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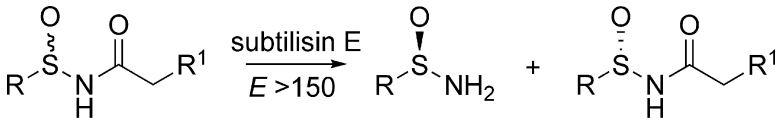
J. Am. Chem. Soc., **2005**, 127 (7), 2104-2113• DOI: 10.1021/ja045397b • Publication Date (Web): 26 January 2005



R = aryl
R^1 = Cl or Bn

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Subtilisin-Catalyzed Resolution of *N*-Acyl Arylsulfinamides

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Abstract: We report the first biocatalytic route to sulfinamides (R−S(O)−NH_2), whose sulfur stereocenter makes them important chiral auxiliaries for the asymmetric synthesis of amines. Subtilisin E did not catalyze hydrolysis of *N*-acetyl or *N*-butanoyl arylsulfinamides, but did catalyze a highly enantioselective ($E > 150$ favoring the (*R*)-enantiomer) hydrolysis of *N*-chloroacetyl and *N*-dihydrocinnamoyl arylsulfinamides. Gram-scale resolutions using subtilisin E overexpressed in *Bacillus subtilis* yielded, after recrystallization, three synthetically useful auxiliaries: (*R*)-*p*-toluenesulfinamide (42% yield, 95% ee), (*R*)-*p*-chlorobenzenesulfinamide (30% yield, 97% ee), and (*R*)-2,4,6-trimethylbenzenesulfinamide (30% yield, 99% ee). Molecular modeling suggests that the *N*-chloroacetyl and *N*-dihydrocinnamoyl groups mimic a phenylalanine moiety and thus bind the sulfinamide to the active site. Molecular modeling further suggests that enantioselectivity stems from a favorable hydrophobic interaction between the aryl group of the fast-reacting (*R*)-arylsulfinamide and the S_1' leaving group pocket in subtilisin E.

Introduction

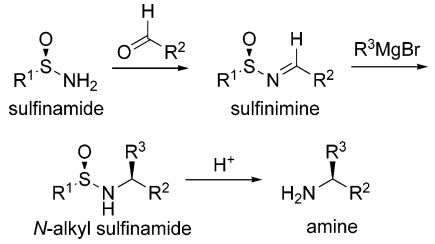
The chiral sulfinyl group (R−S(O)−) is an important functional group for asymmetric synthesis because it effectively transfers chirality to a wide range of centers.[1−3] This efficacy stems from the steric and stereoelectronic differences between the substituents: a lone pair, an oxygen, and an alkyl or aryl group. As well, the sulfinyl group is configurationally stable.[2]

Sulfinamides (R−S(O)−NH_2) are useful sulfinyl chiral auxiliaries for synthesis of amines (Scheme 1).[4,5] When condensed with an aldehyde or ketone to give the sulfinimine, the *N*-sulfinyl group directs nucleophilic addition across the C=N bond. This yields the *N*-alkyl sulfinamide, which upon hydrolysis of the S−N link yields an amine. Enantioselective syntheses using sulfinimines include preparations of amines,[6] α- and β-amino acids,[5] amino alcohols,[7] aziridines,[8] and amino phosphonic acids.[9]

The best route to enantiopure aryl sulfinyl moieties is via menthyl-*p*-toluenesulfinate (Andersen's reagent).[2,10,11] Nucleophilic displacement of menthol at chiral sulfur leads to enantiopure aryl sulfinyl compounds[4] including *p*-toluenesulfinamide.[12] However, preparation of Andersen's reagent relies on the crystallization of diastereomers epimeric at sulfur, and only *p*-toluenesulfinate and close analogues efficiently crystallize as the menthyl derivative.[2,4,13] Thus, this route is limited to *p*-toluenesulfinamide and close analogues. Asymmetric oxidation using 1 equiv of (+)- or (−)-*N*-(phenylsulfonyl)(3,3-dichlorocamphoryl)oxaziridine also yields enantiopure aryl sulfinyl moieties.[14]

The two other routes to enantiopure sulfinamides use either an enantioselective oxidation of *tert*-butyl disulfide[6,15] or a double displacement using a chiral auxiliary derived from indanol.[16] The enantioselective oxidation developed by Ellman

Scheme 1. Synthesis of Enantiopure Amines from Sulfinamides



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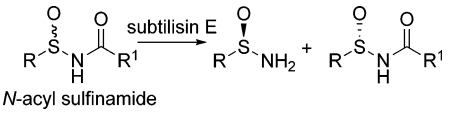
(1) Carreña, M. C. *Chem. Rev.* **1995**, *95*, 1717−1760 and references therein.
(2) Anderson, K. K. In *The Chemistry of Sulphones and Sulphoxides*; Patai, S., Rappoport, Z., Stirling, C. J. M., Eds.; John Wiley & Sons: New York, 1988; pp 55−94.
(3) Walker, A. J. *Tetrahedron: Asymmetry* **1992**, *3*, 961−998.
(4) Nudelman, A. In *The Chemistry of Sulphinic Acids, Esters and their Derivatives*; Patai, S., Ed.; John Wiley & Sons: New York, 1990; pp 35−85.
(5) Davis, F. A.; Zhou, P.; Chen, B.-C. *Chem. Soc. Rev.* **1998**, *27*, 13−18.
(6) Liu, G.; Cogan, D. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1997**, *119*, 9913−9914.
(7) Kochi, T.; Tang, T. P.; Ellman, J. A. *J. Am. Chem. Soc.* **2002**, *124*, 6518−6519.
(8) Davis, F. A.; Zhou, P.; Reddy, G. V. *J. Org. Chem.* **1994**, *59*, 3243−3245.
(9) Lefebvre, I. M.; Evans, S. A. *J. Org. Chem.* **1997**, *62*, 7532−7533.
(10) (a) Phillips, H. *J. Chem. Soc.* **1925**, *127*, 2552−2587. (b) Hulce, M.; Mallamo, J. P.; Frye, L. L.; Kogan, T. P.; Posner, G. A. *Org. Synth.* **1986**, *64*, 196−206.
(11) Drabowicz, J.; Kielbasinski, P.; Mikolajczyk, M. In *The Chemistry of Sulphones and Sulphoxides*; Patai, S., Rappoport, Z., Stirling, C. J. M., Eds.; John Wiley & Sons: New York, 1988; pp 233−378.
(12) Davis, F. A.; Xhang, Y.; Andemichael, Y.; Fang, T.; Fanelli, D. L.; Zhang, H. *J. Org. Chem.* **1999**, *64*, 1403−1406.
(13) Herbranson, H. F.; Cusano, C. M. *J. Am. Chem. Soc.* **1961**, *83*, 2124−2128.
(14) Davis, F. A.; Reddy, R. T.; Reddy, R. E. *J. Org. Chem.* **1992**, *57*, 6387−6389.
(15) Cogan, D. A.; Liu, G.; Kim, K.; Backes, B. J.; Ellman, J. A. *J. Am. Chem. Soc.* **1998**, *120*, 8011−8019.
(16) Han, Z.; Krishnamurthy, D.; Grover, P.; Fang, Q. K.; Senanayake, C. H. *J. Am. Chem. Soc.* **2002**, *124*, 7880−7881.

10.1021/ja045397b CCC: $30.25

Scheme 2. Subtilisin-Catalyzed Kinetic Resolution of Sulfinamides



and co-workers relies on the stability of the oxidation product, thiosulfinate (*t*-Bu-S(O)-S-*t*-Bu), to racemization and is thus limited to *tert*-butylsulfinamide. The double displacement route developed by Senanayake and co-workers uses *N*-sulfonyl-1,2,3-oxathiazolidine-2-oxide intermediates. This route yields a variety of enantiopure sulfinamides, but includes complicated steps requiring low temperatures and moisture- and air-sensitive reagents.

Many groups have reported biocatalytic routes to sulfinyl stereocenters, but not to sulfinamides. A direct biocatalytic route to sulfinyl compounds is oxidation. For example, enantioselective oxidation of unsymmetrical sulfides yields sulfoxides.[11,17] These direct oxidation routes may not be suitable routes to sulfinamides because oxidation of unsubstituted sulfenamides ($RSNH_2$) can involve nitrene intermediates.[18] Another biocatalytic route to enantiopure sulfinyl groups is lipase-catalyzed hydrolysis of a pendant ester group.[19] Enantioselectivity can be high even when the stereocenter is remote from the reacting carbonyl. Ellman and co-workers tested the hydrolase-catalyzed acylation of the sulfinamide amino group, but did not observe any reaction.[15] In this paper, we explore the reverse reaction—hydrolase-catalyzed hydrolysis of *N*-acylsulfinamides (Scheme 2) and find that subtilisin E shows high enantioselectivity toward many arylsulfinamides. Further, molecular modeling suggests that the high enantioselectivity of subtilisin toward sulfinamides compared to the moderate enantioselectivity toward the isosteric secondary alcohols likely stems from the polar nature of the oxygen substituents in sulfinamides (Figure 1).

