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Synthesis of 4-Sulfur-Substituted (2*S*,3*R*)-3-Phenylserines by Enzymatic Resolution. Enantiopure Precursors for Thiamphenicol and Florfenicol

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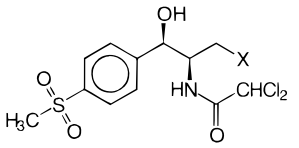
Abstract:

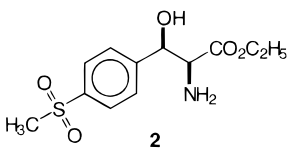
Enantiomerically pure 4-methylthio- and 4-methylsulfonyl-substituted (2*S*,3*R*)-3-phenylserines are prepared by enzymatic resolution of the corresponding racemic *threo* amides using the amidase from *Ochrobactrum anthropi* NCIMB 40321. The unwanted (2*R*,3*S*) enantiomers of the amides are separated from the enantiopure amino acids and easily racemized after Schiff base formation with the corresponding 4-(methylthio)- and 4-(methylsulfonyl)benzaldehyde. The racemization can be combined with the preparation of the racemic amides by aldol reaction under crystallization conditions to yield only the *threo* isomers. Enantiopure (2*S*,3*R*)-3-[4-(methylthio)phenyl]serine and (2*S*,3*R*)-3-[4-(methylsulfonyl)phenyl]serine are thus obtained in 78% and 62% overall yields starting from the corresponding substituted benzaldehydes. (2*S*,3*R*)-3-[4-(Methylthio)phenyl]serine is reduced to (1*R*,2*R*)-2-amino-1-[4-(methylthio)phenyl]propane-1,3-diol with $NaBH_4/H_2SO_4$ and can be used for the synthesis of thiamphenicol and florfenicol.

Introduction

For nearly half a century thiamphenicol (**1a**), a fully synthetic analogue of chloramphenicol, has been known for its antibacterial activity against Gram-positive and Gram-negative bacteria.[1] A more recently developed analogue is florfenicol (**1b**).[2] Currently thiamphenicol is produced in amounts of approximately 100 tons per year and used as a human and veterinary antibiotic. Since only the (1*R*,2*R*) enantiomer is active, early syntheses were developed in which the enantiomerically pure compound was obtained by resolution. In practice two routes are used for the resolution: classical resolution of the *threo*-3-[4-(methylsulfonyl)-phenyl]serine ethyl ester (**2**) using tartaric acid as a resolving agent[3] and resolution by preferential crystallization of *threo*-2-amino-1-[4-(methylthio)phenyl]propane-1,3-diol (**3**).[4] However, as a consequence of both resolution methods the undesired enantiomer remains as waste, though for both methods laborious racamization protocols for the undesired enantiomer are described.[5] Late in the resolution process, an elegant solution is described for the amino diol **3** by Giordano *et al.*,[6] who developed a process based on the inversion of both chiral centers of (1*S*,2*S*)-**3** yielding the required (1*R*,2*R*) enantiomer. More recently, researchers at Celgene described the use of an aldolase to promote the retro-aldol reaction of the (2*R*,3*S*) enantiomer of racemic *threo*-3-[4-(methylthio)phenyl]serine (**4**), leaving the desired (2*S*,3*R*) enantiomer of **4**.[7] Other enzymatic resolution processes are described by Clark *et al.* in which additional reaction steps are necessary.[8,9] Various asymmetric syntheses for florfenicol and thiamphenicol[10] and synthesis from chloramphenicol[11] have been described, but most of these methods lack practical applicability on a large scale.



1a (X = OH) : thiamphenicol
1b (X = F) : florfenicol



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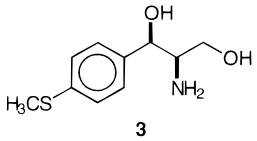

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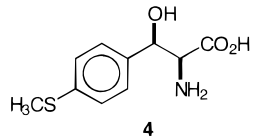

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In developing an economically attractive process we argued that only resolution processes with an easy racem-

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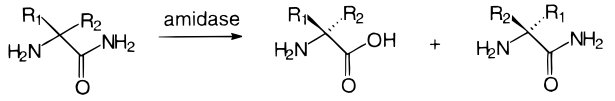
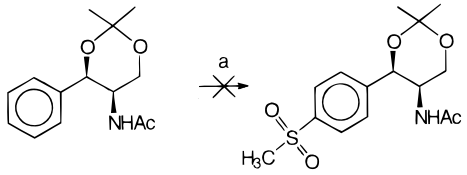
Scheme 1



Scheme 2[a]



[a] (a) CH_3SO_2Cl, Lewis acid (5 equiv), CH_2Cl_2 (Lewis acid: $AlCl_3$, $FeCl_3$, $ZnCl_2$, or $TiCl_4$).

ization of the unwanted isomer or asymmetric syntheses would yield a viable process. For resolution processes it is preferred to perform this step as early as possible in the synthesis to prevent loss of expensive material at the end of the synthesis as well as higher consumption of reagents.

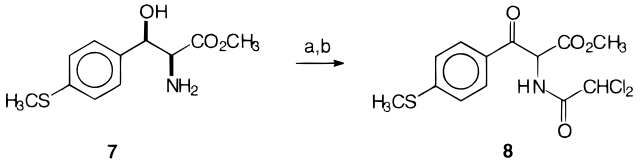
The application of the enzymatic resolution of α-amino acid amides as developed by our group during the last years appears especially attractive (Scheme 1). Amidases from different microorganisms can be used for these resolutions, depending on the substrate: for α-mono-substituted glycinamides the amino peptidase from *Pseudomonas putida* is a suitable choice,[12] while α,α-disubstituted glycinamides can be resolved using the amino amidase from *Mycobacterium neoaurum*.[12,13] More recently we developed a new amidase from *Ochrobactrum anthropi* which can resolve both classes of α-amino acid amides as well as α-hydroxy and α-(*N*-hydroxyamino) carboxamides.[14] Since most processes to thiamphenicol and florfenicol proceed via (racemic) sulfur-substituted phenylserines (**4**),[3,4] the enzymatic resolution seems viable.

