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Design, Synthesis and Evaluation of Transition-state Analogue Inhibitors of *Escherichia coli* γ-Glutamylcysteine Synthetase

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Abstract—Phosphinic acid-, sulfoximine- and sulfone-based transition-state analogues were synthesized and evaluated as inhibitors of *Escherichia coli* γ-glutamylcysteine synthetase. These compounds have a carboxyl function at the β-carbon to the tetrahedral central hetero atom so as to mimic the carboxyl group of the attacking cysteine in the transition state. The phosphinic acid- and the sulfoximine-based compounds were found to be potent ATP-dependent inactivators, both showing a slow-binding kinetics with overall affinities and second-order inactivation rates of one to two orders of magnitude greater than those of L-buthionine (*SR*)-sulfoximine (L-BSO). The sulfone was a simple reversible inhibitor without causing ATP-dependent enzyme inactivation, but its affinity toward the enzyme was still five times greater than that of L-BSO, indicating that the β-carboxyl function plays a key role in the recognition of the inhibitors by the enzyme. The sulfoximine with (*S*)-β-carbon to the sulfur was synthesized stereoselectively, and the two diastereomers with respect to the chiral sulfur atom were separated as a cyclic sulfoximine derivative. The sulfoximine with *R*-configuration around the sulfur served as an extremely powerful ATP-dependent inactivator with an overall inhibition constant of 39 nM and an inactivation rate of 6750 $M^{-1} s^{-1}$, which correspond to 1260-fold higher affinity and almost 1400-fold greater inactivation rate as compared with L-BSO. The sulfoximine with (*S*)-sulfur was a simple reversible inhibitor with an inhibition potency comparable to that of the sulfone. The synthesis and inhibition profile of the *N*-phosphoryl sulfoximine is also described.

Introduction

Glutathione (γ-L-glutamyl-L-cysteinylglycine) is synthesized in living organisms from its constituent amino acids by the consecutive actions of two mechanistically related ATP-dependent peptide ligases, γ-glutamylcysteine synthetase (γ-GCS, EC 6.3.2.2) and glutathione synthetase (EC 6.3.2.3).[1] These enzymes are of particular interest because glutathione plays a pivotal role in the cellular thiol redox system[1b] and constitutes a front line of the detoxification of reactive oxygens and other xenobiotics such as heavy metals and electrophilic alkylating agents.[2] In addition, these enzymes are typical members of the group of ADP-forming peptide ligases such as glutamine synthetase,[3] D-Ala: D-Ala ligase,[4] glutathionylspermidine synthetase,[5] trypanothione synthetase,[5c,6] and a series of amino acid adding enzymes in bacterial peptidoglycan biosynthesis.[7] Thus the design and synthesis of specific inhibitors of glutathione biosynthetic enzymes are of crucial importance not only for use as therapeutic agents, but also for use as mechanistic probes for the ADP-forming peptide ligases, in which an amide bond is formed with concomitant hydrolysis of ATP to ADP and P_i to supply the thermodynamic driving force.

We recently reported the synthesis and characterization of a phosphinic acid transition-state analogue **1** as a potent mechanism-based inactivator of *Escherichia coli* glutathione synthetase.[8] The success in the rational design of the inhibitor of glutathione synthetase has directed our attention to the development of transition-state analogue inhibitors of γ-GCS based on the same rationale. This enzyme, which catalyzes the ATP-dependent coupling of L-Glu and L-Cys to form

Key words: *Escherichia coli* γ-glutamylcysteine synthetase; transition-state analogues; phosphinic acid; sulfoximine; slow-binding inhibition.

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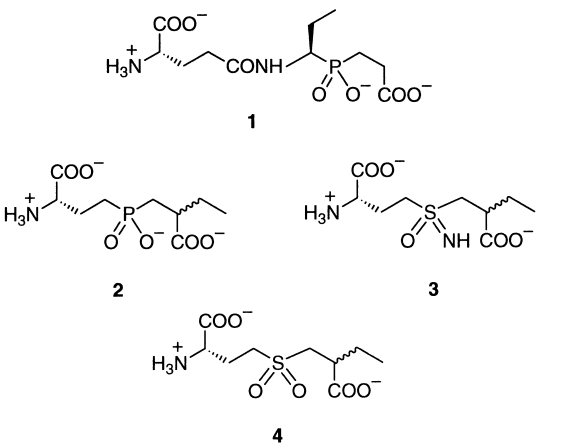
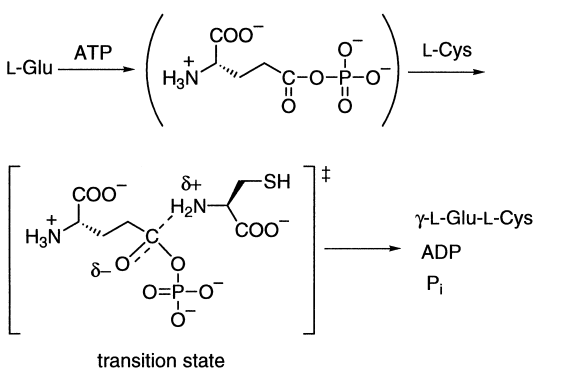
γ-L-Glu-L-Cys, is the rate-limiting enzyme in glutathione biosynthesis, and its inhibition causes glutathione depletion[2a]. Hence its inhibitor is highly important for biochemical and pharmacological studies.[9] Considering that the reaction catalyzed by γ-GCS is thought to proceed through the initial formation of γ-glutamylphosphate intermediate followed by the nucleophilic attack of L-Cys (Scheme 1), we reasoned that tetracoordinated phosphinic acid[8] and sulfoximine[10] moiety would well mimic the partially sp^3-hybridized carbonyl carbon in the transition state and that the carboxyl group of the incoming L-Cys as well as the mercaptomethyl side chain would be a major recognition element by the enzyme. We therefore designed the phosphinic acid-, sulfoximine- and sulfone-based transition-state analogues **2, 3**, and **4** with a carboxyl function at the β-carbon to the central hetero atom.[11]



In this paper, we describe the synthesis and evaluation of these transition-state analogues as inhibitors of *E. coli* γ-GCS and demonstrate that all the compounds showed very high affinity for the enzyme. In particular, the sulfoximine-based compound **3** was an extremely powerful slow-binding inactivator with an unprecedented affinity and inactivation rate. The most potent inhibitor sulfoximine **3** was elaborated further for stereoselective synthesis, and its inhibition behavior is discussed in terms of mechanism-based phosphorylation of the imino nitrogen at the chiral sulfur atom. The inhibition profile and potency of the chemically synthesized *N*-phosphoryl sulfoximine **21** is also described.



Scheme 1. Proposed reaction mechanisms of γ-GCS

Results and Discussion

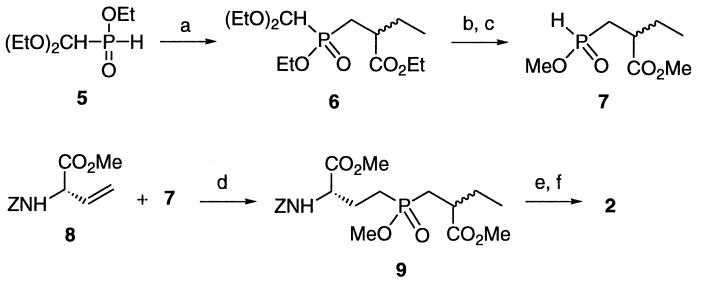
Synthesis of phosphinic acid 2, sulfoximine 3 and sulfone 4

The phosphinic acid **2** was synthesized as shown in Scheme 2. In order to achieve a stepwise construction of two different C-P bonds in the phosphinic acid **2**, we chose ethyl (diethoxymethyl)phosphinate (**5**)[12] as the starting material. The diethoxymethyl group attached to phosphorus serves as 'a masked hydrogen',[13] and after the first C-P bond is formed with the P-H function of **5**, acid hydrolysis unmasks the second P-H for further construction of the second C-P bond. Thus, the compound **5** was subjected to base-catalyzed conjugate addition to ethyl 2-ethylacrylate[14] to give the phosphinate **6**. Complete acid hydrolysis of **6** followed by methylation with diazomethane gave the hydrogen phosphinic acid dimethyl ester **7** as an unstable oil. The construction of the second C-P bond utilized the epimerization-free radical addition of a phosphinate to a vinylglycine derivative reported for the synthesis of enantiomerically pure phosphinothricin.[15] Thus, the hydrogen phosphinate **7** was allowed to react with *N*-(benzyloxycarbonyl)-L-vinylglycine methyl ester (**8**)[16] by using *tert*-butyl peroxybenzoate as a radical initiator to yield the fully protected phosphinate **9**. Alkaline hydrolysis followed by hydrogenolysis afforded the phosphinic acid **2** as a 1:1 mixture of diastereomers (^{31}P NMR).

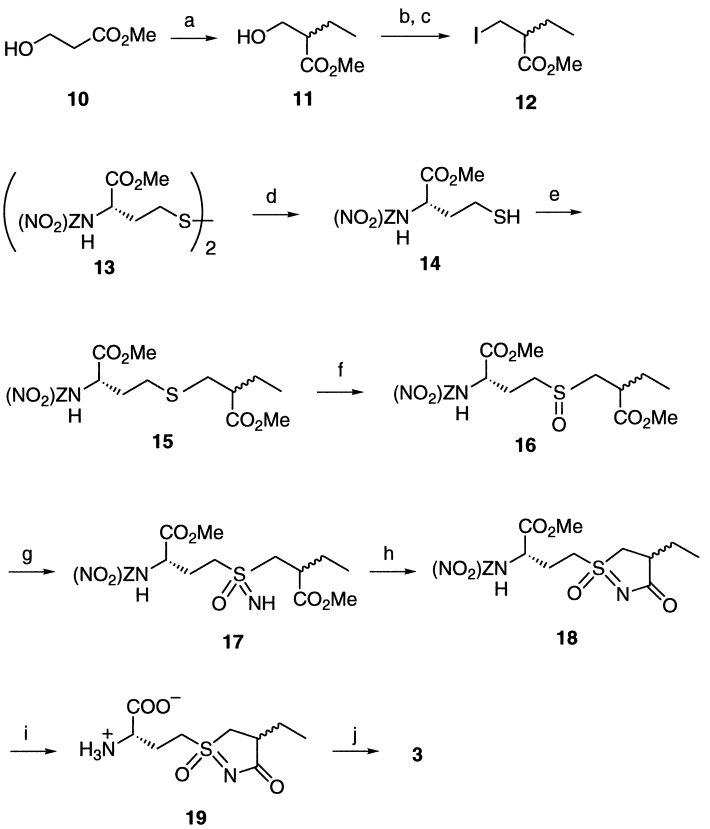
The sulfoximine **3** has two chiral carbons and one chiral sulfur atom. Starting with L-homocystine, we first synthesized a mixture of four diastereomers with respect to the chiral sulfur and the chiral carbon β to the sulfur atom. Scheme 3 outlines the synthesis of the sulfoximine **3**. According to the reported procedure[17] with slight modification, methyl 3-hydroxypropanoate (**10**)[18] was alkylated at the α-position and was converted to the corresponding iodide **12**. *N*-(4-Nitrobenzyloxycarbonyl)-L-homocystine methyl ester (**13**) was reduced by tri-*n*-butylphosphine in the presence of water. The reduction was best carried out in dichloromethane which had been washed to remove a trace of MeOH and saturated with water to give the homocysteine derivative **14** quantitatively. The thiol **14** and the iodide **12** were then coupled to afford the diastereomeric sulfide **15** in 77% yield.

Subsequent oxidation with $NaIO_4$ provided the sulfoxide **16** quantitatively. In this stage, an additional

chiral center was generated at the sulfur atom, but the four diastereomeric mixture of the sulfoxide **16** was treated as such with *O*-mesitylsulfonylhydroxylamine (MSH)[19] to introduce the imino nitrogen at the sulfur atom. A part (ca. 10%) of the product sulfoximine **17** was found to cyclize spontaneously during the reaction to yield the cyclic sulfoximine **18**. This side reaction, however, was successfully utilized to eliminate the need of direct alkaline hydrolysis of the β-methyl ester in **17**, which might risk the C-S bond cleavage by β-elimination.[20] Thus, the sulfoximine **17** was heated in acetic acid to complete the cyclization to yield **18** in 76% yield. In this stage, two sets of diastereomers of **18** could be separated in almost equal amounts by flash column chromatography (*vide*



Scheme 2. Reagents and conditions: (a) Ethyl 2-ethylacrylate, NaOEt, EtOH; (b) 12 M HCl, reflux; (c) CH_2N_2, Et_2O; (d) *tert*-Butyl peroxybenzoate (cat.), xylene; (e) NaOH, THF-H_2O; (f) H_2, 10% Pd on carbon, MeOH–AcOH–H_2O then Dowex 50W×8 (H^+ form), eluted with H_2O.



Scheme 3. Reagents and conditions: (a) LDA, iodoethane, DMPU-THF; (b) CH_3SO_2Cl, Net_3, CH_2Cl_2; (c) NaI, acetone; (d) Tri-*n*-butylphosphine, CH_2Cl_2, H_2O; (e) **12**, K_2CO_3, DMF; (f) $NaIO_4$, THF–H_2O; (g) *O*-Mesitylenesulfonylhydroxylamine (MSH), CH_3CN; (h) AcOH, 50 °C; (i) H_2, 10% Pd on carbon; (j) aq. KOH then Dowex 50W×8 (NH_4^+ form), eluted with H_2O.

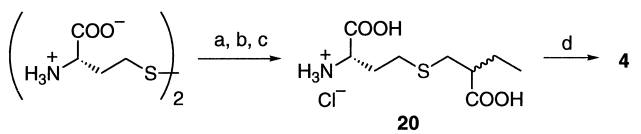
infra), but for the present purpose, all the diastereomers of **18** combined together were subjected to hydrogenolysis. Unexpectedly, the α-methyl ester was also cleaved during the hydrogenolysis to afford the cyclic sulfoximine **19** in one step. The ring opening of **19** by mild alkaline hydrolysis gave the sulfoximine **3** in quantitative yield. The sulfoximine **3** was prone to cyclize under weakly acidic conditions such as in acetic acid or on a silica gel TLC, and therefore the sulfoximine **3** was isolated as an ammonium form to prevent the spontaneous cyclization. The ammonium salt of **3** was stable and could be stored without any extent of cyclization.