Results

Synthesis of Substrates. We prepared racemic sulfinamides **1**−**8** by one of three established routes (Scheme 3). The simplest route, from the corresponding sulfinic acid, yielded *p*-toluenesulfinamide **1** and benzenesulfinamide **2**. Treating the sulfinic acid with oxalyl chloride followed by ammonolysis yielded the sulfinamides in 56−59% yield.[20]

To prepare sulfinamides for which no sulfinic acid precursors were available (*p*-chlorobenzenesulfinamide **3**, *p*-methoxybenzenesulfinamide **4**, 2,4,6-trimethylbenzenesulfinamide **5**, 1-naphthylenesulfinamide **6**, and 2,4,6-triisopropylbenzenesulfinamide **7**), we used the sulfonyl chlorides. Reduction of the sulfonyl chloride with $P(OMe)_3$ in ethanol gave the respective sulfinate ethyl esters.[21] Displacement of ethanol with lithium bis(trimethylsilyl)amide followed by desilylation gave the corresponding sulfinamides in 21−55% overall yield.[20]

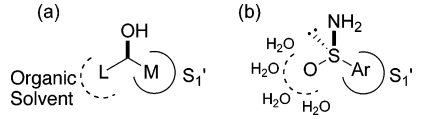


Figure 1. Empirical rules that predict the enantiopreference of subtilisins toward secondary alcohols and sulfinamides. (a) In organic solvent, subtilisins favor the secondary alcohol enantiomer with the shape shown, where L is a large substituent, such as phenyl, and M is a medium substituent, such as methyl. (b) In water, subtilisins favor the sulfinamide enantiomer with the shape shown, where Ar is an aryl substituent. We suggest that a favorable hydrophobic interaction between the aryl substituent and S_1' pocket and good solvation of the polar sulfoxide oxygen in water explain the enantiopreference with sulfinamides.

We prepared *tert*-butylsulfinamide **8** from *tert*-butyl disulfide in three steps. Oxidation of *tert*-butyl disulfide with 3-chloroperoxybenzoic acid followed by addition of sulfuryl chloride gave *tert*-butylsulfinyl chloride,[22] which reacted with NH_4OH to give **8** in 21% overall yield.[20]

To prepare *N*-acylsulfinamides **a**−**m**, we treated the appropriate sulfinamide with 2 equiv of *n*-butyllithium in THF at −78 °C followed by rapid addition of the necessary symmetrical carboxylic acid anhydride (15−90% yield).[20]

Initial Screening. Initial screening of the *N*-butanoyl derivative **1b** with 50 hydrolases revealed no active hydrolases, but screening the more reactive *N*-chloroacetyl derivative **1c** identified four moderately to highly active proteases, one lipase and one esterase (Table 1).[23] To determine enantioselectivities, we carried out small-scale reactions and measured the enantiomeric purity of the starting materials and products by HPLC using a column with a chiral stationary phase.

Proteinase from *Bacillus subtilis* var. biotecus A showed the highest true enantioselectivity ($E_{true} > 150$). At 50% conversion, the product, (*R*)-**1**, had 93% ee while the unreacted starting material, (*S*)-**1c**, had 92% ee corresponding to an apparent enantioselectivity (E_{app}) of 90. However, this substrate also underwent ca. 1−3% spontaneous chemical hydrolysis. After correction for this chemical hydrolysis,[24] the true enantioselectivity (E_{true}) was >150.

A related protease, subtilisin Carlsberg, also catalyzed hydrolysis of **1c** with low apparent enantioselectivity (E_{app}). However, the amount of product sulfinamide was much lower than the amount of **1c** that disappeared. Further analysis revealed that in addition to the expected C−N bond hydrolysis, this protease also catalyzed S−N bond hydrolysis.[25] Details of this unprecedented reaction will be reported separately.

The two other proteases, protease from *Aspergillus oryzae* and protease/acylase from *Aspergillus melletus*, as well as a lipase, *Candida antarctica* lipase A, and bovine cholesterol esterase, also catalyzed the hydrolysis of **1c**, but showed only low to moderate enantioselectivity ($E_{true} = 6-29$). All hydrolases, except lipase A from *Candida antarctica*, favored the (*R*)-enantiomer.

(17) (a) Kagan, H. B. In *Catalytic Asymmetric Synthesis*; Ojima, I., Ed.; VCH: New York, 1993; pp 203−226. (b) Holland, H. L. *Chem. Rev.* **1988**, *88*, 473−485. (c) ten Brink, H. B.; Holland, H. L.; Schoemaker, H. E.; van Lingen, H.; Wever, R. *Tetrahedron: Asymmetry* **1999**, *10*, 4563−4572.

(18) (a) Capozzi, G.; Modena, G.; Pasquato, L. In *The Chemistry of Sulphenic Acids and Their Derivatives*; Patai, S., Ed.; Wiley: Chichester, U.K., 1990; pp 403−516. (b) Atkinson, R. S.; Judkins, B. D. *J. Chem. Soc., Perkin Trans. 1* **1981**, 2615−2619.

(19) (a) Allenmark, S. G.; Andersson, A. C. *Tetrahedron: Asymmetry* **1993**, *4*, 2371−2376. (b) Burgess, K.; Henderson, I. *Tetrahedron Lett.* **1989**, *30*, 3633−3636. (c) Burgess, K.; Henderson, I.; Ho, K.-K. *J. Org. Chem.* **1992**, *57*, 1290−1295. (d) Serreqi, A. N.; Kazlauskas, R. J. *Can. J. Chem.* **1995**, *73*, 1357−1367.

(20) Backes, B. J.; Dragoli, D. R.; Ellman, J. A. *J. Org. Chem.* **1999**, *64*, 5472−5478.

(21) Klunder, J. M.; Sharpless, K. B. *J. Org. Chem.* **1987**, *52*, 2598−2602.

(22) (a) Netscher, T.; Prinzbach, H. *Synthesis* **1987**, 683−686. (b) Gontcharov, A. V.; Liu, H.; Sharpless, K. B. *Org. Lett.* **1999**, *1*, 783−786.

(23) Janes, L. E.; Löwendahl, C. P.; Kazlauskas, R. J. *Chem.—Eur. J.* **1998**, *4*, 2324−2331.

(24) A computer program, Selectivity-KRESH, was used for all calculations. For details, see: Faber, K.; Hönig, H.; Kleewein, A. In *Preparative Biotransformations*; Roberts, S. M., Ed.; John Wiley & Sons: New York, 1995; pp 0.079−0.084.

(25) ^{1}H NMR and HRMS-EI identified *p*-toluenesulfinic acid as the primary product in this enzymatic reaction: Mugford, P. F.; Magloire, V. P.; Kazlauskas, R. J. Manuscript in preparation.

Scheme 3. Synthesis of Sulfinamides **1**−**8** and *N*-Acylsulfinamides

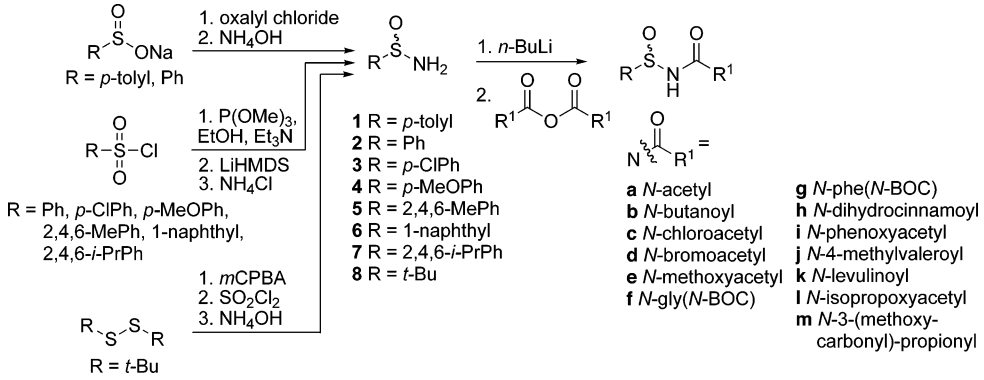


Table 1. Screening of Hydrolases for Enantioselective Hydrolysis of **1c**

hydrolase	wt[a]	%c_{app}[b]	ee$_s$ (%)[c,d]	ee$_p$ (%)[c,d]	E_{app}[d,e]	x(sp)[f] (%)	E_{true}[e,g]	enantiopreference[h]
B. subtilis var. biotecus A protease	16	50	92	93	90	3	>150	R
subtilisin E	n.d.	37[i]	56	97	115	2	>150	R
subtilisin Carlsberg	34	56	82	64	n.d.[j]	n.d.	n.d.[j]	R
A. oryzae protease	90	56	88	68	14	3	17	R
A. melletus protease/acylase	250	29[k]	30	75	9	10	29	R
C. antarctica lipase A	137	14	11	65	5	3	6	S
bovine cholesterol esterase	80	29[k]	27	67	7	10	11	R

[a] Weight of enzyme in milligrams. [b] Conversion %: amount of sulfinamide formed in 6 h, except where noted. [c] Enantiomeric excess %. Enantiomeric excess of substrate and product was determined by HPLC analysis on Daicel Chiralcel OD or AD columns at 238 or 222 nm. [d] The ee$_p$, ee$_s$, and E_{app} (apparent enantioselectivity) are with no correction for chemical hydrolysis; n.d. = not determined. [e] Enantiomeric ratio: the enantiomeric ratio E measures the relative rate of hydrolysis of the fast enantiomer as compared to that of the slow enantiomer as defined by Sih (Chen, C. S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294−7299). [f] The x(sp) refers to % spontaneous chemical hydrolysis. [g] E_{true} is the enantioselectivity corrected for chemical hydrolysis.[24] [h] The absolute configuration was determined by comparison to authentic samples prepared by the method of Davis and coworkers.[12] [i] Reaction for 3 h. [j] This reaction was complicated by competing sulfinyl hydrolysis. Accurate determination of E_{app} and E_{true} was more complicated and will be reported elsewhere. [k] Reaction for 24 h.

