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Matrix Metalloproteinase Inhibitors Based on the 3-Mercaptopyrrolidine Core

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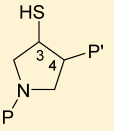
ABSTRACT: New series of pyrrolidine mercaptosulfide, 2-mercaptocyclopentane arylsulfonamide, and 3-mercapto-4-arylsulfonamidopyrrolidine matrix metalloproteinase inhibitors (MMPIs) were designed, synthesized, and evaluated. Exhibiting unique properties over other MMPIs (e.g., hydroxamates), these newly reported compounds are capable of modulating activities of several MMPs in the low nanomolar range, including MMP-2 (~2 to 50 nM), MMP-13 (~2 to 50 nM), and MMP-14 (~4 to 60 nM). Additionally these compounds are selective to intermediate- and deep-pocket MMPs but not shallow-pocketed MMPs (e.g., MMP-1, ~850 to >50 000 nM; MMP-7, ~4000 to >25 000 nM). Our previous work with the mercaptosulfide functionality attached to both cyclopentane and pyrrolidine frameworks demonstrated that the cis-(3*S*,4*R*)-stereochemistry was optimal for all of the MMPs tested. However, in our newest compounds an interesting shift of preference to the trans form of the mercaptosulfonamides was observed with increased oxidative stability and biological compatibility. We also report several kinetic and biological characteristics showing that these compounds may be used to probe the mechanistic activities of MMPs in disease.



INTRODUCTION

Matrix metalloproteinases (MMPs) are a family of metzincin endopeptidases chiefly responsible for remodeling the extracellular matrix (ECM). Further division of the family on the basis of domain arrangement and substrate specificity results in six subgroups: collagenases (MMP-1, -8, -13, and -18); gelatinases (MMP-2 and -9); stromelysins (MMP-3, -10, and -11); matrilysins (MMP-7 and -26); membrane-type MMPs (MMP-14, -15, -16, -17, -24, and -25); and others (MMP-12, -19, -20, -21, -23, -27, and -28). Members of this family also process nonmatrix and cell surface signaling proteins to serve important roles in a number of physiological (e.g., epithelial morphogenesis, neurogenesis) and pathological processes (e.g., cancer, inflammation, cardiovascular disease).[1−3] In light of these pathological roles and in the pursuance of developing targeted treatments, biological and pharmacological regulation of MMPs continues to be extensively studied. Targeting particular MMPs, however, requires careful consideration and places a premium on selectivity so that side effects are minimized. For example in ischemic events (e.g., stroke), proteolytic activities of MMP-2 and -9 result in initial blood−brain barrier disruption,[4] while during later stages MMP-9 may exhibit neuroprotective effects.[5] During atherosclerosis MMP-1 and -8 are suggested to partake in plaque destabilization,[6,7] while MMPs -2 and -9 facilitate smooth muscle cell migration and overall plaque stability.[8] Hence, targeting specific MMPs is disease dependent.

The activity of MMPs is controlled through several mechanisms: gene expression; compartmentalization; zymogen activation; and important to this discussion, enzyme inhibition. Endogenously, the four tissue inhibitors of metalloproteinases (TIMP-1, -2, -3, and -4) are primarily responsible for MMP inhibition. Since TIMPs exhibit picomolar to nanomolar affinities for each of the MMPs, they were initially proposed as the ideal inhibitors to block the pathological activities of MMPs.[9] However, TIMPs are essentially broad-spectrum inhibitors, lacking selectivity for individual MMPs, and themselves are involved in various non-MMP related physiological processes (e.g., apoptosis).[10−13] This led to a decade's endeavor to develop small molecule MMP inhibitors (MMPIs). Generally MMPIs mimic substrate peptide structures, incorporating a noncleavable zinc-binding group in place of the scissile bond, and thus presumably mimic substrate binding with the enzyme active site. Since the catalytic domains of all MMPs present a high degree of homology,[14] the specificity and selectivity of inhibition by these early compounds were minimal, with numerous off-target effects and binding promiscuity observed.[15]

Characterized by our group and others,[16,17] the principal specificity pocket, designated the S_1' pocket, is a hydrophobic cavity positioned to the right (i.e., prime side) of the catalytic zinc. Critical to the shape of this pocket is the identity of one key residue in the catalytic domain's second helix that determines the pocket's relative depth.[18] The S_1' pocket thus may be shallow (MMP-1 and -7), intermediate (MMP-2, -8, -9, and -26), or deep (MMP-3, -12, and -14) and offers a degree of individuality to MMPs that can be exploited to ascertain specificity.[16,17]

The nature of the zinc-binding group (ZBG) is equally important to both potency and specificity of the MMPI. Numerous ZBGs have been utilized, including the widely incorporated hydroxamates and the lesser used carboxylates, phosphinyls, and mercaptans.[19] Hydroxamate is the most

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potent ZBG, coordinating Zn^{2+} in a bidentate fashion to form a complex with distorted trigonal bipyramidal geometry. However, the hydroxamate affinity for Zn^{2+} seems to overwhelm specific protease binding contributions by other groups within the structure. Consequently, the selectivity of hydroxamate-based MMPIs was reduced and resulted in side effects such as tendinitis fibromyalgia and musculoskeletal abnormalities in oncology clinical trials.[20,21] These results stimulated our own interest in developing MMPIs with alternative ZBGs that offer adequate levels of binding without sacrificing specificity.

The popularity of thiol-based angiotensin-converting enzyme inhibitors (e.g., (2*S*)-1-[(2*S*)-2-methyl-3-sulfanylpropanoyl]-pyrrolidine-2-carboxylic acid)[22] and the effectiveness of thiols (-SH) as metal binding ligands, despite some limitations,[23] led us to investigate MMPIs containing the mercaptan ZBG. The earliest of our compounds were peptidomimetic mercaptosulfides; however, these MMPIs were readily deactivated by hydrolysis and by oxidation of the thiol.[24−26] A second generation of mercaptosulfides attached to a cyclopentane or pyrrolidine core was produced.[27−29] Incorporation of the 1,2-mercaptosulfide moiety into a ring system was designed from structure-based computer modeling using MacroModel (version 7) and chemical intuition to increase binding affinity by restricting rotation of the C−C bond connected to the two sulfurs, leading to development of the 1,2-cyclopentanemercaptosulfide moiety as a new pharmacophore. These novel inhibitors with a cyclopentane ring showed moderately improved inhibitory activities and significantly higher oxidation stability. Further modification of the ring from cyclopentane to pyrrolidine was carried out to enhance water solubility and allow for a greater degree of functionality (i.e., so that additional side chains that interact with the nonprime side of the enzyme active site may be appended) (Figure 1).[28,30] This

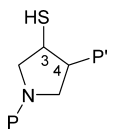

Figure 1. Representation of the pyrrolidine scaffold to functionalize the MMPIs for interaction with the nonprime side of the enzyme active site (P).

structural change improved MMPI stability, leading to enhanced bioavailability and increased potency of inhibition.[17,31,32] A final modification of the peptidomimetic part to a mercaptosulfonamide, again attached to a cyclopentane or pyrrolidine core, led to further increases in ZBG stability and inhibitor potency and selectivity.[33]

An obvious factor determining the effectiveness of these MMPIs, in addition to the nature of the P and P′ residues, is the relative and absolute stereochemistry at C-3 and C-4 needed to maximize MMP binding. Initial work with the mercaptosulfide functionality[26] attached to both cyclopentane and pyrrolidine frameworks demonstrated that the *cis*-(3*S*,4*R*)-stereochemistry was optimal for all of the MMPs tested.[17,31,32] However, in the mercaptosulfonamides the trans stereoisomer turned out to be the most potent, with little preference between absolute configurations.

We have produced an array of mercaptosulfonamide MMPIs capable of modulating the activity of numerous MMPs in the low nanomolar range and report several kinetic and biological characteristics to show how these compounds may be used for probing the mechanistic activities of MMPs in disease.

RESULTS

Structure−Activity Relationship Studies. *Mercaptosulfide Inhibitors.* The synthesis of mercaptosulfide inhibitors with a pyrrolidine pharmacophore is outlined in Scheme 1. Pyrroline (**1**) was Boc protected (**2**) and oxidized with *m*-CPBA to give the pyrrolidine epoxide (**3**) that was treated with thiolbenzoic acid over alumina to afford the benzoylthioalcohol intermediate (**4**). The benzoyl group of **4** was removed with EtONa in EtOH and *t*-Boc protecting group was introduced in one pot, affording compound **5**, which was converted into the differentially protected *cis*-dithio compound (**6**) via the Mitsunobu reaction.[34] The acetyl group of intermediate **6** was selectively removed with $MeNH_2$ in MeOH, and the resulting thiol was coupled with (*S*)-2-bromo-4-methylpentanoic acid to afford the S-alkylated acid intermediate (**7**). The acid (**7**) was coupled with phenylalanine *N*-methylamide to give the key intermediate **8**. *N*-Boc group of **8** was selectively removed in HCl ethyl acetate solution (**9**) and coupled with carboxylic acid to afford intermediate **10** that was treated with HCl in acetic acid to give the mercaptosulfide MMPIs (**11**). A series of pyrrolidine mercaptosulfides with varying N-substituents was initially synthesized to evaluate the ability of the P residue (Table 1, Figure 2) to confer potency and selectivity.[33] These compounds were tested against MMP-1, -2, -3, -7, -9, and -14. As the size of the P residue increased (**11b** vs **11c−e**), potency against the enzymes was generally increased but no substantial changes in selectivity were observed. Notably, potency against MMP-3 (stromelysin 1) was dramatically increased as the chain of the *N*-phthalimidoacyl group (**11c−11e**) was lengthened.

Cyclopentane Mercaptosulfonamide Inhibitors. To enhance the overall biological stability of these compounds, the P′ residue of the 3-mercaptopyrrolidine inhibitors was changed from a peptidomimetic alkylsulfide group to an arylsulfonamide.[35,36] This modification was intended to enhance enzyme−inhibitor binding through hydrogen bonding to the enzyme backbone and guide the hydrophobic substituent into the S_1' pocket.[37] On the nonprime side of the compounds, a cyclopentane scaffold was first employed to determine effective aryl substituents and the stereochemical requirements for inhibition in this new class of compounds.