In addition, two other routes were tested: (a) a dynamic kinetic resolution process by asymmetric hydrogenation of a *β*-keto ester and (b) sulfonation of a readily available intermediate in the chloramphenicol process.

Results and Discussion

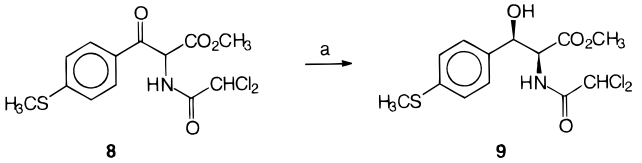
In our first approach to the preparation of enantiomerically pure intermediates for thiamphenicol and florfenicol we tried to sulfonate (1*R*,2*R*)-2-amino-1-phenylpropane-1,3-diol, protected as its *N*-acetyl isopropylidene acetal (**5**), at the para position (Scheme 2). This would offer the advantage of using an enantiopure intermediate used for the preparation of chloramphenicol. However, the use of methanesulfonyl chloride and various Lewis acids[15] did not result in Friedel–Crafts sulfonation. Only deprotection was observed after long reaction times.

Scheme 3[a]



[a] (a) Cl_2CHCO_2Et/MeOH, 70%; (b) DMSO, $(COCl)_2$, 53%.

Scheme 4[a]



[a] (a) 1000 psi of H_2, $Ru_2Cl_4[(R)\text{-BINAP}]_2{\cdot}N(C_2H_5)_3$ (1 mol %), 50 °C, 66 h.

We therefore turned our attention to another approach: the asymmetric hydrogenation of the keto compound **8** into *N*-(dichloroacetyl)-3-[4-(methylthio)phenyl]serine methyl ester (**9**), a method described by Noyori *et al.* for reduction of comparable *β*-keto esters.[16]

In preparing the starting material for this hydrogenation, the racemic methyl ester **7** was acylated with ethyl dichloroacetate and subsequently oxidized at the benzylic alcohol using the Swern oxidation. By this method the *β*-keto ester **8** was obtained in 37% overall yield (Scheme 3).

Like other *β*-keto esters, compound **8** shows a keto–enol equilibrium. In this case the keto–enol ratio is 86:14. The exchange rate of the acidic α-proton was estimated by ^{1}H NMR spectroscopy in $CDCl_3$/10% CD_3OD: a half-life value of $T_{1/2} < 25$ min was found for the deuterium exchange. The rate of exchange of the α-proton by keto–enol equilibration determines the racemization rate of compound **8**. If this racemization is fast enough, asymmetric hydrogenation under dynamic kinetic resolution conditions should be possible, resulting in the highest possible yield of enantio- and diastereomerically pure product.[17] Under conditions for the asymmetric hydrogenation identical to those described by Noyori *et al.*[16] ($Ru_2Cl_4[(R)\text{-BINAP}]_2{\cdot}N(C_2H_5)_3$/1000 psi of H_2), **9** was formed in a *threo:erythro* ratio of ≥95:5. The ee of the *threo* isomer, however, was conversion dependent; at low conversion the ee of the *threo* isomer was 85%, but it decreased to 15% at higher conversion (Scheme 4). The reduction of **8** with $NaBH_4$ or BH_3 resulted in the formation of the *erythro* isomer in excess (*threo:erythro* ratio 1:2 (BH_3) or 1:7 ($NaBH_4$)), together with some overreduction of the ester into the diastereomeric *N*-dichloroacetyl derivatives of amino diol **3**. For this reason we did not study any further asymmetric borane reductions.

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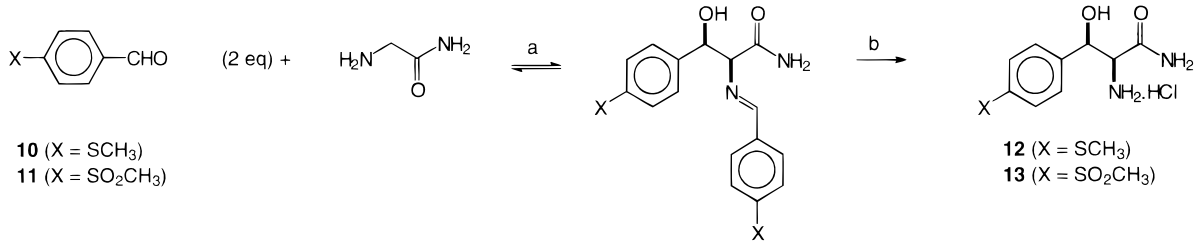
Scheme 5[a]

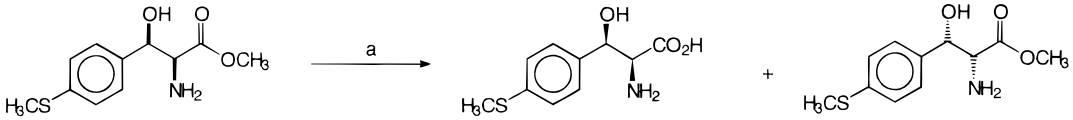


10 (X = SCH_3)
11 (X = SO_2CH_3)

12 (X = SCH_3)
13 (X = SO_2CH_3)

[a] (a) MeOH/H_2O, pH 12−13; (b) HCl/H_2O.

Scheme 6[a]



7 (*2S, 3R*)-**4** (*2R, 3S*)-**7**

[a] (a) 3 w/w % solution, pH 7.5, 0.3 w/w % lipase, 3 h, 30 °C.

Since these (asymmetric) reductions did not result in an applicable route to enantiopure compounds for the thiamphenicol synthesis, we turned our attention to the enzymatic approach. In practice two methods were evaluated: the lipase-catalyzed resolution of *threo*-3-[4-(methylthio)phenyl]-serine methyl ester (**7**) and the amidase-catalyzed resolution of the sulfur-substituted *threo*-3-phenylserinamides **12** and **13**.