Although this initial screen identified *B. subtilis* var. biotecus A as the best commercial enzyme for hydrolysis of **1c**, it is, unfortunately, expensive (U.S. $725/gram), and its amino acid sequence is not available from the supplier. We suspected that subtilisin E might be a similar or even the same enzyme. Subtilisin E is an alkaline serine protease produced by *B. subtilis* that has been cloned and overexpressed,[26] and its structure has been elucidated by X-ray crystallography.[27] Confirming our hunch, subtilisin E showed similar enantioselectivity (E_{true} > 150) to *B. subtilis* var. biotecus A in the hydrolysis of **1c** (Table 1) as well as four other substrates (see below and Supporting Information). We focused further experiments on subtilisin E because it showed high enantioselectivity, it was inexpensive to produce by fermentation, and the X-ray crystal structure permitted a molecular-level interpretation of results.

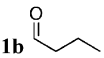
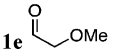
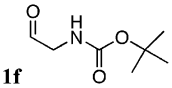
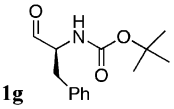
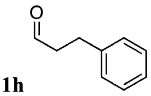
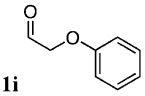
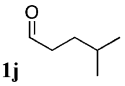
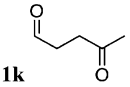
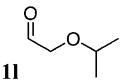
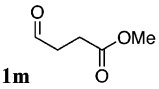
Optimization of *N*-Acyl Group for Enantioselectivity and Reactivity. The *N*-acetyl derivative **1a** and *N*-butanoyl derivative **1b** showed no reaction with subtilisin E,[28] but several acyl groups with electron-withdrawing functional groups similar to those of **1c** showed enantioselective hydrolysis (Table 2). The *N*-bromoacetyl derivative **1d** reacted similarly to **1c**. It showed high enantioselectivity (E_{true} > 150) and conversion (53% c_{app} in 3 h), but also underwent a similar spontaneous chemical hydrolysis (4% after 3 h). The *N*-methoxyacetyl derivative **1e** showed at least 6-fold lower enantioselectivity (E_{true} = 26), 10-fold lower conversion (28% c_{app} after 24 h), and a slower spontaneous chemical hydrolysis (4% after 24 h). Glycine derivative **1f** showed at least 3-fold lower enantioselectivity (E_{true} = 54) than did **1c**, and 11-fold lower conversion (26% c_{app} after 24 h).

Since subtilisin favors hydrolysis of peptides with aromatic or large nonpolar residues at the P_1 position,[29] we prepared several phenylalanine derivatives or analogues. The phenylalanine derivative **1g** demonstrated at least 3-fold lower selectivity (D_{true} = 46) and 6-fold lower conversion (46% c_{app} after 24 h) than **1c**. However, the *N*-dihydrocinnamoyl derivative **1h** showed very high enantioselectivity (E_{true} > 150) similar to that of **1c**, but with 6-fold lower conversion (47% c_{app} after 24 h).[30] Surprisingly, the closely related *N*-phenoxyacetyl derivative **1i** did not react with enzyme.

Three nonpolar substrates structurally related to leucine (**1j**, **1k**, and **1l**) showed at least 6-, 8-, and 21-fold lower enantioselectivity (E_{true} = 7−24) and 24-, 40-, and 60-fold lower conversion (5−13% c_{app} after 24 h), respectively, than did **1c**. A similar compound, **1m**, demonstrated at least 3-fold lower enantioselectivity (E_{true} = 52) and 12-fold lower conversion (24% c_{app} after 24 h) than did **1c**.

(26) (a) Park, S.-S.; Wong, S.-L.; Wang, L.-F.; Doi, R. H. *J. Bacteriol.* **1989**, *171*, 2657−2665. (b) Zhao, H.; Arnold, F. H. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 7997−8000.
(27) Jain, S. C.; Shinde, U.; Li, Y.; Inouye, M.; Berman, H. M. *J. Mol. Biol.* **1998**, *284*, 137−144.
(28) Based on a limit of detection of 0.1%.
(29) (a) Estell, D. A.; Graycar, T. P.; Miller, J. V.; Powers, D. B.; Burnier, J. P.; Ng, P. G.; Wells, J. A. *Science* **1986**, *233*, 659−663. (b) Wells, J. A.; Powers, D. B.; Bott, R. R.; Graycar, T. P.; Estell, D. A. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 1219−1223.
(30) Additional screening with the *N*-dihydrocinnamoyl derivative **1h** showed chymotrypsin from bovine pancreas catalyzed its hydrolysis with good enantioselectivity (E = 45), favoring the (*R*)-enantiomer, and high conversion (c_{app} = 52%) after 6 h. This enzyme was missed in our initial activity screen because it reacted only slowly with the *N*-chloroacetyl derivative **1c**.

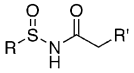
Table 2. Reactivity and Enantioselectivity of Subtilisin E toward Substrates with Differing *N*-Acyl Group (**1a**−**m**)

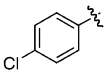
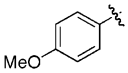
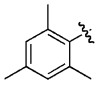
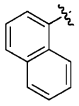
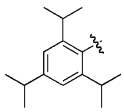
N-acyl group	$\%c_{app}$[a]	relative rate ($\%c_{app}$/h)[b]	ee_s (%)[c]	ee_p (%)[c]	E_{true}[d]
1a 	n.r.	n.a.	n.a.	n.a.	n.a.
1b 	n.r.	n.a.	n.a.	n.a.	n.a.
1c 	36[e]	12	56	97	>150[f]
1d 	53[e]	18	99	89	>150[g]
1e 	28	1.2	33	84	26[g]
1f 	26	1.1	34	95	54
1g 	46	1.9	77	90	44[h]
1h 	47	2.0	89	99	>150
1i 	n.r.	n.a.	n.a.	n.a.	n.a.
1j 	13	0.5	14	91	24
1k 	8	0.3	8	90	20
1l 	5	0.2	4	75	7
1m 	24	1.0	30	95	52

[a] Conversion %: amount of sulfinamide formed in 24 h, except where noted. [b] Conversion % per hour (assumes linear rate throughout course of reaction); n.a. = not applicable. [c] Enantiomeric excess %. Enantiomeric excess of substrate and product was determined by HPLC analysis on Daicel Chiralcel OD or AD columns at 238 or 222 nm; n.d. = not determined. [d] Enantiomeric ratio: the enantiomeric ratio *E* measures the relative rate of hydrolysis of the fast enantiomer as compared to that of the slow enantiomer as defined by Sih (Chen, C. S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294−7299). [e] Reaction for 3 h. [f] Corrected for ca. 2% chemical hydrolysis. [g] Corrected for ca. 4% chemical hydrolysis. [h] Diastereomeric ratio.

The *N*-dihydrocinnamoyl derivative **1h** and *N*-chloroacetyl derivative **1c** were the best acyl groups for *p*-toluenesulfinamide since they showed the highest enantioselectivity and reactivity. Thus, compounds **1c** and **1h**, which contain these groups, reacted at least 100 times faster than the simplest *N*-acylsulfinamide, **1a**.[28] The conversion was 6-fold lower for **1h**, as compared that of **1c**, but it did not suffer spontaneous chemical hydrolysis. Although the reactivity and enantioselectivity of **1d** were comparable to that of **1c**, its synthesis was less efficient (max yield 20% for **1d** vs 50% for **1c**).