The stereoisomeric 2-mercaptocyclopentane arylsulfonamides were synthesized from *trans*-2-azidocyclopentanol as outlined in Scheme 2. Kinetic resolution of racemic *trans*-2-azidocyclopentanol **12** using lipase Amano AK-20 yielded (1*S*,2*S*) **13** and acetylated (1*R*,2*R*)-**14** that was treated with based to give (1*R*,2*R*)-**15** as described similarly by Ami and Ohrui.[38] Each of these chiral *trans*-2-azidocyclopentanols was reduced to amino alcohol, and then the amino group was protected with Boc to afford **16**. Compound **16** was converted through Mitsunobu reaction[34] into compound **17**, which was first treated with HCl in MeOH to remove both protecting groups and then with arylsulfonyl chloride in DCM to give chiral *cis*-mercaptosulfonamide **18**. For the synthesis of chiral *trans*-mercaptosulfonamide, chiral *trans*-2-azidocyclopentanol (**12**) was converted to chiral *cis*-2-azidocyclopentanol (**20**) through Mitsunobu reaction and hydrolysis. The azide group of compound **20** was reduced to amine and subsequently treated with arylsulfonyl chloride to give intermediate **21**. The hydroxyl group of **21** was converted to acetylthio group through

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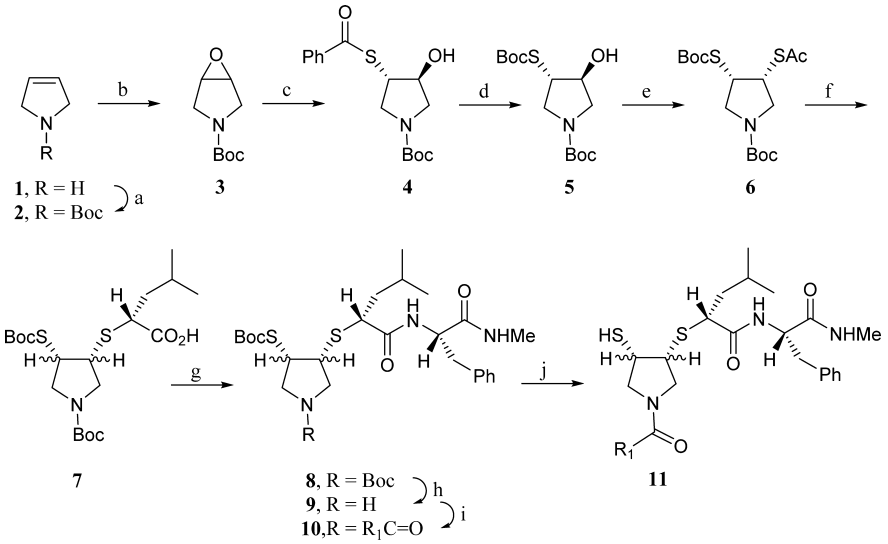
Scheme 1. Synthesis of Pyrrolidine Mercaptosulfide Inhibitors[a]



[a]Reagents and conditions: (a) Boc_2O, CH_2Cl_2, 0 °C; (b) *m*-CPBA, CH_2Cl_2, 0 °C to rt; (c) Al_2O_3, PhCOSH, Et_2O, rt; (d) EtONa/EtOH, Boc_2O, 0 °C; (e) Ph_3P, DEAD, AcSH, THF, 0 °C to rt; (f) (2*S*)-2-bromo-4-methylpentanoic acid, K_2CO_3, DMF, rt; (g) L-Phe-NHMe, EDCI, HOBt, Et_3N, CH_2Cl_2, 0 °C to rt; (h) HCl–EtOAc; (i) R_1CO_2H, DCC, HOBt, Et_3N, CH_2Cl_2, 0 °C to rt; (j) HCl/AcOH, rt.

Table 1. Effect of N-Substituent on MMP Inhibition by Pyrrolidine Mercaptosulfides

	K_i^{app} (nM)					
compd[a]	MMP-1	MMP-2	MMP-3	MMP-7	MMP-9	MMP-14
11a	260	200	4100	230	5.3	310
11b	99	14	990	91	5.7	6.2
11c	110	17	300	50	4.9	70
11d	75	8.5	31	12	3.9	6.0
11e	52	1.7	1.9	11	0.98	7.0

[a]Each inhibitor was a 1:1 mixture of *cis*-(3*R*,4*S*) and *cis*-(3*S*,4*R*) diastereomers.

Mitsunobu reaction to give compound **22**, which was treated with HCl in MeOH to give the desired chiral *trans*-mercaptosulfonamides (**23**). Compound **23f** was obtained directly from *trans*-azidocyclopentanol through azide reduction and amine sulfonation.

With this new set of inhibitors (Table 2, Figure 3), investigations into the effect of P′ aryl group substitution demonstrated that the 4-phenoxyphenyl (**23c**) maximized inhibition of intermediate (MMP-2 and -9) and deep (MMP-13 and -14) S_1' pocket MMPs when compared to those incorporating a phenyl (**23a**) or biphenyl (**23b**) group. In contrast to the preferred cis stereochemistry observed with our

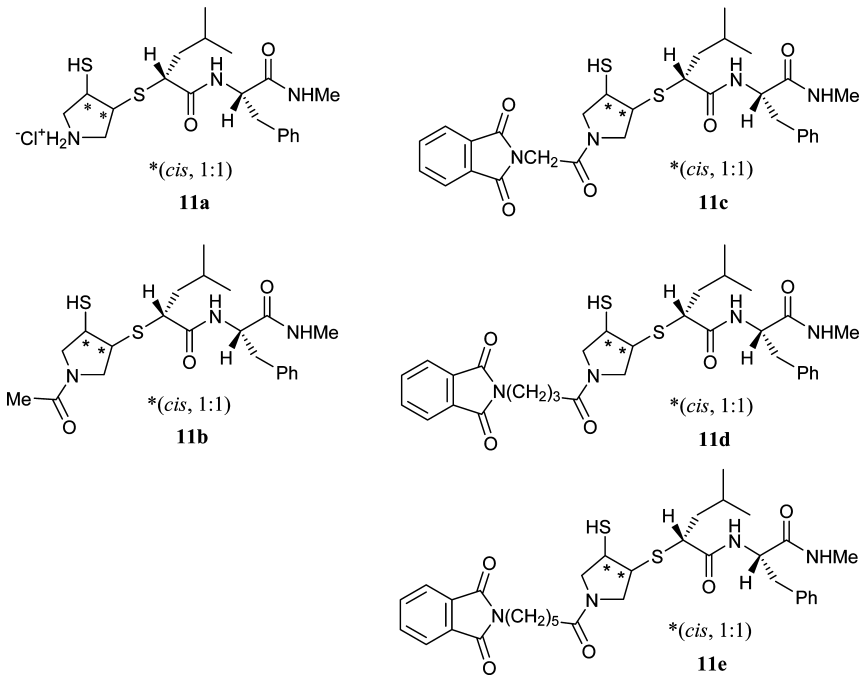


Figure 2. Structural representation of compounds **11a–e** presented in Table 1.

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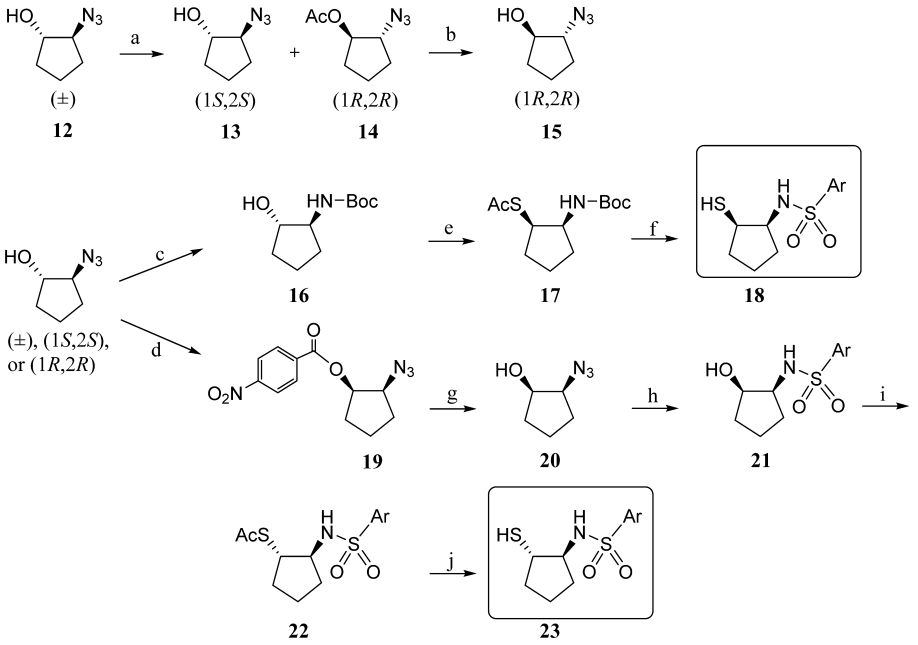
Scheme 2. Stereospecific Synthesis of 2-Mercaptocyclopentane Arylsulfonamides[a]



[a]Reagents and conditions: (a) Amano AK-20 lipase, isopropenyl acetate, *tert*-butyl methyl ether, rt; (b) LiOH, THF−MeOH−H_2O, rt; (c) (1) PPh_3, THF−H_2O, heat; (2) $NaHCO_3$, Boc_2O, 0 °C to rt; (d) 4-nitrobenzoic acid, PPh_3, DEAD, THF, 0 °C to rt; (e) AcSH, PPh_3, DEAD, THF, 0 °C to rt; (f) (1) 2 N HCl in MeOH; (2) $ArSO_2Cl$, Et_3N, CH_2Cl_2, 0 °C to rt; (g) LiOH, THF−MeOH−H_2O, rt; (h) (1) PPh_3, THF−H_20, heat; (2) $NaHCO_3$, $ArSO_2Cl$, 0 °C to rt; (i) AcSH, PPh_3, DEAD, THF, 0 °C to rt; (j) $MeNH_2$, MeOH, rt.