For the synthesis of sulfone **4** (Scheme 4), L-homocystine was reduced by dithiothreitol, followed by base-catalyzed conjugate addition to ethyl 2-ethylacrylate[14] and subsequent hydrolysis in one flask to afford the sulfide **20** in 76% overall yield after chromatography on Dowex 50W (H^+ form). Oxidation with hydrogen peroxide and crystallization from AcOH gave the sulfone **4**. The diastereomeric ratio was 1:1 as judged by the ^1H NMR signal of the α-proton.

Enzyme inhibition

The phosphinic acid **2** and the sulfoximine **3** served as a potent slow-binding inhibitor of *E. coli* γ-GCS (Fig. 1a, b). In view of the mechanism-based phosphorylation of the phosphinic acid **1** by *E. coli* glutathione synthetase[8] and L-buthionine sulfoximine (L-BSO) by rat kidney γ-GCS,[21] the slow-binding inhibition of *E. coli* γ-GCS by **2** and **3** is most likely to reflect a similar inhibition scheme involving the enzyme-catalyzed phosphorylation of the phosphinyl oxygen or the sulfoximine nitrogen by ATP. In fact, both **2** and **3** required ATP for enzyme inactivation: 5′-adenylylimidodiphosphate (AMPPNP), a non-hydrolyzable ATP analogue, failed to cause the inactivation of the enzyme, while the combination of **2** or **3** and ATP resulted in enzyme inactivation (Fig. 2). This is also supported by the fact that the sulfone **4**, which is incapable of phosphorylation at the S=O oxygen, served as a simple reversible inhibitor (Fig. 1). This result is consistent with the reversible inhibition of rat kidney γ-GCS by buthionine sulfone, in which the extent of inhibition did not increase with time.[21]

It is worthy of note, however, that all the inhibitors **2, 3** and **4** were much more potent inhibitors than L-BSO (Table 1). The overall affinity of the phosphinic acid **2** and the sulfoximine **3** was 10- and 500-times greater than that of L-BSO, respectively, and the inactivation rate of enzyme by **2** and **3** was 90- and 290-times, respectively, as fast as that of L-BSO. The two orders increase in the γ-GCS inhibitory activity of the sulfoximine **3** compared to that of L-BSO suggested a significant interaction of the β-carboxyl function in **3** with the enzyme active site. Although the sulfone **4** did not inactivate the enzyme, its inhibition potency was more than five times higher than that of L-BSO as measured by the inhibition constant. The difference is still underestimated because the initial inhibition constant of the sulfone **4** ($K_i = 9.2\,\mu M$) is compared with the overall inhibition constant of L-BSO ($K_i^* = 49\,\mu M$) in which the



Scheme 4. Reagents and conditions: (a) 1,4-Dithio-DL-threitol, NaOH, EtOH-H_2O; (b) Ethyl 2-ethylacrylate; (c) NaOH then Dowex 50W×8 (H^+ form), eluted with 0.7 M HCl; (d) H_2O_2, AcOH.

Table 1. Inhibition of γ-GCS by Phosphinic acid **2**, Sulfoximine **3**, Sulfone **4** and L-buthionine-(*S,R*)-sulfoximine (L-BSO)

Inhibitor	K_i[a] [μM]	k_{inact}[b] [sec^{-1}]	k_{inact}/K_i[c] [$M^{-1}\,sec^{-1}$]	K_i^*[d] [μM]
Phosphinic acid **2**	28.9 ± 6.6	0.0126 ± 0.0014	436 ± 33	4.95 ± 0.27
Sulfoximine **3**	ND[e]	ND[e]	1430 ± 90	0.0989 ± 0.0224
Sulfone **4**	9.23 ± 1.76	—[f]	—[f]	—[f]
L-BSO	ND[e]	ND[e]	4.87 ± 1.01	49.3 ± 7.4

[a]Initial inhibition constant.
[b]Inactivation rate constant.
[c]Second-order rate constant for slow-binding inhibition (see Experimental).
[d]Overall inhibition constant measured after ATP-dependent binding equilibrium was established.
[e]Not determined (see Experimental).
[f]No slow-binding inhibition was observed.

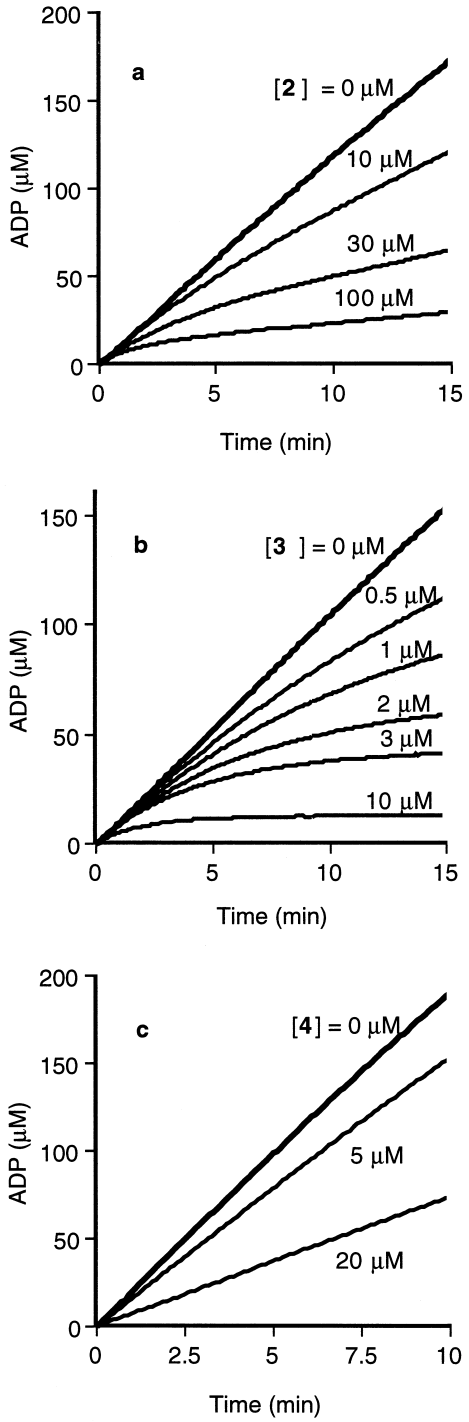


Figure 1. Progress curves for the inhibition of γ-GCS by: (a) the phosphinic acid **2**; (b) the sulfoximine **3**; and (c) the sulfone **4**. The reaction was initiated by adding the enzyme (20–56 nM) to a standard assay mixture (see Experimental) containing each inhibitor at the final concentrations indicated.

ATP-dependent tight binding equilibrium was established. This result again substantiates the importance of the β-carboxyl group in the recognition of the inhibitors by the enzyme. The K_i of the phosphinic acid **2** was also smaller than the K_i^* of L-BSO, suggesting that the phosphinic acid **2** itself is a better transition-state mimic than the putative phosphorylated L-BSO, provided K_i and K_i^* reflect the affinity of the inhibitors before and after phosphorylation, respectively.

Of particular interest is the difference in the inhibition behavior between the phosphinic acid **2** and the sulfoximine

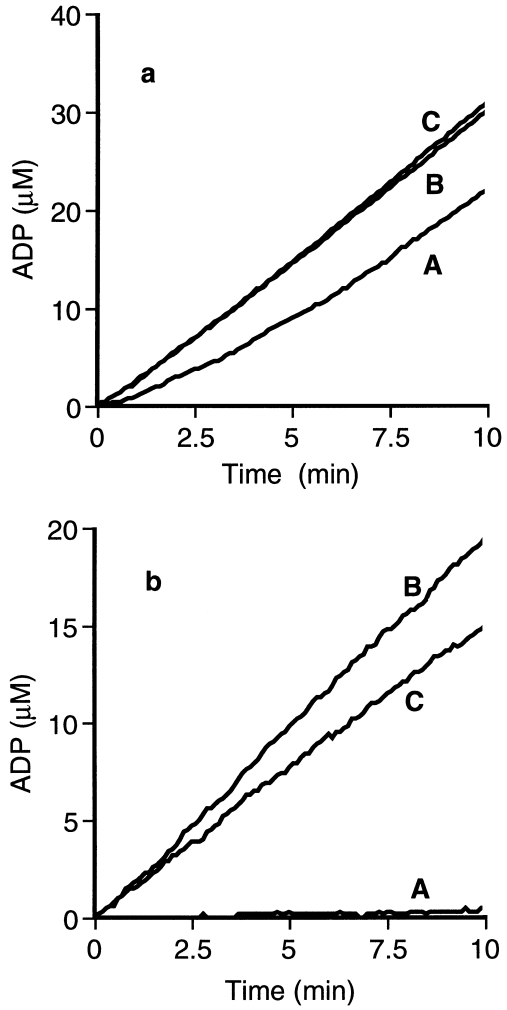


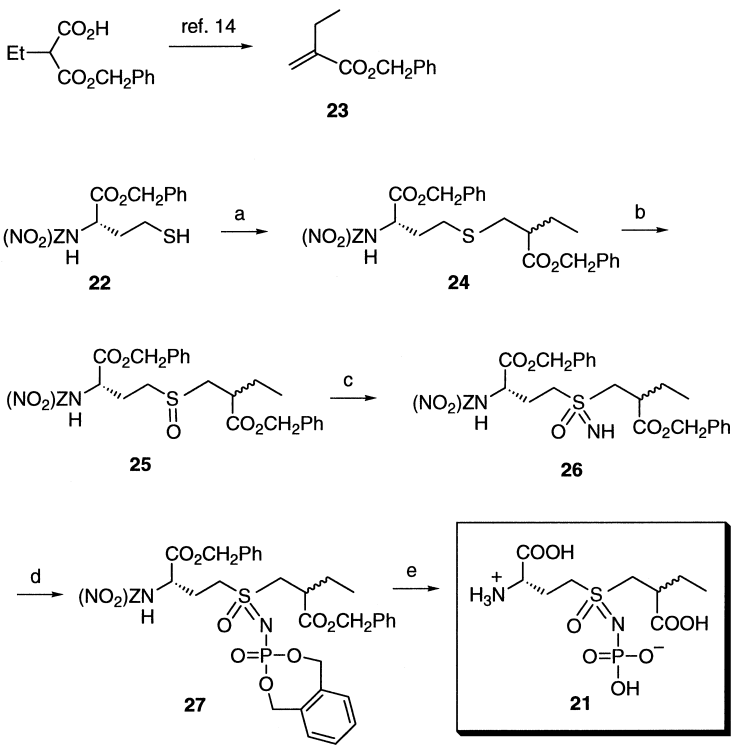
Figure 2. ATP-dependence of enzyme inactivation by: (a) the phosphinic acid **2**; and (b) the sulfoximine **3**. The enzyme (10 μM) was preincubated with the inhibitor in the presence of ATP (1 mM; curve A) or AMP-PNP (10 mM; curve B) at 25 °C for 30 min in Tris–HCl buffer (pH 7.5, 50 mM) containing $MgCl_2$ (2 mM). The remaining activity was then measured after 1000-fold dilution. Curve C (control): no ATP and AMP-PNP was added to the preincubation mixture.

3. First of all, the sulfoximine **3** was a much more potent inactivator than the phosphinic acid **2**: the overall affinity of **3** was 50 times greater than that of **2**, and the sulfoximine **3** inactivated the enzyme more than three times faster than **2** as judged by the second-order rate constant for enzyme inactivation (k_{inact}/K_i). Another interesting facet concerns the regain of enzyme activity. Duration of enzyme inactivation is an important measure relevant to the inhibition potency of slow-binding inhibitors. In this regard, the sulfoximine **3** was also superior to the phosphinic acid **2**. No regain of enzyme activity was observed with **3** under the standard assay conditions (Fig. 2b, curve A), but a significant enzyme reactivation ($t_{1/2} < 3$ min) was noted with the phosphinic acid **2** by 1000-fold dilution as evidenced by a concave upward progress curve (Fig. 2a, curve A).

A question may arise here why the enzyme regained activity so quickly with the phosphinic acid **2**, but not with the sulfoximine **3**, although they are structurally analogous. Since both **2** and **3** failed to inactivate the enzyme with AMPPNP (Fig. 2) and the sulfone **4** did not inactivate the enzyme, the phosphorylation of the phosphinyl oxygen or the sulfoximine nitrogen should be prerequisite for enzyme inactivation. If this is the case, then the chemical stability of the phosphorylated inhibitor may account for the difference. Thus, the putative phosphorylated **2**, a phosphinic–phosphoric anhydride, is potentially unstable and is still capable of transferring the phosphoryl group either to water (hydrolysis) or to ADP (reverse reaction) to release the free phosphinic acid **2**. On the other hand, the phosphoryl group attached to the sulfoximine nitrogen is expected to be stable[22] and remains unchanged, once it is transferred from ATP. To test this notion, we synthesized the *N*-phosphoryl sulfoximine **21**, and its inhibition behavior was examined.

Synthesis and inhibition properties of *N*-phosphoryl sulfoximine 21

The *N*-phosphoryl sulfoximine **21** was synthesized by a similar route to that of the sulfoximine **3** (Scheme 5). A major difference was that the benzyl-based protecting groups were used for the protection of the carboxyl as well as the phosphoryl groups, because the phosphorylated sulfoximine nitrogen would be no longer capable of intramolecular nucleophilic substitution to yield the corresponding cyclic sulfoximine as observed with the cyclization of the sulfoximine **17**. In addition, the simultaneous cleavage of the $(NO_2)Z$ and the α-methyl ester by hydrogenolysis seemed unique to the cyclic



Scheme 5. Reagents and conditions: (a) **23**, $CsCO_3$ (cat.), *i*-PrOH; (b) $NaIO_4$, THF–H_2O; (c) MSH, CH_3CN; (d) *o*-Xylylene *N,N*-diethylphosphoramidite, 1*H*-tetrazole, then *t*-BuOOH; (e) H_2, 10% Pd on carbon, MeOH-H_2O-AcOH, then Dowex 50W×8 (H^+ form), eluted with H_2O.

sulfoximine **18** and in fact was not the case with non-cyclized sulfoximines.[23] Thus, *N*-(4-nitrobenzyloxycarbonyl)-L-homocysteine benzyl ester (**22**) prepared by the reduction of the corresponding disulfide was allowed to react with benzyl 2-ethylacrylate (**23**) prepared from ethylmalonic acid monobenzyl ester.[14] The resulting sulfide **24** was oxidized stepwise with $NaIO_4$ and MSH to afford the sulfoximine **26**. In this case, no spontaneous cyclization of the sulfoximine was observed. The imino nitrogen of **26** was phosphorylated by *o*-xylylene *N,N*-diethylphosphoramidite[24] with 1*H*-tetrazole as a catalyst followed by oxidation to give the *N*-phosphoryl sulfoximine **27**. All benzyl-based protecting groups in **27** were successfully cleaved at once by hydrogenolysis to afford the final product **21**. The N-P bonding was hydrolytically stable even under an acidic condition, and the compound **21** was purified by passing a Dowex-50W column (H^+ form) without any extent of decomposition. The chemical synthesis of **21** thus confirmed the stability of the putative *N*-phosphorylated sulfoximine **3** which is thought to be formed enzymatically within the enzyme active site.