Table 3. Enantioselectivity of Subtilisin E toward Substrates with Differing Sulfinamide Aryl Group

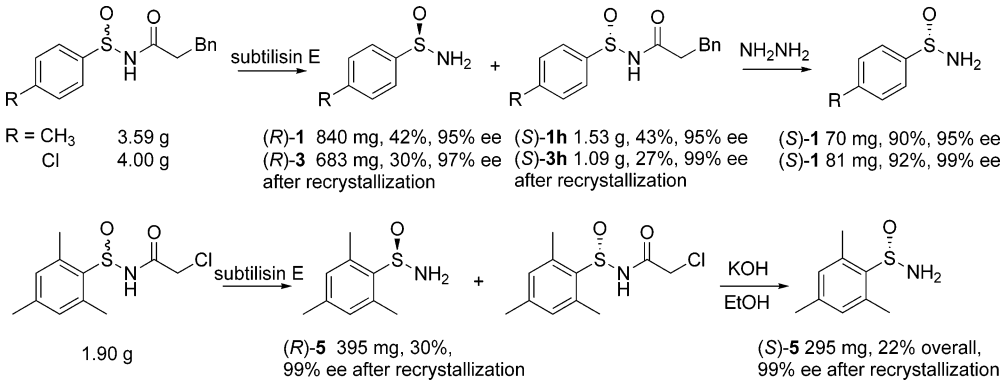


Sulfinamide	R′	$\%c_{app}$[a]	ee_s (%)[b]	ee_p (%)[b]	E_{app}	E_{true}[c]
	**2c** Cl	14	16	97	76	>150[d,e]
	2h Bn	14	16	99	>150	>150
	**3c** Cl	35	52	97	110	>150[d,e]
	3h Bn	9	10	99	>150	>150
	**4c** Cl	21	25	94	41	>150[d,f]
	4h Bn	40	65	98	>150	>150
	**5c** Cl	41	67	94	65	98[d,e]
	5h Bn	0.5	n.d.	n.d.	n.d.	n.d.
	**6c** Cl	19[g]	20	73	13	13
	6h Bn	n.r.	n.a.	n.a.	n.a.	n.a.
	**7c** Cl	30[g]	3	9	1.2	1.2
	**8c** Cl	n.r.	n.a.	n.a.	n.a.	n.a.
	8h Bn	n.r.	n.a.	n.a.	n.a.	n.a.

[a] Conversion %: conversion refers to the amount of sulfinamide formed. All reactions as *N*-chloroacetyl were 3 h and as *N*-dihydrocinnamoyl were 24 h, except where noted; n.r. = no reaction. [b] Enantiomeric excess %. Enantiomeric excess of substrate and product was determined by HPLC analysis on Daicel Chiralcel OD or AD columns at 238 or 222 nm; n.d. = not determined, n.a. = not applicable. [c] Enantiomeric ratio: the enantiomeric ratio *E* measures the relative rate of hydrolysis of the fast enantiomer as compared to the slow enantiomer as defined by Sih (Chen, C. S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294−7299). [d] Similar *E* results were obtained with *B. subtilis* var. biotecus A (see Supporting Information Table S1). [e] Corrected for ca. 1% chemical hydrolysis. [f] Corrected for ca. 3% chemical hydrolysis. [g] Reaction for 24 h.

Sulfinamide Substrate Range and Enantioselectivity. To determine the substrate range of subtilisin E, we tested seven additional arylsulfinamides with enzyme (Table 3). Subtilisin E catalyzed the hydrolysis of **2c** and para-substituted benzenesulfinamides, **3c** and **4c**, with high enantioselectivity (E_{true} > 150), but the enantioselectivity decreased with the more substituted sulfinamide, **5c** (E_{true} = 98), and the more hindered sulfinamides, **6c** (E_{true} = 13) and **7c** (E_{true} = 1.2). Compound **8c** did not react with subtilisin E.

Scheme 4. Gram-Scale Kinetic Resolution of Sulfinamides **1**, **3**, and **5**



Subtilisin E showed moderate to high conversion with **2c**, **3c**, **4c**, and **5c** (c_{app} = 14, 35, 21, and 41%, respectively, after 3 h), but the conversion decreased with the more hindered arylsulfinamides, **6c** and **7c** (c_{app} = 19 and 30%, respectively, after 24 h). Competing chemical hydrolysis (1−3%) occurred with all *N*-chloroacetyl derivatives; however, reducing the reaction temperature to 10 °C reduced spontaneous hydrolysis to <1% after 24 h. *B. subtilis* var. biotecus A gave similar conversions and enantioselectivity (see Supporting Information Table S1).

The *N*-dihydrocinnamoyl sulfinamides showed similar enantioselectivity to the *N*-chloroacetyl sulfinamides, but lower conversion and no spontaneous chemical hydrolysis. *N*-Dihydrocinnamoyl derivatives **2h**, **3h**, and **4h** showed high enantioselectivity (E_{true} > 150) and low to moderate conversion, c_{app} = 14% (8-fold lower than **2c**), 9% (30-fold lower than **3h**), and 40% (4-fold lower than **4c**), respectively, after 24 h. Compound **5h** reacted very slowly (0.5% c_{app} after 24 h), and **6h** and **8h** did not react. This lack of reaction may be due to either poor solubility[31] or poor fit in the enzyme active site. We resolved the more hindered substrates using the more reactive *N*-chloroacetyl group, but used the *N*-dihydrocinnamoyl group for all other sulfinamides to avoid spontaneous chemical hydrolysis.

Comparing the HPLC traces of the product sulfinamides to samples of known absolute configuration, prepared using either Davis and co-workers' method[12] for toluenesulfinamide or Senanayake and co-workers' method[16] for all other sulfinamides (see Supporting Information), established the absolute configurations. Subtilisin E favored the (*R*)-enantiomer in all cases.

Gram-Scale Resolutions. To demonstrate the synthetic usefulness of this reaction, we resolved sulfinamides **1**, **3**, and **5** on a multigram scale with subtilisin E (Scheme 4). We chose these sulfinamides for preparative reactions because sulfinamide **1** is widely used as a chiral auxiliary,[5] the aryl group of **3** has different electronic properties than **1** and might be a useful alternative, and 2,4,6-trimethylbenzenesulfinamide **5** is more hindered than **1** and gives fewer byproducts during nucleophilic addition.[32]

Resolution of **1h** (3.59 g) with subtilisin E gave (*R*)-**1** (840 mg, 42% yield; the maximum yield is 50% in a resolution) with 95% ee and (*S*)-**1h** (1.53 g, 43% yield) with 95% ee at ca. 50% conversion (3 days). If desired, both product and starting material can be recrystallized to >99% ee. The *N*-dihydrocinnamoyl group could be removed with hydrazine hydrate.[33] Treating (*S*)-**1h** (144 mg) with hydrazine hydrate gave (*S*)-**1** (70 mg, 90% yield) with 95% ee.

We resolved **3h** (4.00 g) to give (*R*)-**3** (683 mg, 30% yield) with 97% ee and (*S*)-**3h** (2.42 g, 60% yield) with 64% ee at ca. 40% conversion (3 days). For unknown reasons, this resolution stopped at 40% conversion, but recrystallization of unreacted starting material gave (*S*)-**3h** (1.09 g, 27%) with 99% ee. Treating (*S*)-**3h** (155 mg) with hydrazine hydrate gave (*S*)-**1** (81 mg, 92%) with 99% ee.

We used the *N*-chloroacetyl group to resolve 2,4,6-trimethylbenzenesulfinamide **5** (1.90 g) because the *N*-dihydrocinnamoyl derivative reacted very slowly. The resolution yielded (*R*)-**5** (520 mg, 39% yield) with 90% ee at ca. 48% conversion (6 h). Recrystallization of (*R*)-**5** gave 395 mg (30%) with 99% ee. The unreacted starting material, (*S*)-**5c**, was isolated with 45% yield (860 mg, 82% ee). The *N*-chloroacetyl group cleaved in ethanolic KOH. Hydrolysis, followed by recrystallization, gave (*S*)-**5** (295 mg, 22% yield) with 99% ee. Recrystallization was necessary because 5% of starting material spontaneously hydrolyzed during reaction. However, chemical hydrolysis could be reduced to <1% at 10 °C, while the enzymatic reaction was only 3-fold slower (c_{app} = 16% at 10 °C vs 41% at 37 °C after 3 h).

These enzymatic resolutions are simple, convenient, and avoid costly auxiliaries.[12,16] The current synthetic route to enantiopure **1** relies on the starting material menthyl-*p*-toluenesulfinate (U.S. $15−40/g), and enantiopure **3** and **5** rely on the amino alcohol, *cis*-1-amino-2-indanol (U.S. $30/g) and give low yields of the sterically hindered **5**.[34] This enzymatic resolution yields enantiopure *N*-acylsulfinamides as intermediates. These may be useful for diastereoselective enolate alkylation reactions as a route to enantiopure α-substituted carboxylic acids.[20]

Molecular Basis for Higher Reactivity of 1c and 1h. To understand why (*R*)-**1h** and (*R*)-**1c** reacted with subtilisin E while (*R*)-**1a** did not, we modeled the first tetrahedral intermedi-

(31) The solubility of both **5h** and **6h** in 10% MeCN/buffer was <5 mM. Organic cosolvents (1,4-dioxane, EtOH, DMSO) or additives (10 mM guanidium chloride) did not significantly improve solubility. Performing the reaction experiment at 50 °C or using a biphasic reaction system with *tert*-butylmethyl ether also did not increase conversion. Using MeCN or 1,4-dioxane at higher concentrations (20−90%) improved substrate solubility, but did not increase conversion.

(32) Han, Z.; Krishnamurthy, D.; Pflum, D.; Grover, P.; Wald, S. A.; Senanayake, C. H. *Org. Lett.* **2002**, *4*, 4025−4028.

(33) Keith, D. D.; Tortora, J. A.; Yang, R. *J. Org. Chem.* **1978**, *43*, 3711−3713.