Table 2. MMP Inhibition by 2-Mercaptocyclopentane Arylsulfonamides

	K_i^{app} (nM)						
compd	MMP-1	MMP-2	MMP-3	MMP-7	MMP-9	MMP-13	MMP-14
18a	>1.0 × 10^5	810	>2.0 × 10^5	>2.0 × 10^4	970	200	410
18b	>2.0 × 10^5	~2.5 × 10^4	>2.0 × 10^5	>2.0 × 10^5	>1600	>1.0 × 10^5	~2.5 × 10^4
18c		430		>1.0 × 10^5	280	160	68
23a	>2.0 × 10^4	7200	>2.0 × 10^5	>5.0 × 10^4	9600	3400	3000
23b	~1.0 × 10^4	240	~1.0 × 10^4		630	230	1200
23c	3400	18	>2.0 × 10^5	>2.0 × 10^4	25 ± 7	15	18
23d	2100	4.6	>2.0 × 10^5	>1.0 × 10^5	38 ± 10	17	31
23e		67	>1.0 × 10^5	3.6 × 10^4	42 ± 3	10	10
23f	>2.0 × 10^5	~5.0 × 10^4	>2.0 × 10^5	>2.0 × 10^5	~2.0 × 10^5	~1.0 × 10^5	>2.0 × 10^5
23g	>2.0 × 10^5	5700	>2.0 × 10^5	5700	4300	2.8 × 10^4	>2.5 × 10^4

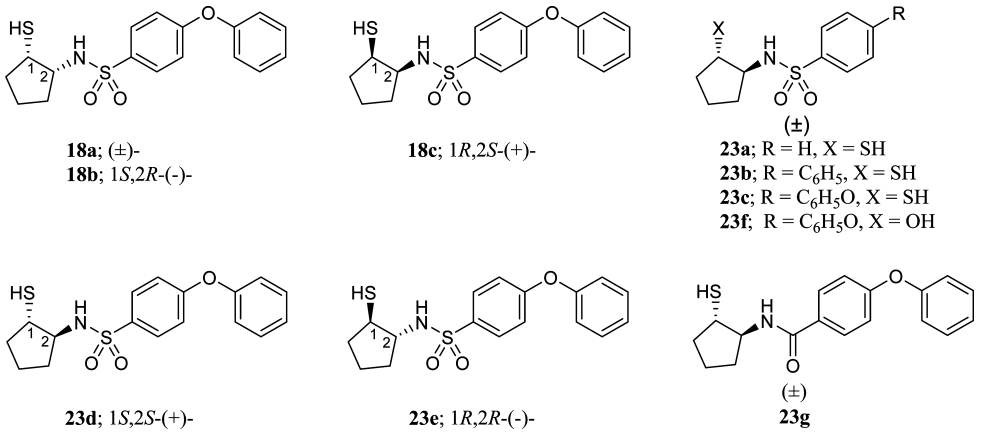


Figure 3. Structural representation of compounds **18a−c** and **23a−g** presented in Table 2.

mercaptosulfide compounds,[17,26,31,32] the trans stereochemistry was more potent (**23c** vs **18a**). Moreover, there was limited enantiomeric stereoselectivity by the *trans*-mercaptosulfonamides (**23c** vs **23d** and **23e**), while there was a distinct

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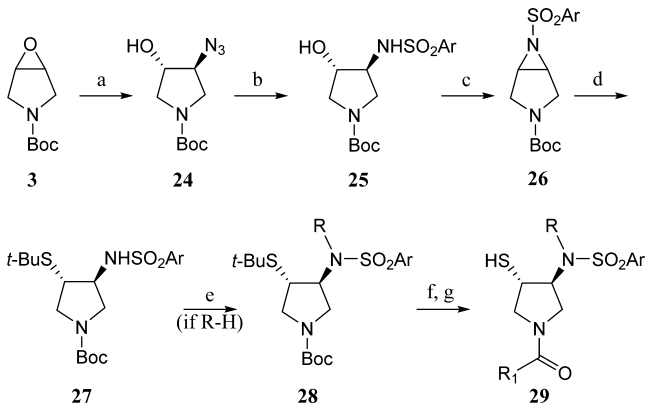
preference in the less potent *cis*-mercaptocyclopentane sulfonamides for the (1*R*,2*S*)-enantiomer (**18c**). The sulfonamide functionality was also evaluated by modifying it to an amido functionality. This resulted in diminished potency as seen when the *trans*-2-(4-phenoxybenzamido)-cyclopentanethiol (**23g**) is compared to the corresponding sulfonamide (**23c**). Expectedly, the structure lacking an effective ZBG (**23f**) showed no significant inhibitory activity when HO- was substituted for HS-.

Pyrrolidine Mercaptosulfonamide Inhibitors. Findings from the cyclopentane series of mercaptosulfonamides were applied to the target pyrrolidine scaffold. The synthetic route to the (±)-*trans*-3-mercapto-4-(4-phenoxybenzenesulfonamido)-pyrrolidine series of inhibitors is summarized in Scheme 3.

Scheme 3. Synthesis of (±)-*trans*-3-Mercapto-4-arylsulfonamidopyrrolidines[a]



[a]Reagents and conditions: (a) NaN_3, NH_4Cl, MeOH/H_2O, 65 °C; (b) PPh_3, THF−H_2O; $NaHCO_3$, $ArSO_2Cl$, 0 °C to rt; (c) PPh_3, DEAD, THF, rt; (d) *t*-BuSH, *t*-BuOK, MeOH, 0 °C to rt; (e) RX, *t*-BuOK, DMF, 0 °C to rt; (f) (1) 1.5 M HCl/EtOAc, rt, (2) R_1COCl, Et_3N, CH_2Cl_2, rt; (g) (1) 2-nitrobenzenesulfenyl chloride, HOAc, rt, (2) tri(carboxyethyl)phosphine, 1 N NaOH, THF, rt.

Pyrrolidine epoxide (**3**) was treated with NaN_3 to give azido alcohol (**24**) that was first reduced to amino alcohol and then coupled with arylsulfonyl chloride to afford compound **25**. *N*-Sulfonylaziridine (**26**), which was obtained from **25** through Mitsunobu reaction,[34] was treated with *t*-BuSH to give compound **27**. *N*-Boc deprotection and subsequent N-acylation followed by removal of *t*-Butyl protecting group afforded the inhibitor **29**. N-Methylated inhibitor **29c** was also made in the same way except for the methylation step of **27**. A different approach was required to prepare the individual enantiomers of these compounds, which is outlined in Scheme 4. Kinetic resolution of the 3,4-hydroxyazidopyrrolidine **24** gave (3*S*,4*S*)-enantiomer **30** and acetylated (3*R*,4*R*)-enantiomer **31** that was treated with base to afford (3*R*,4*R*)-enantiomer **32**. The hydroxy group of each enantiomer was activated by mesylation and then treated with AcOK to give the *cis*-acetoxyazide (**33**). The acetyl group of **33** was removed to give *cis*-3,4-hydroxyazidopyrrolidine **34** that was reduced and coupled with sulfonyl chloride to give compound **35**. Compound **36**, obtained from compound **35** through Mitsunobu reaction, was treated with $MeNH_2$ to remove the acetyl group to give thiol **37**, which was reprotected with *o*-nitrobenzenethio group to afford disulfide **38**. The final compound **39** was obtained by N-acylation after removing the *N*-Boc group and reduction of the disulfide bond with TCEP.

Inhibition values for a selection of mercaptopyrrolidine compounds (Figure 4) are shown in Table 3. As with the *trans*-mercaptosulfonamide cyclopentanes, there was little enantiomeric stereoselectivity (**29a** vs **39a** and **39b**). The *N*-carbamoylpyrrolidine derivative (**29b**) afforded good water solubility without the deleterious effect of the charged amino group of the unsubstituted pyrrolidine (**29a**). Similar to the earlier mercaptosulfides, attachment of a phthalimidoethylaminocarbonyl group to the pyrrolidine nitrogen (**29d**) as a nonprimed side (P_3) residue greatly improved selectivity for MMP-3 and -9 (i.e., roughly 10-fold) and moderately for MMP-1 and -13.

Biological Compatibility. *Compound Stability.* Although structurally distinct from the prototypical hydroxamate MMPIs, the mercaptan-based compounds exhibit comparable potencies, ranging from submicromolar to single digit nanomolar K_i values.[39−41] However, early generations of these compounds suffered from rapid oxidation of the thiol group, resulting in a reduced ability of these compounds to coordinate the active site Zn^{2+} due to disulfide formation.[32] In order to assess the stability of these new compounds, studies were performed to evaluate the thiol's zinc chelation over time.

Compared to the mercaptosulfide inhibitors, the mercaptosulfonamides showed increased oxidative stability in solution, moderately inhibiting MMP-9 for periods 5 times longer than earlier compounds (Figure 5). In addition to increasing potency, incorporation of either the cyclopentyl or pyrrolidinyl ring to the nonprimed side of the inhibitor enhanced resistance of the thiol to air oxidation. However, differences between ring types had no apparent effect on compound stability, as **23c** and **29b** (Figure 5, panels A and B) exhibited indistinguishable stability profiles. Evaluation of longer alkyl additions to the pyrrolidinyl nitrogen (**29f** and **29e**) revealed direct correlation with reduced thiol stability (Figure 5, panels C and D). Methylation of the sulfonamide nitrogen (**29c**) also resulted in reduced thiol stability despite increases in potency toward several MMPs (data not shown). An additional factor affecting the stability of these compounds is the presence of serum. Appearing to reduce oxidative degradation, higher percentages of serum in culture medium prolong inhibitory activity of **23c** and **29f** (Figure 5, panels A and C).

Cytotoxicity. Before use in cellular assays, compound toxicity is assessed in human mesenchymal stem cells (hMSCs). This cell type was selected, as current research surrounds the involvement of MMPs in the proliferation and differentiation of this cell line.[42−44] Consistent with its use in other cell lines in our lab, **23c** exhibited the greatest level of toxicity, reducing the number of cells by 40% at 100 μM (Figure 6, panel A). The decrease in cell number was attributed to MMP inhibition, since the noninhibitory analogue (**23f**) revealed no difference in cell number. Similar trends were observed with **29b**, **29d**, and **29e** (Figure 6, panels B, C, and D).