The *N*-phosphoryl sulfoximine **21** was found to be a potent inactivator of γ-GCS. Interestingly, the extent of inhibition increased with time (Fig. 3), showing a typical slow-binding kinetics as observed for the inhibition by the sulfoximine **3** (Fig. 1b). Since the inhibitor **21** is already phosphorylated, the inhibition scheme involving the enzyme-catalyzed phosphorylation by ATP seems unlikely. However, in view of the X-ray crystal structures of glutathione synthetase[8] and D-Ala: D-Ala ligase[25] complexed with the respective phosphinate, the tight binding of the phosphorylated inhibitor is always accompanied by ADP as a binding partner. In addition, the inhibition of a mechanistically related glutamine synthetase by L-methionine sulfoximine phosphate was increased significantly in the presence of ADP and Mg^{2+}.[22] We reasoned therefore that the tight binding of **21** would also require ADP and that the slow-binding kinetics observed with **21** would reflect the enzymatic formation of ADP during the assay. To test this notion, we examined the effect of ADP on the enzyme inactivation by the *N*-phosphoryl sulfoximine **21** (Fig. 4). As expected, the preincubation of the enzyme and **21** in the presence of ADP caused facile enzyme inactivation, while a very slow decrease of enzyme activity was observed without ADP. These results suggested that the *N*-phosphoryl sulfoximine **21** alone did not cause rapid enzyme inactivation, but the presence of ADP resulted in the formation of a ternary complex consisting of **21**, ADP and Mg^{2+} within the enzyme active site to cause facile enzyme inactivation. If this is the case, then the ternary complex thus formed in the enzyme active site should be identical to the complex formed by the sulfoximine **3**, ATP and Mg^{2+}. We therefore measured the kinetic parameters for the inhibition by **21** and found that the overall inhibition constant (K_i^*) of **21** was 160 nM and the second-order inactivation rate (k_{inact}/K_i)

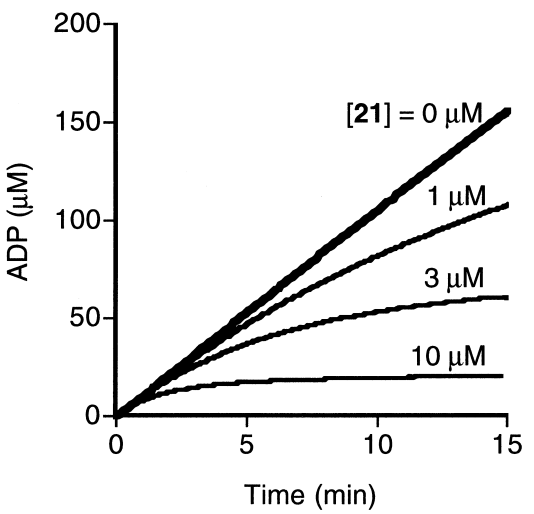


Figure 3. Progress curves for the inhibition of γ-GCS by the *N*-phosphoryl sulfoximine **21**. The reaction was started by adding the enzyme (56 nM) to the standard assay solution (see Experimental) containing **21** at the final concentrations indicated.

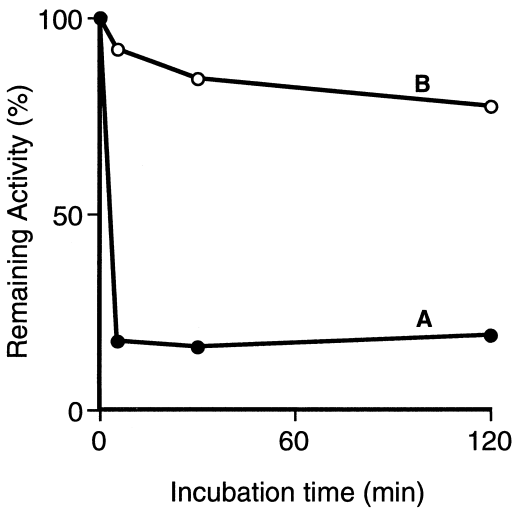


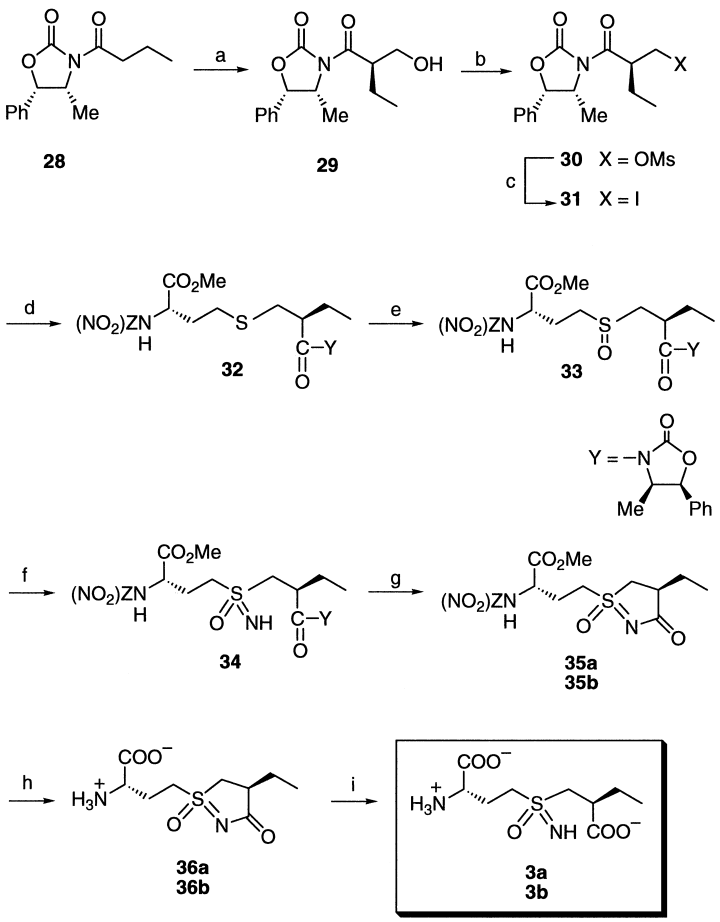
Figure 4. Effect of ADP on the inactivation of γ-GCS by the *N*-phosphoryl sulfoximine **21**. The enzyme (1 μM) was incubated with **21** (2 μM) and $MgCl_2$ (2 mM) in Tris–HCl buffer (pH 7.5, 20 mM) in the presence (curve A) or in the absence (curve B) of ADP (1 mM). Aliquots (50 μL) were withdrawn at the indicated intervals and were diluted with 2 mL of Tris–HCl buffer (pH 7.3, 20 mM) containing $MgCl_2$ (5 mM) and 10% (v/v) glycerol. The mixture was subjected to Centricon-10 to remove unbound **21** and ADP, and the remaining enzyme activity was measured by the standard assay method (see Experimental).

was 1300 $M^{-1} s^{-1}$. As expected, the K_i^* value of **21** was comparable to that of **3** (99 nM), indicating that the enzyme inactivation was most likely to be caused by the formation of the ternary complex comprised of the *N*-phosphoryl sulfoximine **21**, ADP and Mg^{2+}. Interestingly, the k_{inact}/K_i value of **21** (1300 $M^{-1} s^{-1}$) was also comparable to that of **3** (1430 $M^{-1} s^{-1}$). However, we assume this as coincidence, because there is little reason to expect that the rate at which the ternary complex is formed in the enzyme active site by enzymatic phosphorylation of **3** with ATP should bear any relationship to the rate at which the association of the enzymatically formed ADP and **21** in the enzyme active site.

Stereoselective synthesis and inhibition properties of sulfoximine 3

Although the sulfoximine **3** synthesized above was a mixture of four diastereomers with respect to the chiral β-carbon and the chiral sulfur atom, its inhibition potency was much higher than that of L-BSO. Hence the stereoselective synthesis of the sulfoximine **3** was expected not only to produce a γ-GCS inhibitor with much higher potency, but also to yield a transition-state analogue serving as a better mechanistic probe for the enzyme catalysis from a stereochemical point of view. In light of transition-state analogy, the diastereomers with *S*-configuration at the β-carbon would be preferable, since the (*S*)-β-carbon corresponds to the L-configuration of the attacking Cys in the transition state. We therefore synthesized two diastereomeric sulfoximines **3a** and **3b** with (*S*)-β-carbon and (*R*) or (*S*)-chiral sulfur atom, respectively.

Scheme 6 summarizes the stereoselective synthesis of the diastereomeric sulfoximine **3a** and **3b**. The chiral carbon center was constructed by diastereoselective aldol condensation of formaldehyde and the dibutylboron enolate prepared from the chiral *N*-butanoyloxazolidinone **28**.[26] The desired (*R*)-alcohol **29** was isolated, and the



Scheme 6. Reagents and conditions: (a) Bu_2BOTf, NEt_3, CH_2Cl_2, then HCHO (gas); (b) CH_3SO_2Cl, NEt_3, CH_2Cl_2; (c) NaI, acetone; (d) **14**, K_2CO_3, DMF; (e) $NaIO_4$, THF–H_2O; (f) MSH, CH_3CN; (g) K_2CO_3, H_2O_2, THF–H_2O, then 2 M HCl; (h) H_2, 10% Pd on carbon; (i) 1.2 M KOH, H_2O then Dowex 50W×8 (NH_4^+ form), eluted with H_2O.

hydroxyl group was converted to the iodide. The iodide **31** was coupled with the thiol **14** to yield the sulfide **32**. Stepwise oxidation with $NaIO_4$ and MSH gave the sulfoximine **34**. At this stage, another chiral center was generated on the sulfur atom, but the diastereomers of **33** or **34** were not separated by chromatography. We therefore cyclized **34** by removing the chiral oxazolidinone first followed by treatment with dilute acid. Direct cyclization by heating **34** in AcOH as for the cyclization of the sulfoximine **17** was unsuccessful, probably due to the stability of the imide C(O)-N bond compared with the ester C(O)-O bond.

The two diastereomers with respect to the chiral sulfur atom of **35** were separated successfully by flash column chromatography to afford the single stereoisomer **35a** and **35b**. Each stereoisomer was subjected to hydrogenolysis followed by alkaline hydrolysis to yield the sulfoximines **3a** and **3b**. The chirality of the sulfur atom was stable, but the chiral carbon β to the sulfur atom was partially racemized during the alkaline hydrolysis to give ca. 20% of the epimeric isomer as an unseparable byproduct.

In order to determine the absolute stereochemistry of **3a** and **3b**, compound **36b** was crystallized and its absolute configuration was determined by X-ray diffraction analysis (Fig. 5). On the basis of the known L-configuration of the α-amino group and *S*-configuration of the β-carbon, the configuration at sulfur was determined to be *S*. Hence the stereochemistry of the sulfur atom of the sulfoximine **3a** and **3b** is *R* and *S*, respectively.

The (*R*)-sulfoximine **3a** was found to be an extremely powerful slow-binding inhibitor, while the (*S*)-sulfoximine **3b** served as a simple reversible inhibitor with much lower inhibition potency (Fig. 6). Preincubation of the enzyme with (*R*)-**3a** and ATP caused almost complete inactivation of the enzyme (< 2% of residual activity), but the (*S*)-sulfoximine **3b** caused no enzyme inactivation (100% of residual activity) under the same conditions. Table 2 summarizes the kinetic parameters for the enzyme inhibition by (*R*)-**3a**, (*S*)-**3b** and L-BSO for comparison. The overall affinity and the second-order inactivation rate of the (*R*)-sulfoximine **3a** were 1260- and 1390-times, respectively, greater than those of L-BSO. The (*R*)-sulfoximine **3a** was also highly potent in terms of duration of enzyme inactivation: the enzyme inactivated by (*R*)-**3a** and ATP was still totally inactive after gel filtration, and a very slow regain was observed with a half life of recovery ($t_{1/2}$) of 11 days. On the other hand, the inhibition profile of the less inhibitory (*S*)-sulfoximine **3b** was quite similar to that of the sulfone **4** in that both compounds inhibited the enzyme in a reversible manner with comparable inhibition constants ($K_i = 9.23\,\mu M$ for the sulfone **4** and 12.0 μM for the (*S*)-sulfoximine **3b**). Considering that the enzyme inactivation

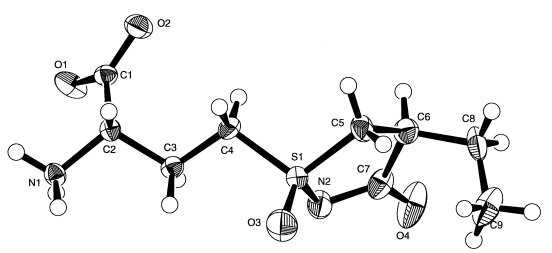


Figure 5. Structure of cyclic sulfoximine **36b** as determined by X-ray diffraction analysis.

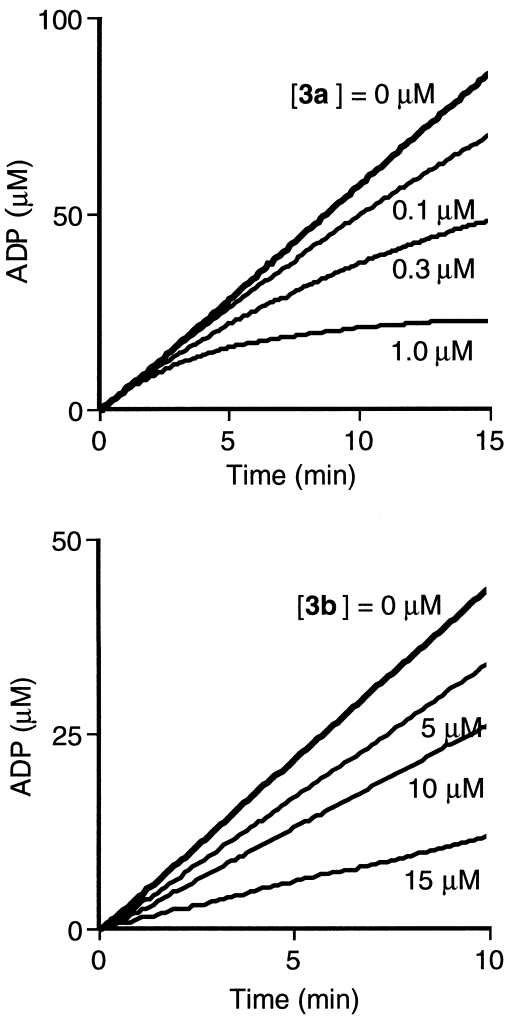


Figure 6. Progress curves for the inhibition of γ-GCS by: (a) (*R*)-sulfoximine **3a**; and (b) (*S*)-sulfoximine **3b**. The reaction was started by adding the enzyme (28 nM) to a standard assay solution (see Experimental) containing each inhibitor at the final concentrations indicated.