(34) In our hands, the sodium bis(trimethylsilyl)amide displacement[16] gave low yields of (*R*)-**2**−**4** and (*R*)-**6** (18−52%) and no yield for (*R*)-**5**. When $LiNH_2$/NH_3 was used in place of sodium bis(trimethylsilyl)amide, (*R*)-**5** formed in low yield (3%) and enantiomeric excess (68% ee).

Table 4. Minimized Structures for the Tetrahedral Intermediate of Subtilisin-E-Catalyzed Hydrolysis of **1h**, **1c**, **1a**, and **7c**

conformation	group in S_1'[a]	H bond $N_{\epsilon 2}$–O_γ distance (Å) ($N-H-O_\gamma$ angle, deg)[b]	comments
(*R*)-**1h** (Figure 2)	tolyl	2.96 (155)	steric contact with Tyr 217, His 64, and Met 222 in S_1' pocket
(*R*)-**1h**	O_S[c]	3.08 (152)	severe steric clash with Gly219 and Asn155
(*S*)-**1h** (Figure 2)	O_S	3.03 (154)	unhindered in S_1' pocket
(*S*)-**1h**	tolyl[c]	2.99 (157)	steric clash with catalytic His 64
(*R*)-**1c** (not shown)	tolyl	2.98 (153)	possible hydrogen bond with Thr 220 ($Cl_\alpha-O_{\gamma 1}$ distance = 3.81 Å)
(*R*)-**1a** (not shown)	tolyl	2.97 (153)	no contact with S_1 residues
(*R*)-**7c** (Figure S2)	none	2.94 (157)	binds above S_1' pocket
(*S*)-**7c** (Figure S2)	none	2.93 (156)	binds above S_1' pocket

[a] Narrow, hydrophobic pocket where leaving group alcohol, amide, or sulfinamide binds. [b] Unless otherwise noted, hydrogen bonds (His64 $N_{\epsilon 2}-N$ or O, Ser221 $NH-O^-$, and Asn155 $NH_{\delta 2}-O^-$) were present in all structures ($N-N$ or O distance 2.7−3.1 Å, $N-H-O$ or $N-H-N$ angle 120−175°). [c] Group is forced out of S_1' because of steric clash with active site residues.

ate for hydrolysis of these substrates (see Experimental Section for modeling details). The modeling identified interactions that bind the *N*-acyl group of (*R*)-**1h** and (*R*)-**1c**, but not (*R*)-**1a**, to the S_1 pocket.

The (*R*)-**1h** tetrahedral intermediate fit well in the active site, and all five catalytically relevant hydrogen bonds were normal length (<3.1 Å) (Table 4). The benzyl moiety of the dihydrocinnamoyl group bound in the S_1 pocket, as expected based on its similarity to phenylalanine.[29,35] This benzyl moiety contacted hydrophobic portions of five out of eight residues lining this pocket (Leu126, Gly127, Gly128, Ala152, and Gly154). Using the incremental Gibbs free energy of transfer from *n*-octanol to water,[36,37] we estimate the energy for this benzyl-S_1 pocket hydrophobic interaction to be 2.7−5.5 kcal/mol.[38] Thus, the hydrophobic interaction between the benzyl moiety and S_1 pocket likely improves binding of (*R*)-**1h**, as compared to (*R*)-**1a**.

The (*R*)-**1c** tetrahedral intermediate also fit well in the active site, and all five hydrogen bonds were normal length (<3.1 Å) (not shown). The α-chloro atom of (*R*)-**1c** was bound in the S_1 pocket near the hydroxyl group ($O_{\gamma 1}-H$) of Thr220 ($O_{\gamma 1}-Cl$ distance = 3.81 Å). This $O_{\gamma 1}-Cl$ distance was closer than the van der Waals contact (3.91 Å),[39] but longer than a hydrogen bond between a hydroxyl group and chlorine (2.91−3.52 Å).[40] A typical hydrogen bond between substrate and protein lowers the energy by 0.5−1.5 kcal/mol.[41] This $O_{\gamma 1}-Cl$ interaction combined with the inductive effects of the α-chlorine atom, which increases the electrophilicity of the carbonyl carbon, may improve the binding and reactivity of (*R*)-**1c**.

Although the tetrahedral intermediate for (*R*)-**1a** also fits in the subtilisin active site and makes all five key hydrogen bonds, the small *N*-acyl group lacks contact with S_1 pocket residues (not shown). This lack of favorable interaction with S_1 residues to improve binding and an electron-withdrawing group to increase reactivity may account for its lack of reaction.[42]

Molecular Basis for Enantioselectivity of Subtilisin E with 1h. Empirical rules based on substituent size can often predict the fast-reacting enantiomer in subtilisin-catalyzed reactions of secondary alcohols (Figure 1a).[43] Sulfinamides mimic secondary alcohols, where the aryl group is the large substituent and the oxygen is the medium substituent, because of their similar shape. However, the solvation of the medium group of a secondary alcohol (e.g., methyl group) and the sulfoxide oxygen differs. The incremental Gibbs free energy of transfer[36,37] from *n*-octanol to water for a methyl group is +0.56 kcal/mol, while that for sulfoxide oxygen is −3.0 kcal/mol.[44] We hypothesized that subtilisin E favors the (*R*)-enantiomer because the sulfoxide oxygen favors a water-exposed orientation, while the aryl group binds in the S_1' leaving group pocket because of a favorable hydrophobic interaction (Figure 1b).

To test this hypothesis, we modeled the first tetrahedral intermediate for hydrolysis of **1h**. The protein modeling molecular mechanics force field (AMBER) did not include parameters for the sulfinamide group, so we estimated these parameters using geometric information for methyl sulfinamide[45]

(35) Lee, T.; Jones, J. B. *J. Am. Chem. Soc.* **1996**, *118*, 502−508.
(36) (a) Fujita, T.; Iwasa, J.; Hansch, C. *J. Am. Chem. Soc.* **1964**, *86*, 5175−5180. (b) Leo, A.; Hansch, C.; Elkins, D. *Chem. Rev.* **1971**, *71*, 525−616. (c) Hansch, C.; Coats, E. *J. Pharm. Sci.* **1970**, *59*, 731−743. (d) Hansch, C.; Leo, A. *Substituent Constants for Correlation Analysis in Chemistry and Biology*; John Wiley and Sons: New York, 1979; pp 48−51.
(37) Fersht, A. *Structure and Mechanism in Protein Science*; W. H. Freeman and Company: New York, 1998; pp 324−348.
(38) A hydrophobic interaction is the tendency of nonpolar compounds to transfer from an aqueous phase to an organic phase. 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.[37] The incremental Gibbs free energy of transfer of a benzyl group from *n*-octanol to water is 2.7 kcal/mol using $\Delta G_{trans} = 2.303RT\pi$, where π is the hydrophobicity constant of the benzyl group ($\pi = 2.01$)[36d] relative to hydrogen. However, binding of the benzyl group in the hydrophobic S_1 cavity removes two water-hydrophobic interfaces (i.e., substrate and protein), so the value might be as high as 5.5 kcal/mol.[37] Since the S_1 pocket lies on the surface of the protein and the benzyl group is not completely buried, we estimate the energy for this hydrophobic interaction to be 2.7−5.5 kcal/mol.[36a,b,37]
(39) Estimated from the van der Waals radii of chlorine (1.75 Å) and hydrogen (1.20 Å) and the O−H bond length (0.96 Å) from: Bondi, A. *J. Phys. Chem.* **1964**, *68*, 441−451.
(40) Jeffrey, G. A.; Saenger, W. *Hydrogen Bonding in Biological Structures*; Springer-Verlag: Berlin, 1991; pp 29−31.
(41) Removal of a hydrogen bond donor or acceptor in an enzyme active site weakens binding by 0.5−1.5 kcal/mol.[37] Although the hydrogen bond inventory is zero (i.e., the number of hydrogen bonds is the same for both the free enzyme and the enzyme−substrate complex), hydrogen bonds between an enzyme and substrate increase the entropy due to the release of bound water molecules.
(42) This reasoning predicts lower K_M values for **1h** and **1c** than for **1a**, but low solubility of these substrates (<5 mM) prevented us from testing this prediction.
(43) (a) Fitzpatrick, P. A.; Klibanov, A. M. *J. Am. Chem. Soc.* **1991**, *113*, 3166−3171. (b) Kazlauskas, R. J.; Weissfloch, A. N. E. *J. Mol. Catal.* **1997**, *3*, 65−72.

from ab initio calculations and from force field parameters for a sulfonamide-based inhibitor[46] also derived from ab initio calculations (see Supporting Information for details). Using these estimates, we expect only a qualitative rationalization of the enantioselectivity of subtilisin E with *N*-acylsulfinamides.

Modeling **1h** with subtilisin E gave one productive conformation for each enantiomer (Table 4). The two other plausible conformations encountered severe steric clash with the protein.[47] The productive complex of (*R*)-**1h** had its *p*-tolyl group bound in the S_1' pocket and the sulfoxide oxygen exposed to solvent water (Figure 2). All hydrogen bond angles were >120°, and all five hydrogen bond lengths were <3.1 Å (Table 4). The *p*-tolyl group appears to just fit in the S_1' pocket: Met222 in the bottom of the S_1' pocket, Tyr217 at the back of the S_1' pocket, and catalytic His64 bumped the *p*-tolyl group.[47] This tight fit suggests a favorable hydrophobic interaction between the tolyl group and the S_1' residues.