DISCUSSION AND CONCLUSIONS

Structure−Activity Relationship Studies. The principle goal of these studies was to develop potent and selective MMPIs that incorporate a thiol ZBG. This strategy was initially selected to circumvent the clinical side effects (e.g., poor plasma bioavailability, target promiscuity) that generally hampered early generation hydroxamate MMPIs, albeit recent hydroxamates are also avoiding such characteristics.[39,45−48] A recent

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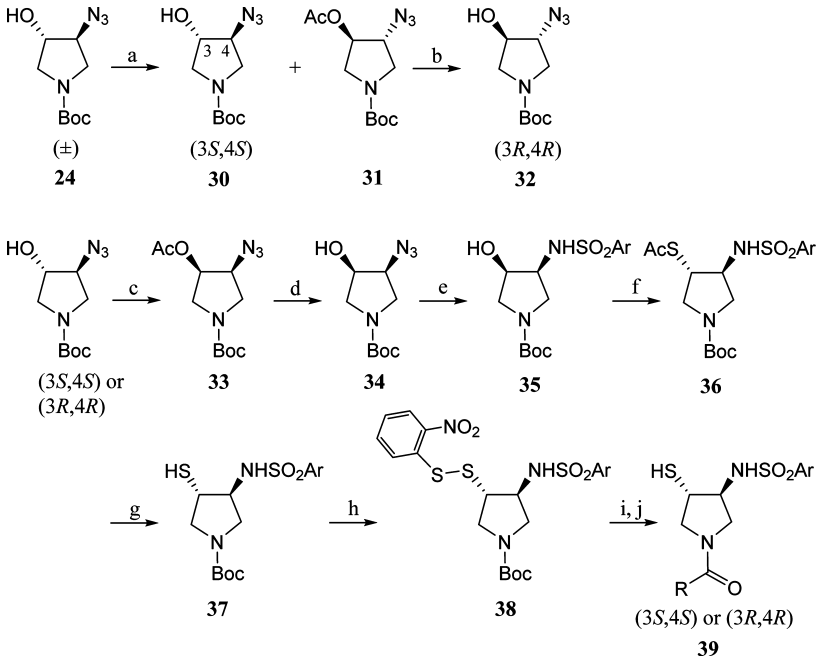
Scheme 4. Stereospecific Synthesis of *trans*-3-Mercapto-4-arylsulfonamidopyrrolidines[a]



[a]Reagents and conditions: (a) Amano AK-20 lipase, *tert*-butyl methyl ether, isopropenyl acetate, rt; (b) LiOH, THF−MeOH−H_2O, rt; (c) (1) $MeSO_2Cl$, Et_3N, $CHCl_3$, rt, (2) AcOK, DMF, 100 °C; (d) LiOH, THF−MeOH−H_2O, rt; (e) PPh_3, THF−H_2O; $NaHCO_3$, $ArSO_2Cl$, 0 °C to rt; (f) AcSH, PPh_3, DEAD, THF, 0 °C to rt; (g) $MeNH_2$, MeOH, rt; (h) 2-nitrobenzenesulfenyl chloride, CH_2Cl_2, rt; (i) (1) TFA, rt, (2) RCOCl, Et_3N, CH_2Cl_2, 0 °C to rt; (j) tri(carboxyethyl)phosphine, 1 N NaOH, THF−H_2O, rt.

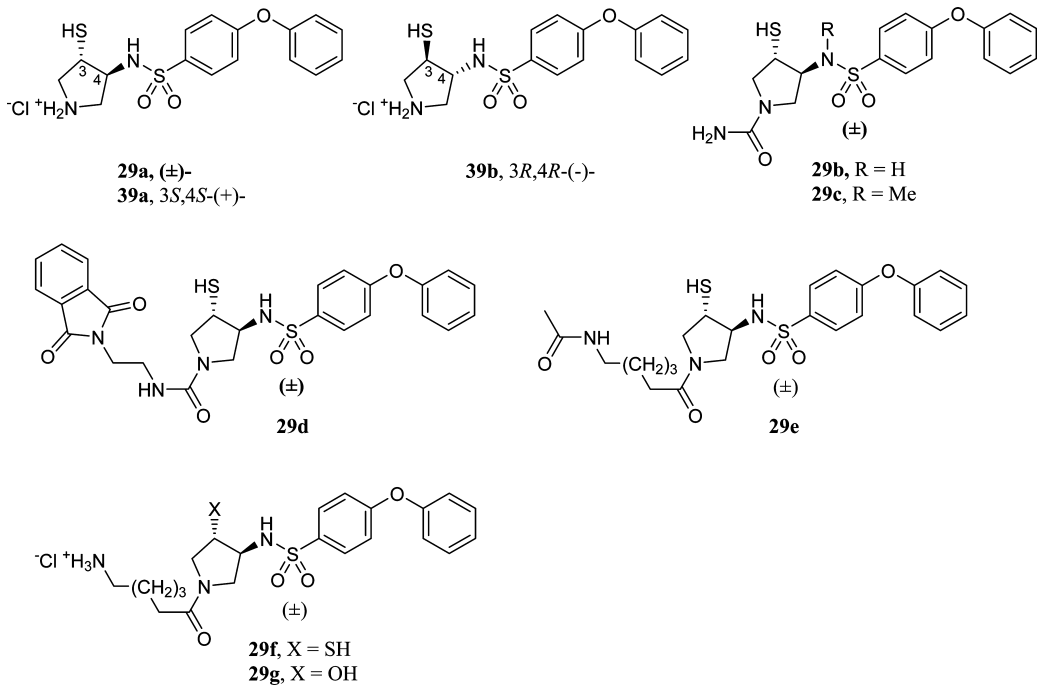


Figure 4. Structural representation of compounds **29a−g**, **39a**, and **39b** presented in Table 3.

report using a molecular dynamics approach to study the docking of various MMPIs with MMP-9 even indicates that thiol-based ZBGs are more energetically favorable than their hydroxamate counterparts for this enzyme.[49]

The inhibition of MMPs by mercaptosulfonamides is predicated upon coordination of the thiol with the enzyme's catalytic zinc. For inhibitor **29c**, where substitution of a methyl group directly upon the sulfonamide nitrogen did not reduce inhibitor potency, we speculate the possibility that hydrogen bonding between the sulfonamide -NH and enzyme is replaced by the methyl group's hydrophobic interaction. In fact, potency was somewhat increased, perhaps by the methyl group interacting with the S_1'* subsite.[50] Although our inhibitors showed somewhat lower binding affinities when compared with known bidentate ZBGs, the lower dissolution cost and

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Table 3. MMP Inhibition by *trans*-3-Mercapto-4-(4-phenoxybenzenesulfonamido)pyrrolidines

	K_i^{app} (nM)						
compd	MMP-1	MMP-2	MMP-3	MMP-7	MMP-9	MMP-13	MMP-14
29a	~2.5 × 10^4	27	2500	>2.5 × 10^4	230		
29b	4100	3.9	460	>2.5 × 10^4	15	50	11
29c	850	2.3	350	>2.5 × 10^4	1.1	28	62
29d	2800	35	37	>1.2 × 10^4	2.4	27	21
29e	>6000	3.8	810	3000	1.5	1.7	4
29f	>3000	3.1	2000	4000	3.6	2.0	6.6
29g	>5.0 × 10^4	~7000	>1.0 × 10^5	>1.0 × 10^5	~3.0 × 10^4	>2.0 × 10^5	8.5 × 10^4
39a	5.0 × 10^4	38	5900	>2.0 × 10^5	210		
39b	6300	49	1600	4100	230		

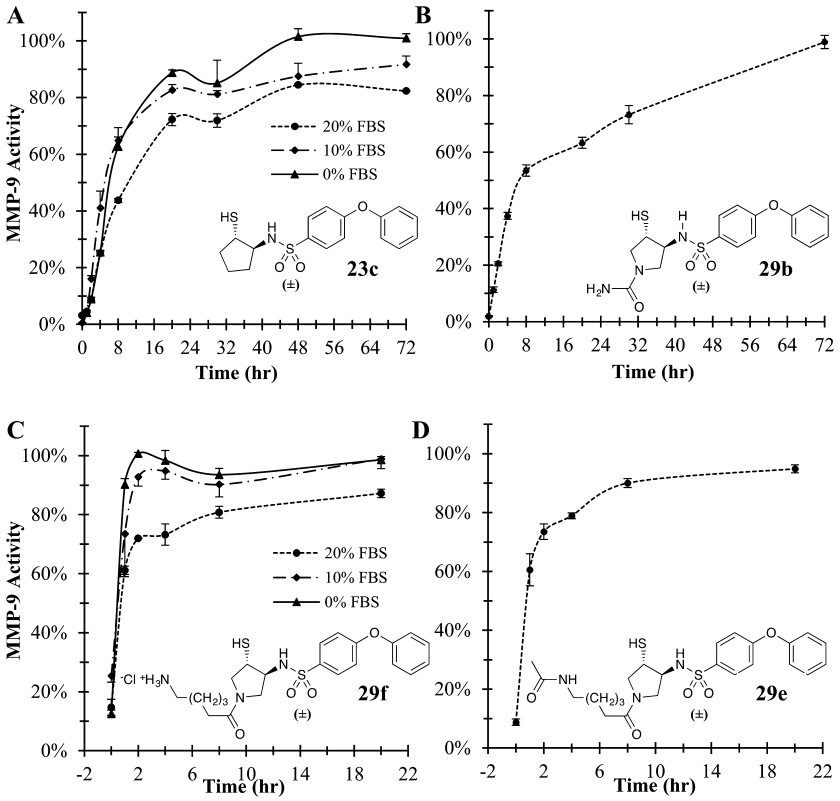

Figure 5. Stability profiles of the mercaptosulfonamide MMPIs in cell culture medium (αMEM). For panels A and C, serum concentration was varied to assess any sequestration of the compounds by albumin. Error bars represent the standard error in the mean (mean ± SEM).

amenable ionization are more favorable, resulting in only slightly lower potencies than hydroxamate-based MMPIs.[51]

Most efforts to establish MMPI selectivity have focused on modifying the prime side of compounds, with little effort evaluating nonprime substituents. The nonprime pockets of MMPs are primarily solvent exposed and display less segregation between subsites.[52] For our purposes, substitution upon the pyrrolidinyl nitrogen was to enhance future functionalization of these compounds. However, exploration of varying N-substituents provided significant changes in potency. For example, addition of alkyl chains terminating with more polar head groups generally increased inhibitor potency (**29e** and **29f**). With particular respect to MMP-3 (stromelysin-1), ring structures attached to long chains had significant effect on selectivity (**11c−e** and **29d**). Because of the S_2 pocket's location immediately adjacent to the catalytic zinc, we hypothesize that the linker and large phthalimido group are likely interacting with the S_3 pocket. As the S_3 pocket is more hydrophobic, it may attract the phthalimido group, which may then position to participate in a previously noted π−π stacking interaction with the pocket's Tyr155.[53] Surprisingly, increased potency of these compounds against MMP-1 (collagenase 1) was considerably less dramatic (i.e., ~5-fold increase) than MMP-3 (i.e., ~2000-fold increase). This was unexpected, as the S_3 pocket in MMP-1, attributed to Ser155, is larger than those in MMP-2, -3, -7, and -9, which all contain Tyr155 and are believed to energetically accommodate larger P residues.[54]

At the prime side, a noticeable trend was observed with modulation of the diphenyl ether and sulfonyl group. Modifying the lone benzene (**23a**) to a biphenyl (**23b**) and finally a diphenyl ether (**23c**) dramatically increased inhibitory potency for MMP-13. Additionally, replacement of the sulfonyl group with a carbonyl (**23g**) not only reduced MMP-13 inhibition but potency toward every MMP. These results are consistent with other reports of thiol-based MMP inhibitors in

