Enzyme-Catalyzed Resolution of *N*-Acylsulfinamides via Hydrolysis at the Carbonyl or Sulfinyl Group

by

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A thesis submitted to the Faculty of Graduate Studies and Research of McGill University in partial fulfillment of degree of Master of Science

Submitted August, 2001

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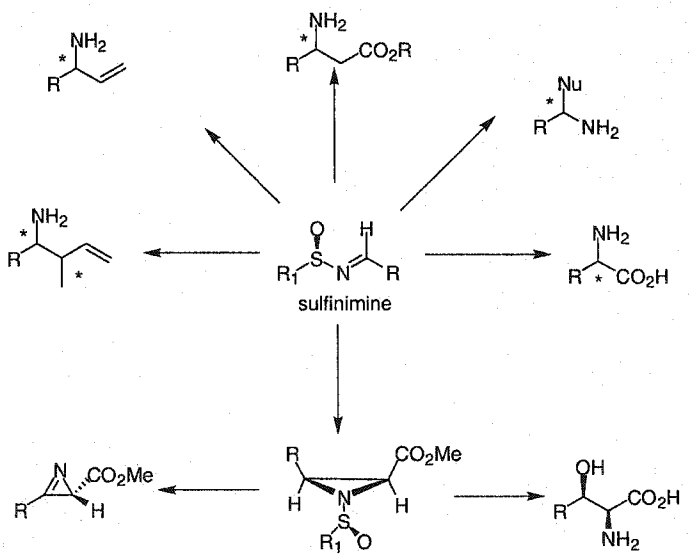
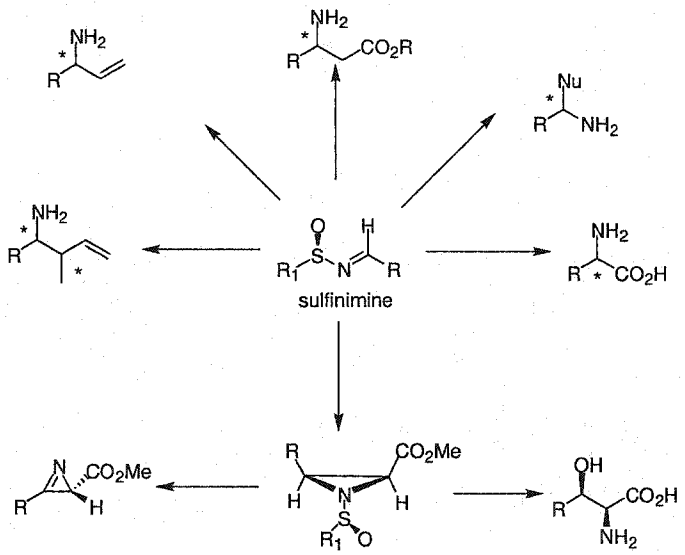
Abstract

Sulfinimines are important chiral auxiliaries for asymmetric synthesis of amines. Enantiopure sulfinimines can be prepared from the direct condensation of enantiopure sulfinamides with aldehydes or ketones. However, few sulfinamides are available in enantiomerically pure form. To make a wider range of sulfinamides, we examined hydrolase-catalyzed resolution of *N*-acylsulfinamides. Using a fast screening method, we identified several hydrolases that catalyzed the hydrolysis of *N*-chloroacetyl-*p*-toluenesulfinamide with high to moderate selectivity. Scale-up reaction identified proteinase from *Bacillus subtilis* var. biotecus A that reacted at the carbonyl position and showed high enantioselectivity (E > 150) favoring the (*R*)-*N*-chloroacetyl-*p*-toluenesulfinamide. This hydrolase provided a new route to enantiopure *p*-chlorobenzenesulfinamide, benzenesulfinamide, *p*-methoxybenzenesulfinamide and 2,4,6-trimethylbenzenesulfinamide that cannot be prepared from current methods. We also identified proteinase, bacterial, which hydrolyzed *N*-acylsulfinamide at the sulfinyl yielding *p*-toluenesulfinic acid. The reaction occurred with enantioselectivity (E' ~ 75) and provided enantiopure *(S)*-*N*-chloroacetyl-*p*-toluenesulfinamide.



Résumé

Les sulfinimines sont d' importants auxiliaires chiraux pour la synthèse asymétrique amines α-substituées. Les sulfinimines énantiomériquement pures peuvent être préparées par la condensation de sulfinamides énantiomériquent pures avec des aldéhydes et des cétones. Toutefois, peu de sulfinamides sont disponibles sous forme énantiomériquement pure. Pour augmenter le nombre de sulfinamides énantiomériquement pures, nous envisageons l' hydrolyse enzymatique de *N*-acylsulfinamides. En utilisant une méthode de criblage rapide, nous avons pu identifier des hydrolases qui hydrolysent le *N*-chloroactéyl-*p*-toluènesulfinamide avec une sélectivité variant de modérée à grande. Des réactions à grande échelle ont identifié la proteinase du *Bacillus subtilis* var. biotecus A comme réagissant au carbonyl avec une grande énantiosélectivité (E > 150) favorisant (*R*)-*N*-chloroacétyl-*p*-toluènesulfinamide. Cette hydrolase a servi de nouvelle route pour l'obtention du *p*-chlorobenzènesulfinamide, du benzènesulfinamide, du *p*-methoxybenzènesulfinamide et du 2,4,6-trimethylbenzènesulfinamide énantiomériquement pure qui ne peuvent être obtenus par d'autre méthodes. Nous avons aussi identifié la proteinase bacterienne qui réagit au sulfinyl donnant l'acide *p*-toluènesulfinique. Cette réaction se produit avec sélectivité donnant le *(S)*-*N*-chloroacétyl-*p*-toluènesulfinamide.



Acknowledgments

Je désire adresser une pensée spéciale aux parents qui m'ont donné la vie et aux parents qui ont fait mon éducation, sans eux rien de tout cela n'aurait été possible. Je désire aussi remercier ma famille et ma fiancé, Nadeige, pour leur support.

I would like to give a special thanks to Dr Romas J. Kazlauskas for his patience and guidance through my research and allowing me to improve my chemistry in his laboratory. I would like to thank Seongsoon Park of sharing ***b***eautiful and ***s***uper ideas for my research. I would also like to thank Ronghua Shu who started the project. A special thanks to all my colleagues, Geoff Horsman, Alessandra Mezzetti and others who have made the laboratory a great working environment.

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Glossary of Symbols and Abbreviations

MeCN	Acetonitrile
°C	Degree Celsius
BES	*N*,*N*-bis[2-hydroxylethyl]-2-aminoethanesulfonic acid
CHES	2-[*N*-cyclohexylamino]ethanesulfonic acid
ε	Extinction coefficient
E	Enantioselectivity
ee	Enantiomeric excess
c	Conversion
GC	Gas chromatography
HPLC	High performance liquid chromatography
NMR	Nuclear magnetic resonance
MS	Mass spectroscopy
HRMS	High resolution mass spectroscopy
m/z	Mass-to-charge ratio
TLC	Thin layer chromatography
DKR	Dynamic kinetic resolution
LiHMSD	Lithium bis(trimethylsilyl)amide
n-Buli	*n*-Butyl lithium
TFA	Trifluoroacetic acid
*p*NP	*p*-Nitrophenol
k'_1(HPLC)	Capacity factor
α (HPLC)	Selectivity factor
THF	Tetrahydrofuran
n.r.	No reaction
n.d.	Not determined
pH	Hydrogen ions concentration
pKa	Negative logarithm of equilibrium constant for association
DAG	Diacetone-D-glucose

Chapter I. Introduction

Chirality

Lord Kelvin defined chirality as follows[1]: "I call any geometric figure or group of points chiral, and say that it has chirality if its image in a plane mirror ideally realized, cannot be brought to coincide with it self." Thus, molecules that are not superimposable with their mirror image are chiral. Chiral molecules have stereogenic centers, axes or planes (one or more) and exist as enantiomers. Enantiomers behave essential identically in achiral environment but can have different response in a chiral environment such as biological systems.

Importance of Chirality in Pharmaceuticals

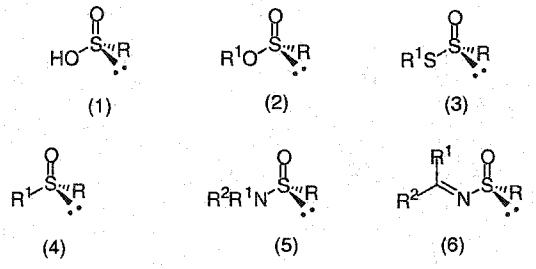
There is lot of attention on interaction of small molecules with biological macromolecules. The search for selective enzyme inhibitors and receptors agonists or antagonists is one of the keys for target research in the pharmaceutical industry. In many cases only one stereoisomer is required as a drug and the other one is either less potent or harmful. For example, captopril is a drug that lowers blood pressure by preventing the conversion of angiotensin I to angiotensin II. The *S* enantiomer is about 100 times more potent than its *R* enantiomer.[2]

Chiral drugs can be prepared by three different routes. First homochiral drugs can be obtained from naturally occurring chiral synthons mainly produced by fermentation processes. Secondly, chiral synthons can be prepared by asymmetric synthesis by either chemical or biocatalytic methods. Finally, resolution of racemic compound can be

achieved by preferential crystallization of one stereoisomer or by kinetic resolution using chemical or biocatalytic methods (this will be discussed on page 6).

Sulfinimines and Sulfinamides in Organic Synthesis

Sulfur forms a variety of organic compounds showing different structural and stereochemical properties. A useful way to classify organosulfur compounds is by the number of ligands on the sulfur.[3] Sulfur compounds with one or two coordinated ligands are achiral and no optical active hexacoordinate sulfur compounds have been described in the literature. Organosulfur compounds with three, four and five coordinates can be chiral depending on the nature of the ligands. Organosulfurs containing three ligands and a lone electron pair that have pyramidal structure are chiral and configurationally stable. Many chiral sulfur compounds of this class are derived from sulfinic acids **(1)**, which are achiral because of rapid proton exchange. Sulfinic acid derivatives include sulfinates **(2)**, thiosulfinates **(3)**, sulfoxides **(4)**, sulfinamides **(5)** and sulfinimines **(6)**; **Scheme 1.1**. All these sulfinic acid derivatives have a stereocenter at the sulfur and can exist as enantiomers and diastereomers depending of the nature of the R groups. These chiral sulfur compounds are important stereocontrolling elements in asymmetric synthesis.[4]

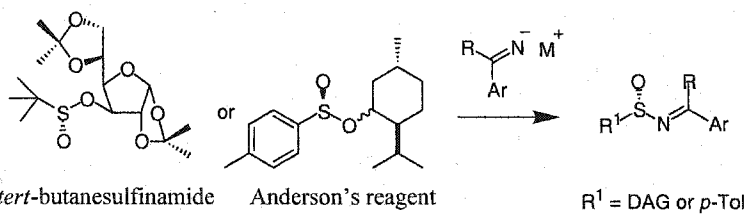


Scheme 1.1. Sulfinic acid and its derivatives.

Researchers often use the sulfinyl group as removable source of diastereoselection in asymmetric synthesis. For example, sulfoxydes were long used for carbon-carbon bond formation reactions[5], while sulfinamides and sulfinimines or *N*-alkylidenesulfinamides[6] are increasingly used as versatile chiral amine synthons (see page 6). Sulfinimines contain an electron withdrawing sulfinyl group making them excellent Michael addition acceptors. They undergo addition reactions with alcohols,[7] thiols,[8] amines,[9] hydrazines,[9] hydrides[10] and also with carbons nucleophiles.[10, 11] In these sulfinimines, the chiral sulfinyl group exerts a powerful stereocontrolling effect on the diastereoselectivity of the reactions.

Synthesis of Enantiopure Sulfinimines

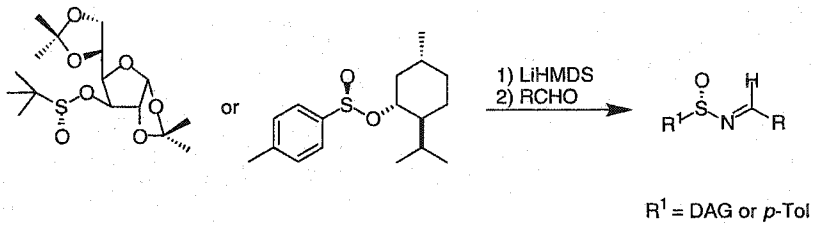
Several methods exist for the synthesis of enantiopure sulfinimines. Optically active sulfinimines were first obtained by asymmetric iminolysis of sulfinate esters with imino-metallo reagents; **Scheme 1.2**. [12, 13] They were formed in moderate to low yields with high stereoselectivity. This reaction takes place with inversion at the sulfur center. Since the imino-metallo reagents were generated *in situ* from aromatic nitriles with lithium or Grignard reagents limited number of derivatives were available from this procedure. This method was applied to Anderson's reagent and DAG-*tert*-butanesulfinate as sulfinate esters.[14] The Anderson reagent, a menthyl aryl sulfinate, is easily prepared via a crystallization step.[15]One the other hand, preparation of diastereomerically pure diacetone-D-glucose (DAG)-*tert*-butanesulfinate requires chromatographic separation.



DAG-*tert*-butanesulfinamide Anderson's reagent

Scheme 1.2. Synthesis of enantiopure sulfinimines from DAG-*tert*-butanesulfinamide or Anderson's reagent and imino-metallo reagents.

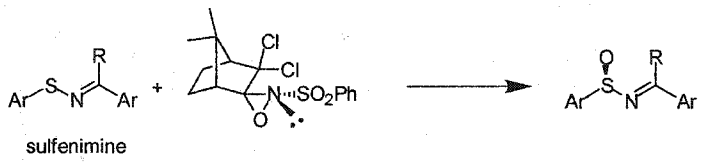
Davis and co-workers described the first preparation of enantiopure sulfinimines using aldehydes; **Scheme 1.3**.[16] This "one pot" method prepared sulfinimines by the treatment of Anderson's reagent with lithium bis(trimethylsilyl)amide (LiHMDS) and *in situ* condensation with aldehydes. Yields were low when aldehydes were enolisable and this reaction didn't work for ketones. DAG-*tert*-butanesulfinate was used in this procedure but again limited by the unwieldy chromatographic purification to obtain diastereomerically pure DAG-*tert*-butanesulfinate.[17]



Scheme 1.3. Synthesis of enantiopure sulfinimines from "one-pot" reaction of sulfinate esters with LiHMDS and aldehydes.

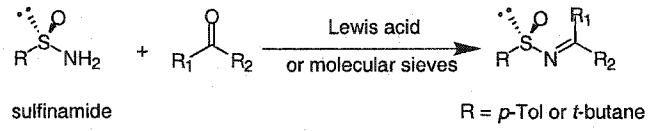
Enantiopure sulfinimines were also obtained from the asymmetric oxidation of sulfenimines derived from aromatic aldehydes or acetophenones with chiral oxaziridines;

Scheme 1.4.[16, 18] However this method required a stoichiometric amount of high molecular weigh chiral oxidant. Also enantiopure aliphatic sulfinimines cannot be obtained from this method because they are not crystalline and the enantiomeric purity cannot be upgraded by recrystallization.



Scheme 1.4. Synthesis of enantiopure sulfinimines from the stereoslective oxidation of sulfenimines.

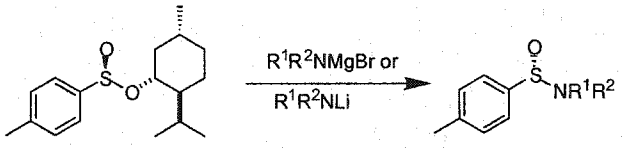
The latest preparation of enantiopure sulfinimines involved enantiopure sulfinamides. This method was an improvement of the first "one pot" preparation of enantiopure sulfinimines developed by Davis; **Scheme 1.5**. Enantiopure *p*-toluenesulfinamide and *t*-butanesulfinamide were used for that procedure.[19, 23] This method provided practical access to more sulfinimines. Importantly ketones-derived sulfinimines were also available from this methodology and encountered less limitation than the previous preparation of sulfinimines. This method was the first general method for synthesis of sulfinimines and the one most widely used today.