The productive complex of the slower-reacting (*S*)-**1h** had its sulfoxide oxygen in the S_1' pocket and the tolyl group exposed to solvent water (Figure 2). All hydrogen bond angles were >120°, and all five hydrogen bond lengths were <3.1 Å (Table 4). The sulfoxide oxygen fits well in the S_1' pocket, and the protein does not hinder the *p*-tolyl group.[47] Unlike the favored enantiomer, the smaller oxygen does not make steric contact with S_1' residues. Although the slow-reacting (*S*)-enantiomer avoids steric hindrances, it also lacks favorable hydrophobic interactions between the tolyl group and the S_1' residues. Using octanol−water partitioning data, we estimate that the hydrophobic interactions favor binding of (*R*)-**1h** by ~6.4 kcal/mol over the (*S*)-enantiomer in water.[44] The (*R*)-enantiomer places the hydrophobic aryl group in the S_1' pocket and the hydrophilic sulfoxide oxygen in the solvent (water), while the (*S*)-enantiomer does the opposite.

Consistent with this explanation, the enantioselectivity of the subtilisin-E-catalyzed hydrolysis of **1h** decreased from $E > 150$ in 9:1 water/acetonitrile to $E = 12$ in 1:9 water/acetonitrile. Similarly, the enantioselectivity with **1c** decreased from $E > 150$ in 9:1 water/acetonitrile to $E = 41$ in 1:9 water/acetonitrile. The high concentration of acetonitrile favors the *p*-tolyl group in the solvent; thus, the enantiomer preference shifts toward the (*S*)-enantiomer, which orients with the oxygen in the S_1' pocket and the tolyl group in solvent. This result suggests that the enantioselectivity for acylation of sulfinamides in organic solvent would also be low, but we did not detect any acylation of *p*-toluenesulfinamide, consistent with Ellman's earlier report of no reaction.[15]

The decreasing enantioselectivity with the larger sulfinamides, **5c** ($E_{true} = 98$) and **6c** ($E_{true} = 13$), and the loss of enantioselectivity with the very large sulfinamide, **7c** ($E_{true} = 1.2$), are also consistent with this explanation for enantioselectivity. Larger aryl substituents increase the steric hindrance in the S_1' pocket, which overwhelms the energy gained through a hydrophobic interaction between the pocket and the aryl group and, therefore, reduces the enantioselectivity. In other words, when either the aryl group or sulfoxide oxygen can fit in the S_1' pocket (**1**−**4**), a hydrophobic interaction favors the aryl group and the enantioselectivity is high. However, as the aryl group becomes larger, increased steric hindrance with S_1' residues oppose the hydrophobic interaction and lower the enantioselectivity (**5**−**7**). Modeling with **7c** suggests that this sulfinamide leaving group is too large for the S_1' pocket and binds above it (see Supporting Information Figure S2).

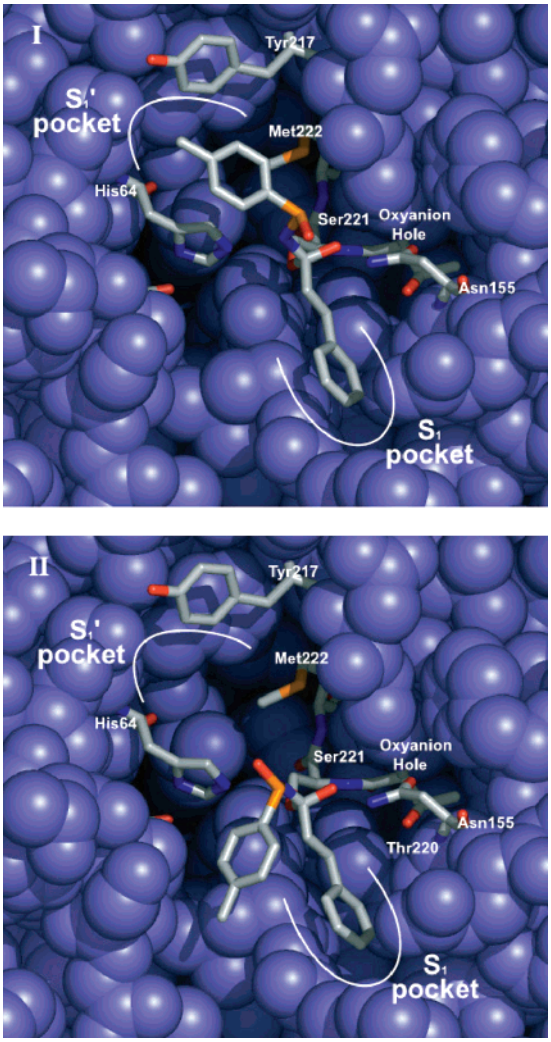


Figure 2. Catalytically productive tetrahedral intermediates for the subtilisin-E-catalyzed hydrolysis of fast-reacting (*R*)-**1h** (I) and slow-reacting (*S*)-**1h** (II), as identified by molecular modeling. The important active site and substrate atoms (sticks) are colored as follows: gray (carbon), red (oxygen), blue (nitrogen), and orange (sulfur). Surrounding atoms (space fill) of subtilisin are shown in blue. For clarity, all hydrogen atoms and water molecules are hidden. Both I and II maintain all catalytically essential hydrogen bonds, and the benzyl moiety of the dihydrocinnamoyl group binds in the S_1 pocket, as expected based on its similarity to phenylalanine.[29] In the fast-reacting enantiomer, (*R*)-**1h** (I), the *p*-tolyl group binds in the hydrophobic S_1' pocket and sulfoxide oxygen is exposed to solvent water. In the slow-reacting enantiomer, (*S*)-**1h** (II), the sulfoxide oxygen binds in the hydrophobic S_1' pocket and the *p*-tolyl group is exposed to solvent water. The nonproductive conformations (not shown) encountered severe steric clash with the active site residues.

(44) The incremental Gibbs free energy of transfer from *n*-octanol to water for the tolyl group was estimated to be ca. +3.4 kcal/mol, and the sulfoxide oxygen was ca. −3.0 kcal/mol using $\Delta G_{trans} = 2.303RT\pi$, where π is the hydrophobicity constant of the group relative to hydrogen.[36a,b,37] Switching from (*S*)-**1h** to (*R*)-**1h** places the tolyl group into a hydrophobic pocket (3.4 kcal/mol for the tolyl group) and removes the sulfoxide group from the hydrophobic pocket (3.0 kcal/mol) for a total of 6.4 kcal/mol in water. The hydrophobicity constant of the tolyl group ($\pi = 2.52$) was estimated by adding π values of a phenyl and a methyl group.[36d] The hydrophobicity constant of the sulfoxide oxygen ($\pi = -2.19$) was estimated by subtracting π values of a methyl sulfide group from a methyl sulfoxide group.[36d] The energy difference may be higher if one includes the hydrophobic surface of the S_1' pocket.

(45) Bharatam, P. V.; Kaur, A.; Kaur, D. *J. Phys. Org. Chem.* **2002**, *15*, 197−203.
(46) Rossi, K. A.; Merz, K. M., Jr.; Smith, G. M.; Baldwin, J. J. *J. Med. Chem.* **1995**, *38*, 2061−2069.
(47) See Supporting Information for complete molecular modeling details.

Discussion

In this paper, we identified subtilisin E as the most suitable hydrolase of those examined for preparation of enantiopure arylsulfinamides. The reactivity and enantioselectivity of subtilisin E toward *N*-acyl arylsulfinamides depend on the *N*-acyl group. Simple *N*-acyl compounds, such as *N*-acetyl and *N*-butanoyl, did not react with subtilisin E. Molecular modeling suggests that the reactive acyl groups may mimic a phenylalanine moiety. Other research groups have also modified unreactive substrates to convert them into good substrates. For example, the fungus *Beauveria bassiane* did not hydroxylate cyclopentanone, but did hydroxylate the *N*-benzoylspirooxazolidine derivative with high yield and diastereoselectivity.[48] In a second example, changing from the 2-pyridylacetyl to the 4-pyridylacetyl increased the rate 8-fold and the enantioselectivity 3-fold for a penicillin G acylase-catalyzed hydrolysis of 1-phenethyl esters.[49] In a third example, the enantioselectivity of *Pseudomonas cepacia* lipase-catalyzed acylation of 2-[(*N*,*N*-dimethylcarbamoyl)methyl)]-3-cyclopenten-1-ol varied from $E = 4$ to 156, depending on the acylating agent.[50]

There are three synthetic routes to enantiopure sulfinamides: the method of Davis and co-workers[12] for *p*-toluenesulfinamide, the method of Ellman and co-workers[6] for *tert*-butylsulfinamide, and the method of Senanayake and co-workers[16] for a variety of alkyl- and arylsulfinamides. Using subtilisin E, we resolved gram quantities of **1h**, **3h**, and **5c**. These resolutions were simple, convenient, and inexpensive. Our strategy does not provide us with as wide a variety of enantiopure sulfinamides as reported by Senanayake, but the selectivity and mildness of the biocatalytic route make it the preferred route when there is a choice. The biocatalytic route is amenable to scale-up, is environmentally acceptable, and is performed under mild conditions. As well, the catalyst, subtilisin E, is inexpensive to produce and could be recycled.