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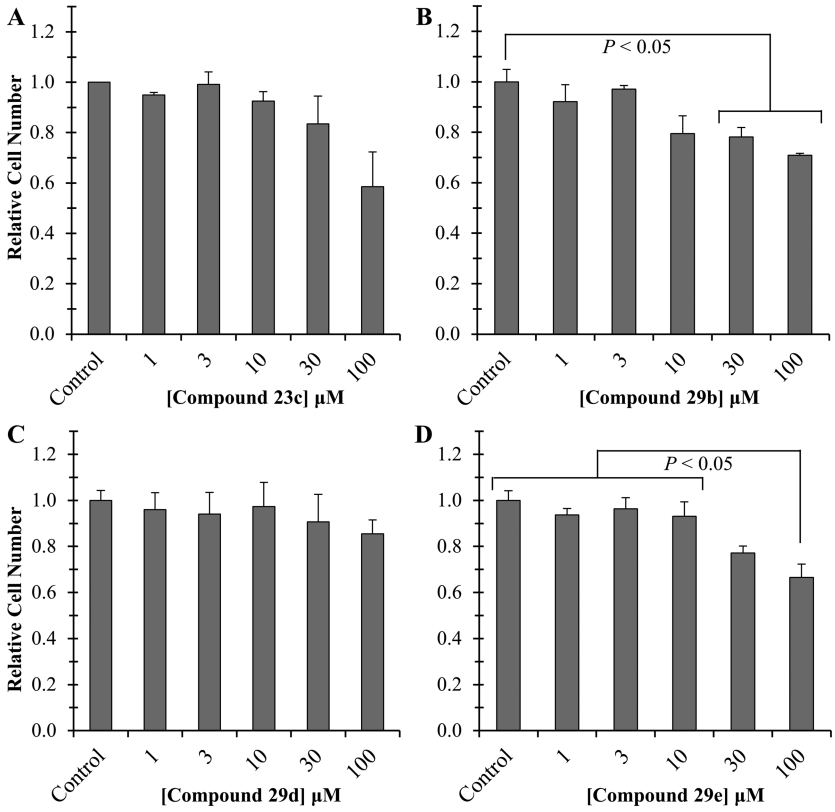

Figure 6. Cytotoxicity profiles of the mercaptosulfonamide MMPIs in hMSCs. Error bars represent standard error in the mean (mean ± SEM).

that the diphenyl ether and sulfonyl groups are critical for maintaining potency.[55]

More surprising was the demonstration that in contrast to the preferred cis stereochemistry observed in our previous work with the mercaptosulfides,[17,26,31,32] *trans*-mercaptosulfonamides were most effective (**18a** vs **23c**). It is possible that replacement of the prime side (i.e., S_1') peptidomimetic groups with 4-phenoxybenzene, as seen in **23c**, necessitates different stereochemistry to maximize binding energy. Such modifications may have arranged the MMPIs in such a way that cis enantiomers are no longer in the correct plain for coordination. We are currently further evaluating this finding.

Biological Studies. Early applications of thiol ZBGs were hampered by their metal-binding promiscuity and susceptibility to metabolic transformations. For example, stability assays with noncyclic mercaptosulfides demonstrated significant sensitivity to air oxidation. Yet the incorporation of cyclopentyl and pyrrolidinyl rings at the P_1 position of mercaptosulfonamides increased inhibitor half-life. When compared to another thiol-based chelator, *N*-(methyl)mercaptoacetamide, where 91% of the thiol was oxidized within the first 8 min,[56] our mercaptosulfonamides appear dramatically more stable. However, in contrast to a previous report indicating that thiol stability varied depending on ring structure, i.e., position of endocyclic heteroatoms, and ring substituents,[56] no preference was discovered during our investigation.

The culture medium utilized with these assays contains approximately 4 times the amount of serum than other typical media. Consequently, it was not surprising to observe a "plateau" toward the later time points in each run. We attribute this to the MMPIs being sequestered by the serum albumin and subsequently released in a dynamic equilibrium of some form. Additional studies are being conducted to further investigate this effect.

Finally, cytotoxicity analyses revealed that these compounds exhibit only moderate toxicity (i.e., killing ≤20% of cells), up to 100 μM. This differs from an earlier report, in part, which indicated that sulfur-containing compounds are generally more cytotoxic.[56] In comparison to a recent study evaluating the effects of the aminobisphosphate alendronate, the mercaptosulfonamides display far less cytotoxicity, as bisphosphonates were toxic to all cells at concentrations 10-fold lower than those typically observed for the mercaptosulfonamides.[57]

Hydroxamate-based inhibitors are quite successful for some other zinc metalloproteinases, like SAHA (suberoylanilide hydroxamic acid) for histone deacetylases.[47,58−62] Even though the popularity of MMPIs as putative therapeutics was diminished because of the notable failures of hydroxamate-based MMPIs in oncology clinical trials, they are still valuable investigative tools for MMP functional studies. For example, preliminary evidence from our lab indicates that several of these mercaptosulfonamides are capable of reducing the adipogenic potential of human mesenchymal stem cells, suggesting unique roles of MMPs in modulating the fate of adult stem cells. Moreover, selectively targeting individual MMPs in a variety of disease models will inevitably provide vital information about the functions of these enzymes, information that may prove to be critical to identifying possible treatment options.

EXPERIMENTAL SECTION

General Information. All chemicals, reagents, and solvents for syntheses were analytical grade, purchased from commercial sources. Solvents were purified according to standard procedures, and all air- and moisture-sensitive reactions were performed under nitrogen. ^{1}H spectra were collected on a Bruker ARX 300 NMR spectrometer with

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chemical shifts in parts per million (ppm) downfield from tetramethylsilane as an internal standard. Mass spectrometry data were collected on a Jeol JMS-600H, applying ESI. Melting points were determined on a Buchi melting point apparatus and are uncorrected. Column chromatography was performed on Merck silica gel 60. The reactions were monitored by TLC using Merck 60 F254 silica gel glass-backed plates; zones were detected visually under UV irradiation (254 nm). Combustion analysis was performed by Atlantic Microlab, Inc., Norcross, GA, U.S. Purity of key compounds was established by elemental analysis and/or Ellman's reagent thiol titration and determined to be >95%.

Pyrrolidine Mercaptosulfides. The inhibitors listed in Table 1 (see Figure 2 for structures) were synthesized as diastereomeric mixtures as described,[17] and as outlined in Scheme 1. Their characterization data are the following:

11a. ^{1}H NMR (300 MHz, CD_3OD) δ 7.25 (m, 5H), 4.66 (m, 1H), 4.05 (m, 0.5H), 3.83−3.00 (m, 7.5H), 2.87 (m, 1H), 2.72 (s, 1.5H), 2.71 (s, 1.5H), 1.57 (m, 1H), 1.26 (m, 1H), 1.16 (m, 1H), 0.76 (m, 6H).

11b. ^{1}H NMR (300 MHz, CD_3OD) δ 7.23 (m, 5H), 4.72 (m, 1H), 4.10−3.20 (m, 7H), 3.11 (m, 1H), 2.78 (m, 1H), 2.72 (m, 3H), 2.03 (m, 3H), 1.59 (m, 2H), 1.25 (m, 1H), 0.77 (m, 6H).

11c. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.87 (m, 2H), 7.74 (m, 2H), 7.40−7.15 (m, 5H), 7.01 (m, 0.5H), 6.93 (m, 0.5H), 6.00−5.78 (m, 1H), 4.67 (m, 1H), 4.40 (s, 1H), 4.36 (s, 1H), 4.00−3.00 (m, 8H), 2.76 (m, 3H), 2.06 (m, 0.5H, SH), 1.95 (m, 0.5H, SH), 1.67 (m, 2H), 1.50 (m, 1H), 0.88 (m, 6H). Anal. Calcd for $C_{30}H_{36}N_4O_5S_2 \cdot {}^1/_4H_2O$: C, 59.93; H, 6.12; N, 9.32; S, 10.66. Found: C, 59.94; H, 5.92; N, 9.10; S, 10.41.

11d. ^{1}H NMR (300 MHz, CD_3OD) δ 7.82 (m, 4H), 7.22 (m, 5H), 4.67 (m, 1H), 3.84−3.0 (m, 9H), 3.10 (m, 1H), 2.85 (m, 1H), 2.70 (s, 1.5H), 2.69 (s, 1.5H), 2.36 (m, 2H), 1.98 (m, 2H), 1.59 (m, 1H), 1.24 (m, 2H), 0.77 (m, 6H).

11e. ^{1}H NMR (300 MHz, CD_3OD) δ 7.80 (m, 4H), 7.24 (m, 5H), 4.69 (m, 1H), 3.85−2.80 (m, 11H), 2.70 (s, 1.5H), 2.69 (s, 1.5H), 2.28 (m, 2H), 1.65 (m, 5H), 1.32 (m, 4H), 0.78 (m, 6H).

2-Mercaptocyclopentane Arylsulfonamides (Scheme 2 and Figure 3). *(1S,2S)-(+)- and (1R,2R)-(−)-trans-2-Azidocyclopentanol.* A modified version of the procedure of Ami and Ohrui was used.[38] To a stirred solution of (±)-*trans*-2-azidocyclopentanol (1.90 g, 14.9 mmol) in *tert*-butyl methyl ether (75 mL) was added isopropenyl acetate (3.3 g, 33.0 mmol) and lipase Amano AK-20 (4.5 g) (Amano Enzyme Inc., Japan), and the mixture was stirred at room temperature for 3 days. The reaction mixture was filtered. The filtrate was evaporated under reduced pressure, and the residue was subjected to flash chromatography on silica gel. Elution with 50% ethyl acetate in hexane gave (1*R*,2*R*)-*trans*-2-azidocyclopentyl acetate (**14**) as an oil (1.26 g, 7.45 mmol, 50%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.96 (dt, *J* = 7, 4, 1H), 3.87 (m, 1H), 2.05 (s, 3H), 1.92−2.18 (m, 2H), 1.57−1.83 (m, 4H); $[\alpha]_D^{20}$ −56.7° (*c* 1.2, CH_2Cl_2). Continued elution with 50% ethyl acetate in hexane afforded (1*S*,2*S*)-*trans*-2-azidocyclopentanol (**13**) as an oil (0.93 g, 49%): ^{1}H NMR δ 4.2 (m, 1H), 3.7 (m, 1H) 1.9−2.2 (m, 2H), 1.5−1.9 (m, 5H); $[\alpha]_D^{20}$ +76.0° (*c* 1.1, CH_2Cl_2), (lit.[63] $[\alpha]_D^{20}$ +84.0°, (*c* 1.1, CH_2Cl_2)).