The amides for the latter resolution method were prepared by aldol reaction of 2 equiv of the para-substituted benzaldehydes **10** and **11**[21] with glycinamide (Scheme 5). In these aldol reactions the Schiff bases of glycinamide are formed initially; these deprotonate at the α-position under the basic conditions and react with a second equivalent of aldehyde. Under the conditions chosen the aldol product is poorly soluble, resulting in the initial (kinetically controlled) crystallization of a mixture of *threo* and *erythro* isomers in a ratio of 1.5:1. However, since in the aldol reaction the Schiff base is in equilibrium with the starting materials, the lower solubility of the *threo* isomer compared to the *erythro* isomer results in a second-order diastereomeric transformation of the *erythro* isomer into the *threo* isomer. After 18 h of reaction the *threo*:*erythro* ratio is ≥95:5. Subsequent acidic hydrolysis of the Schiff bases gave **12** and **13** in 92% and 74% yields, respectively, in the same diastereomeric ratio as the corresponding Schiff bases. A pH of 12−13 in these reactions is critical; lower pH did not result in aldol reaction, and more basic conditions gave decomposition of the Schiff bases. This decomposition was also seen in our initial attempts to transfer the already available methyl ester **7** into amide **12**; amidation in concentrated ammonia resulted in decomposition of **7**, as is known from the literature for identical compounds.[18] Under strongly basic conditions, **7** and other 3-phenylserine derivatives dehydrated to the enamine, which subsequently hydrolyzed to methyl 3-[4-(methylthio)phenyl]-2-oxopropionate.

Table 1

enzyme	conversion %	% ee (2*S*,3*R*)-**4**	% ee (2*R*,3*S*)-**7**	*E*
alcalase (NOVO)	51	82	85	27
lipase PS (Amano)	12	91	12	24
porcine pancreas lipase (PPL, Sigma)	24	94	29	43
lipase hog pancreas (HPL, Fluka)	42	92	67	48

The equilibration of the *threo* and *erythro* isomers of the Schiff bases of **12** and **13** proceeds via a retro-aldol reaction (Scheme 5). This was proven by the racemization of the enantiopure *threo* amide **12** (vide supra) and by reaction of the racemic *threo*-**12** under the reaction conditions in the presence of benzaldehyde. In the latter case a mixture of *p*-(methylthio)-substituted and unsubstituted 3-phenylserinamides was formed as a *threo/erythro* mixture after acidic workup.

For the enzyme-catalyzed resolution of the *threo*-ester **7** (Scheme 6) eight different lipases and one esterase were tested. The most selective lipases for this resolution were porcine pancreas lipase (PPL from Sigma and HPL from Fluka), alcalase, and lipase PS. The best enantioselectivity for HPL was found to be $E = 48$.[19] All of the lipases tested hydrolyzed the (2*S*,3*R*) enantiomer of ester **7**. Identical results were obtained by Clark *et al.*[9] for the hydrolysis of the corresponding ethyl ester: with immobilized protease from *Streptomyces griseus* (2*S*,3*R*)-**4** was obtained with 95% ee. With a best *E*-value of 48 the maximal reachable ee for (2*S*,3*R*)-**4** will be 96%. In practice, to obtain an economically significant yield, this ee will be considerably lower (see Table 1). On the other hand, the remaining (2*R*,3*S*)-ester **7** can be obtained in high ee at conversions higher than 50%. Since the (2*S*,3*R*) enantiomer is more conveniently transformed into thiamphenicol, the ee's obtained for acid **4** are

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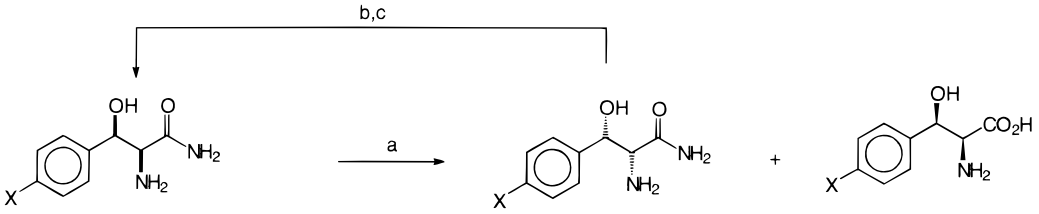
Scheme 7[a]



12 (X = SCH_3)
13 (X = SO_2CH_3)

(*2R, 3S*)-**12**
(*2R, 3S*)-**13**

(*2S, 3R*)-**4**
(*2S, 3R*)-**14**

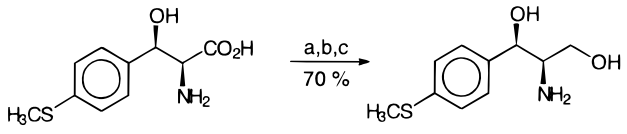
[a] (a) *O. anthropi*, pH 5.6−6.0, 40 °C; (b) 0.5−0.6 equiv of **10** or **11**; (c) MeOH/H_2O, pH 12.5.

too low to be of practical use. Furthermore a reverse enantioselectivity in this kinetic resolution process would be much more advantageous; the remaining ester **7** then could be obtained in high ee while the enantiomerically enriched acid **4** could be racemized.[5b] Because further processing to thiamphenicol proceeds via the ester, additional steps would then not be required. However, none of the tested lipases showed this reverse stereoselectivity. Also enzymatic esterification of racemic **4** in methanol or mixtures of methanol/toluene was not detected. For this reason the lipase route was abandoned.

As mentioned in the Introduction, the use of amidases offers an efficient route for the resolution of amino acid amides. Amide **12** was tested with *P. putida* ATCC 12633[12] and *O. anthropi* NCIMB 40321, which both show amidase activity for amino acid amides. In addition, a screening of other amidases was performed (see Experimental Section). From this screening we concluded that the amidase from *O. anthropi* NCIMB 40321, which was already in our possesion, is the best choice for the resolution.[14] The hydrolysis with *P. putida* is very slow at its pH optimum of 8.5−9. This is mainly due to the insolubility of the substrate at this pH and results only in some decomposition after long reaction times. In contrast, the hydrolysis with *O. anthropi* can be performed at lower pH values. Although the pH optimum for the amidase from this microorganism is pH 8−8.5, it has a broad pH range of activity,[13] so it is possible to perform the resolution at pH < 7. At this pH the solubility of the amide **12** is sufficiently high for reaction (Scheme 7). For both amides **12** and **13** *O. anthropi* is fully stereoselective (E > 200), so the acids **4** and **14** were obtained in ee's higher than 99% at approximately 50% conversion. The remaining amides, which also can be obtained in ee's up to 99% (depending on the conversion), were easily separated from the acids and subsequently racemized (vide infra): addition of the corresponding para-substituted benzaldehyde **10** or **11** to the enzymatic reaction mixture resulted in precipitation of the Schiff bases of the (2*R*,3*S*) amides **12** and **13**. After filtration, the Schiff bases of (2*R*,3*S*)-**12** and -**13** were obtained in 42% and 55% yields, respectively. Further workup of the filtrates gave the enantiopure (2*S*,3*R*) acids **4** and **14** in 48% and 35% direct yields, respectively.

