with CH_2Cl_2 (3×500 mL). The combined organic layers were washed with dH_2O (3×500 mL), saturated NaCl (1×500 mL), dried over $MgSO_4$ and concentrated under vacuum. The crude mixture was dissolved in EtOAc (250 mL) and unreacted starting material was extracted with ice-cold 0.1 N HCl (2×100 mL). The combined aqueous layers were then back-extracted with EtOAc (50 mL). The combined EtOAc layers were washed with saturated $NaHCO_3$ (100 mL), saturated NaCl (100 mL), dried over $MgSO_4$ and concentrated under vacuum to give (*R*)-**1** as a white solid; yield: 4.48 g (41%) with 87% *ee*. The combined aqueous layers were neutralized with solid $NaHCO_3$ and extracted with CH_2Cl_2 (2×200 mL). The combined CH_2Cl_2 layers were washed with sat. $NaHCO_3$ (100 mL), sat. NaCl (100 mL) and dried over $MgSO_4$. The solution was concentrated under vacuum to give (*S*)-**1a**, which was subsequently treated with hydrazine hydrate (35 mL).[19] After stirring for 3 h, the reaction solution was diluted with CH_2Cl_2 (100 mL) and washed with 1 N HCl (50 mL), saturated $NaHCO_3$ (50 mL), saturated NaCl (50 mL) and concentrated under vacuum to give (*S*)-**1**; yield: 4.29 g (40%) with 92% *ee*. Recrystallization from hexanes/ethyl acetate gave (*R*)-**1** (yield: 3.81 g, 35%) with 98% *ee* and (*S*)-**1** (yield: 3.58 g, 33%) with 98% *ee*.

Subtilisin Carlsberg-Catalyzed Resolution of (±)-3a

Protease from *Bacillus licheniformis* (subtilisin Carlsberg; 50 mL of 92 mg/mL solution; 4.5 g) was added to BES buffer (400 mL, 1 mM, pH 7.2) and stirred for 15 min. Substrate **3a** (4.95 g, 20 mmol) was dissolved in MeCN (50 mL) and added to the enzyme solution. The rate of hydrolysis was monitored by pH stat, which maintained the pH at 7.2 by automatic titration with 1 N NaOH. After 2 d, the reaction was terminated by extraction of remaining starting material and product with EtOAc (3×75 mL). The organic layer was extracted with ice-cold 0.1 N HCl (3×50 mL). The combined aqueous layers were then extracted with EtOAc (25 mL). The combined EtOAc layers were dried over $MgSO_4$ and concentrated under vacuum to give (*S*)-**3**; yield: 1.07 g (48%) with 87% *ee*. The aqueous layer was neutralized with solid Na_2HCO_3 and extracted with EtOAc (3×50 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under vacuum to give (*R*)-**3a**, which was subsequently hydrolyzed in ethanolic KOH (1 N, 1:1 ethanol/water) to give (*R*)-**3**; yield: 980 mg (44%) with 89% *ee*.

Aspergillus melleus Protease-Catalyzed Resolution of (±)-4a

Aspergillus melleus protease (1.5 g) was added to BES buffer (180 mL, 1 mM, pH 7.2) and stirred for 30 min to ensure complete dissolution. Substrate **4a** (1.49 g, 5 mmol) was dissolved in DMF (20 mL) and added to the enzyme solution. The rate of hydrolysis was monitored by pH stat, which maintained the pH at 7.2 by automatic titration with 1 N NaOH. After 5 d, the reaction was terminated by extraction of remaining starting material and product with EtOAc (3×50 mL). Product and substrate were difficult to separate *via* acid extraction. Thus, product and remaining starting material were separated using column chromatography (100% hexanes to 4:1 hexanes/acetone) to give (*R*)-**4** (yield: 352 mg, 43%) with 61% *ee* and (*S*)-**4a**, which was subsequently hydrolyzed in ethanolic KOH (1 N, 1:1: ethanol/water) to give (*S*)-**4** (yield: 343 mg, 42%) with 60% *ee*.

Absolute Configuration of 2,2-Dimethylcyclopentanol (3)

The absolute configuration of alcohol **3** was assigned using the configurational correlation model for the corresponding (*R*)-MTPA derivatives, which were synthesized using (*R*)-(+)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride in pyridine.[39] ^{1}H NMR: $\delta=0.91$ (s, 3H, CH_3, *R*-enantiomer),

0.93 (s, 3H, CH_3, *R*-enantiomer), 0.98 (s, 3H, CH_3, *S*-enantiomer), 1.00 (s, 3H, CH_3, *S*-enantiomer), 1.46 (m, 2H, CH_2), 1.57 (m, 2H, CH_2), 1.68 (m, 2H, CH_2), 2.18 (m, 2H, CH_2), 3.54 (s, 3H, OCH_3, *S*-enantiomer), 3.57 (s, 3H, OCH_3, *R*-enantiomer), 4.90 (m, 1H, C*H*), 7.42 (m, 3H, phenyl), 7.52 (m, 2H, phenyl).