Scheme 1.5. Synthesis of enantiopure sulfinimines from the direct condensation of enantiopure sulfinamides with aldehydes or ketones.

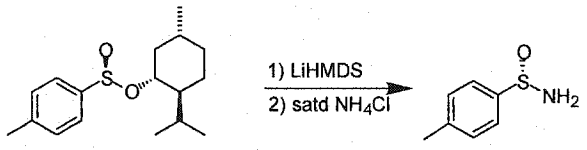
Synthesis of Enantiopure Sulfinamides

The first enantiopure sulfinamides were prepared by the reaction of Anderson's reagent with organometallic reagents containing nitrogen-metal bond. For example, reactions of the Anderson's reagent with dialkylaminomagnesium halides gave optical active sulfinamides in around 60% yields.[20] Primary and secondary lithium amides were also used in preparation of optically active sulfinamides; **Scheme 1.6**.[21] In this case, the stereoselectivity of the conversion was strongly influenced by the nature of the substituents connected to the nitrogen atom. Several groups have demonstrated that the reaction of sulfinamides with organolithium compounds proceeds with inversion at the sulfur center.[20] This reaction could lower the enantiomeric purity of the sulfinamides when the lithium amides are less hindered.



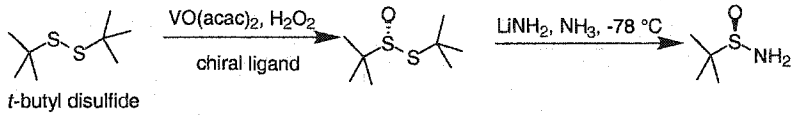
Scheme 1.6. Synthesis of enantiopure *N*-alkylsulfinamides from nitrogen containing organometallic reagents.

All previous methods prepared optically active *N*-alkylsulfinamides. Davis and co-workers prepared *p*-toluenesulfinamide from the Anderson's reagent with bis(trimethylsilyl)amide (LiHMDS); **Scheme 1.7**. Desilylation with NH_4Cl solution gave *p*-toluenesulfinamide in high enantiomeric purity.[19] There are several disadvantages to this method. First, it is limited by the availability of menthyl sulfinates. Only the *p*-tolyl derivative crystallizes easily and thus cannot be used for sulfinates with other alkyl or aryl groups.[15a] Also, Anderson's reagent is fairly expensive. The limitations to the use of sulfinates not only apply to the preparation of enantiopure *p*-toluenesulfinamide but also to the methods cited above using this reagent.[22]



Scheme 1.7. Synthesis of enantiopure *p*-toluenesulfinamide from the Anderson's reagent.

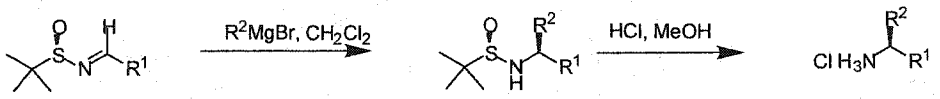
Ellman and co-workers also described the synthesis of enantiopure *t*-butanesulfinamide from the enantioselective oxidation of *t*-butyl disulfide; **Scheme 1.8**. This oxidation was followed by a displacement with lithium amide in liquid ammonia to yield to the sulfinamide.[23, 24] The disadvantage of this method is its limited scope of application. It only works for the *tert*-butyl derivatives because of rapid of racemization of intermediate thiosulfinates when they contain other substituents.



Scheme 1.8. Synthesis of enantiopure *t*-butanesulfinamide from the stereoselective oxidation of *t*-butyl disulfide.

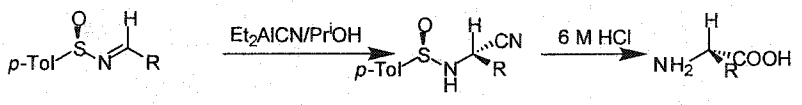
Synthetic Application of Chiral Sulfinimines

There are many examples where sulfiminines are used as chiral auxiliaries. Here, we outline some applications of sulfinimines as chiral synthons for amines. In the first example, Ellman applied *tert*-butanesulfinamide to the asymmetric synthesis of α-branched amines; **Scheme 1.9**.[24] Addition of Grignard reagents to sulfinimines, proceeded to near to quantitative yield and with high diastereoselectivity. Removal of the sulfinyl group using mild acidic conditions provided the α-branched amines.



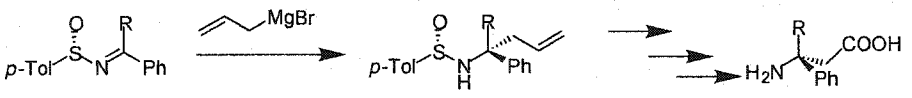
Scheme 1.9. Asymmetric synthesis of α-branched amines from sulfinimines.

Enantiopure sulfinimines were also used for the preparation of α-amino acids. The addition of ethyl(alkoxy)aluminum cyanide to sulfinimines is highly stereoselective; **Scheme 1.10**.[25] The major isomer is isolated by chromatography on silica gel. Gentle reflux in 6 N HCl to remove the sulfinyl group provided the α-amino acids.



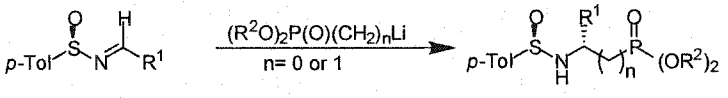
Scheme 1.10. Asymmetric synthesis of α-amino acids from sulfinimines.

Enantiopure sulfinimines were also used for the preparation of β-amino acids. The addition of allylmagnesium bromide to enantiopure sulfinimines produced diastereomeric sulfinamides, which were converted to β-amino acids in multi-steps; **Scheme 1.11**.[26]



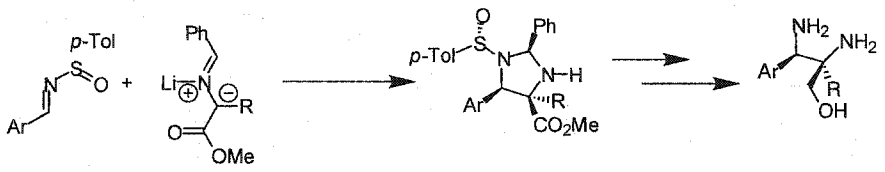
Scheme 1.11. Asymmetric synthesis of β-amino acids from sulfinimines.

Evans investigated the asymmetric synthesis of β and α-aminophosphonic acids via the addition of phosphonic acids to enantiopure sulfinimines; **Scheme 1.12**.[27] α-Aminophosphonic acids usually act as antagonists in the metabolism of amino acids.



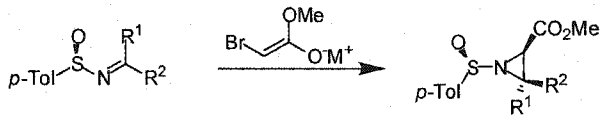
Scheme 1.12. Synthesis of α and β-aminophosphonic acids from enantiopure sulfinimines.

Another interesting reaction, involving sulfinimines as a chiral auxiliary, is the 1,3-dipolar cycloaddition with azomethine ylides reported by André and co-workers; **Scheme 1.13**.[28] In this cycloaddition, the chirality of the sulfur is transferred to three asymmetric centers in a single synthetic operation. Treatment of the cyclic adduct with TFA/MeOH resulted in desulfination and fragmentation to produce vicinal diamines.



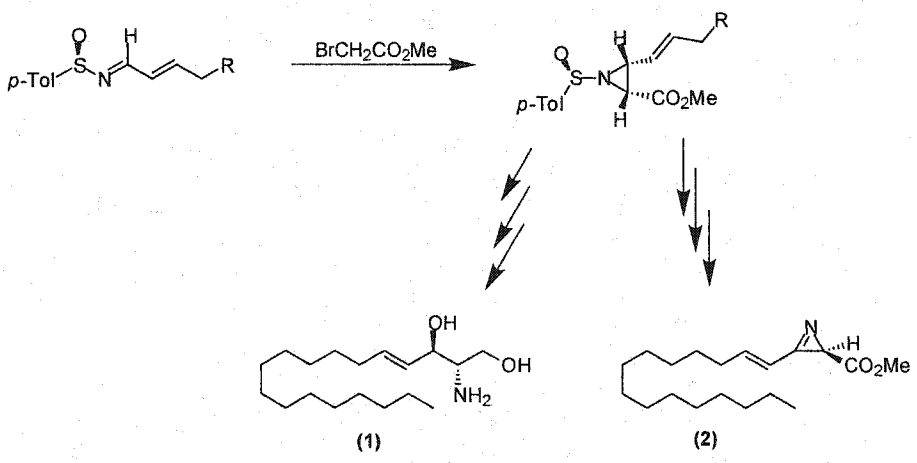
Scheme 1.13. Synthesis of vicinal diamines from enantiopure sulfinimines.

Aziridines were also prepared from enantiopure sulfinimines. Aziridine-2-carboxylate esters are a special class of amino acids. They were obtained from a highly diastereoselective Darzens' type condensation of lithium enolate of α-bromoacetate and sulfinimines; **Scheme 1.14**.[29]



Scheme 1.14. Synthesis of aziridine-2-carboxylate esters from enantiopure sulfinimines.

These aziridines prepared from enantiopure sulfinimines found applications in the synthesis of biologically important compounds. (*R*)-(-)-Dysidaziridine which is an important cytotoxic antitumor antibiotic and D-*erythro*-sphingosine were prepared from enantiomerically pure *N*-sulfinylaziridine. (*R*)-(-)-Dysidaziridine was prepared by the treatment of *N*-sulfinyldizidaziridine with lithium diisopropylamide (LDA).[30] D-*erythro*-Sphingosine was prepared from the same *N*-sulfinyldizidaziridine by a Pummerer-type rearrangement using trifluoroacetic anhydride; **Scheme 1.15**.[31]



Scheme 1.15. Applications of enantiopure sulfinimines in the synthesis of biologically important molecules, D-*erythro*-sphingosine **(1)** and (*R*)-(-)-dysidaziridine **(2)**.

Biocatalytic Kinetic Resolution as a Route to Enantiopure Sulfinamides

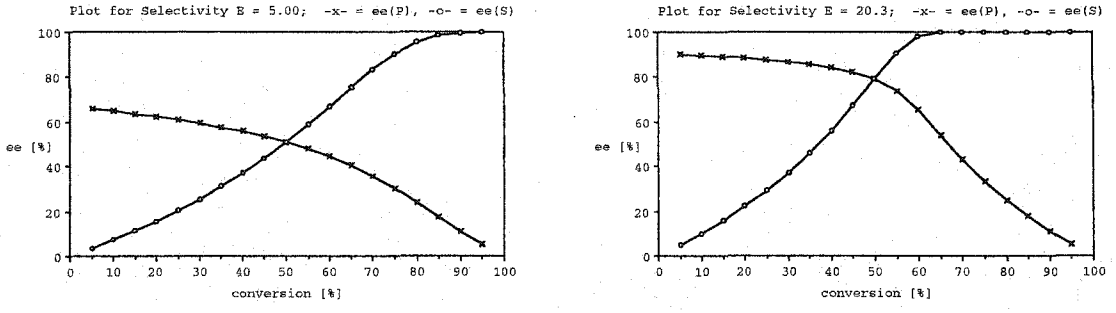
Kinetic Resolution

Biocatalytic resolution of racemates has found widespread applications in preparative organic chemistry for the preparation of enantiopure compounds.[32] Kinetic resolution is a process based on difference in reaction rates between enantiomers. Starting from racemic substrate, the reaction can be catalyzed by an enzyme forming the product with chiral preference for one enantiomer. In the ideal kinetic resolution, reaction rates are very different so one enantiomer reacts quickly and the other one doesn't react at all.[33] The conversion will stop at 50% yield. In practice, the situation of ideal kinetic resolution is not always found.

To describe enzymatic reactions, equations have been developed for convenience of determination of the enantioselectivity of the reaction. This enantioselectivity also called the enantiomeric ratio, E, measures the ability of the enzyme to distinguish

between two enantiomers. The enantioselectivity, E derives from the conversion, c and the enantiomeric excess of the product and the substrate and E remains constant throughout the all reaction, Eq. (1.1).[34] An enantioselectivity of 1 is a non-selective reaction while enantioselectivities of above 20 are useful for synthesis. Two examples of kinetic resolution showing enantioselectivity of 5 and 20 are depicted; **Scheme 1.16**.

$$E = \frac{\ln\left[1 - c\left(1 + ee_p\right)\right]}{\ln\left[1 - c\left(1 - ee_p\right)\right]} \qquad E = \frac{\ln\left[(1-c)(1-ee_s)\right]}{\ln\left[(1-c)(1+ee_s)\right]} \qquad E = \frac{\ln\left[\dfrac{1-ee_s}{1+\left(ee_s / ee_p\right)}\right]}{\ln\left[\dfrac{1+ee_s}{1+\left(ee_s / ee_p\right)}\right]} \qquad \text{Eq. (1.1)}$$



Scheme 1.16. Dependence of optical purities (ee_p and ee_s) of the conversion for two cases, one for enantioselectivity of 5 (left graph) and the other for enantioselectivity of 20 (right graph).

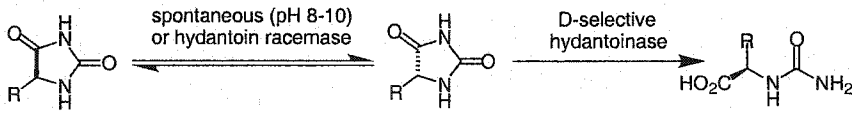
The enantioselectivity of enzymatic reactions is constant throughout the reaction and is not time dependant. However sometimes inhibition, reversible or spontaneous reactions can decrease the observed enantioselectivity and cause it to vary with time. Spontaneous reactions produce racemic product, thereby decreasing the enantiomeric excess of the product. As a consequence, E' is the observed enantioselectivity and E is the

true enantioselectivity, $E' < E$ ($ee_p' < ee_p$, $ee_s' > ee_s$ and $c' > c$). E' is apparent enantioselectivity which is time dependant.

Dynamic Kinetic Resolution

Kinetic resolutions suffer from one major limitation; the maximum yield is 50%. This yield can be improved using dynamic kinetic resolution (DRK).[35] In DRK, the substrate must rapidly racemize during the resolution, the product must not racemize and the enzyme must be highly selective. In such process, the yield of the fast reacting enantiomer can be 100%.

Such dynamic kinetic resolution was performed for the preparation of *N*-carbamoyl D-amino acids by the hydrolysis of 5-monosubstitued hydantoins; **Scheme 1.17**.[36] This DRK involved spontaneous racemization of the substrate at pH > 8 via an enolate or addition of racemization catalyst. Highly enantioselective hydrolysis of the substrate was performed using hydantoinases. It was one of the first examples of DRK reported. This DRK finds application in industrial preparation.

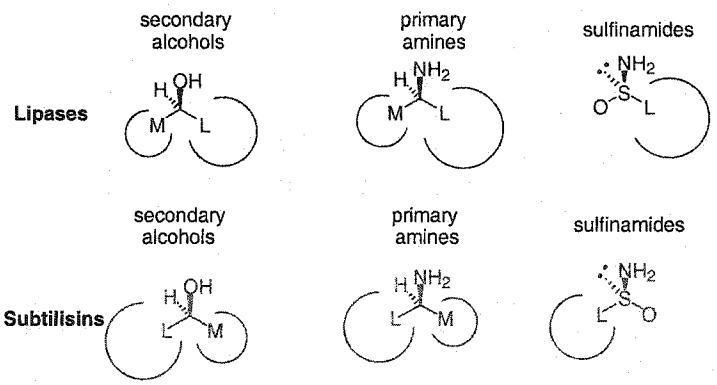


Scheme 1.17. Dynamic kinetic resolution of 5-monosubstitued hydantoins to prepare *N*-carbamoyl D-amino acids.