For the racemization of the Schiff bases of the amides **12** and **13** we looked for conditions identical to those for the synthesis of these amides (see Scheme 5). The racemization of the (2*R*,3*S*) enantiomers of the amides **12** and **13** into the *threo*-amides and the preparation of these amides were both performed in MeOH/H_2O mixtures at pH 12−13. Since these conditions are identical, both steps can be combined in one, making the process economically more attractive. Hence approximately 80−90% of the Schiff base isolated after the enzymatic resolution could be recycled. This resulted in overall yields for the enantiopure (2*S*,3*R*) acids **4** and **14** of 78% and 62%, respectively, over the combined synthesis/racemization step and the resolution step.

Scheme 8[a]



(*2S, 3R*)-**4** (*1R,2R*)-**3**

[a] (a) $NaBH_4/H_2SO_4$; (b) PhCHO; (c) 4 N HCl.

The enzymatic process to enantiopure **4** and **14** as described above can be scaled up without considerable problems for application in multipurpose equipment. For this purpose purification and immobilization of the amidase from *O. anthropi* is not necessary. Crude cell mass of *O. anthropi* can be readily obtained by fermentation and used without any further purification.

The enantiopure amino acid (2*S*,3*R*)-**4** was reduced with $NaBH_4/H_2SO_4$ directly to the known amino diol (1*R*,2*R*)-**3** without prior formation of the ester.[14b, 20] Purification of (1*R*,2*R*)-**3** from the salts formed after hydrolysis was somewhat cumbersome due to its high water solubility. Purification of (1*R*,2*R*)-**3** was accomplished by formation of the benzaldehyde Schiff base (accompanied by formation of some oxazolidines) followed by extraction and acidic workup. No efforts at optimization and scale-up of this reaction were made (Scheme 8). The enantiopure (1*R*,2*R*) amino diol **3**, obtained in 73% yield from **4**, can be transferred into thiamphenicol and florfenicol by methods described in the literature.[3−10]

Environmental Health and Safety Aspects

Caution should be taken in adding H_2SO_4 to a suspension of $NaBH_4$ in THF during scale-up. Considerable amounts of hydrogen gas and heat are released in this reaction step.

(20) Abiko, A.; Masamune, S. *Tetrahedron Lett.* **1992**, *33*, 5517.
(21) 4-(Methylsulfonyl)benzaldehyde (**11**) was prepared in 75% yield from the corresponding sulfide (**10**) by oxidation with *m*-CPBA after protection of the aldehyde and followed by hydrolysis of the protecting group.

On large scale, precautions should be taken to prevent too fast addition of a large amount of H_2SO_4 to the reaction mixture.

Care should be taken in working with enzymes like lipases; especially in dry form, inhalation should be prevented. No health problems were observed for suspensions of *O. anthropi* NCIMB 40321: this strain showed no health effects in animal tests (mice) after oral injection of *O. anthropi* suspensions. However, the usual care should be taken in working with microorganisms.

Conclusions

Several methods were investigated to prepare enantiopure intermediates for thiamphenicol or florfenicol. The direct sulfonylation of protected 2-amino-1-phenylpropane-1,3-diol was unsuccessful. Asymmetric hydrogenation of a 3-oxo-phenylalanine derivative (**8**) did yield the corresponding *threo-N*-(dichloroacetyl)-3-[4-(methylthio)phenyl]serine methyl ester (**9**), but at high conversion the enantiomeric excess was disappointingly low.

The lipase-catalyzed resolution of the methyl ester **7** resulted in (2*S*,3*R*)-3-[4-(methylthio)phenyl]serine (**4**). The best ee's obtained were 94% at only 24% conversion (with PPL from Sigma) or 92% at 42% coversion (with HPL from Fluka). These results will not lead to a viable process since the ee's of (2*S*,3*R*)-**4** are not sufficiently high at workable conversions and additional process steps are needed.

The 4-methylthio- and 4-methylsulfonyl-substituted (2*S*,3*R*)-3-phenylserines **4** and **14** were obtained in excellent enantiomeric excesses (>99%) by enzymatic resolution of the corresponding *threo*-3-phenylserinamides **12** and **13** with the amidase from *O. anthropi* NCIMB 40321. These amides were prepared from 4-(methylthio)- and 4-(methylsulfonyl)-benzaldehyde and glycinamide by an aldol reaction under crystallization conditions (second-order diastereomeric transformation). In this way high *threo:erythro* ratios (>95:5) were obtained. Also the undesired enantiomers of the amides remaining after the enzymatic resolution, isolated as their Schiff bases, were racemized under the same conditions. Altogether this has resulted in a high overall conversion of the amides into the enantiopure (2*S*,3*R*)-3-phenylserines **4** and **14**, of which **4** was directly reduced to the amino diol (1*R*,2*R*)-**3** with $NaBH_4/H_2SO_4$. These compounds can be tranformed into thiamphenicol and florfenicol following known production procedures.

We are currently working on the heterologous overexpression of the gene which is coding for the amidase from *O. anthropi*. Up to now a significant increase of the activity has been reached. These results will be published in more detail in the near future.