Modeling of Tetrahedral Intermediates bound to α-Chymotrypsin

All modeling was performed using *Insight II 2000.1/Discover* (Accelrys, San Diego, USA) on a SGI Tezro UNIX workstation using the AMBER force field.[40] We used a non-bonded cutoff distance of 8 Å, a distance-dependent dielectric of 1.0 and scaled the 1–4 van der Waals interactions by 50%. Protein structures in Figure 1 were created using PyMOL (Delano Scientific, San Carlos, CA, USA). The X-ray crystal structure of α-chymotrypsin (entry 6CHA)[41] is from the Protein Data Bank. The hydrogen atoms were added to correspond to pH 7.0. Histidines were uncharged, aspartates and glutamates were negatively charged, and arginines and lysines were positively charged. The catalytic histidine (His64) was protonated. The positions of the water hydrogens and then the enzyme hydrogens were optimized using a consecutive series of short (1 ps) molecular dynamic runs and energy minimizations.[42] This optimization was repeated until there was <2 kcal mol^{-1} in the energy of the minimized structures. Thereafter, an iterative series of geometry optimizations were performed on the water hydrogens, enzyme hydrogens and full water molecules. Finally, the whole system was geometry optimized.

The tetrahedral intermediates were built manually and covalently linked to Ser195. Non-standard partial charges were calculated using a formal charge of -1 for the substrate oxyanion. Energy minimization proceeded in three stages. First, minimization of substrate with only the protein constrained (25 kcal mol^{-1} Å^{-2}); second, minimization with only the protein backbone constrained (25 kcal mol^{-1} Å^{-2}) and for the final stage the minimization was continued without constraints until the rms value was less than 0.0005 kcal mol^{-1} Å^{-1}. A catalytically productive complex required all five hydrogen bonds within the catalytic machinery. We set generous limits for a hydrogen bond: a donor to acceptor atom distance of less than 3.1 Å with a nearly linear arrangement ($>120°$ angle) of donor atom, hydrogen, and acceptor atom. Structures lacking any of the five catalytically relevant hydrogen bonds or encountering severe steric clash with enzyme were deemed non-productive.

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References

[1] a) K. Faber, *Biotransformations in Organic Chemistry*, 5th edn., Springer-Verlag, Berlin, **2004**; b) K. Drauz, H. Waldman (Eds.), *Enzyme Catalysis in Organic Synthesis* Wiley-VCH, Weinheim, **2002**; c) C.-H. Wong, G. M. Whitesides, *Enzymes in Synthetic Organic Chemistry*, Pergamon, New York, **1994**.

[2] U. T. Bornscheuer, R. J. Kazlauskas, *Hydrolases in Organic Synthesis - Regio- and Stereoselective Biotransformations*, Wiley-VCH, Weinheim, **1999**, pp. 17–18.

[3] M. Breuer, K. Ditrich, T. Habicher, B. Hauer, M. Keßeler, R. Stürmer, T. Zelinski, *Angew. Chem. Int. Ed.* **2004**, *43*, 788–824.

[4] M. Cygler, P. Grochulski, R. J. Kazlauskas, J. D. Schrag, F. Bouthillier, B. Rubin, A. N. Serreqi, A. K. Gupta, *J. Am. Chem. Soc.* **1994**, *116*, 3180–3186.

[5] A. Mezzitti, J. Schrag, C. S. Cheong, R. J. Kazlauskas, *Chem. Biol.* **2005**, *12*, 427–437.

[6] J. D. A. Tyndall, T. Tessa Nall, D. P. Fairlie, *Chem. Rev.* **2005**, *105*, 973–1000.

[7] a) D. C. Muchmore, *US Patent* 5,215,918, **1993**; b) C. K. Savile, V. P. Magloire, R. J. Kazlauskas, *J. Am. Chem. Soc.* **2005**, *127*, 2104–2113.

[8] P. F. Mugford, S. M. Lait, B. A. Keay, R. J. Kazlauskas, *ChemBioChem*, **2004**, *5*, 980–987.

[9] C. K. Savile, R. J. Kazlauskas, *J. Am. Chem. Soc.* **2005**, *127*, 12228–12229.