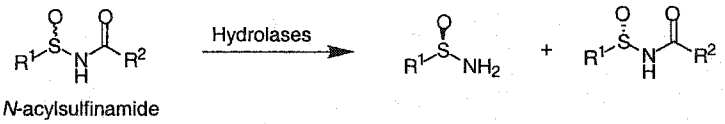
The complete transformation by DRK of a racemic mixture into a single enantiomer is a challenging problem. The most difficult part is the racemization. Examples of DRKs are reported in the literature using organometallic catalysts for racemization of the substrate. As examples, we can site the Williams group example of the first DRK of certain allyl acetates using lipase and a palladium catalyst complex.[37] Also ruthenium catalysts were used in the DRK of secondary alcohols with lipases.[38]

Predictable Use of Hydrolases in the Synthesis of Enantiopure Sulfinamides

There is great value in preparing new enantiopure sulfinamides. If a wider range of sulfinamides were available, chemists could tune reactivity, increase the enantioselectivity or diastereoselectivity of different reactions or even develop new reactions. Hydrolase-catalyzed reactions have already shown their use in organic synthesis. Hydrolases such as lipases and proteases resolved α-methyl amines and secondary alcohols. The three-dimensional shape of sulfinamides resembles that of α-methyl amines and secondary alcohols. The resolution of secondary alcohols with different hydrolases such as lipases or subtilisins is based on the three-dimensional arrangement of the substituents in space. The binding of these substituents to the hydrophobic pockets of the enzymes predicts which enantiomers will react faster. This rule also applied to α-substituted primary amines. This rules work for hydrolysis reaction and in esterification reaction. We believe that lipases and proteases will resolve sulfinamides in similar way; **Scheme 1.18**.



Scheme 1.18. Empirical rules predict the favored enantiomer in lipase- and subtilisins-catalyzed reactions. 'L' represents a large substituent; 'M' represents a medium substituent. Three-dimensional shape of sulfinamide is similar to secondary alcohol and primary amines. Lipases and subtilisin should resolve sulfinamides in a similar way.



Scheme 1.19. Kinetic Resolution of *N*-acylsulfinamides using hydrolases.

The goal in this thesis is to prepare enantiopure sulfinamides through hydrolase-catalyzed reactions; **Scheme 1.19**. Hydrolase-catalyzed resolution of *N*-acylsulfinamide with chiral discrimination for one enantiomer would result in optically active sulfinamides. Using an enzymatic hydrolysis with a higher rate for one enantiomer than the other, a new route to enantiopure sulfinamides will be obtained. Furthermore DKR of *N*-acylsulfinamides will also be envisaged. To provide higher yields, we take the challenge to selectively racemize the substrate, *N*-acylsulfinamide. This racemization should occur rapidly without affecting the enzymatic resolution of the substrate.

Hydrolase-catalyzed reactions can expand the range of enantiopure sulfinamides available as synthetic tools for chemists.

References

[1] Lord Kelvin, *Baltimore Lectures*, Clay and sons, London 1904, p. 436.

[2] (a) Ondetti, M. A.; Ruban, B.; Cushman, D. W. *Science* **1977**, *196*, 441. (b) Cushman, D. W.; Cheung, M. S.; Ondetti, M. A.; Sabo, E. F. *Biochemistry* **1977**, *16*, 5484.

[3] (a) Oae, S. *Organic Sulfur Chemistry: Structure and Mechanism*, CRC Press, Boca Raton, FL, 1991, chap. 3. (b) Laur, P. H. in *Sulfur in Organic and Inorganic Chemistry*, Vol. 3, Senning, A., Ed., Marcel Dekker, New York, 1972.

[4] Mikolajczk, M.; Drabowicz, J.; Kielbasinski, P. *Chiral Sulfur Reagents: Applications in Asymmetric and Stereoselective Synthesis*; CRC Press: Boca Raton, 1997.

[5] (a) Solladié, G. *Synthesis* **1981**, 185. (b) Carreño, M. C. *Chem. Rev.* **1995**, *95*, 1717. (c) Patai, S. *The Chemistry of Sulphinic acids, Esters and their Derivatives*; John Wiley and Sons; Chichester, England, 1990.

[6] Davis, F. A.; Portonovo, P. S.; Reddy, R. E.; Reddy, G. V.; Zhou, P. *Phosphorus, Sulfur, Silicon Relat. Elem.* **1997**, *120 & 121*, 291.

[7] Davis, F. A.; Friedman, A. J.; Kluger, F. *J. Am. Chem. Soc.* **1974**, *96*, 5000.

[8] Burger, K.; Albanbauer, J.; Kafig, F.; Penninger, S. *Liebigs Ann. Chem.* **1977**, 624.

[9] Yoshida, T.; Naruto, S.; Uno, H.; Nishimura, H. *Chem. Pharm. Bull.* **1982**, *30*, 2820.

[10] Hua, D. H.; Miao, S. W.; Chen, J. S.; Iguchi, S. *J. Org. Chem.* **1991**, *56*, 4.

[11] Yang, T. K.; Chen, R. -Y.; Lee, D. -S.; Peng, W. -S.; Jiang, Y. -Z.; Mi, A. -Q.; Jong, T. -T. *J. Org. Chem.* **1994**, *59*, 914.

[12] Khiar, N.; Fernàndez, I.; Alcudia, F. *Tetrahedron Lett.* **1994**, *35*, 5719.

[13](a) Cinquini, M.; Cozzi, F. *J. Chem. Soc. Chem., Commun.* **1977**, 502. (b) Annunziata, R.; Cinquini, M.; Cozzi, F. *J. Chem. Soc. Perkin Trans. 1* **1982**, 339. (c) Hua, D. H.; Mioa, S. W.; Chen, J. S.; Iguchi, S. *J. Org. Chem.* **1991**, *56*, 4.

[14] (a) Fernàndez, I.; Khiar, N.; llera, J. M.; Alcudia, F., *J. Org. Chem.* **1992**, *57*, 6789. (b) Khiar, N.; Fernàndez, I.; Alcudia, F. *Tetrahedron Lett.* **1994**, *35*, 5719.

[15] (a) Anderson, K. K. In *The Chemistry of Sulfones and Sulfoxides*; Patai, S.; Rappoport, Z.; Stirling, C. J. M., Ed.; John Wiley & Sons: New York, 1988; Chap. 3. (b) Kagan, H. B.; Rebiere, F. *Synlett* **1990**, 643. (c) Solladié, G. *Synthesis* **1981**, 185.

[16] Davis, F. A.; Reddy, R. E.; Szewczyk, J. M.; Reddy, G. V.; Portonovo, P. S.; Zhang, H.; Fanelli, D.; Reddy, R. T.; Zhou, P.; Carroll, P. J. *J. Org. Chem.* **1997**, *62*, 2555.

[17] Oppolzer, W.; Froellich, O.; Wiaux-Zamar, C.; Bernardinelli, G. *Tetrahedron Lett.* **1997**, *38*, 2825.

[18] Davis, F. A.; Reddy, R. T.; Reddy, R. E. *J. Org. Chem.* **1992**, *57*, 6387.

[19] Davis, F. A.; Zhang, Y.; Andermicheal, Y.; Fang, D. L.; Zhang, H. *J. Org. Chem.* **1999**, *64*, 1403.

[20] Colonna, S.; Giovimi, R.; Montanari, F. *J. Chem. Soc., Chem. Commun.* **1968**, 865.

[21] Colonna, S.; Germininaroi, G.; Manfredi, A.; Stirling, C. J. M. *J. Chem. Soc. Perkin Trans. 1* **1988**, 1695.

[22] Another enantiomerically pure sulfinate available menthyl 2-methoxy-1-naphthalinesulfinate, it was used for the preparation of enantiopure sulfinimines in the "one pot" procedure. (a) Pyne, S. G.; Hajipour, A. R.; Prabakaran, K. *Tetrahedron Lett.* **1999**, *35*, 645. (b)Davis, F. A.; Zhou, P.; Liang, C. -H.; Reddy, R. E. *Tetrahedron: Asymmetry* **1995**, *6*, 1511.

[23] Liu, G.; Cohan, D. A.; Owens, T. D.; Tang, T. P.; Ellman, J. A. *J. Org. Chem.* **1999**, *64*, 1278.

[24] Liu, G.; Cohan, D. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1997**, *119*, 9913.

[25] Davis, F. A.; Portonovo, P. S.; Reddy, R. E.; Chiu, Y. -H. *J. Org. Chem.* **1996**, *61*, 440.

[26] (a) Hua, D. H.; Miao, S.W.; Iguchi, S. *J. Org. Chem.* **1991**, *56*, 4-6. (b) Tang, T. P.; Ellman, J. A. *J. Org. Chem.* **1999**, *64*, 12.

[27] (a) Mikolajczyk, M.; Lyzwa, P.; Drabowick, J. *Tetrahedron: Asymmetry* **1997**, *8*, 3991. (b) Lefebvre, I.; Evans, S. A., Jr. *J. Org. Chem.* **1997**, *62*, 7532. (c) Mikolajczyk, M.; Lyzwa, P.; Drabowick, J.; Wieczorek, M. W.; Blaszczyk, J. *J. Chem. Soc., Chem. Commun.* **1996**, 1503.

[28] Viso, A.; de la Pradilla, R. F.; Guerrero-Strachan, C.; Alonso, M.; Martinez-Riopoll, M.; André, I. *J. Org. Chem.* **1997**, *62*, 2316.

[29] Davis, F. A.; Zhou, P.; Reddy, G. V. *J. Org. Chem.* **1994**, *58*, 3243.

[30] Davis, F. A.; Reddy, G. V.; Liu, H. *J. Am. Chem. Soc.* **1995**, *117*, 3651.

[31] Davis, F. A.; Zhou, P. *Tetrahedron Lett.* **1996**, *37*, 4349.

[32] Faber, K. *Biotransformations in Organic Chemistry*, 4th ed., Spinger, Heidelberg, 2000, chap. 2.

[33] Klempier, N.; Faber, K.; Griengl, H. *Synthesis*, **1989**, 933.

[34] Chen, C. –S.; Fujimoto, Y.; Girdaukas, G. *J. Am. Chem. Soc.* **1982**, *104*, 7294.

[35] For mathematical treatment of the kinetics in the DKR, see: (a) Kitamura, M.; Tokunaga, M.; Noyori, R. *J. Am. Chem. Soc.***1993**, *115*, 144. (b) Kitamura, M.; Tokunaga, M.; Noyori, R. *Tetrahedron* **1993**, *49*, 1853.

[36] (a) Tsugawa, R.; Okumura, S.; Ito, T.; Katsuga, N. *Agric. Biol. Chem.* **1966**, *30*, 27. (b) Takahashi, S.; Ohashi, T.; Kii, Y.; Kumagai, H.; Yamada, H. *J. Ferment. Technol.* **1979**, *57*, 328. (c) Olivieri, R.; Fascetti, E.; Angelini, L.; Degen, L. *Biotechnol. Bioeng.* **1981**, *23*, 2173.

[37] Allen, J. V.; Wiliams, J. M. *Tetrahedron Lett.* **1996**, *10*, 107.

[38] (a) Persson, B. A.; Larsson, A. L. E.; Ray, M. L.; Baeckvall, J. -E. *J. Am. Chem. Soc.* **1999,** *121*, 1645. (b) Persson, B. A.; Huerta, F. F.; Baeckvall, J. -E. *J. Org. Chem.* **1999**, *64*, 5237.

Chapter II. Enzyme-Catalyzed Kinetic Resolution of *N*-Acylsulfinamide

Abstract

Sulfinimines are important chiral auxiliaries for asymmetric synthesis of amines such as α-branched amines, α- and β-amino acids, aminophosphonic acids, aziridines and other important amine derivatives. Enantiopure sulfinimines can be prepared from the direct condensation of enantiopure sulfinamides with aldehydes or ketones. However, few sulfinamides are available in enantiomerically pure form. To make a wider range of sulfinamides, we examined hydrolase-catalyzed resolution of *N*-acylsulfinamides. Using a fast screening method, we identified several hydrolases that catalyzed the hydrolysis of *N*-chloroacetyl-*p*-toluenesulfinamide with high to moderate enantio- and regioselectivity. Scale-up reaction identified proteinase from *Bacillus subtilis* var. biotecus A that reacted at the carbonyl position and showed high enantioselectivity (E > 150) favoring the (*R*)-*N*-chloroacetyl-*p*-toluenesulfinamide. This hydrolase showed a wider substrate range and provided a new route to enantiopure *p*-chlorobenzenesulfinamide, benzenesulfinamide, *p*-methoxybenzenesulfinamide and 2,4,6-trimethylbenzenesulfinamide that cannot be prepared using current methods. We also identified bacterial proteinase, which hydrolyzed *N*-acylsulfinamide at the sulfinyl yielding *p*-toluenesulfinic acid. The reaction occurred with high enantioselectivity (E' ~ 75) and provided enantiopure *(S)*-*N*-chloroacetyl-*p*-toluenesulfinamide.

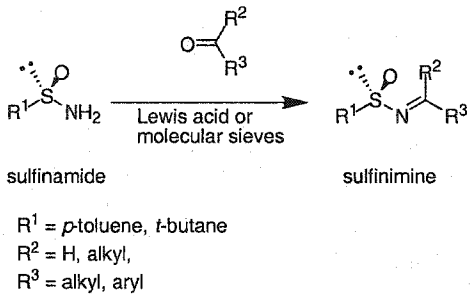
Introduction

Asymmetric synthesis of chiral amines using optically active sulfinimines as a building block is of great value in organic synthesis. In those sulfinimines, the chiral *N*-sulfinyl group exerts a powerful stereo-directing effect, activates the *C-N* bond for nucleophilic additions, and this *N*-sulfinyl group can be easily removed in the products of the reactions. Sulfinimines are used as building blocks in the asymmetric synthesis of α-branched amines,[1] α- and β-amino acids,[2] aminophosphonic acids,[3] aziridines[4] and other important amines derivatives.[5]

Enantiopure sulfinimines can be prepared from the asymmetric oxidation of sulfenimines, which requires stoichiometric amount of chiral oxidant.[6] Enantiopure sulfinimines can also be prepared by reaction of Anderson's reagent (menthyl *p*-toluenesulfinate) or diacetone-D-glucose (DAG) *tert*-butanesulfinate with imino-metallo reagents in moderate to low yields. These reactions are limited in scope because the imine anions are generated *in situ* from aromatic nitrile and Grignard or lithium reagent.[7] Again starting from the Anderson reagent, enantiopure sulfinimines are prepared by reaction with lithium bis(trimethylsilyl)amide and *in situ* condensation with aldehydes but yields are low when aldehydes are enolisable.[6b]

Sulfinimines are also prepared from the direct condensations of enantiopure sulfinamides with aldehydes or ketones[8]; **Scheme 2.1**. This reaction is more versatile for the preparation of a wider range of sulfinimines, because the condensation is possible with aldehydes or ketones. Unfortunately few sulfinamides are available in enantiomerically pure form, since there is no good general method to prepare them. Two

of the mostly use sulfinamides are the *p*-toluenesulfinamide[8a, 6b] and the *t*-butanesulfinamide[8b].