Experimental Section

General Remarks. Commercially available solvents and reagents were used without further purification. All reactions were performed under nitrogen. 4-(Methylthio)benzaldehyde (**10**) was a kind gift of Dr. C. Giordano, Zambon Group, Italy. Racemic *threo*-3-[4-(methylthio)phenyl]serine (**4**) and its methyl ester (**7**) were prepared according to literature procedures.[3,9] Infrared spectra were obtained using a Perkin-Elmer 1600 series FT-IR. ^{1}H NMR spectra were determined in $CDCl_3$ (unless otherwise specified) using a Bruker ACF 200 (200 MHz) spectrometer. ^{13}C NMR (50.31 MHz) spectra were determined on the same apparatus in $CDCl_3$ (unless otherwise indicated). Optical rotations were measured on a Perkin-Elmer 241 spectrophotometer. Thin-layer chromatography (TLC) was performed on silica gel coated glass plates (Merck silica gel 60 F_{254}). Column chromatography refers to flash chromatography using Merck silica gel 60 (230−400 mesh). Melting points are uncorrected. Unless otherwise indicated, the enantiomeric excesses (ee's) of amino acid derivatives were determined by HPLC analysis (column, Daicel Crownpak CR(+); eluent, $HClO_4$ solution (pH 1.3); flow, 0.6 mL/min; temperature, 0 °C; detection, UV 210 nm).

***threo-N*-(Dichloroacetyl)-3-[4-(methylthio)phenyl]-serine Methyl Ester (*rac*-9).** Ethyl dichloroacetate (6.84 g, 44 mmol) was added to a solution of **7** (3.5 g, 14.5 mmol) in 50 mL of MeOH. The solution was stirred for 3 days at rt and was concentrated in vacuo. The remaining semisolid material was recrystallized from $CHCl_3$, yielding 3.55 g (10.1 mmol, 70%) of *rac*-**9** as colorless needles. The crystals contained 30 mol % of $CHCl_3$, which could not be removed after prolonged drying (60 °C/1.0 Torr). Mp: 134−135 °C. ^{1}H NMR (DMSO-d_6): δ 2.44 (s, 3H), 3.69 (s, 3H), 4.54 (dd, 1H, α-CH), 4.16 (t, 1H, β-CH), 6.13 (d, 1H, OH), 6.64 (s, 1H), 7.18 (d, 2H), 7.32 (d, 2H), and 8.90 (d, 1H, NH), + 8.22 (s, 0.30H, $CHCl_3$). ^{13}C NMR (DMSO-d_6): δ 14.37 (q), 52.06 (q), 58.38 (d), 65.52 (d), 71.06 (d), 125.13 (d), 126.48 (d), 136.69 (s), 137.31 (s), 163.52 (s), and 169.41 (s), + 78.76 (d, $CHCl_3$). Anal. Calcd for $C_{13}H_{15}NO_4SCl_2 \cdot 0.3CHCl_3$: C, 41.2; H, 4.0; N, 3.6; S, 8.3; Cl, 26.3. Found: C, 41.4; H, 4.0; N, 3.6; S, 8.2; Cl, 26.3.

***N*-(Dichloroacetyl)-3-oxo-4′-(methylthio)phenylalanine Methyl Ester (8).** A solution of 3.13 g (40 mmol) of DMSO in 15 mL of CH_2Cl_2 was added dropwise in 5 min to a solution of oxalyl chloride (2.79 g, 22 mmol) in 60 mL of CH_2Cl_2 at −50 °C. The solution was stirred for 10 min at −50 to −60 °C. To the solution was then added 7.04 g of *rac*-**9** dissolved in 35 mL of CH_2Cl_2 and 2 mL of DMSO in 10 min. An additional 20 mL of CH_2Cl_2 and 2 mL of DMSO were added, and the solution was stirred for 30 min at −50 °C. Triethylamine (10.1 g, 100 mmol) was then added, the mixture was left to warm to rt, 50 mL of water was added, and the mixture was stirred for 30 min. The aqueous layer was separated, and the organic layer was washed with water and 1 N HCl solution. After drying (Na_2SO_4) and concentration in vacuo, 6.75 g of yellow oil was obtained, which was purified by chromatography (eluent: $CHCl_3$). The product was recrystallized from toluene, resulting in 3.68 g (10.5 mmol, 53%) of **8** as slightly yellow crystals. Mp: 114.5−136.0 °C. R_f: 0.31 ($CHCl_3$). ^{1}H NMR: δ 1.80 (s, 0.14H, OH enol), 2.53 (s, 3H), 3.74 (s, 3H), 6.05 (s, 1H, $CHCl_2$), 6.09 (d, 0.86H, CH keto), 7.30 (d + br s, 2.14H, ArH + NH enol), 7.95 (d, 0.8H, NH keto), and 8.03 (d, 2H). ^{13}C NMR: δ 14.55 (q), 53.57 (q), 58.36 (d), 65.81 (d), 124.89 (d), 129.55 (s), 130.02 (d), 149.03 (s),

163.87 (s), 166.05 (s), and 188.54 (s). Anal. Calcd for $C_{13}H_{13}NO_4SCl_2$: C, 44.6; H, 3.7; N, 4.0; S, 9.2. Found: C, 45.2; H, 3.8; N, 4.0; S, 9.4.

(2*S*,3*R*)-*N*-(Dichloroacetyl)-3-[4-(methylthio)phenyl]-serine Methyl Ester (9). A solution of 1.05 g (3.0 mmol) of **8** and 42 mg (1 mol %) of Ru_2Cl_4·[(*R*)-BINAP]$_2$·N$(C_2H_5)_3$ in 20 mL of CH_2Cl_2 was stirred under 1000 psi of H_2 pressure at 50 °C in an autoclave for 66 h. The reaction mixture was concentrated in vacuo and purified by chromatography (eluent, $CHCl_3$/1% MeOH). Yield: 30 mg (85 μmol, 3%) of **9** as a brownish oil. R_f: 0.22 ($CHCl_3$/1% MeOH). ^{1}H NMR: see *rac*-**9**. Diastereomeric ratio *threo*:*erythro*: ≥95:5. Ee_{threo}: 85% (by HPLC; column, Chiracel OD; eluent, hexane/*i*-PrOH, 90:10; flow, 1.0 mL/min; temperature, 20 °C; detection, UV 210 nm). MS(CI): 334/336 ($M^+ + 1$, $-H_2O$). A 700 mg amount of starting material **8** was recovered.