[10] a) Y. Y. Lin, D. N. Palmer, J. B. Jones, *Can. J. Chem.* **1974**, *52*, 469–476.

[11] a) D. A. Estell, T. P. Graycar, J. V. Miller, D. B. Powers, J. P. Burnier, P. G. Ng, J. A. Wells, *Science* **1986**, *233*, 659–663; b) J. A. Wells, D. B. Powers, R. R. Bott, T. P. Graycar, D. A. Estell, *Proc. Natl. Acad. Sci. USA* **1987**, *84*, 1219–1223.

[12] T. Pohl, H. Waldmann, *Tetrahedron Lett.* **1995**, *36*, 2963–2966.

[13] N. Kundu, S. Roy, F. Maenza, *Eur. J. Biochem.* **1972**, *28*, 311–315.

[14] Based on a limit of detection of 0.1% conversion.

[15] P. F. Mugford, V. P. Magloire, R. J. Kazlauskas, *J. Am. Chem. Soc.* **2005**, *127*, 6536–6537.

[16] a) C. R. Harwood, S. M. Cutting, *Molecular Biological Methods for Bacillus*, John Wiley & Sons, Chichester, U. K., **1990**, pp. 33–35 and 391–402; b) S.-J. Cho, S.-H. Oh, R. D. Pridmore, M. A. Juillerat, C.-H. Lee, *Agric. Food Chem.* **2003**, *51*, 7664–7670.

[17] B. J. Backes, D. R. Dragoli, J. A. Ellman, *J. Org. Chem.* **1999**, *64*, 5472–5478.

[18] L. Crombie, S. H. Harper, *J. Am. Chem. Soc.* **1950**, 2685–2689.

[19] D. D. Keith, J. A. Tortora, R. Yang, *J. Org. Chem.* **1978**, *43*, 3711–3713.

[20] H. C. Brown, B. T. Cho, W. S. Park, *J. Org. Chem.* **1988**, *53*, 1231–1238.

[21] H. C. Brown, M. Srebnik, P. V. Ramachandran, *J. Org. Chem.* **1989**, *54*, 1577–1583.

[22] B. T. Cho, Y. S. Chun, *Tetrahedron: Asymmetry* **1992**, *3*, 1539–1542.

[23] P. Delair, A. M. Kanazawa, M. B. M. de Azevedo, A. E. Greene, *Tetrahedron: Asymmetry* **1996**, *7*, 2707–2710.