Molecular modeling of the first tetrahedral intermediate for subtilisin-E-catalyzed hydrolysis of **1h** suggests that the (*R*)-enantiomer reacts faster because of preferential binding of the nonpolar *p*-tolyl group in the hydrophobic S_1' pocket versus the polar sulfoxide oxygen, which prefers to be exposed to solvent water. Changing the solvent to 1:9 water/acetonitrile decreased the enantioselectivity. A similar decrease in enantioselectivity occurred for subtilisin-catalyzed reactions of amino acid derivatives.[51] In water, subtilisin Carlsberg showed high enantioselectivity for the hydrolysis of a natural L-amino acid ester, but in organic solvent, transesterification was 1−2 orders of magnitude less enantioselective. Klibanov and co-workers suggested that the L-amino acid, but not the D-amino acid, binds to the hydrophobic S_1 pocket. Water as the solvent favors this interaction; thus, the enantioselectivity is higher in water. As expected, the difference in enzyme enantioselectivity was greater for amino acid derivatives with more hydrophobic side chains.

Subtilisins usually show only low to moderate enantioselectivity with secondary alcohols and isosteric amines, but high enantioselectivity with the structurally related sulfinamides. For secondary alcohols and isosteric primary amines, the substituents are usually both hydrophobic, so the hydrophobic binding contribution differences are smaller than those for arylsulfinamides, where the substituents are the polar sulfoxide oxygen and the hydrophobic aryl group. This polarity difference between the two substituents appears to dominate subtilisin E's enantiorecognition of arylsulfinamides, resulting in high enantioselectivity.

Experimental Section

General. ^{1}H and ^{13}C NMR spectra were obtained as dilute $CDCl_3$ solutions at 300 and 75 MHz, respectively. Chemical shifts are expressed in parts per million (δ) and are referenced to tetramethylsilane or trace $CHCl_3$ in $CDCl_3$. Coupling constants are reported in hertz (Hz). HPLC analyses were performed on a 4.6 × 250 mm Daicel Chiralcel OD or Chiralpak AD column (Chiral Technologies, Exton, U.S.A.) and monitored at 238 or 222 nm. Flash chromatography with silica gel (230−400 mesh) or preparative TLC (20 × 20 cm, 1000 μm) was used to purify all intermediates and substrates. Visualization of UV-inactive materials on TLC was accomplished using phosphomolybdic acid or ninhydrin followed by heating. All reagents, buffers, starting materials, and anhydrous solvents were purchased from Sigma-Aldrich Canada (Oakville, Canada) and used without purification. All air- and moisture-sensitive reactions were performed under N_2. The pBE3 *Escherichia coli*−*Bacillus subtilis* shuttle vector,[26b] containing the subtilisin E gene, was kindly provided by Dr. F. Arnold (Caltech), and the *Bacillus subtilis* strain DB104[52] was a gift from Dr. S. L. Wong (University of Calgary).

Synthesis of Substrates. Sulfinamides (1−8). We prepared racemic sulfinamides **1**−**8** using literature procedures (Scheme 3).[20−22] The relevant analytical data are in the Supporting Information.

***N*-Acylsulfinamides.** We prepared *N*-acylsulfinamides by treating sulfinamides **1**−**8** with 2 equiv of *n*-BuLi in THF, followed by rapid addition of the symmetrical anhydride of the appropriate carboxylic acid.[53] The relevant analytical data are given below or in the Supporting Information.

***N*-Chloroacetyl-*p*-toluenesulfinamide (1c)** was obtained as a white solid (186 mg, 50%): mp 119−121 °C; ^{1}H NMR δ 2.43 (s, 3H, PhCH_3), 4.29 (s, 2H, C(O)CH_2Cl), 7.31 (d, $J = 8.1$, 2H), 7.63 (d, $J = 8.1$, 2H); ^{13}C NMR δ 21.8 (PhCH_3), 42.5 (C(O)CH_2Cl), 124.8, 130.6, 139.9, 143.5 (phenyl), 167.2 (*C*═O); HRMS calcd for $C_9H_{10}{}^{35}ClNO_2S$ (M^+) 231.0120, found 231.0123. The enantiomers were separated by HPLC (Daicel Chiralcel OD column, 90:10 hexanes/EtOH, 0.5 mL/min, 238 nm; (*R*)-**1c**, $t_R = 21.3$ min; (*S*)-**1c**, $t_R = 51.4$ min).

***N*-Dihydrocinnamoyl-*p*-toluenesulfinamide (1h)** was obtained as a white solid (550 mg, 59%): mp 85−87 °C (lit.[20] mp 94−96 °C); ^{1}H NMR δ 2.43 (s, 3H, PhCH_3), 2.70 (m, 2H, C(O)CH_2), 3.01 (t, $J = 7.8$, 2H, CH_2Ph), 7.17−7.29 (m, 7H, phenyl), 7.41 (d, $J = 8.1$, 2H, phenyl), 7.81 (br s, 1H, N*H*); ^{13}C NMR δ 21.9 (tolyl *C*H$_3$), 31.2 (*C*H$_2$Ph), 37.9 (C(O)*C*H$_2$), 124.9, 126.6, 128.7, 128.8, 130.1, 140.1, 140.3, 142.6 (phenyl), 173.6 (*C*═O). The enantiomers were separated by HPLC (Daicel Chiralcel OD column, 90:10 hexanes/EtOH, 0.5 mL/min, 238 nm; (*R*)-**1h**, $t_R = 20.4$ min; (*S*)-**1h**, $t_R = 22.4$ min).

Hydrolase Library. All screening was performed at pH 7.2. The hydrolases were dissolved in BES buffer (5.0 mM, pH 7.2) at the concentration listed in Table 1, centrifuged for 10 min at 2000 rpm, and titrated to pH 7.2. The supernatant was used for screening.

Screening of Commercial Hydrolases with pH Indicators. The assay solution was prepared by mixing **1c** (1 mL of a 440 mM solution in CH_3CN) and *p*-nitrophenol (6.71 mL of a 1.0 mM solution in 5.0 mM BES, pH 7.2) with BES buffer (5.14 mL of a 5.0 mM solution, pH 7.2). Hydrolase solutions (10 μL/well) were transferred to a 96-

(48) (a) Braunegg, G.; de Raadt, A.; Feichtenhofer, S.; Griengl, H.; Kopper, I.; Lehman, A.; Weber, H.-J. *Angew. Chem., Int. Ed.* **1999**, *38*, 2763−2766. (b) de Raadt, A.; Griengl, H.; Weber, H. *Chem.—Eur. J.* **2001**, *7*, 27−31.
(49) Pohl, T.; Waldmann, H. *Tetrahedron Lett.* **1995**, *36*, 2963−2966.
(50) Ema, T.; Maeno, S.; Takaya, Y.; Sakai, T.; Utaka, M. *J. Org. Chem.* **1996**, *61*, 8610−8616.
(51) (a) Zaks, A.; Klibanov, A. M. *J. Am. Chem. Soc.* **1986**, *108*, 2767−2768. (b) Margolin, A. L.; Tai, D.-F.; Klibanov, A. M. *J. Am. Chem. Soc.* **1987**, *109*, 7885−7887. (c) Sakurai, T.; Margolin, A. L.; Russell, A. J.; Klibanov, A. M. *J. Am. Chem. Soc.* **1988**, *110*, 7236−7237.
(52) Kawamura, F.; Doi, R. H. *J. Bacteriol.* **1984**, *160*, 442−444.
(53) Chen, F. M. F.; Kuroda, K.; Benoiton, N. L. *Synthesis* **1978**, *12*, 928−930.

well microtiter plate followed by assay solution (90 μL/well). The final concentration in each well was 3.1 mM substrate, 4.65 mM BES, 0.46 mM *p*-nitrophenol in a total volume of 100 μL 7% acetonitrile in buffer. The plate was shaken for 5 s on the microplate reader, and the absorbance decrease was monitored at 404 nm for 1 h. The assay was performed in quadruplicate at 25 and 37 °C.[23] The low pK_a of the *N*-acylsulfinamide NH group (ca. pK_a 6) reduced the sensitivity of the assay by protonating the pH indicator, *p*-nitrophenoxide, but a decrease in the absorbance could still be observed at pH 7.2.

Small-Scale Reactions with Commercial Hydrolases to Determine Enantioselectivity. Reactions of *N*-chloroacetylsulfinamides with commercial enzymes were carried out at 25 °C. For example, proteinase from *B. subtilis* var. biotecus A (16 mg) and substrate (5 mM in CH_3CN) in a total volume of 10 mL of buffer (20 mM BES, pH 7.2) were stirred together in reaction vials for 3−6 h. Upon completion, the aqueous phase was extracted with CH_2Cl_2 (2 × 10 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was dissolved in 1 mL of EtOH, filtered through a nylon filter (0.45 μm), and analyzed by HPLC (Daicel OD column, 90:10 hexanes/EtOH, 1.0 mL/min, 238 nm).