To a stirred solution of (1*R*,2*R*)-(−)-*trans*-2-azidocyclopentyl acetate (**14**) (610 mg, 3.61 mmol) in THF−MeOH (3:1, 15 mL) at 0 °C was added 1 N LiOH (6 mL) dropwise. The reaction mixture was stirred at room temperature until no starting material was detectable by TLC. The reaction mixture was evaporated under reduced pressure, and ethyl ether (50 mL) was added to the residue. The solution was washed with water, dried over Na_2SO_4, and evaporated. The residue was purified by flash chromatography on silica gel (50% ethyl acetate in hexane) to give (1*R*,2*R*)-*trans*-2-azidocyclopentanol (**15**) as an oil (450 mg, 98%): ^{1}H NMR identical to that of the (1*S*,2*S*) enantiomer; $[\alpha]_D^{20}$ −84.3° (*c* 1.1, CH_2Cl_2).

(1R,2S)-(+)- and (1S,2R)-(−)-cis-2-Azidocyclopentanol. To a stirred solution of Ph_3P (1.55 g, 5.91 mmol) in THF (25 mL) at 0 °C was added DEAD (1.03 mL, 6.54 mmol) dropwise. After 10 min, a solution of (1*S*,2*S*)-(+)-*trans*-2-azidocyclopentanol (**13**) (500 mg, 3.93 mmol) and *p*-nitrobenzoic acid (985 mg, 5.89 mmol) in THF (5 mL) was added dropwise and the reaction mixture was stirred at room temperature overnight. The mixture was concentrated under reduced pressure and the residue was purified by flash chromatography on silica gel (20% ethyl acetate in hexane) to give (1*R*,2*S*)-*cis*-2-azidocyclopentyl *p*-nitrobenzoate (**19**) as a solid (1.00 g, 92%): mp 51.5−52.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.33 (d, *J* = 9, 2H), 8.27 (d, *J* = 9, 2H), 5.42 (dt, *J* = 6, 5, 1H), 4.00 (dt, *J* = 5, 6, 1H), 1.88−2.25 (m, 5H), 1.65−1.83 (m, 1H); $[\alpha]_D^{20}$ −97.3° (*c* 0.59, CH_2Cl_2).

To a stirred solution of (1*R*,2*S*)-(−)-*cis*-2-azidocyclopentyl *p*-nitrobenzoate (**19**) (500 mg, 1.81 mmol) in THF−MeOH (3:1, 10 mL) at 0 °C was added 1 N LiOH (3 mL) dropwise. The reaction mixture was stirred at room temperature until no starting material was detectable by TLC. The reaction mixture was evaporated under reduced pressure, and ethyl ether (25 mL) was added to the residue. The solution was washed with water, dried over Na_2SO_4, and evaporated. The residue was purified by flash chromatography on silica gel (50% ethyl acetate in hexane) to give (1*R*,2*S*)-*cis*-2-azidocyclopentanol (**20**) as an oil (225 mg, 98%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.13 (br p, *J* = 5, 1H), 3.78 (dt, *J* = 5, 6, 1H), 1.78−2.00 (m, 4H), 1.53−1.75 (m, 3H); $[\alpha]_D^{20}$ +66.7° (*c* 1.44, CH_2Cl_2).

By use of the same procedures, (1*R*,2*R*)-(−)-*trans*-2-azidocyclopentanol (**15**) was converted to (1*S*,2*R*)-*cis*-2-azidocyclopentyl *p*-nitrobenzoate (**19** enantiomer) (mp 51.5 °C, $[\alpha]_D^{20}$ +97.9° (*c* 1.22, CH_2Cl_2)) and then to (1*S*,2*R*)-*cis*-2-azidocyclopentanol (**20** enantiomer): ^{1}H NMR identical to that of the (1*R*,2*S*) enantiomer; $[\alpha]_D^{20}$ −64.4° (*c* 1.12, CH_2Cl_2).

(±)-, (1S,2S)-(−)-, and (1R,2R)-(+)-trans-2-(tert-Butoxycarbonylamino)cyclopentanol. To a stirred solution of (±)-*trans*-2-azidocyclopentanol (**12**) (1.27 g, 10.0 mmol) in THF−H_2O (4:1, 10 mL) was added Ph_3P (2.88 g, 12.0 mmol), and the mixture was stirred at room temperature for 2 h and at 65 °C for 2 h. The solution was cooled with stirring to 0 °C, and Et_3N (1.4 mL, 10.0 mmol) was added. Then a solution of di-*tert*-butyl dicarbonate (2.18 g, 10.0 mmol) in THF (5 mL) was added dropwise. The reaction mixture was warmed to room temperature and was stirred for 3 h. The mixture was concentrated under reduced pressure. Ethyl acetate was added to the residue, and the organic layer was washed with 10% citric acid and saturated aqueous $NaHCO_3$. Then it was dried over Na_2SO_4 and evaporated. The residue was purified by flash chromatography on silica gel (50% ethyl acetate in hexane) to give (±)-*trans*-2-(*tert*-butoxycarbonylamino)cyclopentanol ((±)-**16**) (1.8 g, 90%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.67 (br s, 1H), 3.98 (m, 2H), 3.62 (m, 1H), 1.93−2.18 (m, 2H), 1.59−1.85 (m, 3H), 1.45 (s, 9H), 1.27−1.41 (m, 1H).

By the same procedure, (1*S*,2*S*)-(+)-*trans*-2-azidocyclopentanol (**13**) gave (1*S*,2*S*)-*trans*-2-(*tert*-butoxycarbonylamino)cyclopentanol (**16**) as a solid (370 mg, 73%): mp 81 °C; ^{1}H NMR (300 MHz, $CDCl_3$) identical to that of (±)-**16**; $[\alpha]_D^{20}$ −23.6° (CH_2Cl_2) (lit.[63] mp 87 °C, $[\alpha]_D^{20}$ −21.0° (*c* 1.0, CH_2Cl_2)).

By the same procedure, (1*R*,2*R*)-(−)-*trans*-2-azidocyclopentanol (**15**) (160 mg, 1.26 mmol) afforded (1*R*,2*R*)-*trans*-2-(*tert*-butoxycarbonylamino)cyclopentanol (**16** enantiomer) (180 mg, 71%): mp 81 °C; ^{1}H NMR (300 MHz, $CDCl_3$) identical to that of (±)-**16**; $[\alpha]_D^{20}$ +23.4° (CH_2Cl_2) (lit.[63] mp 87 °C, $[\alpha]_D^{20}$ +21.0° (*c* 1.0, CH_2Cl_2)).

*(1R,2S)-(+)-cis-2-(4-Phenoxybenzenesulfonamido)-cyclopentanethiol (**18c**).* To a stirred solution of Ph_3P (393 mg, 1.50 mmol) in THF (5 mL) at 0 °C was added DEAD (236 μL, 1.50 mmol). After 10 min, solutions of (1*S*,2*S*)-(−)-*trans*-2-(*tert*-butoxycarbonylamino)cyclopentanol (**16**) (166 mg, 0.826 mmol) and thiolacetic acid (107 μL, 1.50 mmol) in THF (2 mL) were added. The reaction mixture was stirred at room temperature overnight. Then it was concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (10% ethyl acetate in hexane) to give (1*R*,2*S*)-(+)-*cis*-2-(*tert*-butoxycarbonylamino)-cyclopentanethioacetate (**17**) (170 mg, 79%) as a solid: mp 72 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.57 (br s, 1H), 4.18 (br s, 1H), 3.95 (q, *J* = 6, 1H), 2.33 (s, 3H), 2.09−2.21 (m, 1H), 1.93−2.09 (m, 1H), 1.59−1.81 (m, 3H), 1.44 (s, 9H), 1.35−1.53 (m, 1H); $[\alpha]_D^{20}$ +34.7° (CH_2Cl_2).

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To a stirred solution of **17** (35 mg, 0.135 mmol) in THF (0.5 mL) was added 40% aqueous $MeNH_2$ (120 μL, 3.47 mmol). After 20 min the reaction mixture was concentrated under reduced pressure and the residue was dissolved in trifluoroacetic acid (0.5 mL). The mixture was stirred for 2 h. The solvent was removed under reduced pressure, and the residue was dissolved in CH_2Cl_2 (2 mL). The solution was cooled to 0 °C, and Et_3N (38 μL, 0.273 mmol) and 4-phenoxybenzenesulfonyl chloride (31.5 mg, 0.135 mmol) were added successively. The reaction mixture was stirred at 0 °C for 3 h. Then it was concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (20% ethyl acetate in hexane) to give **18c** as a solid (41 mg, 87%): mp 112−113 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (d, *J*= 9, 2H), 7.42 (t, *J* = 8, 2H), 7.23 (t, *J* = 8, 1H), 7.06 (m, 4H), 5.02 (d, *J* = 8, 1H), 3.62 (m, 1H), 3.23 (m, 1H), 2.06 (m, 1H), 1.5−1.8 (m, 5H), 1.33 (d, *J* = 7, 1H); $[\alpha]_D^{20}$ +1.30° (CH_2Cl_2); HRMS (ESI) calculated for $C_{17}H_{19}NO_3S_2Na^+$ 372.0699, found 372.0703.

*(1S,2R)-(−)-cis-2-(4-Phenoxybenzenesulfonamido)-cyclopentanethiol (**18b**).* Following the Ph_3P/DEAD procedure described above, (1*R*,2*R*)-(+)-*trans*-2-(*tert*-butoxycarbonylamino)-cyclopentanol (151 mg, 0.75 mmol) was converted to (1*S*,2*R*)-*cis*-2-(*tert*-butoxycarbonylamino)cyclopentanethioacetate as a solid (177 mg, 91%): mp 72 °C; ^{1}H NMR (300 MHz, $CDCl_3$) identical to that of the (1*R*,2*S*) enantiomer **17**; $[\alpha]_D^{20}$ −34.8° (CH_2Cl_2).