Table 2. Inhibition of γ-GCS by the (*R*)-sulfoximine **3a**, (*S*)-sulfoximine **3b** and L-buthionine-(*S,R*)-sulfoximine (L-BSO)

Inhibitor	K_i^* [μM]	k_{inact}/K_i [$M^{-1}\,sec^{-1}$]	Type of inhibition
(*R*)-Sulfoximine **3a**	0.0392 ± 0.0090	6750 ± 200	slow-binding
(*S*)-Sulfoximine **3b**	12.0 ± 1.7	—[a]	reversible
L-BSO	49.3 ± 7.4	4.87 ± 1.01	slow-binding

[a]No slow-binding inhibition was observed.

by the sulfoximine **3** was due to the mechanism-based phosphorylation of the sulfoximine nitrogen by ATP, the active site geometry of *E. coli* γ-GCS is most likely to allow phosphorylation of (*R*)-**3a** solely, while the (*S*)-sulfoximine **3b** was devoid of enzymatic phosphorylation probably due to the unfavorable orientation of the sulfoximine nitrogen in the enzyme active site. Griffith et al. reported the separation of the diastereomers of L-buthionine (*SR*)-sulfoximine and examined the inhibitory activity of each diastereomer towards rat kidney γ-GCS.[27] In this case, L-buthionine-(*S*)-sulfoximine served as a tight-binding, mechanism-based inactivator of rat kidney γ-GCS, while L-buthionine-(*R*)-sulfoximine was a relatively weak inhibitor without causing enzyme inactivation. Of particular interest is that the relative configuration of the chiral sulfur atom of (*R*)-**3a** is the same as that of L-buthionine-(*S*)-sulfoximine, although the sequence rule dictates the opposite sign for these two chiral sulfur atoms. As far as the mechanism-based phosphorylation of the sulfoximine nitrogen is concerned, the *E. coli* γ-GCS and rat kidney γ-GCS require the same relative configuration of the chiral sulfoximine nitrogen to transfer the γ-phosphate of ATP in the enzyme active site.

Recent elucidation of the crystal structures of glutathione synthetase[28] and D-Ala: D-Ala ligase[25] has revealed a marked structural similarity between these two mechanistically related ligases.[29] A common fold may also be found for other members of ADP-forming peptide ligases such as γ-GCS. In this context, the (*R*)-sulfoximine **3a** should find an important use as a ligand for structural determination of *E. coli* γ-GCS. This aspect of study is in progress.

Conclusion

The results presented in this paper demonstrate that the rational design of γ-GCS inhibitors on the basis of the proposed transition state is highly effective in producing potent γ-GCS inhibitors. A key was the introduction of a carboxyl function at the β-carbon to the central hetero atom so as to mimic the carboxyl group of the attacking L-Cys in the transition state. Among three types of inhibitors, phosphinic acid **2**, sulfoximine **3** and sulfone **4**, the sulfoximine **3** served as the most potent, ATP-dependent inactivator of γ-GCS. In particular, the sulfoximine **3a** with (*S*)-β-carbon and the (*R*)-sulfur atom was an extremely powerful mechanism-based inactivator with an overall affinity and a second-order inactivation rate of more than 1000 times greater than those of L-BSO. As far as we know, this is the most potent transition-state analogue inhibitor of γ-GCS. In this study, the inhibitory activity was examined only with the *E. coli* enzyme, but γ-GCS from other sources should also be inactivated by the sulfoximine **3** more effectively than L-BSO, because the structure of **3** is structurally much closer to the transition state. Another important facet is the stereochemistry of the chiral sulfur atom. The *E. coli* enzyme was rapidly inactivated by the (*R*)-sulfoximine **3a**, but not by the (*S*)-sulfoximine **3b**. Since the stereochemical preference depends on the active site geometry of individual enzyme, the chiral sulfoximine **3a** and **3b** may serve as a lead for a species-selective drug for regulating the physiological level of glutathione.

Experimental

General methods

THF was dried by distillation from Na/benzophenone ketyl. CH_2Cl_2, CH_3CN and NEt_3 were distilled from CaH_2. DMF was distilled from CaH_2 under reduced pressure and stored over 4 Å molecular sieves. Analytical TLC was performed on a silica gel plate (Merck 5715, 0.25 mm). Flash column chromatography was performed on silica gel 60 (Merck 9385, 230–400 mesh). Medium-pressure column chromatography was performed using an ULTRA PACK® silica gel column (Yamazen Co.) with a medium pressure of 2–3 kg/cm^2. Melting points were recorded on a Mettler FP62 and were corrected. Elemental analyses were performed on a Yanaco MT-5. ^1H NMR and ^{31}P NMR were recorded on a Varian VXR-200 (200 MHz) or JEOL JNM-AL400 (400 MHz) with tetramethylsilane or 3-(trimethylsilyl)-propanesulfonic acid sodium salt (for D_2O) as internal standard. Eighty five per cent phosphoric acid was used as external standard for ^{31}P NMR. Infrared spectra were recorded on a Hitachi U-215 spectrophotometer. Optical rotations were measured on a Perkin-Elmer 241

polarimeter. Mass spectra were obtained on a JEOL JMS700 spectrometer.

Ethyl (2*R*,*S*)-3-[ethoxy(diethoxymethyl)phosphinyl]-2-ethylpropanoate (6). A solution of NaOEt prepared from Na (2.04 g, 88.9 mmol) in dry EtOH (200 mL) was added dropwise via cannula to ethyl (diethoxymethyl)phosphinate[12] (15.9 g, 80.9 mmol) in dry EtOH (100 mL) at 0 °C. The mixture was stirred at 0 °C for 10 min, and ethyl 2-ethylacrylate[14] (11.4 g, 88.9 mmol) was added dropwise to the mixture at 0 °C over 20 min. The mixture was stirred at 0 °C for 1 h and at room temperature for 2 h. The reaction was quenched by adding AcOH (7.2 g, 121 mmol), and the reaction mixture was evaporated. The residue was dissolved in EtOAc (200 mL), washed with satd $NaHCO_3$ (200 mL) and satd NaCl (200 mL), successively, and dried over Na_2SO_4. Solvent was evaporated, and the product was purified by flash column chromatography (6:1, EtOAc/hexane) to give the phosphinate **6** (19.7 g, 75%, diastereomer mixture) as a colorless oil: IR (NaCl, neat) ν_{max} 1725, 1200, 1040 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.68 (d, J = 7.8 Hz) and 4.65 (d, J = 7.2 Hz) [1H, PCH], 4.3–4.0 (m, 4H, $POCH_2$ and $COOCH_2$), 3.9–3.6 [m, 4H, $(CH_3CH_2O)_2C$], 2.8 (m, 1H, C*H*$COOCH_2CH_3$), 2.3 and 1.9 (m, 2H, CH_2P), 1.7 (m, 2H, CH_2CH_3), 1.4–1.2 (m, 12H, $OCH_2CH_3\times4$), 0.92 (t, 3H, J = 7.4 Hz, CH_2CH_3); ^{31}P NMR ($CDCl_3$) δ +44.4. MS (EI) 324 (M^+).

Methyl (2*R*,*S*)-2-ethyl-3-(methoxyphosphinyl)propanoate (7). Compound **6** (4.40 g, 13.6 mmol) was heated in 12 M HCl (50 mL) under reflux for 4 h. The reaction mixture was evaporated to dryness to give a light brown syrup. H_2O (50 mL) was added to the syrup and evaporated again. The residual syrup was dried in a desiccator over powdered $CaCl_2$ and NaOH to remove trace HCl to give 2-carboxybutylphosphorous acid (2.42 g) as a pale brown syrup: ^1H NMR (D_2O) δ 7.10 (d, $^1J_{HP}$ = 549 Hz, 1H, PH), 2.7 (m, 1H, C*H*COOH), 2.3–1.8 (m, 2H, CH_2P), 1.7 (m, 2H, CH_2CH_3), 0.92 (t, 3H, J = 7.4 Hz, CH_2CH_3). MS (EI) 166 (M^+).

The crude phosphorous acid (2.42 g) was treated with an ethereal solution of CH_2N_2 until yellow color persisted. Excess CH_2N_2 was decomposed by adding AcOH, and the reaction mixture was evaporated below 40 °C. The product was unstable when heated above 50 °C and was quickly purified by flash column chromatography (2:3, acetone/hexane) to give the phosphinate **7** (1.54 g, 59%, diastereomer mixture) as a colorless oil: ^1H NMR ($CDCl_3$) δ 7.15 (d, $^1J_{HP}$ = 552 Hz, 1H, PH), 3.79 (d, $^3J_{HP}$ = 11.8 Hz, 3H, $POCH_3$), 3.73 (2×s, 3H, $COOCH_3$), 2.8 (m, 1H, C*H*$COOCH_3$), 2.4–1.8 (m, 2H, CH_2P), 1.7 (m, 2H, CH_2CH_3), 0.92 (2×t, 3H, J = 7.4 Hz, CH_2CH_3). HRMS (EI) calcd for $C_7H_{15}O_4P$ (M^+) 194.0709, found 194.0708. The product **7** was partially decomposed during purification and was used as such for the next reaction.

Methyl (2*S*)-2-(*N*-benzyloxycarbonylamino)-4-[(*R*,*S*)-(methoxy)[(2*R*,*S*)-2-(methoxycarbonyl)butyl]phosphinyl]-butanoate (9). The phosphinate **7** (1.53 g, 7.88 mmol) and (*N*-benzyloxycarbonyl)-L-vinylglycine methyl ester (**8**)[16] (1.16 g, 4.65 mmol) were dissolved in xylene (15 mL), and Ar gas was passed through the solution for 10 min to purge oxygen. To this solution was added *tert*-butyl peroxybezoate (12.8 mg, 0.066 mmol), and the mixture was heated at 110 °C for 4 h under an Ar atmosphere. During this period, additional *tert*-butyl peroxybenzoate (12.8 mg, 0.066 mmol) was added in every 1 h (total 38.3 mg, 0.20 mmol), and the mixture was heated at 110 °C for 11 h. Another portion of *tert*-butyl peroxybenzoate (25.6 mg, 0.132 mmol) was added, and the mixture was heated at 120 °C for 6 h. The reaction mixture was evaporated, and the residual oil was purified by flash column chromatography (4:1 to 2:1, benzene/acetone) to give the compound **9** (454 mg, 22%, diastereomer mixture): $[\alpha]_D^{25}$ +8.76° (*c* 1.05, $CHCl_3$); IR (NaCl, neat) 3250 (br), 1720, 1525, 1203, 1035 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.36 (s, 5H, arom), 5.7 (m, 1H, NH), 5.12 (s, 2H, PhCH_2), 4.40 (m, 1H, α-proton), 3.8–3.6 (m, 9H, $POCH_3$ and $COOCH_3\times2$), 2.7 (m, 1H, C*H*$COOCH_3$), 2.2 and 2.0 (2×m, 2H, CH_2P), 1.9–1.7 (m, 4H, CH_2CH_2P), 1.7 (m, 2H, CH_2CH_3), 0.90 (t, 3H, J = 7.4 Hz, CH_2CH_3); ^{31}P NMR δ + 55.9 (47%), +56.4 (27%), +56.6 (26%). HRMS (EI) calcd for $C_{20}H_{30}NO_8P$ (M^+) 443.1710, found 443.1730.

(2*S*)-2-Amino-4-[(2*R*,*S*)-(2-carboxybutyl)(hydroxy)phosphinyl]butanoic acid (2). Compound **9** (381 mg, 0.86 mmol) was dissolved in THF (2 mL), and 3 M NaOH (1.72 mL, 5.15 mmol) was added to the solution. The mixture was stirred at 25 °C for 7 h. The reaction mixture was made acidic (pH∼1) by adding 3 M HCl (1.8 mL), and the mixture was evaporated to dryness to give a colorless foam. The residue was extracted with acetone (15 mL), and insoluble NaCl was removed by filtration through Celite. The filtrate was evaporated to give the *N*-protected free acid as a colorless foam (346 mg): ^1H NMR (acetone-d_6) δ 9.50 (br s, 3H, POH and $COOH\times2$), 7.3 (m, 5H, arom), 6.75 (br s, 1H, NH), 5.10 (s, 2H, PhCH_2), 4.3 (m, 1H, α-proton), 2.8 (m, 1H, C*H*COOH), 2.3–1.8 (m, 6H, $CH_2CH_2PCH_2$), 1.7 (m, 2H, CH_2CH_3), 0.93 (t, 3H, J = 7.4 Hz, CH_2CH_3); ^{31}P NMR (acetone-d_6) δ +58.95.

The product (320 mg) was dissolved in MeOH–AcOH–H_2O (8:2:1, 11 mL). Hydrogen gas was passed through the solution in the presence of 10% Pd on carbon (150 mg) at room temperature. The hydrogenolysis was monitored with silica gel TLC (*n*-BuOH–AcOH–H_2O,

5:2:2) and was completed in 20 min. The reaction mixture was filtered through Celite, and the filtrate was evaporated to give crude **2** as a colorless syrup. The crude **2** was applied to a Dowex 50WX8 column (H^+ form, 35 cm^3), and the column was eluted with H_2O. A strongly acidic impurity passed through first, and the eluate became neutral (75 mL of H_2O). The eluent was changed to 6% pyridine, and the column was eluted until the solvent front reached 4 cm above the bottom of the column (*ca.* 50 mL of 6% pyridine). The eluent was changed again to H_2O, and the product **2** was eluted as a mildly acidic eluate (pH 3–4). Ninhydrin positive fractions were collected (*ca.* 70 mL) and lyophilized to give the phosphinic acid **2** (184 mg, 80%, diastereomer mixture) as a colorless non-hygroscopic solid: $[\alpha]_D^{25}$ +11.8° (*c* 0.53, H_2O); IR (KBr) ν_{max} 3400 (br), 2950 (br), 1710, 1638, 1510, 1220, 1100, 1008 cm^{-1}; ^1H NMR (400 MHz, D_2O) δ 3.99 (2×t, 1H, *J* = 6.05 Hz, α-proton), 2.7 (m, 1H, C*H*COOH), 2.1 (m, 2H, C$H_2$$CH_2$P), 2.0 and 1.7 (m, 2H, PCH_2CH), 1.8 (m, 2H, CH_2CH_2P), 1.6 (m, 2H, C$H_2$$CH_3$), 0.91 (t, 3H, *J* = 7.33 Hz, CH_2CH_3); ^{31}P NMR (D_2O) δ +45.6. HRMS (FAB, glycerol) calcd for $C_9H_{19}NO_6P$ (MH^+) 268.0951, found 268.0972.