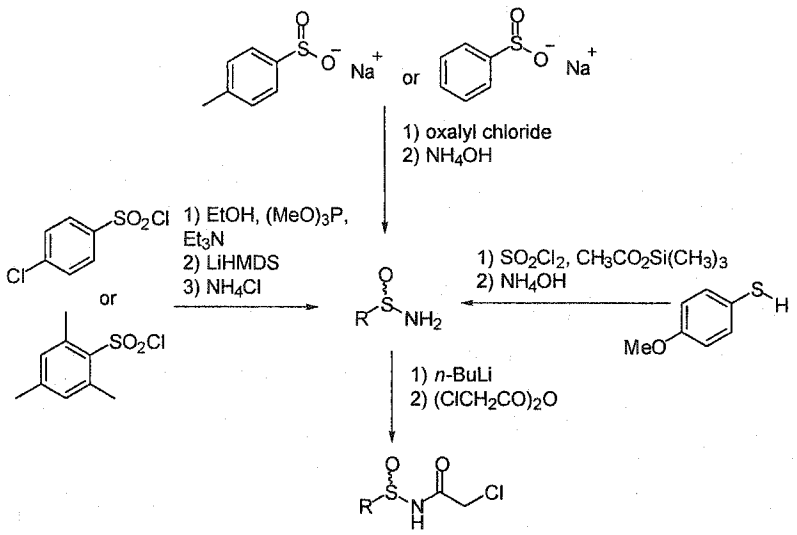


Scheme 2.1. Synthesis of enantiopure sulfinimines via the direct condensation of enantiopure sulfinamides with aldehydes or ketones.

A wider range of enantiopure sulfinamides could be achieved by kinetic resolution of inexpensive racemic starting material. Ellman tried to resolve *t*-butanesulfinamide by oxidation of racemic sulfinamide, by crystallization with chiral acids and by enzyme-catalyzed acylation of the sulfinamide, but was not successful.[9,10] Here we outline the use of hydrolase-catalyzed resolution of *N*-acylsulfinamides, which leads to enantiopure sulfinamides. Those hydrolase-catalyzed reactions occurred at the carbonyl or the sulfinyl center with regioselectivity and enantioselectivity using different hydrolases. Reaction at the carbon resulted in enantiopure sulfinamide and enantiopure *N*-acylsulfinamide and reaction at the sulfur resulted in achiral *p*-toluenesulfinic acid and enantiopure *N*-chloroacetyl-*p*-toluenesulfinamide.

Results

The racemic sulfinamides and *N*-acylsulfinamides were prepared following literature procedures from inexpensive starting materials; **Scheme 2.2**. *p*-Toluenesulfinamide and benzenesulfinamide were prepared in 60% yield from their corresponding sulfinic acids, which were converted to sulfinyl chlorides by treatment with oxalyl chloride and to the sulfinamides by aminolysis in NH_4OH.[11] The ehyl *p*-chlorobenzenesulfinate and the ethyl 2,4,6-trimethylbenzenesulfinate were prepared by reduction of their corresponding sulfonyl chlorides and trapping of the sulfinate esters with trimethyl phosphite, Et_3N and EtOH.[11, 12] The sulfinamides were prepared from these intermediates by treatment of the sulfinate esters with LiHMDS at -78°C and quenching with saturated NH_4Cl solution respectively in 53% and 56% yield.[8b] *p*-Methoxybenzenethiol was oxidized with sulfuryl chloride in the presence of trimethylsilyl acetate, which served as the oxygen donor and the chloride acceptor.[13] Treatment of the sulfinyl chloride with NH_4OH gave the *p*-methoxybenzenesulfinamide in 57% yield. All *N*-acylsulfinamides were prepared by acylation using *n*-BuLi as a base at -78°C followed by addition of the symmetrical anhydride.[11, 14]



Scheme 2.2. Synthesis of sulfinamides and *N*-chloroacetylsulfinamides.

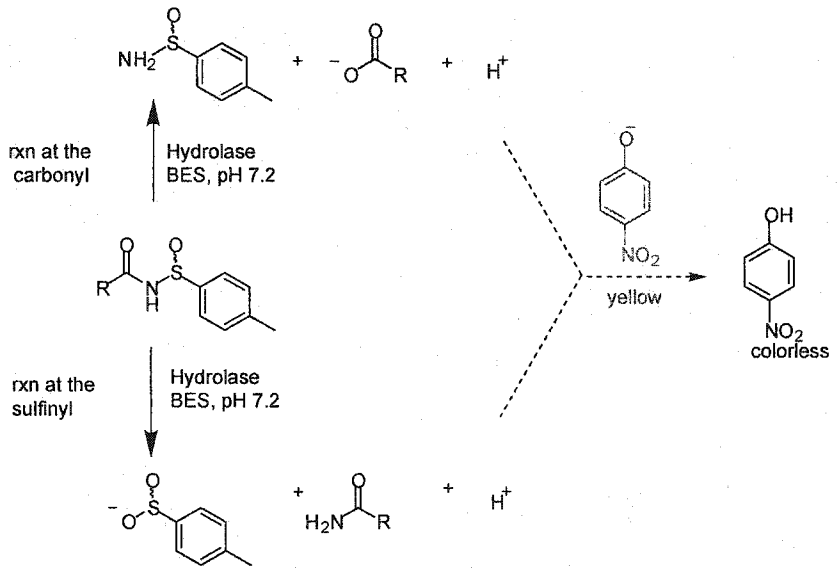
Table 2.1. Yields of reactions and separation of enantiomers of sulfinamides and *N*-acylsulfinamides by HPLC.

R	yield[1],%	yield[2],%	k'$_1$(*S*)	k'$_2$(*R*)	k'$_3$(*S*)	k'$_4$(*R*)	$\alpha_{1,2}$	$\alpha_{3,4}$
p-tol	60	50	5.0	6.9	8.3	16.6	1.4	2.0
C_6H_4	60	34	5.0	5.8	7.9	15.7	1.2	2.0
2,4,6-$Me_3C_6H_2$	56	96	6.8	7.5	4.8	5.8	1.1	1.2
p-ClC_6H_4	53	93	6.0	7.6	11.4	20.5	1.3	1.8
p-MeO-C_6H_4	57	38	10.0	14.5	18.4	30.9	1.4	1.7

The substrates (except R = 2,4,6-$Me_3C_6H_2$) were analyzed by HPLC on a chiralcel AD column. (±)-2,4,6-Trimethylbenzenesulfinamide and (±)-*N*-choloacetyl-2,4,6-trimethylbenzenesulfinamide were analyzed on the chiralcel OD column. Yield[1] is the yield to the sulfinamides, yield[2] is the yield to the *N*-acylsulfinamides, k'$_1$(*S*) and k'$_2$(*R*) are the capacity factors of the sulfinamides and k'$_3$(*S*) and k'$_4$(*R*) are the capacity factors of the *N*-acylsulfinamides. $\alpha_{1,2}$ is the selectivity factor of the sulfinamides and $\alpha_{3,4}$ of the *N*-acylsulfinamides.

N-chloroacetyl-*p*-toluenesulfinamide was screened against a library of fifty hydrolases in an activity-screening assay; **Scheme 2.3**. The absorbance at 404 nm decreased when a proton was taken by *p*-nitrophenoxide.[15] Several hydrolases were identified as possible catalyst for hydrolysis of the substrate (more details in supplementary material, p. 34). In addition, the activity-screening assay identified a slow spontaneous chemical hydrolysis for *N*-chloroacetyl-*p*-toluenesulfinamide. However, this

activity-screening assay didn't identify if the hydrolysis occurred at the carbonyl or sulfinyl position.



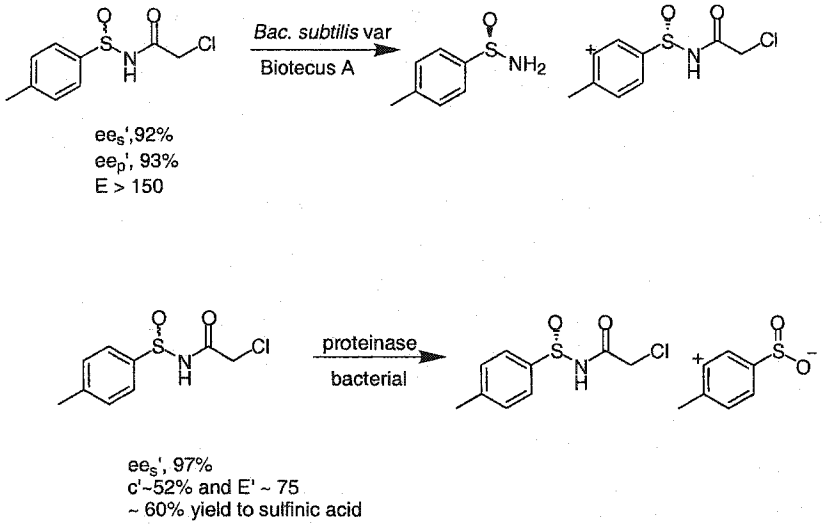
Scheme 2.3. Activity-screening assay detect the hydrolysis at carbonyl or sufinyl position using a pH indicator.

The ee_p and ee_s were determined by HPLC on chiral column and the enantioselectivity of the active hydrolases were determined by end-point method **(Table 2.2)**.[16] The results revealed that the lipase from wheat germ and the acylase I from hog kidney did not catalyze the hydrolysis, only chemical hydrolysis was observed. Lipase A from *Candida antarctica* catalyzed the hydrolysis but with low enantioselectivity and showed enantiopreference for the (*S*)-*N*-chloroacetyl-*p*-toluenesulfinamide. All the proteases favored the (*R*)-*N*-chloroacetyl-*p*-toluenesulfinamide. Cholesterol esterase from bovine pancreas also showed enantiopreference for the (*R*)-enantiomer. In all cases, the enantioselectivity was low except for bacterial proteinase and proteinase from *Bacillus subtilis* var. biotecus.

Table 2.2. Kinetic resolution of *N*-acylsulfinamides.

Source of active hydrolases	R	wt[a]	time[b]	ee_s' %	ee_p' %	%c' %	E'	E	enantio-preference[c]
Lipases									
Lipase from wheat germ, type I	*p*-tol	90	6			n.r.			
Candida antarctica lipase A	*p*-tol	137	24	65%	11%	14%	5	n.d.	*S*
Proteases-subtilisins									
Bacillus licheniformis	*p*-tol	60	6	74	66	53	10	11	*R*
subtilisin Carlsberg	*p*-tol	34	6	82	64	56	11	12	*R*
Aspergillus oryzae	*p*-tol	90	6	88	68	56	15	17	*R*
proteinase, bacterial	*p*-tol	64	6	97	n.d.	~ 52	~ 75	n.d.	*R*
Bac. subtilis var. biotecus A	*p*-tol	16	6	92	93	50	88	>150	*R*
Bac. subtilis var. biotecus A	C_6H_6	16	6	81	90	47	48	>200	*R*
Bac. subtilis var. biotecus A	2,4,6-$Me_3C_6H_2$	16	6	66	94	41	64	>200	*R*
Bac. subtilis var. biotecus A	*p*-$MeOC_6H_4$	16	6	87	90	49	52	>200	*R*
Bac. subtilis var. biotecus A	*p*-ClC_6H_4	16	3	77	96	45	103	>150	*R*
Proteases-acylases									
hog kidney	*p*-tol	20	6			n.r.			
Aspergillus melletus	*p*-tol	250	24	75%	30%	29%	9	29	*R*
Esterases									
bovine cholesterol	*p*-tol	80	24	67%	27%	29%	7	n.d.	*R*

Enantiomeric excess of product and substrate were analyzed by HPLC on Daicel Chiralcel OD and AD . n.r.: no reaction; n.d.: not determined. [a]Weigh of solid enzyme in mg used to perform the hydrolysis. [b]Time in h to perform to hydrolysis. E', ee_p' and ee_s' refer to the parameter with no correction for chemical hydrolysis and E is the enantioselectivity corrected for chemical hydrolysis.

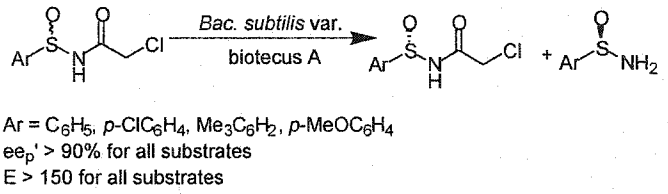


Scheme 2.4. Resolution of *N*-chloroacetyl-*p*-toluenesulfinamide using proteinase from *Bacillus subtilis* var. biotecus A and bacterial proteinase.

Bacterial proteinase gave 97% ee (E' ~ 75) but the expected product was not found. The only trace of *p*-toluenesulfinamide resulted from chemical hydrolysis (< 3%). The enzymatic hydrolysis occurred at the sulfinyl position and gave achiral *p*-toluenesulfinic acid; **Scheme 2.4**. This product stayed in the buffer layer (pH 7.2) after extraction with methylene chloride. When the aqueous layer was acidified, *p*-toluenesulfinic acid was identified by TLC and isolated in 60% yield. ^{1}H NMR identified the characteristic aromatic protons of *p*-toluenesulfinic acid and this was supported by exact mass of *p*-toluenesulfinic acid measured by mass spectrometry.

Proteinase from *Bacillus subtilis* var. biotecus A showed very high entioselectivity in the hydrolysis of *N*-chloroacetyl-*p*-toluenesulfinamide with 93% ee$_p$ and 92% ee$_s$ and 50% conversion; **Scheme 2.4**. This kinetic resolution was still affected

by chemical hydrolysis (< 3%) and when we corrected for that factor, the true enantioselectivity (E) was greater than 150.[17]



Scheme 2.5. Resolution of *N*-chloroacetylsulfinamide using proteinase *Bacillus subtilis* var. biotecus A.

This enzyme could provide a route to new sulfinamides that were not available in enantiomerically pure form; **Scheme 2.5**. At the same time, using different *N*-acylsulfinamides, we could also investigate the substrate specificity of the enzyme.[18] When performing the reactions with the enzyme, the substrates were resolved efficiently in all cases. Ee_p were greater than 90% in all cases with high conversion and less than 3% chemical hydrolysis. When we corrected for this spontaneous hydrolysis, the enantioselectivity was greater than 150 for all the substrates. Thus, the substrate specificity is such that a broad range of aryl substituents can be used.

The absolute configuration of *p*-toluenesulfinamide was determined by chemical correlation. For this purpose, enantiopure *p*-toluenesulfinamide [8a] was prepared from Anderson's reagent and was than acylated to give *N*-chloroacetyl-*p*-toluenesulfinamide (< 40% ee) (details in supplementary section, page 46). These compounds of known absolute configuration were compared to those from enzymatic reaction. Since the

relative elution order was the same on the Chiralcel AD column, we assumed that the favored enantiomer had the same absolute configuration in all cases **(Table 2.2)**.

Discussion

The activity-screening assay quickly identified two lipases, one esterase and seven proteases as possible catalysts for the hydrolysis of *N*-chloroacetyl-*p*-toluenesulfinamide.[19] This screening assay didn't give any information about the enantioselectivity or regioselectivity of the reactions or on which enantiomer reacted faster. We used the slower end-point method to determine the enantioselectivity and the regioselectivity expressed of each hydrolase. In the same way, we could determine the enantiopreference of the enzymes according to the enantiomeric excess of the product and the substrate.

Using end-point method, the enantioselectivity of the lipase A from *Candida antarctica* was found to be 5 favoring the *(S)*-enantiomer. The cholesterol esterase from bovine pancreas showed an enantioselectivity of 7 for the other enantiomer. This is unusual because lipases and esterases are α/β hydrolases.[20] The connectivity of the β-sheets and the α-helices is the same in those so most likely they should favor the same enantiomer. On the other hand the proteases found to catalyze the reaction showed enantiopreferences for the *(R)*-enantiomer.