To a stirred solution of 1*S*,2*R*-(−)-*cis*-2-(*tert*-butoxycarbonylamino)cyclopentanethioacetate (50 mg, 0.193 mmol) in MeOH (0.5 mL) was added 2 N HCl in MeOH (2 mL), and the mixture was stirred at room temperature for 2 h. The solvent was removed under reduced pressure, and the residue was dissolved in CH_2Cl_2 (2 mL). The solution was cooled to 0 °C, and Et_3N (65 μL, 0.466 mmol) and 4-phenoxybenzenesulfonyl chloride (45.0 mg, 0.193 mmol) were added successively. The mixture was stirred at 0 °C for 3 h. Then it was concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (20% ethyl acetate in hexane) to give **18b** as a solid (52 mg, 77%): mp 112−113 °C; ^{1}H NMR identical to that of **18c**; $[\alpha]_D^{20}$ −1.30° (CH_2Cl_2); HRMS (ESI) calculated for $C_{17}H_{19}NO_3S_2Na^+$ 372.0699, found 372.0703.

*(±)-cis-2-(4-Phenoxybenzenesulfonamido)cyclopentanethiol (**18a**).* By the same procedures, (±)-*trans*-2-azidocyclopentanol (**12**) afforded the racemic thiol **18a** as a solid: mp 113−114 °C; ^{1}H NMR (300 MHz, $CDCl_3$) identical to that of each of the enantiomers.

*(±)-trans-2-(Benzenesulfonamido)cyclopentanethiol (**23a**).* To a stirred solution of Ph_3P (1.4 g, 5.3 mmol) in THF (25 mL) at 0 °C was added DEAD (0.87 g, 5.0 mmol) dropwise. After 10 min, (±)-*trans*-2-(*tert*-butoxycarbonylamino)cyclopentanol ((±)-**16**) (1.0 g, 5.0 mmol) was added dropwise, and the reaction mixture was stirred at room temperature overnight. The mixture was concentrated under reduced pressure and the residue was purified by flash chromatography on silica gel (10% ethyl acetate in hexane) to give the aziridine *N*-*tert*-butoxycarbonyl-6-azabicyclo[3.1.0]pentane as an oil (0.80 g, 87%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.04−2.16 (m, 2H), 1.53−1.64 (m, 4H), 1.46 (s, 9H), 1.15−1.32 (m, 2H).

To a stirred solution of the aziridine (550 mg, 3.0 mmol) in MeOH (20 mL) were added *tert*-butyl thiol (0.5 mL, 4.4 mmol) and a solution of sodium methoxide (162 mg, 3.0 mmol) in MeOH (5 mL), and the reaction mixture was stirred at room temperature overnight. Water was added to the solution, and the mixture was evaporated under vacuum. The residue was dissolved in ethyl acetate (50 mL) and the organic layer was washed with water, dried over Na_2SO_4, and evaporated to give the crude (±)-*trans*-*S*-*tert*-butyl-2-(*tert*-butoxycarbonylamino)-cyclopentanethiol as an oil (820 mg, 100%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.60 (broad, 1H), 3.64 (m, 1H). 2.80 (m, 1H), 2.10−2.25 (m, 2H), 1.60−1.75 (m, 4H), 1.45 (s, 9H), 1.36 (s, 9H).

A sample of the oil (82 mg, 0.30 mmol) was dissolved in trifluoroacetic acid (1 mL), and the solution was stirred at room temperature until no starting material remained by TLC analysis. The trifluoroacetic acid was evaporated under vacuum, and the residue was dissolved in CH_2Cl_2 (5 mL). The solution was cooled to 0 °C, and Et_3N (124 μL, 0.88 mmol) and benzenesulfonyl chloride (42 μL, 0.33 mmol) were added. The reaction mixture was warmed to room temperature and was stirred for 3 h. The mixture was concentrated under reduced pressure. Ethyl acetate was added to the residue, and the organic layer was washed with 10% citric acid and saturated aqueous $NaHCO_3$. Then it was dried over Na_2SO_4 and evaporated. The residue was purified by flash chromatography on silica gel (50% ethyl acetate in hexane) to give (±)-*trans*-*S*-*tert*-butyl-2-(benzenesulfonamido)cyclopentanethiol (90 mg, 100%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.85−7.89 (m, 2H), 7.48−7.61 (m, 3H), 4.75 (br d, *J* = 3, 1H, NH), 3.01 (dq, *J* = 4, 8, 1H), 2.81 (q, *J* = 8, 1H), 2.00−2.22 (m, 2H), 1.44−1.70 (m, 4H), 1.28 (s, 9H).

To a solution of the (±)-*trans*-*S*-*tert*-butyl-2-(benzenesulfonamido)-cyclopentanethiol (90 mg, 0.30 mmol) in AcOH (1 mL) was added 2-nitrobenzenesulfenyl chloride (62 mg, 0.33 mmol), and the mixture was stirred at room temperature for 2 h. The AcOH was evaporated under vacuum, and to the residue (82 mg, ∼0.2 mmol) were added THF (2 mL), 1 N NaOH (0.4 mL), and tris(2-carboxyethyl)-phosphine (TCEP, 63.1 mg, 0.22 mmol). The mixture was stirred at room temperature under argon for 3 h. Then it was condensed under vacuum. The residue was dissolved in ethyl acetate, dried over Na_2SO_4, and filtered. The solvent was evaporated and the residue was purified by flash chromatography (25% ethyl acetate in hexane) to give the thiol **23a** (45 mg, 58%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90−7.94 (m, 2H), 7.50−7.63 (m, 3H), 4.67 (br d, *J* = 6, 1H, NH), 3.25 (p, *J* = 7.5, 1H), 2.90 (p, *J* = 7.5, 1H), 1.99−2.19 (m, 2H), 1.63−1.74 (m, 2H), 1.59 (d, *J* = 7, 1H, SH), 1.33−1.52 (m, 2H); HRMS (ESI) calculated for $C_{11}H_{15}NO_2S_2Na^+$ 280.0436, found 280.0442.

*(±)-trans-2-(4-Phenylbenzenesulfonamido)cyclopentanethiol (**23b**).* A sample of the crude (±)-*trans*-*S*-*tert*-butyl-2-(*tert*-butoxycarbonylamino)cyclopentanethiol (59 mg, 0.214 mmol) from the previous experiment was dissolved in trifluoroacetic acid (1 mL) and was stirred at room temperature until no starting material remained by TLC analysis. The trifluoroacetic acid was evaporated under vacuum, and the residue was dissolved in CH_2Cl_2 (5 mL). The solution was cooled to 0 °C, and Et_3N (65.6 μL, 0.47 mmol) and 4-phenylbenzenesulfonyl chloride (46.6 mg, 0.214 mmol) were added. The reaction mixture was warmed to room temperature and was stirred for 3 h. The mixture was concentrated under reduced pressure. Ethyl acetate was added to the residue, and the organic layer was washed with 10% citric acid and saturated aqueous $NaHCO_3$. Then it was dried over Na_2SO_4 and evaporated. The residue was filtered through a short column of silica gel (50% ethyl acetate in hexane). The resulting crude product was dissolved in AcOH (1 mL). 2-Nitrobenzenesulfenyl chloride (41 mg, 0.24 mmol) was added, and the mixture was stirred at room temperature for 2 h. The AcOH was evaporated under vacuum to give the crude disulfide that was used without further purification. To the crude disulfide was added THF (1 mL), 1 N NaOH (0.23 mL), and TCEP (63.1 mg, 0.22 mmol). The mixture was stirred at room temperature under argon for 3 h. Then it was condensed under vacuum. To the residue was added ethyl acetate, and the mixture was dried over Na_2SO_4 and filtered. The solvent was evaporated and the residue was purified by flash chromatography (25% ethyl acetate in hexane) to give **23b** (66 mg, 0.198 mmol, 93%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.96 (d, *J* = 8, 2H), 7.74 (d, *J* = 8, 2H), 7.62 (d, *J* = 8, 2H), 7.39−7.52 (m, 3H), 4.69 (br d, *J* = 5, 1H, NH), 3.29 (p, *J* = 8, 1H), 2.93 (p, *J* = 8, 1H), 2.03−2.22 (m, 2H), 1.64−1.75 (m, 2H), 1.62 (d, *J* = 7, 1H, SH), 1.36−1.54 (m, 2H); HRMS (ESI) calculated for $C_{17}H_{19}NO_2S_2Na^+$ 356.0749, found 356.0755.

(1R,2S)-(−)- and (1S,2R)-(+)-cis-2-(4-Phenoxybenzenesulfonamido)cyclopentanol. To a stirred solution of (1*R*,2*S*)-(+)-*cis*-2-azidocyclopentanol (**20**) (414 mg, 3.26 mmol) in THF−H_2O (4:1, 5 mL) was added Ph_3P (897 mg, 3.42 mmol), and the mixture was stirred at room temperature for 2 h and at 65 °C for 2 h. The solution was cooled with stirring to 0 °C, and $NaHCO_3$ (310 mg, 3.69 mmol) was added. Then a solution of 4-phenoxybenzenesulfonyl chloride (760 mg, 3.26 mmol) in THF (5 mL) was added dropwise. The reaction mixture was warmed to room temperature and was stirred for 3 h. The mixture was concentrated under reduced pressure. Ethyl acetate was added to the residue, and the organic layer was washed with 10% citric acid and saturated aqueous $NaHCO_3$. Then it was dried over Na_2SO_4 and evaporated. The residue was

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purified by flash chromatography on silica gel (50% ethyl acetate in hexane) to give (1*R*,2*S*)-*cis*-2-(4-phenoxybenzenesulfonamido)-cyclopentanol as a solid (420 mg, 75%): mp 110 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (d, J = 9, 2H), 7.42 (t, J = 8, 2H), 7.23 (t, J = 8, 1H), 7.06 (m, 4H), 4.87 (d, J = 8, 1H), 4.03 (m, 1H), 3.42 (m, 1H), 1.7−1.9 (m, 4H), 1.4−1.7 (m, 3H); $[\alpha]_D^{20}$ −22.0° (c 1.00, CH_2Cl_2).

Application of the same procedure to (1*S*,2*R*)-(−)-*cis*-2-azidocyclopentanol (**20** enantiomer) afforded (1*S*,2*R*)-*cis*-2-(4-phenoxybenzenesulfonamido)cyclopentanol: mp 110 °C; ^{1}H NMR identical to that of the (1*R*,2*S*) enantiomer; $[\alpha]_D^{20}$ +22.3° (c 1.05, CH_2Cl_2).