Methyl 2-ethyl-3-hydroxypropanoate (11). This compound was prepared by the alkylation[17] of methyl 3-hydroxypropanoate (**10**) in the presence of two equiv of *N,N′*-dimethylpropyleneurea (DMPU) and was purified by flash column chromatography (1:2, EtOAc/hexane) to give a colorless liquid (56%): IR (NaCl, neat) ν_{max} 3350 (br), 2940, 1710, 1430, 1370, 1180, 1040 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.8 (m, 2H, OCH_2), 3.73 (s, 3H, $COOCH_3$), 2.5 (m, 1H, CH), 2.08 (br s, 1H, OH), 1.6 (m, 2H, C$H_2$$CH_3$), 0.95 (t, 3H, *J* = 7.5 Hz, CH_2CH_3). HRMS (FAB, glycerol) calcd for $C_6H_{13}O_3$ (MH^+) 133.0865, found 133.0874.

Methyl 2-(iodomethyl)butanoate (12). The alcohol **11** was converted to the iodide **12** via the mesylate by the general method (NaI, reflux in acetone). Purification by flash column chromatography (1:9, Et_2O/hexane) gave **12** as a colorless liquid (70%): IR (NaCl, neat) ν_{max} 2950, 1735, 1200 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.74 (s, 3H, $COOCH_3$), 3.36 (dd, 1H, *J* = 8.0 and 9.8 Hz, CH_2I), 3.26 (dd, 1H, *J* = 5.6 and 9.8 Hz, CH_2I), 2.7 (m, 1H, CH), 1.7 (m, 2H, C$H_2$$CH_3$), 0.93 (t, 3H, *J* = 7.5 Hz, C$H_2$$CH_3$). HRMS (EI) calcd for $C_6H_{11}IO_2$ (M^+) 241.9806, found 241.9807.

(*N*-4-Nitrobenzyloxycarbonyl)-L-homocystine methyl ester (13). (*N*-4-Nitrobenzyloxycarbonyl)-L-homocystine was treated with methanol containing 1% (v/v) H_2SO_4 (room temperature, 12 h). Purification by flash column chromatography (1:1, EtOAc/hexane) afforded the methyl ester **13** (65%) as white powder: $[\alpha]_D^{27}$ +12.82° (*c* 1.17, $CHCl_3$); mp 104.5–105.2 °C; IR (KBr) ν_{max} 3340, 2960, 1740, 1695, 1520, 1340, 1215 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.21 (d, 4H, *J* = 8.6 Hz, arom), 7.50 (d, 4H, *J* = 8.8 Hz, arom), 5.60 (d, 2H, *J* = 8.6 Hz, NH×2), 5.21 (s, 4H, ArCH_2×2), 4.5 (m, 2H, α-proton×2), 3.78 (s, 6H, $COOCH_3$×2), 2.73 (t, 4H, *J* = 7.4 Hz, CH_2CH_2S×2), 2.2 (m, 4H, C$H_2$$CH_2$S×2). Anal. calcd for $C_{26}H_{30}N_4O_{12}S_2$: C, 47.70; H, 4.62; N, 8.56. Found: C, 47.80; H, 4.62; N, 8.57.

(*N*-4-Nitrobenzyloxycarbonyl)-L-homocysteine methyl ester (14). The homocystine derivative **13** (2.30 g, 3.51 mmol) was dissolved in CH_2Cl_2 (30 mL) which had been washed and saturated with H_2O. To this solution was added tri-*n*-butylphosphine (0.71 g, 3.51 mmol) at once at room temperature. The mixture was stirred for 20 min at room temperature and was concentrated to dryness. The residue was purified by flash column chromatography (3:2, hexane/EtOAc) to give the thiol **14** (2.36 g, 100 %) as a yellow oil: ^1H NMR ($CDCl_3$) δ 8.23 (d, 2H, *J* = 8.8 Hz, arom), 7.52 (d, 2H, *J* = 8.8 Hz, arom), 5.45 (d, 1H, *J* = 7.6 Hz, NH), 5.22 (s, 2H, ArCH_2), 4.5 (m, 1H, α-proton), 3.78 (s, 3H, $COOCH_3$), 2.6 (m, 2H, CH_2SH), 2.0 (m, 2H, C$H_2$$CH_2$S), 1.56 (t, 1H, *J* = 8.3 Hz, SH).

Methyl (2*S*)-4-[(2*R*,*S*)-2-(methoxycarbonyl)butylthio]-2-(*N*-4-nitrobenzyloxycarbonylamino)butanoate (15). The thiol **14** (8.17 g, 24.9 mmol) and the iodide **12** (7.23 g, 29.9 mmol) were dissolved in dry DMF (70 mL). To the solution was added powdered K_2CO_3 (3.78 g, 27.4 mmol), and the mixture was stirred at 60 °C for 1 h. The reaction mixture was poured into H_2O (600 mL) and extracted with EtOAc (100 mL×3). A dilute solution of I_2 in EtOAc was added to the combined EtOAc extracts until brown color persisted to oxidize the residual thiol. The mixture was then washed with 5% $Na_2S_2O_3$, satd NaCl, successively, and dried over Na_2SO_4. Evaporation gave a syrup. The product was purified by flash column chromatography (1:2, EtOAc/hexane) to give the sulfide **15** (8.50 g, 77%, diastereomer mixture) as a pale yellow syrup: IR (NaCl, neat) ν_{max} 3300, 2930, 1720, 1600, 1510, 1430, 1340, 1210, 1045, 740 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.23 (d, 2H, *J* = 8.8 Hz, arom), 7.52 (d, 2H, *J* = 8.8 Hz, arom), 5.53 (d, 1H, *J* = 7.8 Hz, NH), 5.21 (2×d, 2H, *J* = 13.9 Hz, ArCH_2), 4.48 (dt, 1H, *J* = 5.1 and 7.8 Hz, α-proton), 3.77 (s, 3H, NHCHCO_2CH_3), 3.71 (s, 3H, CO_2CH_3), 2.77 (dd, *J* = 2.7 and 13.2 Hz) and 2.75 (dd, *J* = 2.9 and 13.2 Hz) [1H, SCH_2CH], 2.64 (dd, *J* = 3.9 and 13.2 Hz) and 2.63 (dd, *J* = 3.7 and 13.2 Hz) [1H, SCH_2CH], 2.6–2.5 (m, 3H, CH_2CH_2S and SCH_2C*H*), 2.2 and 2.0 (2×m, 2H, C$H_2$$CH_2$S), 1.7 (m, 2H, C$H_2$$CH_3$), 0.91 (t, 3H, *J* = 7.6 Hz, CH_2CH_3). Anal. calcd for $C_{19}H_{26}N_2O_8S$: C, 51.57; H, 5.92; N, 6.33. Found: C, 51.43; H, 5.90; N, 6.34.

Methyl (2*S*)-4-[(2*R*,*S*)-2-(methoxycarbonyl)butyl-(*R*,*S*)-sulfinyl]-2-(*N*-4-nitrobenzyloxycarbonylamino)butanoate (16). A solution of $NaIO_4$ (4.87 g, 22.7 mmol) in H_2O (50 mL) was added to a solution of the sulfide **15** (8.40 g, 18.9 mmol) in THF–H_2O (7:2, 90 mL) at 0 °C. The mixture was stirred at 25 °C for 24 h and evaporated to remove THF, and the resulting mixture was extracted with EtOAc (100 mL×2). The combined extracts were washed twice with H_2O and satd NaCl, successively, and dried over Na_2SO_4 and concentrated. This residual syrup was purified by flash column chromatography (2:3, acetone/hexane) to give the sulfoxide **16** (7.93 g, 91%, diastereomer mixture) as a pale yellow syrup: IR (NaCl, neat) ν_{max} 3220, 2950, 1720, 1600, 1520, 1430, 1340, 1210, 1010, 740 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.23 (d, 2H, J = 8.6 Hz, arom), 7.52 (d, 2H, J = 8.6 Hz, arom), 6.0 (m) and 5.9 (d, J = 7.1 Hz) [1H, NH], 5.21 (2×d, 2H, J = 13.6 Hz, ArCH_2), 4.5 (m, 1H, α-proton), 3.79 (s, 3H, NHCHCO$_2$CH_3), 3.74–3.72 (4×s, 3H, CO_2CH_3), 3.1 and 2.7 (2×m, 2H, SCH_2CH), 3.0 and 2.9 (2×m, 1H, SCH$_2$C*H*), 2.82 (t, 2H, J = 7.6 Hz, CH_2CH_2S), 2.4 and 2.2 (2×m, 2H, CH_2CH$_2$S), 1.8 (m, 2H, CH_2CH_3), 0.95 (4×t, 3H, J = 7.4 Hz, CH_2CH_3). Anal. calcd for $C_{19}H_{26}N_2O_9S$: C, 49.78; H, 5.72; N, 6.11. Found: C, 50.12; H, 5.70; N, 6.06. HRMS (FAB, glycerol) calcd for $C_{19}H_{27}N_2O_9S$ (MH^+) 459.1437, found 459.1428.

Methyl (2*S*)-4-[(2*R*,*S*)-2-(methoxycarbonyl)butyl-(*R*,*S*)-sulfonimidoyl]-2-(*N*-4-nitrobenzyloxycarbonylamino)butanoate (17). To a solution of the sulfoxide **16** (7.35 g, 16.0 mmol) in dry CH_3CN was added *O*-mesitylenesulfonylhydroxylamine (MSH; CAUTION!: explosive especially when stored in a glass vial) (3.45 g, 16.0 mmol) at once under an Ar atmosphere, and the mixture was stirred at room temperature for 24 h. An additional portion of MSH (1.03 g, 4.78 mmol) was added, and the solution was stirred for 72 h to complete the reaction. The reaction mixture was evaporated, and the residual colorless foam was dissolved in EtOAc. The solution was washed with satd $NaHCO_3$, H_2O, and satd NaCl, successively, and was dried over Na_2SO_4 and concentrated. The residual syrup was purified by medium-pressure column chromatography (3–5% MeOH in $CHCl_3$) to give the sulfoximine **17** (4.95 g, 65%, diastereomer mixture) as a colorless foam: IR (NaCl, neat) ν_{max} 3270, 2950, 1720, 1600, 1510, 1430, 1340, 1200, 1050, 990, 730 cm^{-1}; ^1H NMR δ 8.22 (d, 2H, J = 8.6 Hz, arom), 7.52 (d, 2H, J = 8.6 Hz, arom), 6.3–6.0 (m, 1H, CHN*H*), 5.21 (s, 2H, ArCH_2), 4.46 (m, 1H, α-proton), 3.78 (s, 3H, NHCHCO$_2$CH_3), 3.74 (s, 3H, CO_2CH_3), 3.7 (m, 1H, SCH_2CH), 3.2–2.8 (m, 5H, CH_2SCH*H*CH and S = NH), 2.5 and 2.3 (2×m, 2H, CH_2CH$_2$S), 1.7 (m, 2H, CH_2CH_3), 0.95 (t, 3H, J = 7.5 Hz, CH_2CH_3). HRMS (FAB, glycerol) calcd for $C_{19}H_{28}N_3O_9S$ (MH^+) 474.1546, found 474.1551.

Diastereomers of methyl (2*S*)-4-[(1*RS*,4*RS*)-4-ethyl-4,5-dihydro-1,3-dioxo-1,2-thiazol-1-yl]-2-(*N*-4-nitrobenzyloxycarbonylamino)butanoate (18a and 18b). A solution of the sulfoximine **17** (1.10 g, 2.32 mmol) in AcOH (30 mL) was stirred at 50 °C for 5 h and was concentrated. The residue was purified by medium-pressure column chromatography (6% *i*-PrOH in $CHCl_3$) to give the diastereomeric **18a** (382 mg, 37%) and **18b** (393 mg, 38%) as a colorless foam and solid, respectively. Each compound was a mixture of two diastereomers. Compound **18a**: IR (KBr) ν_{max} 3280, 2950, 1700 (br), 1600, 1510, 1440, 1340, 1220 (br), 1055, 1000, 740 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.23 (d, 2H, J = 8.4 Hz, arom), 7.52 (d, 2H, J = 8.4 Hz, arom), 5.96 and 5.90 (2×br s, 1H, NH), 5.2 (m, 2H, ArCH_2), 4.5 (m, 1H, α-proton), 3.81 and 3.80 (2×s, 3H, CO_2CH_3), 3.67 and 3.43 (2×m, 2H, SCH_2CH), 3.6 (m, 2H, CH_2CH_2S), 3.2 (m, 1H, SCH$_2$C*H*), 2.6 and 2.3 (2×m, 2H, CH_2CH$_2$S), 2.1 and 1.6 (2×m, 2H, CH_2CH_3), 1.00 and 0.98 (2×t, 3H, J = 7.2 Hz, CH_2CH_3). Anal. calcd for $C_{18}H_{23}N_3O_8S$: C, 48.97; H, 5.25; N, 9.52. Found: C, 48.79; H, 5.25; N, 9.31. Compound **18b**: IR (KBr) ν_{max} 3300, 2930, 1725, 1665, 1600, 1510, 1430, 1340, 1220, 1060, 1000, 740 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.23 (d, 2H, J = 8.7 Hz, arom), 7.52 (d, 2H, J = 8.7 Hz, arom), 5.63 and 5.98 (2×br s, 1H, NH), 5.2 (m, 2H, ArCH_2), 4.5 (m, 1H, α-proton), 3.9 (m, 1H, SCH_2CH), 3.81 and 3.80 (2×s, 3H, CO_2CH_3), 3.6 (m, 2H, CH_2CH_2S), 3.2 (m, 1H, SCH_2CH), 3.0 (m, 1H, SCH$_2$C*H*), 2.5 and 2.3 (2×m, 2H, CH_2CH$_2$S), 2.0 and 1.8 (2×m, 2H, CH_2CH_3), 1.00 (t, 3H, J = 7.5 Hz, CH_2CH_3). Anal. calcd for $C_{18}H_{23}N_3O_8S$: C, 48.97; H, 5.25; N, 9.52. Found: C, 48.79; H, 5.19; N, 9.49.