When the reaction was repeated with freshly prepared Ru_2Cl_4·[(*R*)-BINAP]$_2$·N$(C_2H_5)_3$, **9** was obtained in 67% yield and ee_{threo} 15%.

4-(Methylsulfonyl)benzaldehyde (11). To a solution of **10** (50 g, 0.33 mol) and $HC(OCH_3)_3$ in 700 mL of MeOH was added *p*-TsOH (0.5 g, 3 mmol). After 1.5 h of stirring at rt, KO-*t*-Bu (0.40 g, 3 mmol) was added. The mixture was concentrated in vacuo to give the dimethyl acetal in quantitative yield as a viscous colorless oil. ^{1}H NMR: δ 2.45 (s, 3H), 3.30 (s, 6H), 5.35 (s, 1H), 7.25 (d, 2H), and 7.35 (d, 2H).

To an ice-cooled solution of the acetal (65 g, 0.32 mol) in 600 mL of CH_2Cl_2 was added dropwise over 30 min a solution of *m*-CPBA in CH_2Cl_2 (prepared by dissolving 235 g of 50% *m*-CPBA in 1200 mL of CH_2CL_2, extraction with 300 mL of water, and drying over Na_2SO_4) in such a way that the solvent did not reflux. The reaction mixture was stirred for 16 h. The mixture was filtered and washed with $CHCl_3$. The filtrate was washed with 1 N NaOH solution, 0.25 M NaI solution, 1 N NaOH solution, 0.25 M $Na_2S_2O_3$ solution, and water. After drying (Na_2SO_4) and concentration in vacuo, the sulfone acetal (59.8 g, 0.26 mol, 81%) was obtained as an oil, which crystallized on standing. ^{1}H NMR: δ 3.10 (s, 3H), 3.35 (s, 6H), 5.52 (s, 1H), 7.65 (d, 2H), and 7.95 (d, 2H).

To a solution of the sulfone acetal (59.7 g, 0.26 mol) in THF (600 mL) was added 600 mL of a 4% H_2SO_4 solution in 45 min. The mixture was stirred for 16 h at rt, 300 mL of saturated $NaHCO_3$ was added, and the product was filtered off. Yield of **11**: 44.6 g (0.24 mol, 93%) as a white crystalline solid. Mp: 158−159 °C. ^{1}H NMR: δ 3.21 (s, 3H), 7.99 (d, 2H), 8.18 (d, 2H), and 10.15 (s, 1H). (Overall yield: 75%.)

***threo*-3-[4-(Methylthio)phenyl]serinamide Hydrochloride Salt (12).** A solution of 221 g (2 mol) of glycinamide hydrochloride salt in 1500 mL of water was brought to pH 12.5 with 570 mL of 4 N NaOH solution. To this solution at 25 °C was added dropwise a solution of 608 g (4 mol) of **10** in 1500 mL of MeOH at such a speed that the temperature rose to 35−40 °C. To the suspension formed was added an additional 100 mL of MeOH. After 2−3 h a thick suspension was formed, which was stirred for 18 h at rt. Then 600 mL of 4 N HCl solution was added in portions. After 4 h of stirring, all solids were dissolved and the solution was extracted twice with toluene (2 and 1 L, respectively). After evaporation of the toluene layers, 400 g (66%) of **10** was recovered. The aqueous phase was concentrated to a thick suspension of 500 mL. The solid product was filtered, washed with acetone, and dried. Yield: 330 g of **12** (63% yield, 92% on used aldehyde). *Threo*:*erythro*: 97:3. Mp: >250 °C. ^{1}H NMR (D_2O) δ 2.47 (s, 3H), 4.19 (d, 1H), 5.10 (d, 1H), and 7.35 (2d, 4H) [*erythro* isomer 4.31 (d) and 5.20 (d)]. In DMSO-d_6 the amide protons are observed at δ 7.43 and 7.96. ^{13}C NMR (DMSO-d_6): δ 14.54 (q), 58.20 (d), 71.10 (d), 125.41 (d), 127.36 (d), 136.56 (s), 137.51 (s), and 167.83 (s). IR (cm^{-1}): 1699, 1486, 1039, 813, 540. HRMS: calcd for $C_{10}H_{15}N_2O_2SCl$ (M^+ − HCl) 226.0776, found: 226.0780. Anal. Calcd for $C_{10}H_{15}N_2O_2SCl$: C, 45.7; H, 5.8; N, 10.7. Found: C, 45.4; H, 5.8; N, 10.6.

***threo*-3-[4-(Methylsulfonyl)phenyl]serinamide Hydrochloride Salt (13).** A solution of 4.4 g (40 mmol) of glycinamide hydrochloride salt in 5 mL of water was made alkaline (pH 12.5) with 12 mL of 4 N NaOH solution. To this solution was added 15 mL of MeOH, followed by 14.75 g (80 mmol) of **11**. An additional 20 mL of MeOH was added to this reaction mixture. The heterogeneous mixture was stirred for 40 h at rt. The thick suspension was acidified with 15 mL of 4 N HCl solution and stirred for 1 h. The remaining solid was filtered, resuspended in 100 mL of water, stirred again for 1 h, and filtered off (7.5 g of impure **11**). The combined filtrates were washed with $CHCl_3$ and concentrated to a thick suspension. The solid was filtered, washed with water and MeOH, and dried. Yield: 8.7 g of **13** (29.5 mmol, 74%). Mp: 223−225 °C dec. *Threo*:*erythro*: >95:5. ^{1}H NMR (D_2O): δ 3.27 (s, 3H), 4.26 (d, 1H), 5.31 (d, 1H), 7.75 (d, 2H), and 8.03 (d, 2H), [*erythro* signals at 4.39 (d) and 5.48 (d)]. In DMSO-d_6 the amide signals are observed at δ 7.52 and 8.03. ^{13}C NMR (D_2O): δ 43.88 (q), 59.08 (d), 71.52 (d), 128.23 (d), 128.29 (d), 139.68 (s), 145.03 (s), and 169.59 (s). IR (cm^{-1}): 1702, 1282, 1145, 544. HRMS: calcd for $C_{10}H_{15}N_2O_4SCl$ 241.0647 (M^+, −HCl, $-H_2O$), found 241.0666. Anal. Calcd for $C_{10}H_{15}N_2O_4SCl$: C, 40.7; H, 5.1; N, 9.5. Found: C, 40.3; H, 5.1; N, 8.9.