[24] K. Naemura, M. Murata, R. Tanaka, M. Yano, K. Hirose, Y. Tobe, *Tetrahedron: Asymmetry* **1996**, *7*, 3285–3294.
[25] The sulfoxide oxygen of non-productive (*R*)-**1a** bumped catalytic His57 (O–$C_{\delta 2}$ distance = 3.04 Å) and the *p*-tolyl group was hindered by Met192 (C_{ortho}–C_{γ} distance = 3.33 Å and C_{meta}–C_{γ} distance = 3.73 Å), Gly193 (C_{para}–N distance = 3.96 Å) and Phe41 (CH_3–C_{ε} distance = 3.75 Å). As well, the *p*-tolyl group forms an unfavorable *syn*-pentane interaction with the oxyanion. This intramolecular interaction and severe steric clash between *p*-tolyl group and active site residues significantly distorts the substrate and results in the loss of two catalytically relevant hydrogen bonds ($N_{\delta 2}$–O_{γ} distance = 4.49 Å and $N_{\delta 2}$–$N_{sulfinamide}$ distance = 5.13 Å). The *p*-tolyl group of non-productive (*S*)-**1a** was hindered by catalytic His57 (C_{ortho}–$N_{\varepsilon 2}$ distance = 3.38 Å, C_{ortho}–$C_{\delta 2}$ distance = 3.27 Å, C_{meta}–$C_{\delta 2}$ distance = 3.73 Å and C_{meta}–C_{γ} distance = 3.90 Å) and the sulfinamide bumped the Met192 ($S_{sulfinamide}$–S_{δ} distance = 3.33 Å and $S_{sulfinamide}$–C_{ε} distance = 3.52 Å). The steric hindrance between *p*-tolyl group and active site residues results in the loss of one catalytically relevant hydrogen bond ($N_{\delta 2}$–$N_{sulfinamide}$ distance = 3.20 Å).
[26] S. G. Cohen, *Trans. N. Y. Acad. Sci.* **1969**, *31*, 705–719.
[27] The van der Waals distance for CH–C = 3.99 Å, CH–N = 3.84 Å, CH–S = 4.09 Å and O–HC = 3.81 Å. These were estimated from the van der Waals radii of carbon (1.70 Å), nitrogen (1.55 Å), sulfur (1.80 Å) or oxygen (1.52 Å) and hydrogen (1.20 Å) and the C–H bond length (1.09 Å) from: A. Bondi, *J. Phys. Chem.* **1964**, *68*, 441–451.
[28] The energy of a hydrophobic interaction is from the regaining of entropy by water after it is removed from a hydrophobic group and can be estimated using the incremental Gibbs free energy of transfer. The incremental Gibbs free energy of transfer of the *p*-tolyl group from *n*-octanol to water is estimated to be *ca.* +3.4 kcal mol^{-1}, using $\Delta G_{trans} = 2.303RT\pi$, where π is the hydrophobicity constant of the *p*-tolyl group [$\pi = 2.5$, using *ClogP* (BioByte, Claremont, USA)] relative to hydrogen, whereas the sulfoxide oxygen is *ca.* -1.8 kcal mol^{-1}. Switching from (*S*)-**1a** to (*R*)-**1a** places the *p*-tolyl group into the hydrophobic leaving group pocket and removes the sulfoxide oxygen for a total of *ca.* 5.2 kcal mol^{-1} in favor of placing the *p*-tolyl group in the hydrophobic leaving group pocket when in an aqueous medium. This estimate neglects the hydrophobic surface of the leaving group pocket, which would increase the value, and neglects that the pocket does not completely surround the *p*-tolyl group, which would decrease the value. It is likely that these two omissions cancel out. For details, see: a) A. Fersht, *Structure and Mechanism in Protein Science* W. H. Freeman and Company, New York, **1998**, pp. 324–348; b) A. Leo, C. Hansch, D. Elkins, *Chem. Rev.* **1971**, *71*, 525–616.
[29] P. A. Fitzpatrick, A. M. Klibanov, *J. Am. Chem. Soc.* **1991**, *113*, 3166–3171.
[30] F. A. Davis, P. Zhou, B.-C. Chen, *Chem. Soc., Rev.* **1998**, *27*, 13–18.
[31] P. Moreau, M. Essiz, J.-Y. Merour, D. Bouzard, *Tetrahedron: Asymmetry* **1997**, *8*, 591–598.
[32] F. A. Davis, P. Zhou, G. V. Reddy, *J. Org. Chem.* **1994**, *59*, 3243–3245.
[33] I. M. Lefebvre, S. A. Evans, *J. Org. Chem.* **1997**, *62*, 7532–7533.
[34] a) K. K. Anderson, in: *The Chemistry of Sulphones and Sulphoxides*, (Eds.: S. Patai, Z. Rappoport, C. J. M. Stirling), John Wiley & Sons, New York, **1988**, pp. 55–94; b) H. J. Phillips, *J. Chem. Soc.,* **1925**, *127*, 2552–2587; c) M. Hulce, J. P. Mallamo, L. L. Frye, T. P. Kogan, G. A. Posner, *Org. Synth.* **1986**, *64*, 196–206.
[35] Z. Han, D. Krishnamurthy, P. Grover, Q. K. Fang, C. H. Senanayake, *J. Am. Chem. Soc.* **2002**, *124*, 7880–7881.
[36] T. Omata, N. Iwamoto, T. Kimura, A. Tanaka, S. Fukui, *Eur. J. Appl. Microbiol. Biotechnol.* **1981**, *11*, 199–204.
[37] S. Fukui, A. Tanaka, *Methods Enzymol.* **1987**, *136*, 293–302.
[38] F. A. Walker, M. Benson, *J. Am. Chem. Soc.* **1980**, *102*, 5530–5538.
[39] J. A. Dale, H. S. Mosher, *J. Am. Chem. Soc.* **1973**, *95*, 512–519.
[40] a) S. J. Weiner, P. A. Kollman, D. A. Case, U. C. Singh, C. Ghio, G. Alagona, S. Profet, P. Weiner, *J. Am. Chem. Soc.* **1984**, *106*, 765–784; b) S. J. Weiner, P. A. Kollman, D. T. Nguyen, D. A. Case, *J. Comp. Chem.* **1986**, *7*, 230–252.
[41] A. Tulinsky, R. A. Blevins, *J. Biol. Chem.* **1987**, *262*, 7737–7743.
[42] S. Raza, L. Fransson, K. Hult, *Protein Sci.* **2001**, *10*, 329–338.
[43] M. M. Bradford, *Anal. Biochem.* **1976**, *72*, 248–254.
[44] C. S. Chen, Y. Fujimoto, G. Girdaukas, C. J. Sih, *J. Am. Chem. Soc.* **1982**, *104*, 7294–7299.