(2*S*)-2-Amino-4-[(1*RS*),(4*RS*)-4-ethyl-4,5-dihydro-1,3-dioxo-1,2-thiazol-1-yl]butanoic acid (19). The combined sulfoximine **18a** and **18b** (1:1, 709 mg, 1.61 mmol) were dissolved in AcOH (15 mL). To the solution was added 10% Pd on carbon (Aldrich, wet, Degussa type E101 NE/W), and H_2 gas was passed through the mixture at 30 °C for 2.5 h. The reaction mixture was filtered through Celite, and the filtrate was concentrated to dryness. The residue was applied to a Dowex 50W×8 column (H^+ form, 25 cm^3). The column was washed with H_2O (250 mL) and 5% pyridine (200 mL), successively, and was eluted with 3% NH_4OH. The ninhydrin positive fractions were collected and lyophilized to give the cyclic sulfoximine **19** (349 mg, 88%, diastereomer mixture) as a colorless powder: IR (KBr) ν_{max} 3350 (br), 2930 (br), 1665, 1650–1600, 1400, 1210, 1000 cm^{-1}; ^1H NMR (400 MHz, D_2O) δ 4.4, 4.0 and 3.6 (3×m, 2H, SCH_2CH), 3.9–3.8 (m, 3H, α-proton and CH_2CH_2S), 3.4 and 3.2 (2×m, 1H, SCH$_2$C*H*), 2.4 (m, 2H, CH_2CH$_2$S), 1.8 (m, 2H, CH_2CH_3), 0.93 (t, 3H, J = 7.5 Hz, CH_2CH_3). HRMS (FAB, glycerol) calcd for $C_9H_{17}N_2O_4S$ (MH^+) 249.0909, found 249.0915.

(2*S*)-2-Amino-4-[(2*R*,*S*)-2-carboxybutyl-(*R*,*S*)-sulfonimidoyl]butanoic acid (3). To a solution of the cyclic sulfoximine **19** (326 mg, 1.31 mmol) in H_2O (20 mL) was added dropwise 1 M KOH (3.28 mL, 3.28 mmol) at 0 °C. The mixture was stirred for 5 days at 4 °C and was applied to a Dowex 50W×8 column (NH_4^+ form, 25 cm^3). The column was eluted with H_2O, and the ninhydrin positive fractions were collected and lyophilized to give the ammonium salt of the sulfoximine **3** (370 mg, 100%) as a hygroscopic solid: IR (KBr) ν_{max} 3600–2400 (br), 1580 (br), 1390, 1190, 1000 cm^{-1}; ^1H NMR (400 MHz, D_2O) δ 3.9–3.8 (4×t, 1H, J = 6.4 Hz, α-proton), 3.6 (m, 1H, SCH_2CH), 3.4–3.2 (m, 3H, CH_2CH_2SCHH), 2.7 (m, 1H, SCH_2CH), 2.4–2.2 (m, 2H, CH_2CH_2S), 1.6 (m, 2H, CH_2CH_3), 0.89 (t, 3H, J = 7.6 Hz, CH_2CH_3). HRMS (FAB, glycerol) Calcd for $C_9H_{19}N_2O_5S$ (MH^+) 267.1016, found 267.1019.

(2*S*)-2-Amino-4-[(2*R*,*S*)-2-carboxybutylthio]butanoic acid hydrochloride (20). L-Homocystine (3.50 g, 13.0 mmol) was dissolved in degassed EtOH–H_2O (1:1, 50 mL) containing NaOH (11.4 g, 27.4 mmol). 1,4-Dithio-DL-threitol (DTT; 2.41 g, 15.7 mmol) was added to the solution at once, and the mixture was stirred at room temperature for 1 h under an Ar atmosphere. Ethyl 2-ethylacrylate[14] (4.01 g, 31.3 mmol) was added to the solution with vigorous stirring, and a small amount of NaOH (ca. 50 mg) was added as a catalyst. The mixture was stirred under an Ar protection for 2 h at room temperature. The reaction mixture was evaporated, and the residue was dissolved in 2 M NaOH (60 mL). The solution was washed with ether (50 mL×3), and the aqueous solution was allowed to stand at room temperature for 18 h. After the hydrolysis was completed (TLC; *n*-BuOH:AcOH:H_2O = 5:2:2, ninhydrin), the mixture was made acidic (pH = 2) by adding 6 M HCl. The mixture was evaporated to dryness, and the residual semisolid was triturated with EtOH (200 mL). Insoluble salt (NaCl) was filtered off, and the filtrate was evaporated to give crude **20** as a pale yellow semisolid. The crude product was applied to a Dowex 50W×8 column (H^+ form, 100 cm^3), and the column was washed with H_2O. The column was then eluted with 0.7 M HCl, and ninhydrin positive fractions were collected and evaporated to dryness to give the sulfide **20** (5.39 g, 76%) as a colorless solid: mp 172.7–173.9 °C; $[\alpha]_D^{24}$ +11.3° (*c* 1.01, H_2O); IR (KBr) ν_{max} 3200–2300 (br), 1720, 1695, 1604, 1490, 1215 cm^{-1}; ^1H NMR (D_2O) δ 4.17 and 4.16 (2×t, 1H, J = 6.4 Hz, α-proton), 2.8 (m, 4H, CH_2SCH_2), 2.7 (m, 1H, C*H*COOH), 2.3 (m, 2H, CH_2CH_2S), 1.65 (quintet, 2H, J = 7.4 Hz, CH_2CH_3), 0.92 (t, 3H, J = 7.4 Hz, CH_2CH_3). Anal. calcd for $C_9H_{19}Cl_2NO_4S$: C, 35.07; H, 6.21; N, 4.54. Found: C, 35.24; H, 5.90; N, 4.64. HRMS (EI) calcd for $C_9H_{17}NO_4S$ (M^+) 235.0879, found 235.0909.

(2*S*)-2-Amino-4-[(2*R*,*S*)-(2-carboxybutyl)sulfonyl]butanoic acid (4). The sulfide **20** (830 mg, 3.05 mmol) was oxidized with 30% H_2O_2 (1.07 mL, 13.8 mmol) in AcOH (6.5 mL) at 50 °C for 7 h. After the oxidation was completed (TLC, *n*-BuOH:AcOH:H_2O = 5:2:2, ninhydrin), the reaction mixture was evaporated to give a colorless syrup. AcOH (5 mL) was added to the syrup, and the solution was allowed to stand at room temperature for 30 min and then at 4 °C overnight to give the sulfone **4** (144 mg, 23%) as colorless crystals complexed with ca. 1 equiv of AcOH: mp 210.1–211.4 °C (decomp.); IR (KBr) ν_{max} 3600–3200 (br), 3100–2700 (br), 1710, 1620, 1460, 1285, 1223, 1105 cm^{-1}; ^1H NMR (D_2O + CF_3COOD) δ 4.28 (t, 1H, J = 6.2 Hz, α-proton), 3.73 (dd, J = 10.3 and 14.7 Hz) and 3.45 (dd, J = 3.3 and 14.7 Hz) [2H, SO_2CH_2CH], 3.5 (m, 2H, $CH_2CH_2SO_2$), 3.0 (m, 1H, C*H*COOH), 2.5 (m, 2H, $CH_2CH_2SO_2$), 2.08 (s, 3H, CH_3COOH), 1.74 (quintet, 2H, J = 7.3 Hz, $CHCH_2CH_3$), 0.96 (t, 3H, J = 7.5 Hz, CH_2CH_3). Anal. calcd for $C_9H_{17}NO_6S{\cdot}CH_3COOH{\cdot}0.3H_2O$: C, 39.71; H, 6.54; N, 4.21. Found: C, 39.73; H, 6.43; N, 4.44. HRMS (FAB, glycerol) calcd for $C_9H_{18}NO_6S$ (MH^+) 268.0855, found 268.0864.

(*N*-4-Nitrobenzyloxycarbonyl)-L-homocysteine benzyl ester (22). (*N*-4-Nitrobenzyloxycarbonyl)-L-homocystine benzyl ester was reduced by tri-*n*-butylphosphine as for the synthesis of the thiol **14**. Purification with flash column chromatography (1:2, EtOAc/hexane) gave the thiol **22** (2.10 g, 91%) as a pale yellow oil: ^1H NMR ($CDCl_3$) δ 8.21 (d, 2H, J = 8.6 Hz, arom), 7.50 (d, 2H, J = 8.8 Hz, arom), 7.36 (s, 5H, Ph), 5.4 (m, 1H, NH), 5.2 (m, 4H, $ArCH_2$×2), 4.5 (m, 1H, α-proton), 2.6 (m, 2H, CH_2CH_2S), 2.1 (m, 2H, CH_2CH_2S), 1.52 (t, 3H, J = 8.2 Hz, SH).

Benzyl 2-ethylacrylate (23). Compound **23** was prepared from benzyl hydrogen ethylmalonate according to the reported procedure.[14] The crude product was purified by flash column chromatography (5:95, Et_2O/hexane) to afford the acrylate **23** (81%) as a colorless oil: IR (NaCl, neat) ν_{max} 2970, 1715, 1150 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.3 (m, 5H, Ph), 6.2 (m, 1H, C = CH_2), 5.6 (m, 1H, C = CH_2), 5.20 (s, 2H, $PhCH_2$), 2.4 (m, 2H, CH_2CH_3), 1.09 (t, 3H, J = 7.4 Hz, CH_2CH_3). Anal. calcd for $C_{12}H_{14}O_2$: C, 75.76; H, 7.42. Found: C, 75.45; H, 7.57. HRMS (EI) calcd for $C_{12}H_{14}O_2$ (M^+) 190.0994, found 190.1010.

Benzyl (2*S*)-4-[(2*R*,*S*)-2-(benzyloxycarbonyl)butylthio]-2-(*N*-4-nitrobenzyloxycarbonylamino)butanoate (24). The thiol **22** (4.0 g, 9.89 mmol), benzyl 2-ethylacrylate (**23**) (2.63 g, 13.5 mmol) and $CsCO_3$ (48.3 mg, 0.148 mmol) were mixed in degassed *i*-PrOH (30 mL) under an Ar atmosphere. The resulting mixture was stirred for 1.5 h at room temperature under an Ar atmosphere. The reaction was quenched by adding AcOH (0.32 g,

5.3 mmol), and the solvent was removed by evaporation. The residue was dissolved in EtOAc (150 mL), washed with H_2O (150 mL×2) and sat. NaCl (100 mL), successively, dried over Na_2SO_4, and the solvent was evaporated to dryness. The crude product was purified by flash column chromatography (7:3 to 2:1, EtOAc/hexane) to give the sulfide **24** (5.60 g, 95%, diastereomer mixture) as a yellow oil: $[\alpha]_D^{27}$ −1.24° (*c* 1.13, $CHCl_3$); IR (NaCl, neat) ν_{max} 3320, 3030, 2950, 1720, 1510, 1340, 1215 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.18 (d, 2H, *J* = 8.3 Hz, arom), 7.47 (d, 2H, *J* = 8.3 Hz, arom), 7.3 (m, 10H, Ph×2), 5.59 (d, 1H, *J* = 8.3 Hz, NH), 5.23–5.10 (m, 6H, ArC*H*$_2$×3), 4.5 (m, 1H, α-proton), 2.74 (dd, *J* = 8.7 and 12.8 Hz) and 2.73 (dd, *J* = 8.9 and 13.1 Hz) [1H, SC*H*$_2$CH], 2.6–2.5 (m, 4H, C*H*$_2$SCH*HCH*), 2.1 and 2.0 (2×m, 2H, C*H*$_2$CH$_2$S), 1.7 (m, 2H, C*H*$_2$CH$_3$), 0.87 (t, 3H, *J* = 7.6 Hz, CH$_2$C*H*$_3$). Anal. Calcd for $C_{31}H_{34}N_2O_8S$: C, 62.61; H, 5.76; N, 4.71. Found: C, 62.61; H, 5.79; N, 4.74.

Benzyl (2*S*)-4-[(2*R*,*S*)-2-(benzyloxycarbonyl)butyl-(*R*,*S*)-sulfinyl]-2-(*N*-4-nitrobenzyloxycarbonylamino)butanoate (25). Compound **25** was prepared by the same procedure as for the sulfoxide **16**. Purification by flash column chromatography (3:2 to 3:1, EtOAc/hexane) gave **25** (85%, diastereomer mixture) as a yellow oil: $[\alpha]_D^{27}$ −4.24° (*c* 0.99, $CHCl_3$); IR ν_{max} (NaCl, neat) 3250, 3040, 2940, 1720, 1520, 1340, 1215, 1020 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.2 (m, 2H, arom), 7.5 (m, 2H, arom), 7.3 (m, 10H, Ph×2), 6.1–5.9 (m, 1H, NH), 5.2–5.1 (m, 6H, ArC*H*$_2$×3), 4.5 (m, 1H, α-proton), 3.1–2.8 (m, 2H, SCH*HCH*), 2.7–2.6 (m, 3H, C*H*$_2$SC*H*HCH), 2.4 and 2.2 (2×m, 2H, C*H*$_2$CH$_2$S), 1.8 (m, 2H, C*H*$_2$CH$_3$), 0.9 (m, 3H, CH$_2$C*H*$_3$). Anal. Calcd for $C_{31}H_{34}N_2O_9S$: C, 60.97; H, 5.61; N, 4.59. Found: C, 60.25; H, 5.53; N, 4.69. HRMS (FAB, NBA) Calcd for $C_{31}H_{35}N_2O_9S$ (MH^+) 611.2063, found 611.2067.

Benzyl (2*S*)-4-[(2*R*,*S*)-2-(benzyloxycarbonyl)butyl-(*R*,*S*)-sulfonimidoyl]-2-(*N*-4-nitrobenzyloxycarbonylamino)butanoate (26). Compound **26** was synthesized by the same procedure as for the sulfoximine **17**. The product was purified by flash column chromatography (2:3, acetone/hexane) to give **26** (3.22 g, 68%, diastereomer mixture) as a pale yellow syrup: $[\alpha]_D^{26}$ −2.45° (*c* 0.94, $CHCl_3$); IR (NaCl, neat) ν_{max} 3300, 3040, 2940, 1720, 1520, 1340, 1200, 1050, 1010 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.2 (m, 2H, arom), 7.5 (m, 2H, arom), 7.3 (m, 10H, Ph×2), 6.2–6.0 (m, 1H, NH), 5.2–5.1 (m, 6H, ArC*H*$_2$×3), 4.5 (m, 1H, α-proton), 3.7 (m, 1H, SC*H*$_2$CH), 3.2–3.0 (m, 4H, C*H*$_2$SC*H*HC*H*), 2.54 (br s, 1H, S=NH), 2.4 and 2.2 (2×m, 2H, C*H*$_2$CH$_2$S), 1.71 (m, 2H, C*H*$_2$CH$_3$), 0.90 (m, 3H, CH$_2$C*H*$_3$). Anal. Calcd for $C_{31}H_{35}N_3O_9S$: C, 59.51; H, 5.64; N, 6.72. Found: C, 59.06; H, 5.64; N, 6.71. HRMS (EI) Calcd for $C_{31}H_{35}N_3O_9S$ (M+) 625.2094, found 625.2080.