In the hydrolases identified, only one lipase, lipase A from *Candida antarctica* was found to catalyze the reaction. The activity-screening assay identified two lipases as possible catalyst but only one was found to show activity in the scale-up with 14% conversion. Lipases favor water insoluble substrates and are used to cleave triglyceride

and long chain fatty acids.[20] They are not good catalysts for the hydrolysis of normal amides. We expected that the *N*-acylsulfinamide would be more reactive, more like an imide bond. *N*-chloroacetyl-*p*-toluenesulfinamide is a polar molecule, which is water-soluble. Proteases are known to like water-soluble substrates and to cleave peptide or amide bond and the *N*-acylsulfinamide has amide functionality.[20] Perhaps it was why more proteases were identified to catalyze the hydrolysis of the substrate.

Hydrolases showed high enantioselectivity toward a broad range of substrates. Empirical rules predict which enantiomer of secondary alcohols and primary amines would be favored by lipases and subtilisins. These rules are based on how the chirality of the substrate complements the three-dimensional structure of the active site of the enzymes.[21] According to empirical rules and based on similarity in the three-dimensional structure of sulfinamides with primary amines and secondary alcohols that have already been resolved by lipase and proteases, lipases should prefer the *(R)*-enantiomer of the sulfinamide and proteases the *(S)*-enantiomer. The rule suggests that size of substituents is the most important characteristic and does not account of electronic effects, solvent, subtituent orientation or any other conditions that can affect the enantioselectivity. This rationale considers that the *p*-toluene group in the sulfinamide should fit in the large pocket but does not account for possible electronic effects due to the presence of oxygen. The experimental results found were opposite to the predictions but still lipases and proteases favored opposite enantiomers. We are currently modeling with subtilisin Carlsberg to understand the enantiopreference of the proteases. Perhaps electronic effects such as hydrogen bonds could be an important factor in the binding of the sulfinamides with hydrolases.

Bacterial proteinase was found to hydrolyze the *N*-chloroacetyl-*p*-toluenesulfinamide at the sulfur center. Hydrolysis at the sulfur was reported previously for sulfite esters.[22] In some case, this enzyme-catalyzed hydrolysis using pepsin occurred with stereo-specificity in resolution of alkyl phenyl sulfites[22b] To our knowledge, it is the first time that hydrolysis at the sulfur is reported expressing both regioselectivity and enantioselectivity.

Proteinase from *Bacillus subtilis* var. biotecus A was found to be a very powerful enzyme for the synthesis of new enantiopure sulfinamides. Hydrolysis of *N*-acylsulfonamides groups in human plasma was previously reported.[23] Steric or electronic effects on the benzene ring of the sulfinamides had no significant effect on the resolution. This enzyme is versatile catalyst but also fairly expensive. This enzyme-catalyzed resolution of *N*-acylsulfinamide leads to a new route to enantiopure sulfinamides that are not available from other methods. This new enzymatic hydrolysis of *N*-acylsulfinamide using proteinase from *Bacillus subtilis* var. biotecus A is very interesting since it occurred with high chiral discrimination. Still the enantiomeric excess obtained for the sulfinamides could be increase by recrystallization.

In future work, we could also try the reverse reaction with this bacterial proteinase. One can imagine starting from achiral sulfinic acid and by aminolysis obtain sulfinamides, in up to 100% yield. This could give enantiopure sulfinamides since in the hydrolysis reaction, the enzyme showed high enantiopreference for the *(R)-N*-chloroacetyl-*p*-toluenesulfinamide. Proteinase from *Bacillus subtilis* var. biotecus A is a variant of subtilisin Carlsberg, and is also commercially available. We could use the parent enzyme to engineer a similar catalyst by means of directed evolution.[24] Modeling

results could also be used to identify key residues and modify those in saturation mutagenesis experiments to provide mutant enzymes.[25] We obtained the (*R*)-enantiomer in good ee and were left with the other. It is difficult to hydrolyze *N*-acylsulfinamide because they are stable under basic condition, racemize under acidic conditions and the chemical hydrolysis with water is very slow. We could use low enantioselectivities obtain for other hydrolases to catalyze the hydrolysis of the (*S*)-*N*-chloroacetyl-*p*-toluenesulfinamide after the first kinetic resolution performed with proteinase from *Bacillus subtilis* var. biotecus A to obtain the other enantiomer with high enantiomeric excess.

In conclusion, enantiopure sulfinamide, which are attractive building blocks for organic synthesis, have been prepared for the fist time by hydrolase-catalyzed reactions in nearly enantiomerically pure form. Bacterial proteinase was found to be an enzyme expressing unique regio- and stereoselectivity for *N*-chloroacetyl-*p*-toluenesulfinamide with hydrolysis at the sulfinyl position. This hydrolase-catalyzed reaction gave achiral sulfinic acid and enantiopure (*S*)-*N*-chloroacetyl-*p*-toluenesulfinamide. On the other hand, proteinase from *Bacillus subtilis* var. biotecus A was found to catalyze the reaction at the carbonyl position. This hydrolase provided a route to enantiopure sulfinamide for synthetic applications that are not available from other synthetic methods.

Supplementary Material

This section is complementary to the enzyme-catalyzed kinetic resolution of *N*-acylsulfinamides. It contains additional details about the activity-screening assay. We also report in this section attempts to increase the apparent enantioselectivity, E' in the kinetic resolution of *N*-chloroacetyl-*p*-toluenesulfinamide using proteinase from *Bacillus subtilis* var. biotecus A. The effect of pH was investigated to try to control the chemical hydrolysis and increase the enantioselectivity. It also contains preliminary investigation about dynamic kinetic resolution of *N*-acylsulfinamides. Attempts to racemize *N*-chloroacetyl-*p*-chlorobenzenesulfinamide and *p*-chlorobenzenesulfinamide are also found in this section.

Determination of Active Hydrolases

Hydrolase-catalyzed reactions have solved lot of problems encountered by chemists.[26] The limitation to the use of hydrolases is finding the best one for a particular synthetic application, in this case, hydrolysis of *N*-acylsulfinamide. To identify selective hydrolases, one can run small-scale reactions (end-point method) and determine the enantiomeric excess of product and substrate by the use of HPLC, GC, and NMR.[16] We decided to use a fast screening assay to find the active hydrolases for the hydrolysis of *N*-acylsulfinamide.[15] This assay doesn't give information about the enantioselectivity or regioselectivity of the reactions. Regioselectivity refers to reaction at either reaction at the carbonyl or the sulfinyl position and enantioselectivity refers to the chiral sulfinyl group (refer to **Scheme 2.3**). The regioselectivity and enantioselectivity of the reactions were determined later by end-point method.

This activity-screening assay uses a pH indicator, *p*-nitrophenol (*p*NP) and measures the rate of proton release. This way, we can quickly determine active hydrolases that catalyze the reaction. The substrate was prepared as a racemic mixture and used in this colorimetric assay. This assay is performed in 96-well plates and the pH indicator (*p*NP) monitors the appearance of the acidic proton at 404 nm. The reaction is performed in a BES buffer with 7% acetonitrile to dissolve the substrate. Since BES and *p*-nitrophenol have the same affinity for the proton (pKa's within 0.1 unit), the relative amount of buffer and indicator protonated stays the same during the hydrolysis when the proton is release from the reaction. The proportionality factor between indicator absorbance and reaction rate is given by the buffer factor, Q[27]. Q is described in Eq. (2.1). Where $[BES]$ is the buffer concentration, $[pNP]$ is the indicator concentration, ε_{404} is the difference in extinction coefficient between the protonated and deprotonated form of the indicator and l is the path length.

$$Q = \frac{[BES] + [pNP]}{[pNP]} \qquad \text{Eq (2.1)}$$

The true reaction rate is described in equation (**2**) where dA_{404}/dT expresses the rate of indicator absorbance change.

$$rate_{substrate}\left(\frac{\mu mol}{\min}\right) = \frac{dA_{404}}{dt} \times \frac{1}{\varepsilon_{404} \times \ell} \times V \times \frac{10^6 \mu mol}{mol} \times Q \qquad \text{Eq. (2.2)}$$

Both the *N*-butanoyl-*p*-toluenesulfinamide and the *N*-chloroacetyl-*p*-toluenesulfinamide are screened via this activity-screening assay in a 96 well microplates at two different temperatures. When the temperature is increased the reaction rate should also increase. By screening at higher temperature, we could identify also slow hydrolases.

Table 2.3. Activity screening of *N*-butanoyl-*p*-toluenesulfinamide toward a library of hydrolases at 25 °C and 37 °C.

Source of active hydrolases[a]	Wt[b]	supplier	activity[c] T (25°C)	activity[c] T (37°C)
Lipases				
Aspergillus niger	167	d	1.25E-04	n.r.
Candida lipolytica	78	d	7.66E-05	6.84E-05
Candida rugosa (*cylindracea*)	240	h	1.26E-04	2.62E-04
Mucor miehei	**9.2**	**d**	**1.24E-03**	
Penicillium camemberti	340	d	5.49E-05	8.98E-05
Penicillium roqueforti	200	d	1.95E-04	2.33E-04
Pseudomonas cepacia	**70**	**d**	**2.65E-04**	**1.18E-03**
***Pseudomonas sp.*, lipoprotein lipase**	**7**	**e**	**1.17E-03**	**1.05E-03**
Pseudomonas sp. type B, lipoprotein lipase	7	e		
Rhizopus arrhizus	64	e	7.81E-04	n.r.
Rhizopus javanicus	130	d	7.68E-05	1.05E-04
Rhizopus niveus	65.3	f	9.03E-05	8.93E-05
Rhizopus oryzae	162	d	n.r.	2.15E-04
Rhizopus stolonifer	89	d	7.19E-05	7.40E-05
Thermus aquaticus	1	e	1.66E-04	7.48E-04
Thermus thermophilus	10	e	n.r.	1.32E-04
Thermus flavus	5.5	e	9.32E-04	7.95E-04
Lipoprotein lipase from *Chromobacterium viscosum*	8	e	2.00E-04	3.61E-04
Pseudomonas fluorescens	14	h	3.90E-04	3.64E-04
Lipase B from *Candida antarctica*	**38**	**e**	**2.87E-04**	**1.17E-03**
Porcine pancreatic lipase	180	h		
Lipase from wheat germ, type I	**112**	**g**	**4.92E-04**	**2.03E-03**
Esterases				
Bacillus thermoglycosidasius	2.4	e	4.53E-04	1.10E-03
bovine cholesterol esterase	23	i	5.07E-04	
Candida lipolytica	3	e	n.r.	n.r.
horse liver esterase	14	e	3.10E-04	4.14E-04
Mucor miehei	38	e	1.87E-04	9.94E-05
Saccharomyces cerevisiae	15	e	3.39E-04	2.26E-04
Thermoanaerobium brockii	13	e	n.r.	n.r.
Proteases-subtilisins				
Aspergillus oryzae	300	g	6.86E-04	4.58E-04
Bacillus polymyxa	20	g	1.27E-04	2.90E-04
Bac. subtilis var. biotecus A	26	e	1.30E-04	6.03E-04
α-chymotrypsin	46.5	g	4.15E-06	4.04E-04
Proteinase, bacterial	57.6	e	2.13E-04	6.04E-04

Proteinase K	3.1	e	1.84E-05	1.23E-04
subtilisin from *Bacillus licheniformis*	18	e	3.04E-04	3.64E-04
subtilisin Carlsberg	32	g	1.85E-04	4.79E-04
Protease from papaya	200	g	n.r.	1.69E-04
Rennin from *Mucor Meihei*	90.9	e	4.79E-05	1.22E-04
Optimase L-660	2000ul	j	2.64E-04	5.30E-04
Proteinase N from *Bacillus subtillis*	20	e	2.15E-04	5.53E-04
Proteases-acylases				
Aspegillus melleus	92.3	e	1.54E-0.4	2.54E-0.4
hog kidney	24	e	6.56E-04	6.88E-04
Carbonic anhydrase	22	g	n.r.	n.r.

[a]Activity of hydrolases toward *N*-butanoyl-*p*-toluenesulfinamide. [b]Amount (mg) of solid enzyme per mL of buffer. [c]Observed rate[28] of hydrolysis in μmol/min. n.r.: no reaction. [d]Amano Enzyme USA Co., Ltd. (Troy, Va). [e]Fluka Chemie (Oakville, On). [f]Boerhinger-Mannheim (Mannheim, Germany). [g]Sigma-Aldrich (Oakville, On). [h]Biocataysts Ltd. (Pontypridd, Mid Glam, Wales, UK.). [i]Genzyme (Cambridge, MA). [j]Genenco. This activity screening-assay was performed at pH 7.2 since most hydrolases have maximum activity at that pH.

Screening the *N*-butanoyl-*p*-toluenesulfinamide toward the enzyme library revealed low activity for most hydrolases. All proteases and esterases showed either slow reaction with the substrate or no reaction. The increase in temperature didn't really change the activities, the reactions were still very slow. In some cases like for lipase from *Aspergillus niger* or lipase from *Rhizopus arrhizus*, it resulted in lost of activity but still the first reaction rates where too slow to say that the substrate was getting hydrolyzed. It is possible that those enzymes denatured when the temperature was increased because of thermal instability. Lipase from *Mucor miehei*, lipase from *Pseudomonas cepacia*, lipoprotein lipase from *Pseudomonas sp.*, lipase B from *Candida antarctica*, lipase from wheat germ, type I were identified to catalyze the hydrolysis of *N*-butanoyl-*p*-toluenesulfinamide **(Table 2.3)**. Even for those enzymes the reaction rates were slow but fast enough to identify them as possible catalyst. In general, we can say that the substrate reacted slowly with the hydrolases.

To try to increase the activity of the substrate, we prepared *N*-chloroacetyl-*p*-toluenesulfinamide. The carbonyl functionality of this substrate is activated for nucleophilic attack because of the chlorine atom α to the carbonyl. This new substrate was also screened toward the same enzyme library at 25 °C and 37 °C. For the *N*-chloroacetyl-*p*-toluenesulfinamide; the first observation was that the substrate was undergoing spontaneous chemical hydrolysis whereas this wasn't observed for the *N*-butanoyl-*p*-toluenesulfinamide. Generating a more reactive *N*-acylsulfinamide for enzymatic hydrolysis also resulted in undesired chemical hydrolysis. This spontaneous reaction produced racemic *p*-toluenesulfinamide and lowered the enantioselectivity of the reactions. In this case, E is the true enantioselectivity and E' the observed enantioselectivity, which includes chemical hydrolysis (E > E') and is time dependant. The challenge was not only to find a selective enzyme but also to find an enzyme with sufficient rate acceleration to make background hydrolysis negligible by comparison. Reduced selectivity of biocatalytic transformations through spontaneous reaction was observed for protease-catalyzed hydrolysis of oxazolones[29], in the hydrolysis of epoxides catalyzed by fungal epoxide hydrolases[30] and in the acyl transfer reactions in the resolution of secondary amines[31].

When the *N*-chloroacetyl-*p*-toluenesulfinamide was screened against the enzyme library, the lipases found for the first substrate were no longer active except for lipase from wheat germ, type I. Six proteases were found to show activity. Thus, the two substrates show different activities for the lipases and the proteases **(Table 2.4)**. Modification of the substrate influenced the enzymatic reaction. Increase in activity was found for the proteases but all the lipases identified with *N*-butanoyl-*p*-

toluenesulfinamide as a substrate were no longer showing activity. The chlorine atom α to the carbonyl decreases activity for the lipases. The *N*-chloroacetyl-*p*-toluenesulfinamide is more hydrophilic, and the *N*-butanoyl-*p*-toluenesulfinamide, which have an alkyl chain α to the carbonyl making it more hydrophobic. Lipases like water-insoluble substrate and most lipases have a lid that needs interfacial activation promoted by hydrophobic molecules. Proteases like water-soluble molecules and usually a cosolvent can be added to help the solubility of substrates. The different activities observed for both substrates toward lipases and proteases are a direct consequence of their properties.[20]

Table 2.4. Activity screening of *N*-chloroacetyl-*p*-toluenesulfinamide toward a library of hydrolases at 25 °C and 37 °C.