The identical compound was made by oxidation of *threo*-3-[4-(methylthio)phenyl]serinamide hydrochloride salt (**12**): 570 mg of **12** was suspended in 10 mL of AcOH. To this was added 12 mL of peracetic acid (made by mixing 3 parts of AcOH with 1 part of 30% H_2O_2). After 2 h reaction time excess peroxides were decomposed with 10% Pd/C by stirring for 2 h at 40 °C. The Pd/C was filtered off, and the solution was concentrated. The residue was washed with MeOH and dried. Yield: 400 mg (62%) of a white solid, identical to the material described above.

Lipase-Catalysed Resolution of 7

To a solution of 100 mg of **7** in 3 mL of 50 mM KH_2PO_4/K_2HPO_4 buffer (pH 7.5) was added 10 mg of lipase (20 μL in the case of alcalase). The mixtures were stirred for 3

Table 2

microorganism	% ee (2*R*,3*S*)-**12**	% ee (2*S*,3*R*)-**4**
O. anthropi NCIMB 40321	99	>99
Achromobacter group VD	91	>99
Klebsiella oxytoca NCIMB 40322	46	>99
Pseudomonas carophylli NRRL-B 11257	26	>95
Klebsiella sp.	19	>99
P. putida	6	84

h at 30 °C, acidified with 1 N HCl, and analyzed with chiral HPLC. Eight different lipases and one esterase were tested. The best results obtained are found in Table 1. Addition of an organic solvent had a negative effect on the enzyme activity.

Enzymatic Screening of Amidases for the Hydrolysis of Amide 12. For this screening different enzyme samples were tested (microorganisms as well as commercially available enzymes). In part microorganisms from soil and sewage samples were obtained by enrichment on YCB medium with racemic **4** as the sole nitrogen source. Microorganisms growing on **4** were isolated and determined via the API test. These microorganisms were also tested for their stereoselectivity. Therefore 0.5 mL of a 1% solution of **4** in 50 mM phosphate buffer (pH 6.6) was incubated with 0.1 mL of cell suspension during 3 h at 28 °C. After acidification with 1 N HCl solution the ee's of the amide and the acid were determined by HPLC (see Table 2).

Enzymatic Resolution of *threo*-3-[4-(methylthio)phenyl]serinamide (12). To a solution of 15.3 g (58 mmol) of **12**·HCl in 150 mL of water at pH 5.5 (brought to pH with 2 mL of 4 N NaOH solution) was added 7.7 g of amidase suspension from *O. anthropi* NCIMB 40321 (±10% dry weight). The solution was stirred in an orbital shaker at 200 rpm for 18 h at 37 °C. The conversion during the reaction was determined with an ammonia-sensitive electrode. At a conversion of 47% the reaction was stopped by addition of 3 mL of 4 N HCl solution, and the mixture was centrifuged at 40 °C. The pellet was resuspended in 150 mL of water and centrifuged again. The combined supernatants were neutralized to pH 7, and 4.6 g (30 mmol) of aldehyde **10** was added. The mixture was stirred for 15 h at rt, followed by filtration to obtain the white solid: 8.8 g (24.4 mmol, 42%) of (2*R*,3*S*)-*N*-[4-(methylthio)benzylidene]-3-[4-(methylthio)phenyl]serinamide. ^{1}NMR: δ 2.44 (s, 3H), 2.52 (s, 3H), 4.05 (d, 1H), 4.16 (br d, 1H), 5.22 (br t, 1H), 6.05 (br d, 1H), 6.75 (br d, 1H), 7.16 (2d, 4H), 7.23 (d, 2H), 7.59 (d, 2H), and 7.83 (s, 1H). Ee 94% (HPLC after hydrolysis with dilute HCl). The filtrate was concentrated in vacuo to 30 mL, and after cooling the white solid was filtered off: 6.2 g (27.3 mmol, 48%) of (2*S*,3*R*)-**4**. Mp: >200 °C dec. ^{1}NMR (D_2O/DCl): δ 2.44 (s, 3H), 4.22 (d, J = 4.0 Hz, 1H), 5.34 (d, J = 4.0 Hz, 1H), 7.30, and 7.35 (2d, 4H) [*erythro* signals at 4.31 (d) and 5.34 (d)]. ^{13}C NMR (D_2O/DCl): δ 15.09 (q), 59.48 (d), 70.74 (d), 127.01 (d), 127.19 (d), 135.16 (s), 138.93 (s), and 170.29(s). Ee: >99.7% (HPLC). $[\alpha]^{20}_D$: −45.4 (*c* 1, 1 N HCl).

The (2*R*,3*S*) Schiff base [containing 5% of (2*S*,3*R*)-**4**] was transformed into (2*R*,3*S*)-**12**·HCl: Thus 8.5 g (22 mmol) of Schiff base was suspended in 200 mL of $CHCl_3$/200 mL of 1 N HCl solution and stirred for 20 h at rt. The aqueous layer was concentrated in vacuo. Yield: 5.4 g (20.5 mmol, 93%) of (2*R*,3*S*)-**12**·HCl as a yellow solid. Ee: 93% (HPLC). A small part was recrystallized from *i*-PrOH/MeOH, yielding (2*R*,3*S*)-**12**·HCl as white crystals. Mp: >250 °C. Ee: 99.3% (HPLC). $[\alpha]^{20}_D$: −9.3 (*c* 1, 1 N HCl). For spectroscopic data, see racemic **12**.