Benzyl (2*S*)-2-(*N*-4-nitrobenzyloxycarbonylamino)-4-[(2*R*,*S*)-2-(benzyloxycarbonyl)butyl-(*R*,*S*)-*N*-(*o*-xylylene-phosphoryl)-sulfonimidoyl]butanoate (27). The sulfoximine **26** was dried by co-evaporation with dry CH_3CN. To a solution of the sulfoximine **26** (2.73 g, 4.36 mmol) and *o*-xylylene *N,N*-diethylphosphoramidite (1.04 g, 4.35 mmol) in dry CH_2Cl_2 (50 mL) was added 1*H*-tetrazole (351 mg, 5.01 mmol). The mixture was stirred for 3 h at room temperature under an Ar atmosphere. *tert*-Butyl hydroperoxide (70% aqueous solution, 600 μL, 4.38 mmol) was then added to the solution. The resulting solution was stirred for 20 min at room temperature. The reaction mixture was washed with satd $NaHCO_3$ (100 mL), H_2O (100 mL×2) and satd NaCl (100 mL), successively, dried over Na_2SO_4, and the solvent was evaporated to dryness. The crude product was purified by flash column chromatography (1:1, acetone/hexane) to give the *N*-phosphoryl sulfoximine **27** (2.88 g, 81.7%, diastereomer mixture) as a pale yellow syrup: $[\alpha]_D^{26}$ −7.87° (*c* 0.94, $CHCl_3$); IR (NaCl, neat) ν_{max} 3250, 3040, 2950, 1720, 1520, 1340, 1240, 1050, 1020 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.1 (m, 2H, arom), 7.5 (m, 2H, arom), 7.3 (m, 12H, Ph), 7.2 (m, 2H, Ph), 6.5 (m, 1H, NH), 5.2–5.0 [m, 10H, ArC*H*$_2$×3 and Ph(C*H*$_2$)$_2$], 4.4 (m, 1H, α-proton), 3.8 (m, 1H, SC*H*$_2$CH), 3.4 (m, 2H, CH$_2$C*H*$_2$S), 3.2–3.1 (m, 2H, SCH*HCH*), 2.5 and 2.4 (2×m, 2H, C*H*$_2$CH$_2$S), 1.7 (m, 2H, C*H*$_2$CH$_3$), 0.9 (m, 3H, CH$_2$C*H*$_3$); ^{31}P NMR ($CDCl_3$) δ −0.045 (53%), −0.428 (47%). Anal. calcd for $C_{39}H_{42}N_3O_{12}PS$: C, 57.99; H, 5.24; N, 5.20. Found: C, 57.82; H, 5.31; N, 5.22. HRMS (EI) calcd for $C_{39}H_{42}N_3O_{12}PS$ (M^+) 807.2227, found 807.2234.

(2*S*)-2-Amino-4-[(2*R*,*S*)-2-carboxybutyl-(*R*,*S*)-(*N*-phosphoryl)-sulfonimidoyl]butanoic acid (21). The *N*-phosphoryl sulfoximine **27** (1.24 g, 2.80 mmol) and 10% Pd on carbon (Aldrich, wet, Degussa type E101 NE/W, 1.36 g) were suspended in a mixture of MeOH–H_2O–AcOH (5:3:2, 40 mL), and H_2 gas was passed through the suspension for 24 h at 35 °C. The resulting mixture was filtered through Celite, and the filtrate was evaporated to dryness. The residual colorless syrup was diluted with H_2O (40 mL), washed with Et_2O (20 mL×2), and concentrated to dryness. The residue was applied to a Dowex 50W×8 column (H^+ form, 60 cm^3), and the column was washed with H_2O (200 mL). The eluent was changed to 5% pyridine, and the column was eluted until the solvent front reached 6 cm above the bottom of the column (ca. 100 mL of 5% pyridine). The eluent was changed again to H_2O, and the acidic fractions (ca. pH < 3.0) were combined, concentrated and lyophilized to afford the *N*-phosphoryl sulfoximine **21** (369 mg, 67%, diastereomer mixture) as a colorless powder: $[\alpha]_D^{26}$ +3.48° (*c* 1.04, H_2O); IR (KBr) ν_{max} 3600–2300 (br), 1720, 1520, 1340, 1240, 1050, 1020 cm^{-1}; ^1H NMR (400 MHz, D_2O) δ 4.07 (m, 1H, α-proton), 3.9 (m, 1H,

SCH_2CH), 3.7–3.4 (m, 3H, CH_2SCHH), 3.1 (m, 1H, SCH_2CH), 2.5 (m, 2H, CH_2CH_2S), 1.74 (q, 2H, $J=7.3$ Hz, CH_2CH_3), 0.95 (t, 3H, $J=7.4$ Hz, CH_2CH_3); ^{31}P NMR (200 MHz, $D_2O+K_2CO_3$, pH 8.9) δ −1.31 (36%), −1.46 (19%), −1.73 (21%), −1.97 (24%). HRMS (FAB, glycerol) calcd for $C_9H_{20}N_2O_8PS$ (MH^+) 347.0678, found 347.0687.

(4*R*,5*S*)-3-[(2*R*)-2-Ethyl-3-hydroxypropanoyl]-4-methyl-5-phenyl-2-oxazolidinone (29). The oxazolidinone **28** (4.0 g, 16.2 mmol) prepared from (1*S*,2*R*)-norephedrine[26b] was dissolved in dry CH_2Cl_2 (40 mL), and the solution was cooled in an ice salt bath at −15 °C. Dibutylboron triflate (1.0 M solution in CH_2Cl_2, 19.4 mL, 19.4 mmol) was added via syringe to the solution under an Ar protection, followed by the addition of dry NEt_3 (2.12 g, 21.0 mmol). The resulting solution was stirred for 20 min at −15 °C and then cooled to −78 °C. Formaldehyde gas, which was generated by heating paraformaldehyde (2.43 g, 80.9 mmol), was passed through the solution with a stream of Ar gas for 10 min. After the resulting mixture was stirred for 20 min at −78 °C, the flask was warmed to 0 °C with continuous stirring. After 1 h, the reaction was quenched by adding a phosphate buffer (pH 7.0, 1.0 M, 18.0 mL) and MeOH (54 mL). A mixture of MeOH–30% H_2O_2 (2:1, 60 mL) was added to the mixture at such a rate as to keep the internal temperature below 10 °C. After the reaction mixture was stirred for additional 1 h, the solvent was removed by evaporation. The residue was extracted with Et_2O (50 mL×3), and the combined Et_2O layers were washed with satd $NaHCO_3$ (100 mL) and satd NaCl (100 mL), successively, dried over Na_2SO_4, and was concentrated to dryness. The crude adduct had a diastereomeric purity of 75.3% as determined by HPLC (ChemcoPak®, silica gel column 4.6 mm×250 mm, 3:4, EtOAc/hexane). The desired diastereomer was purified by flash column chromatography (1:2, EtOAc/hexane) to afford the alcohol **29** (2.25 g, 50%) as a colorless oil: $[\alpha]_D^{22}$ +27.7° (*c* 1.00, $CHCl_3$); IR (NaCl, neat) ν_{max} 3430 (br), 2960, 1780, 1690, 1450, 1340, 1230, 1120 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.4 (m, 5H, Ph), 5.70 (d, 1H, $J=7.4$ Hz, OC*H*Ph), 4.85 (dq, 1H, $J=6.6$ and 7.4 Hz, CH_3CHN), 4.0–3.8 (m, 3H, COCH and CH_2OH), 2.66 (br s, 1H, OH), 1.7 (m, 2H, CH_2CH_3), 0.98 (t, 3H, $J=7.4$ Hz, CH_2CH_3), 0.90 (d, 3H, $J=6.6$ Hz, $CHCH_3$). Anal. calcd for $C_{15}H_{19}NO_4$: C, 64.97; H, 6.91; N, 5.05. Found: C, 65.00; H, 6.96; N, 4.93.

(4*R*,5*S*)-3-[(2*R*)-2-Ethyl-3-(methylsulfonyloxy)propanoyl]-4-methyl-5-phenyl-2-oxazolidinone (30). The alcohol **29** (1.19 g, 4.29 mmol) was mesylated by the standard procedure and purified by flash column chromatography (1:2, EtOAc/hexane) (100%): IR (NaCl, neat) ν_{max} 2970, 1780, 1695, 1345, 1210, 1170, 960 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.4 (m, 5H, Ph), 5.74 (d, 1H, $J=7.2$ Hz, OC*H*Ph), 4.82 (dq, 1H, $J=6.6$ and 7.2 Hz, CH_3CHN), 4.54 (dd, $J=9.4$ and 9.4 Hz) and 4.34 (dd, $J=4.2$ and 9.6 Hz) [2H, CH_2SO_2], 4.2 (m, 1H, $CHCH_2$), 3.05 (s, 3H, SO_2CH_3), 1.8 (m, 2H, CH_2CH_3), 1.01 (t, 3H, $J=7.5$ Hz, CH_2CH_3), 0.90 (d, 3H, $J=6.6$ Hz, $CHCH_3$). Anal. calcd for $C_{16}H_{21}NO_6S$: C, 54.07; H, 5.96; N, 3.94. Found: C, 54.24; H, 6.11; N, 3.79.

(4*R*,5*S*)-3-[(2*S*)-2-(Iodomethyl)butanoyl]-4-methyl-5-phenyl-2-oxazolidinone (31). The mesylate **30** (1.52 g, 4.3 mmol) was converted to the iodide by the general method (NaI, reflux in acetone) and purified by flash column chromatography (1:9, EtOAc/hexane) to give the iodide **31** (84%) as a colorless oil: $[\alpha]_D^{21}$ −57.1° (*c* 1.00, MeOH); IR (NaCl, neat) ν_{max} 2960, 1780, 1690, 1240, 1120 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.3 (m, 5H, Ph), 5.72 (d, 1H, $J=7.4$ Hz, OC*H*Ph), 4.86 (dq, 1H, $J=6.8$ and 6.8 Hz, CH_3CHN), 4.2 (m, 1H, $CHCH_2$), 3.43 (dd, $J=9.6$ and 9.6 Hz) and 3.28 (dd, $J=4.6$ and 9.6 Hz) [2H, CH_2I], 1.7 (m, 2H, CH_2CH_3), 0.98 (t, 3H, $J=7.5$ Hz, CH_2CH_3), 0.91 (d, 3H, $J=6.6$ Hz, $CHCH_3$). Anal. calcd for $C_{15}H_{18}INO_3$: C, 46.53; H, 4.69; N, 3.62. Found: C, 46.45; H, 4.68; N, 3.50.

Methyl (2*S*)-4-[(2*S*)-2-[[(4*R*,5*S*)-4-methyl-5-phenyl-2-oxazolidinone-3-yl]carbonyl]butylthio]-2-(*N*-4-nitrobenzyloxycarbonylamino)butanoate (32). The sulfide **32** was prepared from thiol **14** and the iodide **31** by the same procedure as for the sulfide **15**. The crude product was purified by flash column chromatography (1:4, EtOAc/hexane) (100%): $[\alpha]_D^{26}$ −52.8° (*c* 1.04, MeOH); IR (NaCl, neat) ν_{max} 3340, 2940, 1780, 1720, 1695, 1605, 1520, 1340, 1220 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.18 (d, 2H, $J=8.8$ Hz, arom), 7.50 (d, 2H, $J=8.8$ Hz, arom), 7.4 (m, 3H, Ph), 7.3 (m, 2H, Ph), 5.72 (d, 1H, $J=7.2$ Hz, OC*H*Ph), 5.64 (d, 1H, $J=8.0$ Hz, NH), 5.21 (s, 2H, $ArCH_2$), 4.86 (dq, 1H, $J=6.8$ and 7.0 Hz, CH_3CHN), 4.5 (m, 1H, α-proton), 4.1 (m, 1H, SCH_2CH), 3.78 (s, 3H, $COOCH_3$), 2.86 (dd, 1H, $J=9.6$ and 13.4 Hz, SCH_2CH), 2.7–2.5 (m, 3H, CH_2SCHH), 2.1 (m, 2H, CH_2CH_2S),1.7 (m, 2H, CH_2CH_3), 1.0 (m, 6H, $CHCH_3$ and CH_2CH_3). Anal. calcd for $C_{28}H_{33}N_3O_9S$: C, 57.23; H, 5.66; N, 7.15. Found: C, 56.98; H, 5.61; N, 7.13.

Methyl (2*S*)-4-[(2*S*)-2-[[(4*R*,5*S*)-4-methyl-5-phenyl-2-oxazolidinone-3-yl]carbonyl]butyl-(*R*,*S*)-sulfinyl]-2-(*N*-4-nitrobenzyloxycarbonylamino)butanoate (33). The sulfide **32** was oxidized with $NaIO_4$ by the same procedure as for the synthesis of **16**. The product was purified by flash column chromatography (8:1, EtOAc/hexane) to give the sulfoxide **33** (84%, diastereomer mixture) as a pale yellow oil: IR (NaCl, neat) ν_{max} 3300, 2960, 1780, 1720, 1695, 1520, 1340, 1200, 1040 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.20 (d, 2H, $J=8.6$ Hz, arom), 7.51 (d, 2H, $J=8.6$ Hz, arom), 7.4 (m, 3H, Ph), 7.3 (m, 2H, Ph), 6.07

(d, $J=7.8$ Hz) and 5.80 (d, $J=7.6$ Hz) [1H, NH], 5.72 (d, $J=7.4$ Hz) and 5.70 (d, $J=7.4$ Hz) [1H, OC*H*Ph], 5.2 (m, 2H, ArCH_2), 4.8 (m, 1H, CH_3CHN), 4.5 (m, 1H, α-proton), 4.3 (m, 1H, SCH_2CH), 3.80 (s) and 3.79 (s) [3H, $COOCH_3$], 3.41 (dd, $J=10.6$ and 13.0 Hz) and 3.35 (dd, $J=10.2$ and 12.8 Hz) [1H, SCH_2CH], 3.0–2.7 (m, 3H, CH_2SCHH), 2.3 (m, 2H, CH_2CH_2S), 1.8 (m, 2H, CH_2CH_3), 1.00 (t, 3H, $J=7.4$ Hz, CH_2CH_3), 0.89 (d, 3H, $J=6.6$ Hz, $CHCH_3$). Anal. calcd for $C_{28}H_{33}N_3O_{10}S$: C, 55.71; H, 5.51; N, 6.96. Found: C, 55.53; H, 5.62; N, 6.76.