Source of active hydrolases[a]	Wt[b]	supplier	activity[c] T(25°C)	activity[c] T(37°C)
Lipases				
Aspergillus niger	167	d	4.55E-05	n.r.
Candida antarctica lipase A	75	f		
Candida lipolytica	78	d	9.73E-05	n.r.
Candida rugosa (*cylindracea*)	240	h	n.r.	n.r.
Mucor miehei	9.2	d	n.r.	n.r.
Penicillium camemberti	340	d	7.56E-06	n.r.
Penicillium roqueforti	200	d	2.03E-04	n.r.
Pseudomonas cepacia	70	d	6.28E-04	8.77E-04
Pseudomonas sp., lipoprotein lipase	7	e	5.83E-04	6.90E-04
Pseudomonas sp. type B, lipoprotein lipase	7	e		
Rhizopus arrhizus	64	e	1.11E-04	n.r.
Rhizopus javanicus	130	d	n.r.	n.r.
Rhizopus niveus	65.3	f	1.28E-04	n.r.
Rhizopus oryzae	162	d	n.r.	n.r.
Rhizopus stolonifer	89	d	1.25E-06	n.r.
Thermus aquaticus	1	e		n.r.
Thermus thermophilus	10	e	n.r.	n.r.
Thermus flavus	5.5	e	1.75E-04	2.08E-04
Lipoprotein lipase from *Chromobacterium viscosum*	8	e	2.04E-04	3.03E-04
Pseudomonas fluorescens	14	h	3.68E-04	9.48E-04
Lipase B from *Candida antarctica*	38	e	5.19E-04	5.55E-04
Porcine pancreatic lipase	180	h		
Lipase from wheat germ, type I	**112**	**g**	**7.23E-04**	**1.07E-03**
Esterases				
Bacillus thermoglycosidasius	2.4	e	6.13E-04	7.40E-04
bovine cholesterol esterase	23	i	7.01E-04	7.97E-04
Candida lipolytica	3	e	1.75E-04	2.94E-04
horse liver esterase	14	e	3.16E-04	5.81E-04
Mucor miehei	38	e	1.15E-04	1.23E-04
Saccharomyces cerevisiae	15	e	1.42E-04	3.19E-04
Thermoanaerobium brockii	13	e	1.56E-04	1.94E-04
Proteases-subtilisins				
Aspergillus oryzae	300	g	9.69E-04	8.91E-04
Bacillus polymyxa	20	g	n.r.	n.r.
***Bac. subtilis* var. Biotecus A**	**26**	**e**	**8.79E-04**	**1.16E-03**
α-chymotrypsin	46.5	g	3.71E-04	3.21E-04

proteinase, bacterial	**57.6**	**e**	**1.46E-03**	**1.93E-03**
proteinase K	3.1	e	1.66E-04	n.r.
Subtilisin from *Bacillus licheniformis*	**18**	**e**	**1.14E-03**	**1.87E-03**
Subtilisin Carlsberg	**32**	**g**	**1.39E-03**	**1.98E-03**
Protease from papaya	200	g	1.66E-04	3.24E-05
Rennin from *Mucor Meihei*	90.9	e	1.01E-04	1.12E-04
Optimase L-660	2000ul	j	4.23E-04	3.58E-04
Proteinase N from *Bacillus subtillis*	20	e	n.r.	n.r.
Acetylesterase from orange peel	**7**	**g**	**1.33E-03**	**2.33E-03**
Proteases-acylases				
Aspegillus melleus	92.3	e	2.81E-04	n.r.
hog kidney	**24**	**e**	**1.06E-03**	**1.89E-03**
Carbonic anhydrase	22	g	1.34E-04	n.r.

[a]Activity of hydrolases toward *N*-chloroacetyl-*p*-toluenesulfinamide. [b]Amount (mg) of solid enzyme per mL of buffer. [c]Observed rate[28] of hydrolysis in μmol/min. n.r.: no reaction. [d]Amano Enzyme USA Co., Ltd. (Troy, Va). [e]Fluka Chemie (Oakville, On). [f]Boerhinger-Mannheim (Mannheim, Germany). [g]Sigma-Aldrich (Oakville, On). [h]Biocataysts Ltd. (Pontypridd, Mid Glam, Wales, UK.). [i]Genzyme (Cambridge, MA). [j]Genenco. This activity screening-assay was performed at pH 7.2 since most hydrolases have maximum activity at that pH.

Using the activity-screening assay, we identified active enzymes that could catalyze the hydrolysis of the *N*-acylsulfinamides. This assay still doesn't identify enantioselective enzymes. This could be done by estimated E or Quick E[32], which are fast colorimetric screening-assay used to determine the enantioselectivity of reactions. These assays require enantiopure starting material. Unfortunately, following literature procedure,[14, 11] all attempts to prepare enantiopure *N*-acylsulfinamides were unsuccessful. Nevertheless, the reaction rates observed with the two substrates are slow and background chemical hydrolysis with *N*-chloroacetyl-*p*-toluenesulfinamide would make it more difficult to have accurate E-values. Still both those assay are unable to study the regioselectivity of the reaction. If the hydrolysis occurs at the acyl or the sulfinyl position, those assay are only able to detect release of acidic proton. They make no distinction between protons coming from the sulfinic acid or the carboxylic acid release

after reaction. Only the slower end-point method can determine the enantioselectivity or regioselectivity of the reactions.

Kinetic Resolution of *N*-Chloroacetyl-*p*-toluenesulfinamide with pH Variations

Kinetic resolution of *N*-chloroacetyl-*p*-toluenesulfinamide was done at pH 7.2 in BES buffer since most hydrolases have maximum activity at this pH. During the enzyme hydrolysis of that substrate, we encountered a problem of spontaneous chemical hydrolysis. A way to minimize the effect of that chemical hydrolysis on the enantioselectivity of the reaction using proteinase from *Bacillus subtilis* var. biotecus A is to use more enzyme and have a faster enzymatic reaction with the substrate. The enzyme is very expensive ($ 500 for 500 mg of enzyme) and to make a large-scale resolution a lot of enzyme would be required. We could also try to reduce the chemical hydrolysis by changing the pH of the reaction but first, we need to make sure that the enzyme is still effective with pH variation in **Table 2.5** summarized preliminary assays.

Tableau 2.5. Kinetic resolution of *N*-chloroacetyl-*p*-toluenesulfinamide with pH variations.

	wt[a]	time[b]	ee_s' %	ee_p' %	E'	%c %
pH 9 (CHES)	16	6	85	90	49	49
pH 8 (phosphate)	16	6	94	85	44	52
pH 7.2 (BES)	16	6	92	93	88	49
pH 6 (phosphate)	16	6	90	#	#	#

Kinetic resolution of *N*-chloroacetyl-*p*-toluenesulfinamide with pH variations was analyzed by end-point methods. The buffer solutions were adjusted to pH using pHstat. . [a]Weigh of solid enzyme in mg used to perform the hydrolysis .[b]Time in h to perform to hydrolysis. E', ee_p' and ee_s' refer to the parameter with no correction for chemical hydrolysis. CHES: 2-[*N*-cyclohexylamino]ethanesulfonic acid. The buffer solution were prepared from BES: *N*,*N*-bis[2-hydroxylethyl]-2-aminoethanesulfonic acid and phosphate: K_2HPO_4/KH_2PO_4.

Using different buffer solutions, the enzymatic reactions were performed pH 6-9. In all cases, the enzyme-catalyzed hydrolysis of the *N*-chloroacteyl-*p*-toluenesulfinamide occurred with high enantioselectivity. Ee_p of greater than 85% were obtained at different pH (**Table 2.5**). The enzyme is quite robust to pH variations. This is not surprising since subtilisins have a broad pH optimum and some subtilisins have maximum activity at high pH.[33]

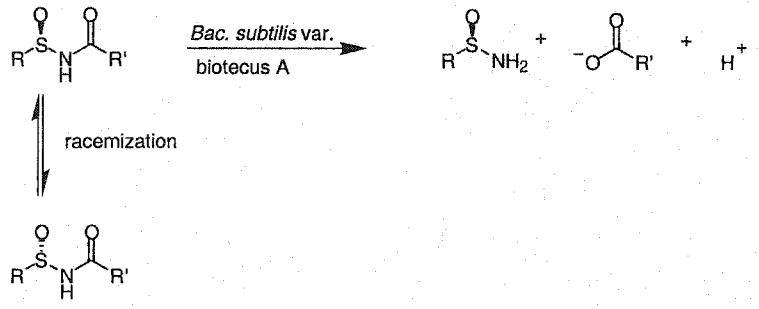
Unfortunately the E' is still affected by the chemical hydrolysis. In all cases, less than 3% chemical hydrolysis was found. The pH variation didn't solve the chemical hydrolysis problem. The most important figure of the investigation is E', which is the apparent enantioselectivity. Overall, it is possible to say that the enantioselectivity, E' did not change when the pH of the enzymatic reactions was changed.

On the other hand, one interesting feature is that at pH 6, the product of the reaction vanished. As a consequence, the enantioselectivity of the reaction could not be determined. The starting material is showing high enantiomeric excess with almost complete conversion of the *(R)*-*N*-chloroacetyl-*p*-toluenesulfinamide. At the end of the

reaction when the buffer layer was extracted with CH_2Cl_2, the product of the reaction staid in solution in the buffer layer. The *N*-chloroacetyl-*p*-toluenesulfinamide was found in the organic layer. This separation of sulfinamides and *N*-acylsulfinamides can be useful in large-scale reaction for separation of the products obtained after kinetic resolution by a simple acid-base extraction.

Dynamic Kinetic Resolution of *N*-acylsulfinamide

Kinetic resolution of racemic mixture based on hydrolase catalysis provides a useful methodology for the synthesis of optically active compounds. In order to have very efficient kinetic resolution, one enantiomer must react must faster than the other one. Even under those conditions, the maximum yield is 50%. The counterpart for improving the yield is the dynamic kinetic resolution of racemic starting material.[34] In this process the slower reacting enantiomer is converted *in situ* into the faster reacting enantiomer **(Scheme 2.6)**. A consequence of this racemization is the possibility to obtain 100% yield from the enzyme resolution. Reactions of this type are rare in the literature. Several examples have been reported which use enzymes.[35]



Scheme 2.6. Dynamic kinetic resolution of *N*-acylsulfinamides.

The kinetic resolution of *N*-acylsulfinamide was performed efficiently using proteinase from *Bacillus subtilis* var. biotecus A. We have demonstrated that the(*R*)-enantiomer reacted must faster than the (*S*)-enantiomer. Racemization of *N*-acylsulfinamide should allow the dynamic kinetic resolution, thereby allowing us to make enantiopure sulfinamide in greater than 50% yield. First to be able to perform such dynamic kinetic resolution, we need to find racemization conditions. This is what we have attempted to do with the *N*-acylsulfinamide.

Table 2.6. Racemization of *N*-chloroacetyl-*p*-chlorobenzenesulfinamide and *p*-chlorobenzenesulfinamide under basic and acidic conditions.

ee_p' %	ee_s' %	Conditions
88	80	after kinetic resolution[a]
86	78	NH_4Cl, MeCN (7%), 48h
88	74	NH_4Cl, MeCN (7%), SiO_2 (10%), 48h
n.d.	81	HCl, MeCN (7%), 48h
n.d.	82	HCl, MeCN (7%), SiO_2 (10%), 48h
89	73	NaOH, MeCN (7%), 48h
87	82	NaOH, MeCN (7%), SiO_2 (10%), 48h

[a]Racemization of *N*-chloroacetyl-*p*-chlorobenzenesulfinamide and *p*-chlorobenzenesulfinamide were performed after kinetic resolution of the racemic *N*-chloroacetyl-*p*-chlorobenzenesulfinamide. N.d.: not determine Kinetic resolution with this substrate was performed on 6 h. The ee_p' and ee_s' refer to the parameter with no correction for chemical hydrolysis.

According to literature, *N*-acylsulfinamides racemize under acidic conditions and on silica gel **(Table 2.6)**.[14] Also, the pKa of *N*-acylsulfinamide was determined to be 6.0-6.5.[36] The stability of *N*-acylsulfinamides was investigated under acidic and basic conditions. Optically active *N*-chloroacetyl-*p*-chlorobenzenesulfinamide and *p*-chlorobenzenesulfinamide were obtained from kinetic resolution of the racemic substrate.

We were hoping to find conditions at which the *N*-chloroactetyl-*p*-chlorosulfinamide would racemize and not the *p*-chlorosulfinamide. This substrate was chosen because it was the most active one. Under all conditions investigated no racemization was observed of neither the substrate nor the product. When we tried to make enantiopure *N*-chloroacetyl-*p*-toluenesulfinamide starting from enantiopure *p*-toluenesulfinamide, we obtained product with low enantiomeric excess. The reaction was performed under basic condition but racemization of *N*-acylsulfinamide is believed to go through formation of *meso* bis-sulfinylamines and attack of the anion of sulfinamide results in inversion at the sulfur.[14] It would have been very interesting to have racemization with pH variation because we have demonstrated previously that it was possible to use proteinase from *Bacillus subtilis* var. biotecus A to resolve *N*-chloroacetyl-*p*-toluenesulfinamide at different pH. Still we can continue, racemization investigation of *N*-acylsulfinamide using different substrate.

Experimental Section

General. Chemicals were purchased from Sigma-Aldrich and used with no further purification. Enzyme suppliers are noted in footnotes of **Table 2.3**. THF was distilled from Na/benzophenone ketyl. The reactions were carried out under inert nitrogen atmosphere. Chromatography was performed on preparative TLC (20 × 20 cm, 1000 micron). HPLCs were performed on a Spectra Physics HPLC (Model SP8800) and the Daicel Chiralcel OD and AD column (250 × 4.6 mm) were purchased from (Chiral Technologies, Exton, PA). The first eluted enantiomer is reported. All NMR was performed in $CDCl_3$ at room temperature. All microplate assays were performed on a Spectramax 340 microplate reader with SOFTmax PRO 2.2.1 software (Molecular Device, Sunnvale, CA). Polystyrene 96-well flat-bottomed microplates (Corning Costar, Acton, MA) were filled using Eppendorf 8-channel pipettes (5-100 μL, 50-1200 μL). A Radiometer RTS882 pHstat was used to measure pH.

(±)-*p*-Toluenesulfinamide.[11] *p*-Toluenesulfinic acid sodium salt (7.5 g, 42 mmol) dried overnight under high vacuum, was suspended in toluene (20 mL) under N_2, cooled with an ice bath and stirred. Oxalyl chloride (0.37 mL, 4.3 mmol) was added dropwise via a syringe through a rubber septum. After 1 hour of reaction, a second flask was charged with 29% NH_4OH (50 mL) and cooled in an ice bath. The sulfinyl chloride solution was transferred into NH_4OH solution via a cannula. The reaction was stirred for 30 minutes and was extracted with ethyl acetate (3 × 60 mL). The organic layers were combined, dried over anhydrous $MgSO_4$, evaporated. The solid obtained was washed with hexane; a

white powder obtained: 4.0 g, 60% yield. mp 108-110 °C. ^{1}H NMR (300 MHz) δ 2.4 (s, 3H), 4.3 (s, 2H), 7.3 (d, J = 8.1, 2H), 7.6 (d, J = 8.1, 2H). ^{13}C NMR (101 MHz) 21.5, 125.6, 129.8, 141.6, 143.6. MS (EI): *m/z* : M^+ 156, 139 (100). The enantiomers were separated HPLC (Diacel Chiralcel AD column, 95: 5 hexanes/ethanol, 1 mL/min, 238 nm) with $k'_s = 5.0$ and $\alpha = 1.4$.