Cultivation and Isolation of Subtilisin E. Protease-deficient *B. subtilis* DB104 was transformed with vector pBE3, as previously described.[26b,54] Protease-deficient *B. subtilis* DB104 secretes proteases into the culture medium and accumulates them to a high level. Endogenous protease activity is less than 3%.[52] Cells were grown in Schaeffer's sporulation medium (2XSG) supplemented with kanamycin (50 μg/mL). Enzyme activity was monitored by adding 90 μL of assay solution [0.2 mM succinyl-AAPF-*p*-nitroanilide (suc-AAPF-*p*NA)/100 mM Tris pH 8.0/10 mM $CaCl_2$] to 10 μL of supernatant and following the reaction at 410 nm at 37 °C for 15 min. When maximal activity was reached (48 h), the cells were centrifuged, and the supernatant was filter sterilized and lyophilized. The residue was dissolved in 10 or 50 mM Tris buffer (pH 8.0, 10 mM $CaCl_2$), and the initial activity was assessed as above. Typical activity of the supernatant or prepared solution was 10−20 U/mL, with suc-AAPF-*p*NA.

Hydrolysis of *N*-Acylsulfinamides with Subtilisin E. Reactions of *N*-acylsulfinamides with subtilisin E were carried out at 37 °C. Each mixture consisted of subtilisin E (solution in 10 mM Tris buffer (pH 8.0, 1 mM $CaCl_2$), 178 μL), $CaCl_2$ (1 M, 2 μL), and substrate (50 mM in CH_3CN, 20 μL). The reaction was allowed to continue for 3 h with *N*-bromoacetyl and *N*-chloroacetyl sulfinamides and for 24 h with all other substrates. The reaction was diluted with dH_2O (400 μL) and terminated with the addition of CH_2Cl_2 (500 μL). The organic layer was removed, and the aqueous layer was twice extracted with CH_2Cl_2 (500 μL). The phases were separated by centrifugation, and the organic layers were collected. The combined organics were evaporated under a stream of N_2, and the residue was diluted with EtOH (150 μL) for analysis by HPLC (Brownlee Si-10 silica column followed by Daicel OD column, 90:10 hexanes/EtOH, 0.6 mL/min, 238 or 222 nm). Although the chiral column separated enantiomers, it did not separate the substrate and product. This two-column system separated the substrate and product in the silica column and the enantiomers of each in the chiral column.

Gram-Scale Resolution of *N*-Acylsulfinamides. 1h. Substrate (3.59 g, 12.5 mmol) in CH_3CN (50 mL) was added to enzyme solution (450 mL, ca. 20 U/mL with suc-AAPF-*p*NA) and incubated at 37 °C until ca. 50% conversion (3 days), as determined by HPLC. The mixture was filtered through Celite, extracted with CH_2Cl_2 (3 × 200 mL), dried over Na_2SO_4, and concentrated in vacuo. Separation of substrate and product on silica (1:1 EtOAc/hexanes to EtOAc) gave (*R*)-**1** (840 mg, 42%, 95% ee) and (*S*)-**1h** (1.53 g, 43%, 95% ee), as determined by HPLC. Remaining starting material (*S*)-**1h** (144 mg, 0.5 mmol) was treated with hydrazine hydrate (1 mL) at 0 °C followed by stirring at RT until the reaction was complete by TLC (3 h). The reaction mixture was diluted with CH_2Cl_2 (15 mL) and washed with 1 N HCl (2 × 10 mL), saturated $NaHCO_3$ (2 × 10 mL), and saturated NaCl (10 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo to give (*S*)-**1** (70 mg, 90%, 95% ee).

3h. Substrate (4.00 g, 13.0 mmol) in MeCN (100 mL) was added to enzyme solution (900 mL, ca. 15 U/mL with suc-AAPF-*p*NA) and incubated at 37 °C until ca. 40% conversion (3 days), as determined by HPLC. The mixture was filtered through Celite, extracted with CH_2Cl_2 (3 × 200 mL), dried over Na_2SO_4, and concentrated in vacuo. Separation of substrate and product on silica (1:1 EtOAc/hexanes to EtOAc) gave (*R*)-**3** (683 mg, 30%, 97% ee) and (*S*)-**3h** (2.42 g, 60%, 64% ee). Recrystallization from EtOAc/hexanes gave (*S*)-**3h** (1.09 g, 27%, 99% ee), as determined by HPLC. Remaining starting material (*S*)-**3h** (155 mg, 0.5 mmol) was treated with hydrazine hydrate (1 mL) at 0 °C followed by stirring at RT until the reaction was complete by TLC (3 h). The reaction mixture was diluted with CH_2Cl_2 (15 mL) and washed with 1 N HCl (2 × 10 mL), saturated $NaHCO_3$ (2 × 10 mL), and saturated NaCl (10 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo to give (*S*)-**3** (81 mg, 92%, 99% ee).

5c. Substrate (1.90 g, 7.30 mmol) in CH_3CN (30 mL) was added to enzyme solution (270 mL, ca. 20 U/mL with suc-AAPF-*p*NA) and incubated at 37 °C until ca. 49% conversion (6 h), as determined by HPLC. The mixture was filtered through Celite, extracted with CH_2Cl_2 (3 × 200 mL), dried over Na_2SO_4, and concentrated in vacuo to give the crude mixture of substrate and product. Separation of substrate and product on silica (2:1 EtOAc/hexanes to EtOAc) gave (*R*)-**5** (520 mg, 39%, 90% ee) and (*S*)-**5c** (860 mg, 45%, 82% ee). Recrystallization from EtOAc/hexanes gave (*R*)-**5** (395 mg, 30%, 99% ee), as determined by HPLC. Remaining starting material was hydrolyzed in 1 N KOH (1:1 H_2O/EtOH) for 1 h at RT. The reaction mixture was extracted with CH_2Cl_2 (3 × 15 mL), dried over Na_2SO_4, and concentrated in vacuo to give (*S*)-**5** (295 mg, 22%, 99% ee) after recrystallization.

Modeling of Tetrahedral Intermediates Bound to Subtilisin E. All modeling was performed using Insight II 2000.1/Discover (Accelrys, San Diego, CA) on an SGI Octane UNIX workstation using the AMBER force field.[55] We used a nonbonded cutoff distance of 8 Å and a distance-dependent dielectric of 1.0, and we scaled the 1−4 van der Waals interactions by 50%. Protein structures in Figures 2 and S1 (Supporting Information) were created using PyMOL (Delano Scientific, San Carlos, CA). The X-ray crystal structure of subtilisin E (entry 1SCJ)[27] from the Protein Data Bank is a Ser221Cys subtilisin E−propeptide complex. Using the Builder module of Insight II, we replaced the Cys221 with a serine and removed the propeptide region. The hydrogen atoms were added to correspond to pH 7.0. Histidines were uncharged, aspartates and glutamates negatively charged, and arginines and lysines 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.[56] This optimization was repeated until there was <2 kcal/mol 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 Ser221. Since the parameters for the sulfinamide group were not included in the AMBER force field, they were assigned in analogy to existing parameters.[45−47] The geometric properties of the sulfinamide

(54) Harwood, C. R.; Cutting, S. M. *Molecular Biological Methods for Bacillus*; John Wiley & Sons: Chichester, U.K., 1990; pp 33−35 and 391−402.

(55) (a) Weiner, S. J.; Kollman, P. A.; Case, D. A.; Singh, U. C.; Ghio, C.; Alagona, G.; Profeta, S.; Weiner, P. *J. Am. Chem. Soc.* **1984**, *106*, 765−784. (b) Weiner, S. J.; Kollman, P. A.; Nguyen, D. T.; Case, D. A. *J. Comput. Chem.* **1986**, *7*, 230−252.

(56) Raza, S.; Fransson, L.; Hult, K. *Protein Sci.* **2001**, *10*, 329−338.

moiety of minimized **1h** were compared to an available X-ray crystal structure[57] and adjusted as necessary. Nonstandard 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 root mean square 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 nonproductive.

Acknowledgment. We thank the donors of the Petroleum Research Fund administered by the American Chemical Society, and McGill University for financial support. C.K.S. thanks the Natural Sciences and Engineering Research Council (Canada) for a postgraduate fellowship. We thank Dr. Frances Arnold (California Institute of Technology, USA) for the subtilisin E plasmid, Dr. S. L. Wong (University of Calgary, Canada) for the *B. subtilis* DB104 cells, Dr. Fred Schendel and Rick Dillingham (University of Minnesota Biotechnology Institute, USA) for the large-scale fermentation and purification of subtilisin E, Dr. Ronghua Shu (McGill University) for the initial substrate synthesis and screening experiments, Linda Fransson (Royal Institute of Technology (KTH), Sweden), and the Minnesota Supercomputing Institute (University of Minnesota, USA) for assistance with the modeling software.

Supporting Information Available: Synthesis and characterization data for compounds **1**−**8**, enantioselectivity of *Bacillus subtilis* var. biotecus A with **2c**−**5c**, complete molecular modeling details, and the modeling figures for **7c**. This material is available free of charge via the Internet at http://pubs.acs.org.

JA045397B

(57) Robinson, P. D. *Acta Crystallogr.* **1991**, *C47*, 594−596.