Methyl (2*S*)-4-[(2*S*)-2-[[(4*R*,5*S*)-4-methyl-5-phenyl-2-oxazolidinone-3-yl]carbonyl]butyl-(*R*,*S*)-sulfonimidoyl]-2-(*N*-4-nitrobenzyloxycarbonylamino)butanoate (34). The sulfoxide **33** was treated with 1.8 equiv of MSH as for the synthesis of **17** to give the sulfoximine **34** (100%, diastereomer mixture) as a colorless foam: ^{1}H NMR ($CDCl_3$) δ 8.18 (d, 2H, $J=8.8$ Hz, arom), 7.51 (d, 2H, $J=8.6$ Hz, arom), 7.4 (m, 3H, Ph), 7.3 (m, 2H, Ph), 6.07 (d, $J=8.0$ Hz) and 5.85 (d, $J=8.0$ Hz) [1H, NH], 5.73 (d, 1H, $J=7.2$ Hz, OCH*P*h), 5.2 (m, 2H, ArCH_2), 4.8 (m, 1H, CH_3CHN), 4.5 (m, 1H, α-proton), 4.3 (m, 1H, SCH_2CH), 3.80 (s) and 3.79 (s) [3H, $COOCH_3$], 3.39 (dd, 1H, $J=10.7$ and 13.1 Hz, SCH_2CH), 3.1–2.6 (m, 3H, CH_2SCHH), 2.3 (m, 2H, CH_2CH_2S), 1.8 (m, 2H, CH_2CH_3), 1.00 (t, 3H, $J=7.4$ Hz, CH_2CH_3), 0.89 (d, 3H, $J=6.6$ Hz, $CHCH_3$). The sulfoximine **34** was used for the next reaction without purification.

Methyl (2*S*)-4-[(1*R*,4*S*)-4-ethyl-4,5-dihydro-1,3-dioxo-1,2-thiazol-1-yl]-2-(*N*-4-nitrobenzyloxycarbonylamino)butanoate (35a)

Methyl (2*S*)-4-[(1*S*,4*S*)-4-ethyl-4,5-dihydro-1,3-dioxo-1,2-thiazol-1-yl]-2-(*N*-4-nitrobenzyloxycarbonylamino) butanoate (35b). A solution of K_2CO_3 (616 mg, 4.46 mmol) in H_2O (4 mL) was added to a solution of the sulfoximine **34** (6.90 g, 11.1 mmol) in THF–H_2O (10:3, 130 mL) at 0 °C. After 20 min, 30% H_2O_2 aqueous solution (2 mL) was added to the mixture at 0 °C. The resulting mixture was stirred for 3 h at 0 °C. To the solution was added 2 M HCl (10 mL) dropwise, and the mixture was evaporated. The residue was dissolved in EtOAc (200 mL), and the solution was washed with 0.3 M HCl (100 mL×3), H_2O (100 mL) and satd NaCl (100 mL), successively, dried over Na_2SO_4, and was evaporated to dryness to give the cyclic sulfoximine **35a** and **35b**, as a colorless foam. The two diastereomers were separated by flash column chromatography (7:1, EtOAc/hexane, then 2:1, EtOAc/acetone) to afford the cyclic sulfoximine **35a** (1.15 g, 23.4%) and the cyclic sulfoximine **35b** (1.35 g, 27.5%) as colorless crystals: **35a**; $[\alpha]_D^{25}$ +51.9° (*c* 1.03, $CHCl_3$); mp 142.3–143.3 °C; IR (KBr) ν_{max} 3360, 2960, 1735, 1705, 1680, 1540, 1350, 1205, 1010 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (d, 2H, $J=8.8$ Hz, arom), 7.52 (d, 2H, $J=8.8$ Hz, arom), 6.12 (d, 1H, $J=7.7$ Hz, NH), 5.21 (2×d, 2H, $J=13.6$ Hz, ArCH_2), 4.5 (m, 1H, α-proton), 3.80 (s, 3H, $COOCH_3$), 3.68 (dd, $J=8.8$ and 13.9 Hz) and 3.48 (dd, $J=8.4$ and 13.9 Hz) [2H, SCH_2CH], 3.6 (m, 2H, CH_2CH_2S), 3.1 (m, 1H, SCH_2CH), 2.6 and 2.3 (2×m, 2H, CH_2CH_2S), 2.1 and 1.6 (2×m, 2H, CH_2CH_3), 0.97 (t, 3H, $J=7.3$ Hz, CH_2CH_3). Anal. calcd for $C_{18}H_{23}N_3O_8S$: C, 48.97; H, 5.25; N, 9.52. Found: C, 49.01; H, 5.24; N, 9.54. **35b**; $[\alpha]_D^{25}$ +50.3° (*c* 0.99, $CHCl_3$); mp 140.3–141.4 °C; IR (KBr) ν_{max} 3340, 2940, 1740, 1680, 1520, 1350, 1205, 1010 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (d, 2H, $J=8.8$ Hz, arom), 7.51 (d, 2H, $J=8.8$ Hz, arom), 6.40 (d, 1H, $J=8.0$ Hz, NH), 5.20 (s, 2H, ArCH_2), 4.5 (m, 1H, α-proton), 4.01 (dd, $J=9.9$ and 14.3 Hz) and 3.19 (dd, $J=4.4$ and 14.3 Hz) [2H, SCH_2CH], 3.78 (s, 3H, $COOCH_3$), 3.7 (m, 2H, CH_2CH_2S), 3.0 (m, 1H, SCH_2CH), 2.5 and 2.3 (2×m, 2H, CH_2CH_2S), 1.9 and 1.7 (2×m, 2H, CH_2CH_3), 0.98 (t, 3H, $J=7.3$ Hz, CH_2CH_3). Anal. calcd for $C_{18}H_{23}N_3O_8S$: C, 48.97; H, 5.25; N, 9.52. Found: C, 48.84; H, 5.23; N, 9.49.

(2*S*)-2-Amino-4-[(1*R*,4*S*)-4-ethyl-4,5-dihydro-1,3-dioxo-1,2-thiazol-1-yl]butanoic acid (36a). The cyclic sulfoximine **35a** (1.24 g, 2.80 mmol) and 10% Pd on carbon (123 mg) were suspended in a mixture of MeOH–EtOAc (1:1, 100 mL). A few drops of AcOH was added to the solution, and H_2 gas was passed through the mixture at room temperature. After 4 h, the solution was filtered through Celite, and the filtrate was evaporated to dryness. The pale yellow residue was applied to a Dowex 50W×8 column (H^+ form, 30 cm^3), and the column was eluted successively with H_2O, 5% pyridine and 3% NH_4OH. The ninhydrin positive fractions eluted with 3% NH_4OH were combined and lyophilized to afford the crude product **36a** (634 mg) as a colorless powder. This material was recrystallized from H_2O (8 mL)–EtOH (3 mL) to give the pure cyclic sulfoximine **36a** (479 mg, 69%): $[\alpha]_D^{25}$ −77.71° (*c* 0.96, H_2O); mp 203.1–203.5 °C (dec.); IR (KBr) ν_{max} 3100–2400 (br), 2960, 1670, 1580, 1390, 1220, 1010 cm^{-1}; ^{1}H NMR (400 MHz, D_2O) δ 4.1–3.8 (m, 5H, α-proton and CH_2SCH_2), 3.4 (m, 1H, SCH_2CH), 2.5 (m, 2H, CH_2CH_2S), 1.9 and 1.6 (2×m, 2H, CH_2CH_3), 0.94 (t, 3H, $J=7.4$ Hz, CH_2CH_3). Anal. calcd for $C_9H_{16}N_2O_4S$: C, 43.54; H, 6.50; N, 11.28. Found: C, 43.28; H, 6.51; N, 11.30. HRMS (FAB, glycerol) calcd for $C_9H_{17}N_2O_4S$ (MH^+) 249.0909, found 249.0933.

(2*S*)-2-Amino-4-[(1*S*,4*S*)-4-ethyl-4,5-dihydro-1,3-dioxo-1,2-thiazol-1-yl]butanoic acid (36b). Compound **36b** was synthesized according to the same procedure as for the compound **36a**. After passing through a Dowex 50W×8 column (H^+ form, 30 cm^3) in the same way, the ninhydrin positive fractions eluted with 3% NH_4OH were combined and lyophilized to afford the crude product **36b** (490 mg) as a colorless powder. This material was

recrystallized from 0.5 M HCl (5 mL)–EtOH (3 mL) to give the pure cyclic sulfoximine **36b** (265 mg, 35%): $[\alpha]_D^{25}$ +78.02° (*c* 1.01, 0.5 N HCl); IR (KBr) ν_{max} 3100–2300 (br), 2960, 1680, 1580, 1520, 1400, 1220, 1010 cm^{-1}; ^1H NMR (400 MHz, D_2O) δ 4.42 (dd, J = 10.1 and 15.2 Hz) and 3.64 (dd, J = 4.6 and 15.4 Hz) [2H, SCH_2CH], 4.0–3.8 (m, 3H, α-proton and CH_2CH_2S), 3.3 (m, 1H, SCH_2CH), 2.4 (m, 2H, CH_2CH_2S), 1.8 (m, 2H, CH_2CH_3), 0.94 (t, 3H, J = 7.4 Hz, CH_2CH_3). Anal. calcd for $C_9H_{16}N_2O_4S$: C, 43.54; H, 6.50; N, 11.28. Found: C, 43.30; H, 6.50; N, 11.29.

(2*S*)-2-Amino-4-[(2*S*)-2-carboxybutyl-(*R*)-sulfonimidoyl] butanoic acid (3a). To a cooled solution of the cyclic sulfoximine **36a** (152.1 mg, 0.613 mmol) in H_2O (2 mL) was added 1.16 M KOH aqueous solution (1.06 mL, 1.23 mmol) dropwise at 0 °C. The mixture was stirred for 16 h at 4 °C. The reaction mixture was directly applied to a Dowex 50W×8 column (NH_4^+ form, 20 cm^3). The column was eluted with H_2O, and the ninhydrin positive fractions were collected and lyophilized to afford the sulfoximine **3a** (172 mg, 99%) as a colorless powder: IR (KBr) ν_{max} 3500–2400 (br), 1595 (br),1400, 1200, 1000 cm^{-1}; ^1H NMR (400 MHz, D_2O) δ 3.89 (t, 0.8H, J = 6.2 Hz, α-proton in major isomer), 3.85 (t, 0.2H, J = 6.6 Hz, α-proton in minor isomer), 3.68 (dd, 0.2H, J = 10.1 and 14.7 Hz, SCH_2CH in minor isomer), 3.63 (dd, 0.8H, J = 9.5 and 14.7 Hz, SCH_2CH in major isomer), 3.5–3.2 (m, 3H, CH_2SCHH), 2.7 (m, 1H, SCH_2CH), 2.4 (m, 2H, CH_2CH_2S), 1.6 (m, 2H, CH_2CH_3), 0.92 (t, 3H, J = 7.6 Hz, CH_2CH_3). HRMS (FAB, glycerol) calcd for $C_9H_{19}N_2O_5S$ (MH^+) 267.1016, found 267.1013.

(2*S*)-2-Amino-4-[(2*S*)-2-carboxybutyl-(*S*)-sulfonimidoyl] butanoic acid (3b). Compound **3b** was synthesized according to the same procedure as for the compound **3a**. The crude product was purified by a Dowex 50W×8 column (NH_4^+ form, 20 cm^3) eluted with H_2O, and the ninhydrin positive fractions were combined and lyophilized to afford the sulfoximine **3b** (43 mg, 97%) as a colorless powder: IR (KBr) ν_{max} 3500–2400 (br), 1590 (br), 1400, 1200, 1000 cm^{-1}; ^1H NMR (400 MHz, D_2O) δ 3.89 (t, 0.8H, J = 6.2 Hz, α-proton in major isomer), 3.85 (t, 0.2H, J = 6.4 Hz, α-proton in minor isomer), 3.68 (dd, 0.8H, J = 10.2 and 14.6 Hz, SCH_2CH in major isomer), 3.62 (dd, 0.2H, J = 9.8 and 14.6 Hz, SCH_2CH in minor isomer), 3.4–3.2 (m, 3H, CH_2SCHH), 2.7 (m, 1H, SCH_2CH), 2.4 (m, 2H, CH_2CH_2S), 1.6 (m, 2H, CH_2CH_3), 0.92 (t, 3H, J = 7.5 Hz, CH_2CH_3). HRMS (FAB, glycerol) calcd for $C_9H_{19}N_2O_5S$ (MH^+) 267.1016, found 267.1023.

Enzyme assay. Enzyme activity was determined by measuring ADP formation with a pyruvate kinase (PK)-lactate dehydrogenase (LDH) coupled enzyme assay.[30] A standard assay mixture consisted of 0.75 mM L-Glu, 150 mM L-2-aminobutyric acid, 1 mM ATP, 10 mM $MgSO_4$, 1 mM phosphoenolpyruvate, 0.24 mM NADH, 10 unit PK, 25 unit LDH, 0.1 M KCl in 0.1 M Tris–HCl buffer (pH 7.5). The second-order rate constants for slow-binding inhibition (k_{inact}/K_i) were calculated from a plot of the pseudo first-order rate constants (k_{obs}) of enzyme inactivation versus inhibitor concentration ([I]) by regression to $k_{obs} = k_{inact}[I]/(K_i + [I])$ or $k_{obs} = (k_{inact}/K_i)[I]$ when saturation or no saturation was observed, respectively. In the case of the phosphinic acid **2**, saturation was observed and the values of K_i and k_{inact} were determined separately. On the other hand, no saturation was observed with the sulfoximine **3** and L-BSO up to 10 μM and 1 mM, respectively, beyond which rapid enzyme inactivation did not enable the initial reaction rate to be measured by the conventional method. The values of k_{obs} were calculated from the progress curves[31] in which the reaction was initiated by adding the enzyme to the standard assay solution containing varying concentrations of the inhibitor. The overall inhibition constants (K_i^*) for slow binding were obtained from a plot of the steady state inhibited velocities (v_s) versus [I] by nonlinear least-squares analysis. The values of v_s were obtained by incubating the enzyme and various concentrations of the inhibitor in the standard assay mixture without L-Glu at 37 °C for 30 min to establish the binding equilibrium, followed by adding L-Glu (final concn 0.75 mM) to start the assay for enzyme activity.

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