(±)-Benzenesulfinamide. This substrate was prepared following same procedure used for the preparation of *p*-toluenesulfinamide starting from benzenesulfinic acid (7.0 g, 43 mmol)

Trituration in hexanes gave white crystals. 3.6 g, 60% yield. mp 105-108 °C. ^{1}H NMR (300 MHz) δ 4.3 (s, 2H), 7.5 (m, 2H), 7.7 (m, 3H). ^{13}C NMR (101 MHz) δ 125.6, 129.1, 131.3, 146.6. MS (EI) *m/z:* M^+ 141, 77 (100). The enantiomers were separated HPLC (Diacel Chiracel AD column, 95: 5 hexanes/ethanol, 1 mL/min, 238 nm) with $k'_s = 5.0$ and $\alpha = 1.2$.

(±)-*p*-Methoxybenznesulfinamide. *p*-Methoxybenzenesulfinyl chloride was prepared by a known procedure.[13] *p*-Methoxybenzenethiol (3.53 g, 25.0 mmol) and trimethylsilyl acetate (0.025 mmol, 3.8 mL) were added to a round bottom flask under N_2. The temperature was chilled with an ice bath. Sulfuryl chloride (25 mmol, 4.0 mL) was added dropwise via a syringe. The mixture was allowed to reach at room temperature and the reaction stirred 5 hours. This intermediate sulfinyl chloride was not isolated. A second round bottom flask was charged with 29% NH_4OH (130 mL) and chilled with an ice bath. The *p*-methoxybenzenesulfinyl chloride solution was added to the flask via a cannula and

the reaction was stirred for 30 minutes. The solution was saturated with NaCl and extracted with CH_2Cl_2 (2 × 150 mL). The organic phase were combined, dried with $MgSO_4$ and concentrated. Trituration in hexane gave white solid. 2.5 g, 57% yield. mp 120-123 °C. ^{1}H NMR (300 MHz) δ 3.9 (s, 3H), 4.3 (s, 2H), 7.0 (d, J = 9, 2H), 7.6 (d, J = 9, 2H). ^{13}C NMR (75 MHz) δ 55.8, 114.5, 127.3, 162.1, 174.1. MS (FAB) *m/z*: [M + H]$^+$ 172, 154 (100). The enantiomers were separated by HPLC (Diacel Chiracel AD column, 95: 5 hexanes/ethanol, 1 mL/min, 238 nm) with k'_s = 10.0 and α = 1.4.

(±)-2,4,6-Trimethylbenzenesulfinamide.[11] A three neck round bottom flask fitted with a reflux condenser under N_2 was charged with 2,4,6-trimethylbenzenesulfonyl chloride (11.8 g, 54.0 mmol), absolute EtOH (2.9 mL, 49 mmol) and CH_2Cl_2 (200 mL). Triethylamine (10.4 ml, 75.0 mmol) and trimethyl phosphite (11.6 mL, 98.0 mmol) were added via a syringe through a rubber septum. A glass stopper replaced the septum and the reaction was heated to reflux. The reaction was followed on TLC and when the 2,4,6-trimethylbenzenesulfonyl chloride disappeared, the reaction was cooled and poured into a separatory funnel containing ether (150 mL), EtOAc (150 mL) and 1 N HCl (250 mL). The organic layer was washed with $NaHCO_3$, brine, dried over anhydrous $MgSO_4$ and concentrated to give yellow oil. Part of the oil was purified by preparative TLC (80:20 hexanes/EtOAc) R_f = 0.6; ^{1}H NMR (300 MHz) δ 1.4 (t, J = 7.2, 3H), 2.3 (s, 3H), 2.6 (s, 6H), 4.10-4.18 (m, 2H), 6.68 (s, 2H); MS (FAB) *m/z*: [M + H]$^+$ 213. The rest of the oil was used immediately in the next step because ethyl sulfinate is not very stable. A 500 mL round bottom flask under N_2 was charged with the unpurified ethyl sulfinate (8.9 g, 42 mmol) and THF (100 mL). The reaction mixture was cooled in a dry ice/acetone bath

and LiHMDS (1.3-1.5 equiv., 63 mL) was added drop wise via a rubber septum. The reaction was warmed overnight to room temperature and NH_4Cl (200 mL) was added to the reaction mixture and stirred for 30 minutes. The reaction was poured in a mixture of EtOAc (75 mL) and ether (75 mL) and extracted. The organic layer was washed with brine, dried over anhydrous $MgSO_4$ and concentrated. Recrystallization in hexanes/EtOAc gave 4.6 g of crystals. 57% yield, mp 116-118 °C. ^{1}H NMR (300 MHz) δ 2.3 (s, 3H), 2.6 (s, 6H), 4.4 (s, 2H), 6.9 (s, 2H) .^{13}C NMR (75 MHz) 19.5, 21.2, 31.2, 131.1, 136.4, 141.0. MS (FAB) *m/z*: [M + H]$^+$ 184, 154 (100). The enantiomers were separated HPLC (Diacel Chiralcel OD column, 95: 5 hexanes/ethanol, 1 mL/min, 238 nm) with $k'_s = 6.8$ and $\alpha = 1.1$.

(±)-*p*-Chlorobenzenesulfinamide. This substrate was prepared following procedure of preparation of (±)-2,4,6-trimethylbenzenesulfinamide starting from *p*-chlorobenzenesulfonyl chloride (30.0 g, 142 mmol). Part of the ethyl sulfinate was purified on preparative TLC (90:10 hexanes/EtOAc) $R_f = 0.5$; ^{1}H NMR (300 MHz) δ 1.3 (t, *J* = 7.2, 3H), 3.70-3.76 (m, 1H), 4.09-4.11 (m, 1H), 7.5 (d, *J* = 7, 2H), 7.6 (d, *J* = 7, 2H); MS (EI) *m/z*: M$^+$ 204, 159 (100).

Trituration in hexanes gave white solid. 9.9 g, 53% yield. mp 126-128 °C. ^{1}H NMR (400 MHz) δ 4.4 (S, 2H), 7.5 (d, *J* = 8, 2H), 7.7 (d, *J* = 8, 2H). ^{13}C NMR (101 MHz) 127.3.129.4, 137.8, 145.1. MS (EI) *m/z*: M$^+$ 175, 159 (100). The enantiomers were separated HPLC (Diacel Chiracel AD column, 95: 5 hexanes/ethanol, 1 mL/min, 238 nm) with $k'_s = 6.0$ and $\alpha = 1.3$.

(±)-*N*-Chloroacetyl-arenesulfinamide.[11, 14] Sulfinamides (0.250 g) were added to a three neck round bottom flask under N_2. Dried THF was added via a syringe through a rubber septum and the temperature was cooled in an acetone/dry ice bath. *n*-BuLi in hexane (2.5 equiv, 1.6 M) was added dropwise followed by rapid addition of the chloroacetic anhydride (2 equiv). The reactions were monitored on TLC and an aqueous solution saturated with NH_4Cl was added after disappearance of the sulfinamides. The aqueous layers were extracted with EtOAc (3 × 20 mL). The combined organic layers were dried over anhydrous $MgSO_4$ and concentrated. The residues were purified on preparative TLC.

(±)-*N*-Chloroacetyl-*p*-toluenesulfinamide. The solid was purified on preparative TLC, hexanes/MeOH 98:2, R_F = 0.4. Recrystallization of the crude solid in benzene gave white crystals. 186 mg, 50% yield. mp 98-102 °C. ^{1}H NMR (300 MHz) 2.5 (s, 3H), 4.2 (s, 2H), 7.4 (d, J = 8, 2H), 7.6 (d, J = 8, 2H). ^{13}C NMR δ (75 MHz) 21.8, 42.5, 124.8, 130.6, 134.0, 143.5, 167.2. MS (EI) *m/z*: M^+ 231, 139 (100). HRMS-EI (*m/z*): M^+ calcd for $C_9H_{10}SNO_2{}^{35}Cl$, 231.01208 found 231.01238. The enantiomers were separated HPLC (Diacel Chiralcel AD column, 95:5 hexanes/ethanol, 1 mL/min, 238 nm) with k'_s = 8.3 and α = 2.0.

(±)-*N*-Chloroacetylbenzenesulfinamide. The solid was purified on preparative TLC, hexanes/MeOH 98:2, R_F = 0.4. Trituration in hexanes gave white solid. 216 mg, 56% yield. mp 91-94 °C. ^{1}H NMR (300 MHz) δ 4.2 (s, 2H), 7.7 (m, 3H), 7.8 (m, 2H), 8.1 (s, 1H). ^{13}C NMR (75 MHz) δ 42.4, 124.9, 129.8, 143.0, 167.3. MS (EI) *m/z*: M^+ 217, 125

(100). HRMS-EI (*m/z*): M^+ calcd for $C_8H_8SNO_2{}^{35}Cl$, 216.99643 found 216.99727. The enantiomers were separated HPLC (Diacel Chiracel AD column, 95: 5 hexanes/ethanol, 1 mL/min, 238 nm) with $k'_s = 7.9$ and $\alpha = 2.0$.

(±)-*N*-Chloroacetyl-*p*-chlorobenzenesulfinamide. The solid was purified on preparative TLC, hexanes/MeOH 95:5, $R_F = 0.5$. Recrystallization of the crude solid in EtOAc/hexanes gave white crystals. 337 mg, 94% yield. mp 110-114 °C. ^{1}H NMR (400 MHz) 4.1 (s, 2H), 7.6 (d, $J = 8$, 2H), 7.7 (d, $J = 8$, 2H). ^{13}C NMR (75 MHz) δ 42.4, 126.3, 130.2, 139.3, 141,5, 167.0. MS (EI) *m/z*: M^+ 251, 159 (100). HRMS-EI (*m/z*): M^+ calcd for $C_8H_7SNO_2{}^{35}Cl_2$, 250.95746 found 250.95776. The enantiomers were separated HPLC (Diacel Chiracel AD column), 95: 5 hexanes/ethanol, 1 mL/min, 238 nm) with $k'_s = 11.4$ and $\alpha = 1.8$.

(±)-*N*-Chloroacetyl-*p*-methoxybenzenesulfinamide. The crude product was purified on preparative TLC (hexanes/MeOH 98:2) $R_f = 0.4$; and trituration in hexanes gave white crystals. 152 mg, 42% yield. mp 79-83 °C. ^{1}H NMR (300 MHz) δ 3.9 (s, 3H), 4.2 (s, 2H), 7.1 (d, $J = 9$, 2H), 7.7 (d, $J = 9$, 2H), 8.2 (s, 1H). ^{13}C NMR (75 MHz) δ 42.4, 55.9, 115.2, 126.6, 133.9, 163.13, 167.2. MS (EI) *m/z*: M^+ 247, 155 (100). The enantiomers separated HPLC (Diacel Chiracel AD column, 95: 5 hexanes/ethanol, 1 mL/min, 238 nm) with $k'_s = 18.4$ and $\alpha = 1.7$.

(±)-*N*-Chloroacetyl-2,4,6-trimethylbenzenesulfinamide. The solid was purified on preparative TLC, hexanes/MeOH 98:2, $R_F = 0.4$. Recrystallization of the crude solid in

EtOAc/hexanes gave white crystals. 340 mg, 96% yield, mp 96-98 °C; ^{1}H NMR (400 MHz) δ 2.3 (s, 3H), 2.6(s, 6H), 4.1-4.2 (m, 2H), 6.9 (s, 2H). ^{13}C NMR (75 MHz) δ 19.4, 21.4, 42.6, 131.5, 135.8, 138.1, 142.0, 166.7. MS (EI) *m/z*: M^+ 259, 105 (100). HRMS-EI (*m/z*): M^+ calcd for $C_{11}H_{14}SNO_2{}^{35}Cl$, 259.04338 found 259.04307. The enantiomers were separated HPLC (Diacel Chiracel OD column, 95: 5 hexanes/ethanol, 1 mL/min, 238 nm) with $k'_s = 4.8$ and $\alpha = 1.2$.

Scale-up reactions with *N*-chloroacetyl-*p*-toluenesulfinamde.[37] In a 100 mL volumetric flask, (±)-*N*-chloroacetyl-*p*-toluenesulfinamide (0.10 g, 0.45 mmol) was dissolved in CH_3CN (7%, 7 mL) and the volume was made up to 100 mL with BES buffer (20 mM, pH 7.2). In a vial (20 mL) equipped with a magnet, the solid enzyme was added. The *N*-chloroacetyl-*p*-toluenesulfinamide solution was added to the vials (10 mL). The reactions were stirred (reaction time are listed in **Table 2.2**). The aqueous phase was extracted with CH_2Cl_2 (2 × 10 mL). The organic layers were combined, dried over anhydrous $MgSO_4$ and concentrated. The solid was dissolved in 1 mL of EtOH and filtered through a filter (nylon, 4 mm, 0.45 μm) via a syringe. The samples were analyzed by HPLC on a Chiralcel AD column (250 × 4.6 mm; Hex: EtOH, 95:5; temperature: 25 °C, injection volume: 20 μL, flow rate =1.0 mL/min; detection UV = 238 nm).

Scale-up reaction with proteinase from *Bacillus subtilis* var. biotecus A and the other *N*-acylsulfinamides. Same conditions were used than for *N*-chloroacetyl-*p*-toluenesulfinamide. In a 20 mL vial, (±)-*N*-chloroacetylsulfinamide (0.045 mmol) was dissolved in CH_3CN (7%, 7mL) and 10 mL of BES buffer (20 mM, pH 7.2) was added.

The samples were analyzed by HPLC on a chiralcel AD column (250 × 4.6 mm; Hex: EtOH, 95:5; temperature: 25 °C, injection volume: 20 μL, flow rate =1.0 mL/min; detection UV = 238 nm).

(±)-2,4,6-trimethylbenzenesulfinamide and (±)-*N*-chloroacetyl-2,4,6-trimethylbenzene-sulfinamide were analyzed by HPLC on a chiralcel OD column (250 × 4.6 mm; Hex: EtOH, 95:5; temperature: 25°C, injection volume: 20 μL, flow rate = 1.0 mL/min; detection UV = 238 nm).

Scale-up reaction with bacterial proteinase. The same procedure used for scale-up reaction was used. After extraction with methylene chloride, the buffer layer was acidified with 0.1 N HCl to a pH of 2 to protonate the *p*-toluenesulfinic acid. Than the aqueous layer was extracted with 2×15 mL ether. The ethereal layer was dried over $MgSO_4$ and concentrated. A white powder was isolated in 60% yield, 2.1 mg. TLC (90:10 $CHCl_3$ /HCOOH) R_f= 0.3; ^{1}H NMR (400 MHz) δ 2.45 (s, 3H), 7.4 (d, J = 8,2H), 7.6 (d, J = 8, 2H); HRMS-EI (*m/z*): M^+ calcd for $C_7H_8O_2S$, 156.02450 found 156.02450.

Hydrolase library. Since many hydrolases have maximal activity at pH 7, pH 7.2 was used to screen the enzyme library. The hydrolases were dissolved in BES buffer (5.0 mM, pH 7.2) at the concentration listed in **Table 2.2**. The solutions were centrifuge (10 min, 2000 rpm) and titrated to a pH of 7.2. The supernatant was used in the screening assays.