Enzymatic Resolution of *threo*-3-[4-(Methylsulfonyl)-phenyl]serinamide (13). To a solution of 7.5 g (25.5 mmol) of *threo*-amide **13**·HCl in 75 mL of water at pH 6.0 (1 N NaOH solution) was added 3.0 g of amidase suspension from *O. anthropi* NCIMB 40321 (±10% dry weight). The solution was stirred in an orbital shaker at 200 rpm for 22 h at 40 °C. At 46% conversion the reaction mixture was neutralized to pH 7 with 4 N NaOH solution, and the mixture was centrifuged at 40 °C. The pellet was resuspended twice with 15 mL of water and centrifuged again. To the combined supernatants was added 2.4 g (13 mmol) of aldehyde **11**, and the mixture was stirred for 5 h at rt. The solid material was filtered off: 5.6 g (±14 mmol, 55%) of (2*R*,3*S*)-*N*-[4-(methylsulfonyl)benzylidene]-3-[4-(methylsulfonyl)phenyl]-serinamide. ^{1}H NMR (DMSO-d_6): δ 3.17 (s, 3H), 3.21 (s, 3H), 3.88 (d, 1H), 5.10 (t, 1H), 5.72 (d, 1H), 7.18 (br d, 1H), 7.29 (d, 2H), 7.57 (d, 2H), 7.70 (d, 2H), 7.83 (d, 2H), 7.94 (br d, 1H), and 8.01 (s, 1H). A small fraction of the Schiff base was hydrolyzed with dilute HCl solution and analyzed with NMR and chiral HPLC: ee 83%.

The filtrate was concentrated in vacuo to 30 mL and cooled to rt. The white solid was filtered off and dried, yielding 2.3 g (8.9 mmol, 35%) of (2*S*,3*R*)-**14**. Mp: 225−227 °C dec. ^{1}H NMR (1 N DCl) δ 3.29 (s, 3H), 4.45 (d, 1H), 5.62 (d, 1H), 7.80 (d, 2H), and 8.03 (d, 2H) [*erythro* signals at 4.55 (d) and 5.55 (d)]. ^{13}C NMR (1 N DCl): δ 43.73 (q), 59.15 (d), 70.32 (d), 127.79 (d), 128.25 (d), 139.41 (s), 145.41 (s), 170.02 (s). Ee: 99% (HPLC). $[\alpha]^{20}_D$: −5.6 (*c* 1, 1 N HCl).

Racemization of (2*R*,3*S*)-3-[4-(methylthio)phenyl]serinamide [(2*R*,3*S*)-12]. To a solution of 1.31 g (5.0 mmol) of (2*R*,3*S*)-**12**·HCl (ee 99.3%) in 12 mL of 1:1 MeOH/water was added 770 mg (5.05 mmol) of aldehyde **10**. The mixture was brought to pH 12.5 by addition of 4 N NaOH solution. The suspension was stirred for 24 h at 20 °C, acidified to pH 2 with 4 N HCl solution, and stirred for 1 h. The solution was washed with toluene (2 × 10 mL) and concentrated in vacuo to yield 1.15 g (4.4 mmol, 88%) of **12** as a cream-coloured solid. *Threo*:*erythro*: 97:3. Ee: 9% (2*R*,3*S*) enantiomer.

Racemization of (2*R*,3*S*)-3-[4-(methylsulfonyl)phenyl]-serinamide [(2*R*,3*S*)-13]. A suspension of 5.1 g (12 mmol) of (2*R*,3*S*)-*N*-[4-(methylsulfonyl)benzylidene]-3-[4-(methylsulfonyl)phenyl]serinamide (ee 83%) was racemized under identical conditions and workup as described for (2*R*,3*S*)-**12**. Yield: 2.6 g (8.8 mmol, 74%) of **13**·HCl as a yellowish solid. For spectroscopic data, see above. *Threo*:*erythro*: >98:2. Ee: <5%.

Combined Synthesis and Racemization of *threo*-3-[4-(Methylthio)phenyl]serinamide (12). A suspension of 5.0 g (13.9 mmol) of (2*R*,3*S*)-*N*-[4-(methylthio)benzylidene]-3-[4-(methylthio)phenyl]serinamide (ee 94%) and 3.07 g (27.8 mmol) of glycinamide hydrochloride in 30 mL of water was brought to pH 12.9 with 4 N NaOH solution. A solution of 8.1 g (53 mmol) of aldehyde **10** in 20 mL of MeOH was added, and the mixture was stirred for 20 h at rt. The thick suspension was worked up by the same procedure as mentioned above for the synthesis of **12**. Yield: 6.85 g (26.1 mmol, 65%) of **12**·HCl as a white solid (93% yield based on recovered aldehyde **10**). Ee: < 5%.

(1*R*,2*R*)-2-Amino-1-[4-(methylthio)phenyl]propane-1,3-diol [(1*R*,2*R*)-3]. To a mixture of 2.27 g (10 mmol) of (2*S*,3*R*)-**4** in 15 mL of dry THF was added 1.0 g (26 mmol) of $NaBH_4$ followed by 6 mL of THF. The thick suspension was cooled in ice, and in 20 min a solution of 1.23 g (12.5 mmol) of H_2SO_4 in 3 mL of diethyl ether was added dropwise. After 3 h of stirring at rt, the solution was carefully acidified with 4 N HCl solution and washed with $CHCl_3$. The aqueous layer was brought to pH 10 with 4 N NaOH solution, and 1.05 g (10 mmol) of benzaldehyde was added. The mixture was stirred for 2 h at rt and extracted with $CHCl_3$ (3 × 25 mL). The $CHCl_3$ solution contained a mixture of Schiff base and the two oxazolidines as a diastereomeric mixture, as was determined by ^{1}H NMR. The $CHCl_3$ solution was stirred for 2 h at 60 °C with 30 mL of 4 N HCl solution (repeated twice). The aqueous layers were concentrated in vacuo to yield 1.80 g (7.3 mmol, 73%) of (1*R*,2*R*)-**3**·HCl salt as a yellow solid. Mp: 230 °C dec. ^{1}H NMR (D_2O): δ 2.41 (s, 3H), 3.35−3.62 (m, 3H), 4.75 (d, 1H), 7.25 (d), and 7.31 (d, together 4H). ^{13}C NMR (D_2O): δ 14.80 (q), 58.23 (d), 58.75 (t), 70.77 (d), 126.84 (d), 127.71 (d), 136.09 (s), and 138.97 (s). $[\alpha]^{20}_D$: −27 (*c* 2, 1 N HCl). Optical purity: 95%.[6]

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