Activity screening-assays. The assay solution was mixing (±) *N*-chloroacetyl-*p*-toluenesulfinamide (0.10 g, 0.44 mmol) or (±) *N*-butanoyl-*p*-toluenesulfinamide (0.10 g, 0.44 mmol) in acetonitrile (1000 μL), *p*-nitrophenol (6714 μL of a 1.0 mM solution in 5.0 mM BES, pH 7.2), and BES buffer (5143 μL of 5.0 mM solution, pH 7.2). The solution was vortexed to have complete mixing. Hydrolases solutions (10 μL/ well) were transferred to a 96 well microplate using a micropipette. Assay solution (90 μL/well) was transferred to each well using a 1200 μL eight-channel pipette. The final concentrations in each well were 3.1 mM substrate, 4.65 mM BES, 0.46 mM *p*-nitrophenol, and 7% acetonitrile. The plate was placed in the microplate reader and shaken for 5 s; the decrease in absorbance was monitored at 404 nm. This assay was done twice when the temperature was fixed at 25 °C and 37 °C as permitted by the microplate software. The data were collected for one hour in quadruplicate and averaged.

Experimental Section (Supplementary Material)

(±)-*N*-Butanoyl-*p*-toluenesulfinamide.[11, 14] *p*-Toluenesulfinamide (3.9 mmol, 0.6 g) was added to a three neck round bottom flask under N_2. Dried THF was added via a syringe through a rubber septum and the temperature was cooled in an acetone/dry ice bath. *n*-BuLi in hexane (6.0 mL, 1.6 M) was added dropwise followed by rapid addition of the butyric anhydride (2 equiv, 1.3 mL). The reactions were monitored on TLC and an aqueous solution saturated with $NaHCO_3$ solution was added after disappearance of the *p*-toluenesulfinamide. The aqueous layers were extracted with CH_2Cl_2 (3 × 20mL). Recrystallization of the crude solid in EtOAc/hexanes gave white crystals. 0.78 g, 90% yield; ^{1}H NMR (300 MHz) 1.0 (t, J = 7, 3H), 1.7 (m, 2H), 2.2 (m, 2H), 2.4 (s, 3H), 7.3

(d, J = 8, 2H), 7.6 (d, J = 8, 2H).. ^{1}MS (FAB) *m/z*: [M + H]$^+$ 226, 139 (100). The enantiomers were separated HPLC (Diacel Chiracel OD column, 95: 5 hexanes/ethanol, 1 mL/min, 238 nm) with k'_s = 7.4 and α = 2.2.

(*R*)-(+)-*p*-Toluenesulfinamide.[8a] In a 100 mL three neck round-bottomed flask equipped with magnetic stirring bar, rubber septum, and nitrogen inlet was placed 1.28 g (4.35mmol) of (*1S,2R,5S*)-(+)-menthyl-(*R*)-p-tolunesulfinate in 20 mL of dry THF. The reaction was cooled in a dry ice/acetone bath, and 13 mL of LiHMDS (1 M) was added dropwise via a syringe. The reaction was warmed to room temperature, stirred for 3 hours and TLC followed the disappearance of the starting material. The mixture was quenched with NH_4Cl sat (30 ml) an additional 10 mL of water and extracted with ethyl acetate (3×15 mL). The organic phase was dried with $MgSO_4$ and concentrated. The crude solid obtained was wash with hexanes to give 0.50 g (75%) of solid. mp 111-110 °C. ^{1}H NMR (300 MHz) δ 2.4 (s, 3H), 4.3 (s, 2H), 7.3 (d, J = 8.1, 2H), 7.6 (d, J = 8.1, 2H). ^{13}C NMR (101 MHz) 21.5, 125.6, 129.8, 141.6, 143.6. MS (EI) *m/z*: 156, 139 (100). The enantiomeric excess was determine by HPLC (Diacel Chiralcel AD column, 95: 5 hexanes/ethanol, 1 mL/min, 238 nm) with k'_s = 5.0 and α = 1.4. 96% ee.

(*R*)-*N*-Chloroacetic-*p*-toluenesulfinamide. The procedure for preparation of racemic (±)-*N*-chloroacetyl-p-toluenesulfinamide was used. The enantiomeric excess was determine by HPLC HPLC (Diacel Chiralcel AD column, 95:5 hexanes/ethanol, 1 mL/min, 238 nm) with k'_s = 8.3 and α = 2.0, 40% ee.

Scale-up reaction with proteinase from *Bacillus subtilis* var. biotecus A and pH variations. Same conditions were used than for *N*-chloroacetyl-*p*-toluenesulfinamde. Phosphate, CHES and BES buffer were prepared and adjusted to pH using the Radiometer RTS882 pHstat. In a 20 mL vial, (±)-*N*-chloroacetylsulfinamide (0.045 mmol) was dissolved in CH_3CN (7%, 7 mL) and 10 mL of the buffer was added.

Racemization of *N*-chloroacetyl-*p*-chlorobenzenesulfinamide and *p*-chlorobenzenesulfinamide. Kinetic resolution of *N*-chloroacetyl-*p*-chlorobenzenesulfinamide was performed using proteinase from *Bacillus subtilis* var. biotecus A (refer to procedure for scale-up reactions with *N*-acylsulfinamides). After kinetic resolution, the residue was dissolved in 0.5 mL of MeCN. Acetonitrile solution, 70 μL was dissolved in 1 mL of HCl (1N), NaOH (1N) or saturated NH_4Cl. This was also done with same solution containing 10% SiO_2. The reactions were stirred for 48 h, extracted with (1 mL×2) CH_2Cl_2, dried over $MgSO_4$, and concentrated. The solid was dissolved in 1 mL of EtOH and filtered through a filter (nylon, 4 mm, 0.45 μm) via a syringe. The samples were analyzed by HPLC on a chiralcel AD column (250 × 4.6 mm; Hex: EtOH, 95:5; temperature: 25 °C, injection volume: 20 μL, flow rate =1.0 mL/min; detection UV = 238 nm).

References

[1] (a) Liu, G.; Cohan, D. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1997**, *119*, 9913. (b) Hua, D. H.; Miao, S. W.; Chen, J. S.; Iguchi, S. *J. Org. Chem.* **1991**, *56*, 5. (c) Moreau, P.; Essiz, M.; Mérour, J. -Y.; Bouzard, D. *Tetrahedron: Asymmetry* **1997**, *8*, 591.

[2] (a) Davis, F. A.; Zhou, P.; Chen, B. -C. *Chem. Soc. Rev.* **1998**, *27*, 13. (b) Hua, D. H.; Miao, S.W.; Iguchi, S. *J. Org. Chem.* **1991**, *56*, 4. (c) Tang, T. P.; Ellman, J. A. *J. Org. Chem.* **1999**, *64*, 12.

[3] (a) Lefevre, I.; Evans, A. *J. Org. Chem.* **1997**, *62*, 7532. (b) Mikolajczyk, M.; Lyzwa, P.; Drabowick, J. *Tetrahedron: Asymmetry* **1997**, *8*, 3991. (c) Mikolajczyk, M.; Lyzwa, P.; Drabowick, J.; Wieczorek, M. W.; Blaszczyk, J. *J. Chem. Soc., Chem. Commun.* **1996**, 1503.

[4](a) Davis, F. A.; Zhou, P.; Reddy, G. V. *J. Org. Chem.* **1994**, *59*, 3243. (b) Ruano, J. L. G.; Fernández, I.; Catalina, M. D.; Cruz, A. A. *Tetrahedron: Asymmetry* **1996**, *7*, 3407-3414. (c) Davis, F. A.; Zhou, P. *Tetrahedron Lett.* **1994**, *35*, 7525. (d) Davis, F. A.; Zhou, P.; Liang, C. -H. *Tetrahedron Lett.* **1997**, *38*, 5139.

[5] Refer to chapter 1 for applications of sulfinimines in organic synthesis.

[6](a) Davis, F. A.; Reddy, R. T.; Reddy, R. E. *J. Org. Chem.* **1992**, *57*, 6387. (b) Davis, F. A.; Reddy, R. E.; Szewczyk, J. M.; Reddy, G. V. Portonovo, P. S.; Zhang, H.; Fanelli, D.; Reddy, R. T.; Zhou, P.; Carroll, P. J. *J. Org. Chem.* **1997**, *62*, 2555.

[7] (a) Cinquini, M.; Cozzi, F. *J. Chem. Soc., Chem. Commun.* **1977**, 502. (b) Davis, F. A.; Portonovo, P. S.; Reddy, R. E.; Szewczyk, J. M. *Tetrahedron Lett.* **1993**, *34*, 6229. (c) Khiar, N.; Fernàndez, I.; Alcudia, F. *Tetrahedron Lett.* **1994**, *35*, 5719.

[8] (a) Davis, F. A.; Zhang, Y.; Andermicheal, Y.; Fang, D., L.; Zhang, H. *J. Org. Chem.* **1999**, *64*, 1403. (b) Liu, G.; Cohan, D. A.; Owens, T. D.; Tang, T. P.; Ellman, J. A. *J. Org. Chem.* **1999**, *64*, 1278.

[9] Cohan, D. A.; Liu, G.; Kim, K.; Backes, B. J.; Ellman, J. A. *J. Am. Chem. Soc.* **1998**, *120*, 8011.

[10] The acylation of *p*-toluenesulfinamide in organic solvents with proteinase from *Bacillus subtilis* var. biotecus A was tried using vinyl acetate but no reaction was observed.

[11] Backes, B. J.; Dragoli, D. R.; Ellman, J. A. *J. Org. Chem.* **1999**, *64*, 5472.

[12] Klunder, J. M.; Sharpless, K. B. *J. Org. Chem.* **1987**, *52*, 2598.

[13] Drabowicz, J.; Bujnicki, B.; Dudzinski, B. *Synth. Comm.* **1994**, *24*, 1207.

[14] Ruano-García, J. L.; Alonso, L.; Zarzuelo, M. M.; Noheda, P. *Tetrahedron: Asymmetry* **1995**, *6*, 1133.

[15] Janes, L. E.; Löwendahl, C. P.; Kazlauskas, R. J. *Chem. Eur. J.* **1998**, *4*, 2324.

[16] The enantioselectivity was calculated from the following equations: $E = \ln[1-c(1+ee_p)]/\ln[1-c(1-ee_p]$; $E = \ln[(1-c)(1-ee_s)]/\ln[(1-c)(1+ee_s]$; c = conversion, ee = enantiomeric excess of substrate(s) or product (p), E = Enantioselectivity; Chen, C. S.; Fujimoto, Y.; Girdaukas, G.; Sih, J. *J. Am. Chem. Soc.* **1982**, *104*, 7294.

[17] A computer program, *Selectivity-Kresh* available from http//www.orgc.tu-graz.ac.at was used, this program develop by Kurt Faber correct from spontaneous reaction.

[18] We are currently optimizing the conditions to resolve 250 mg of 2,4,6-trimethylbenzenesulfinamide.

[19] None of proteases showed activity toward *N*-chloroacetyl-*p*-toluenesulfinamide but five of them showed activity toward *N*-chloroacetyl-*p*-toluenesulfinamide.

[20] Bornscheuer, U. T.; Kazlauskas, R. J. *Hydrolases in Organic Synthesis. Stereo- and Regioselectivity Biotransformations*, Wiley-VCH, Weinheim, 1999.

[21] Kazlauskas, R. J.; Weissfloch, A. N. E. *J. Mol. Catal. B Enz.* **1997**, *3*, 65-72.

[22] (a) Reid, T. W.; Fahmey, D. *J. Am. Chem. Soc.* **1967**, *89*, 3941. (b) Reid, T. W.; Stein, T. P.; Fahmey, D. *J. Am. Chem. Soc.* **1967**, *89*, 7125. (c) Chen, H. J.; Kaiser, E. T. *J. Am. Chem. Soc.* **1974**, *96*, 625.

[23] (a) Larsen, J. D.; Bumdgaard, H. *Int. J. Phar.* **1987**, *37*, 87. (b) Larsen, J. D.; Bumdgaard, H.; Lee, V. H. L. *Int. J. Phar.* **1988**, *47*, 103. (c) Larsen, J. D.; Bumdgaard, H. *Int. J. Phar.* **1989**, *51*, 27.

[24] Arnold, F. A.; Zhao, H. *Protein Eng.* **1999**, *12*, 47.

[25] Miyazaki, K.; Arnold, F. A. *J. Mol. Evol.* **1999**, *49*, 716.

[26] (a) Roberts, S. M. *Preparative Biotransformations*, Wiley, Chichester, 1992-1997. (b) Faber, K. *Biotransformations in Organic Chemistry*, 3rd ed., Springer, Berlin, 1997.

[27] (a) Rosenberg, R. M.; Herreid, R. M.; Piazza, G. J.; O'Leary, M. H. *Anal. Biochem.* **1992**, 81. (b) Gibbons, B. H.; Edsall, J. T. *J. Biol. Chem.* **1963**, *238*, 3502.

[28] Calculated from Eq. 2.1 and Eq 2.2.

[29] Daffe, V.; Fastrez, J. *J. Am. Chem. Soc.* **1980**, *102*, 3601.

[30] Pedragoza-Moreau, S.; Archelas, A; Furstoss, R. *Tetrahedron* **1996**, *52*, 4593.

[31] Orsat, B.; Alper, P. B.; Moree, W.; Mak, C. -P.; Wong, C. -H. *J. Am. Chem. Soc.* **1996**, *118*, 712.

[32] (a) Janes, L. E.; Kazlauskas, R. J. *J. Org. Chem.* **1997**, *62,* 4560. (b) Janes, L. E.; Kazlauskas, R. J. *J. Org. Chem.* **1999**, *64*, 9019.

[33] Shirai, T.; Suzuki, A; Yamane, T.; Ashida, T.; Kobayashi, T.; Hitomi, J.; Ito, S. *Protein Eng.* **1997**, *10*, 627.

[34] Ward, R. S. *Tetrahedron: Asymmetry* **1995**, *6*, 1475.

[35] Meijer, E. M.; Boestan, W. H. J.; Shoemaker, H. E.; an Blaken, J. A. M. In *Biocatalysis in Organic Synthesis*, Tramper, J., Van Der Plas, H. C.; Linko, P.; Eds.; Elsevier Publishing: Amsterdam, 1985; p. 135.

[36] pKa determination was done by titration of the *N*-acylsulfinamides with 0.01 N NaOH using a pHstat.

[37] The chemical hydrolysis was determined by running scale-up reactions with no enzymes. This was done for all scale-up reactions.

Conclusions

Kinetic resolution of *N*-acylsulfinamide using hydrolases catalyzed reactions has been achieved successfully. Using a fast activity-screening assay, we were able to quickly identified promising hydrolases. Most were found to give moderate enantioselectivity for the *N*-chloroacetyl-*p*-toluenesulfinamide. Two proteases were identified to catalyze the hydrolysis of the substrate with high enantioselectivity. More importantly these proteases also showed regioselectivity with regard to the sulfinyl and carbonyl position. Proteinase from *Bacilus subtilis* var. biotecus A provided enantiopure (*R*)-*p*-toluenesulfinamide as well as other (*R*)-arylsulfinamides in high enantiomeric excess. This bacterial proteinase showed high enantioselectivity for (*R*)-*N*-chloroacetyl-*p*-toluenesulfinamide and catalyzed the formation of *p*-toluenesulfinic acid.

Proteinase from *Bacilus subtilis* var. biotecus A provides new enantiopure sulfinamides that cannot be prepared from current methods. The enzyme was only used for arylsulfinamide; the substrate specificity of this protease may be pushed further by investigating different alkylsulfinamides. Also this enzyme is a variant of Subtilisin Carlberg. Protein engineering of subtilisin could result in improve catalyst for the resolution of sulfinamides. Bacterial proteinase has been identified to catalyze the hydrolysis of *N*-acylsulfinamide at the chiral sulfinyl center. In the reverse reaction starting with achiral sulfinic acids, enantiopure sulfinamides may be obtained, with yield up to 100%.

We succeeded to extend the use of hydrolases to resolution of *N*-acylsulfinamides. It new route provides enantiopure sulfinamide that are not currently available in enantiomerically pure form.