*(±)-, (1S,2S)-(+)-, and (1R,2R)-(−)-trans-2-(4-Phenoxybenzenesulfonamido)cyclopentanethiol (**23c, 23d, and 23e**).* To a stirred solution of Ph_3P (262 mg, 1.0 mmol) in THF (5 mL) at 0 °C was added DEAD (157 μL, 1.0 mmol). After 10 min, a solution of (1*R*,2*S*)-(−)-*cis*-2-(4-phenoxybenzenesulfonamido)-cyclopentanol (166 mg, 0.50 mmol) and thiobenzoic acid (118 μL, 1.0 mmol) in THF (2 mL) was added. The reaction mixture was stirred at room temperature overnight. Then it was concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (20% ethyl acetate in hexane) to give (1*S*,2*S*)-*trans*-2-(4-phenoxybenzenesulfonamido)-cyclopentanethiobenzoate as a solid (180 mg, 79%): mp 132−133 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.82 (d, J = 8, 2H), 7.72 (d, J = 9, 2H), 7.59 (t, J = 8, 1H), 7.43 (t, J = 8, 2H), 7.36 (t, J = 8, 2H), 7.19 (t, J = 8, 1H), 6.93 (d, J = 9, 2H), 6.77 (d, J = 9, 2H), 5.54 (br d, 1H), 3.73 (q, J = 9, 1H), 3.42 (m, 1H), 2.1−2.3 (m, 2H), 1.6−1.9 (m, 4H); $[\alpha]_D^{20}$ −140.6° (c 0.65, CH_2Cl_2).

To a stirred solution of (1*S*,2*S*)-(−)-*trans*-2-(4-phenoxybenzenesulfonamido)cyclopentanethiobenzoate (80 mg, 0.176 mmol) in THF (1 mL) under N_2 was added 40% aqueous $MeNH_2$ (900 μL, 10.5 mmol), and the reaction mixture was stirred for 20 min. The solution was concentrated under reduced pressure and the residue was purified by flash chromatography on silica gel (20% ethyl acetate in hexane) to give (1*S*,2*S*)-(+)-*trans*-2-(4-phenoxybenzenesulfonamido)cyclopentanethiol (**23d**) as a solid (60 mg, 98%): mp 133−134 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (d, J = 9, 2H), 7.42 (t, J = 8, 2H), 7.23 (t, J = 8, 1H), 7.06 (m, 4H), 4.53 (br d, 1H), 3.23 (pentet, J = 7.5, 1H), 2.91 (p, J = 7.5, 1H), 2.03−2.21 (m, 2H), 1.64−1.76 (m, 2H), 1.60 (d, J = 7, 1H), 1.34−1.53 (m, 2H); $[\alpha]_D^{20}$ +16.9° (c 1.00, CH_2Cl_2).

By the same procedure, (1*S*,2*R*)-(+)-*cis*-2-(4-phenoxybenzenesulfonamido)cyclopentanol (180 mg, 0.54 mmol) was converted to (1*R*,2*R*)-*trans*-2-(4-phenoxybenzenesulfonamido)-cyclopentanethiobenzoate (170 mg, 69%): mp 132−133 °C; $[\alpha]_D^{20}$ +142.4° (1.05, CH_2Cl_2). NMR analysis of the product in the presence of the chiral shift reagents (europium[3] tris-trifluoromethylhydroxymethylene-(+)-camphorate and europium[3] tris-heptafluoropropylhydroxymethylene-(+)-camphorate) at both 300 and 400 MHz indicated it to be in >90% ee.

The (1*R*,2*R*)-(+)-*trans*-2-(4-phenoxybenzenesulfonamido)-cyclopentanethiobenzoate (85 mg, 0.187 mmol) was hydrolyzed as above to give (1*R*,2*R*)-(−)-*trans*-2-(4-phenoxybenzenesulfonamido)-cyclopentanethiol **23e** as a solid (61 mg, 93%): mp 133−134 °C; ^{1}H NMR (300 MHz, $CDCl_3$) identical to that of **23d**; $[\alpha]_D^{20}$ −17° (c 1.00, CH_2Cl_2).

By the same procedures, (±)-*trans*-2-azidocyclopentanol afforded the racemic thiol **23c** as a solid: mp 156−157 °C; ^{1}H NMR (300 MHz, $CDCl_3$) identical to that of each of the enantiomers; HRMS (ESI) calculated for $C_{17}H_{19}NO_3S_2Na^+$ 372.0699, found 372.0704.

*(±)-trans-2-(4-Phenoxybenzamido)cyclopentanethiol (**23g**).* To a stirred solution of cyclopentane oxide (1.57 g, 18.7 mmol) in MeOH (20 mL) were added *t*-Bu thiol (2.1 mL, 18.7 mmol) and a solution of sodium methoxide (1.01 g, 18.7 mmol) in MeOH (10 mL). The reaction mixture was stirred at room temperature overnight. Water was added to the solution, and the mixture was evaporated under vacuum. The residue was dissolved in ethyl acetate (50 mL) and the organic layer was washed with water, dried over Na_2SO_4, and evaporated to give (±)-*trans*-2-*tert*-butylmercaptocyclopentanol (3.00 g, 92%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.90 (q, J = 7, 1H), 2.84 (dt, J = 7, 8, 1H), 2.24 (m, 1H), 1.99 (m, 1H), 1.94 (br s, 1H, OH), 1.73 (m, 2H), 1.61 (m, 2H), 1.36 (s, 9H).

To a stirred solution of Ph_3P (2.7 g, 10.3 mmol) in THF (25 mL) at 0 °C was added DEAD (1.8 g, 10.3 mmol) dropwise. After 10 min, (±)-*trans*-2-*tert*-butylmercaptocyclopentanol (1.64 g, 9.36 mmol) in THF (5 mL) and hydrazoic acid (1.0 g in 10 mL of benzene, 25 mmol) were added dropwise, and the reaction mixture was stirred at room temperature overnight. The mixture was concentrated under reduced pressure and the residue was purified by flash chromatography on silica gel (20% ethyl acetate in hexane) to give (±)-*trans*-2-*tert*-butylmercaptocyclopentylazide as an oil (1.3 g, 70%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.75 (m, 1H), 2.98 (m, 1H), 2.25 (m, 1H), 2.02 (m, 1H), 1.70 (m, 4H), 1.37 (s, 9H).

To a stirred solution of the azide (470 mg, 2.35 mmol) in THF−H_2O (4:1, 10 mL) was added Ph_3P (696 mg, 2.65 mmol), and the mixture was stirred at room temperature for 2 h and at 65 °C for 2 h. The solution was cooled with stirring to 0 °C, and Et_3N (3.93 μL, 4.79 mmol) was added. Then a solution of 4-phenoxybenzoyl chloride (546 mg, 2.35 mmol) in THF (5 mL) was added dropwise. The reaction mixture was warmed to room temperature and was stirred for 3 h. The mixture was concentrated under reduced pressure. Ethyl acetate was added to the residue, and the organic layer was washed with 10% citric acid and saturated aqueous $NaHCO_3$. Then it was dried over Na_2SO_4 and evaporated. The residue was purified by flash chromatography on silica gel (50% ethyl acetate in hexane) to give (±)-*trans*-*S*-*tert*-butyl-2-(4-phenoxybenzamido)cyclopentanethiol (0.67 g, 77%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.73 (d, J = 9, 2H), 7.38 (t, J = 8, 2H), 7.17 (t, J = 7, 1H), 7.04 (d, J = 8, 2H), 7.00 (d, J = 9, 2H), 6.10 (br d, J = 4, 1H, NH), 4.06 (p, J = 7, 1H), 3.08 (q, J = 7, 1H), 2.20−2.40 (m, 2H), 1.66−1.87 (m, 3H), 1.52−1.66 (m, 1H), 1.38 (s, 9H).

To a solution of (±)-*trans*-*S*-*tert*-butyl-2-(4-phenoxybenzamido)-cyclopentanethiol (211 mg, 0.57 mmol) in AcOH (1 mL) was added 2-nitrobenzenesulfenyl chloride (108 mg, 0.57 mmol), and the mixture was stirred at room temperature for 2 h. The AcOH was evaporated under vacuum, and to the residue (212 mg, 0.455 mmol) were added THF (4 mL), 1 N NaOH (0.91 mL), and TCEP (144 mg, 0.50 mmol). The mixture was stirred at room temperature under argon for 3 h. Then it was condensed under vacuum. To the residue was added ethyl acetate, and it was dried over Na_2SO_4 and filtered. The solvent was evaporated and the residue was purified by flash chromatography (25% ethyl acetate in hexane) to give **23g** (135 mg, 76%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (d, J = 9, 2H), 7.38 (t, J = 8, 2H), 7.17 (t, J = 7.5, 1H), 7.02 (m, 4H), 6.01 (br d, J = 7, 1H, NH), 4.19 (p, J = 7.5, 1H), 3.10 (dt, J = 7, 8, 1H), 2.30−2.42 (m, 1H), 2.15−2.29 (m, 1H), 1.88 (d, J = 6, 1H, SH), 1.45−1.85 (m, 4H); HRMS (ESI) calculated for $C_{18}H_{19}NO_2SNa^+$ 336.1029, found 336.1034.

*(±)-trans-2-(4-Phenoxybenzenesulfonamido)cyclopentanol (**23f**).* To a stirred solution of (±)-*trans*-2-azidocyclopentanol (553 mg, 4.35 mmol) in THF−H_2O (4:1, 10 mL) was added Ph_3P (1.26 g, 4.79 mmol), and the mixture was stirred at room temperature for 2 h and at 65 °C for 2 h. The solution was cooled with stirring to 0 °C, and Et_3N (0.67 mL, 4.79 mmol) was added. Then a solution of 4-phenoxybenzenesulfonyl chloride (1.01 g, 4.35 mmol) in THF (5 mL) was added dropwise. The reaction mixture was warmed to room temperature and was stirred for 3 h. The mixture was concentrated under reduced pressure, and ethyl acetate was added. The organic layer was washed with 10% citric acid and saturated aqueous $NaHCO_3$. Then it was dried over Na_2SO_4 and evaporated. The residue was purified by flash chromatography on silica gel (50% ethyl acetate in hexane) to give **23f** as a solid (0.93g, 71%): mp 98 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (d, J = 9, 2H), 7.42 (t, J = 8, 2H), 7.24 (t, J = 8, 1H), 7.06 (m, 4H), 4.8 (br, 1H, NH), 4.06 (q, J = 6, 1H), 3.24 (m, 1H), 1.88−2.03 (m, 2H), 1.49−1.72 (m, 4H), 1.30−1.43 (m, 1H); HRMS (ESI) calculated for $C_{17}H_{19}NO_4SNa^+$ 356.0927, found 356.0933.

***trans*-3-Mercapto-4-(4-phenoxybenzenesulfonamido)-pyrrolidines (Scheme 3 and Figure 4, Ar = -C_6H_4-O-C_6H_5).** *(±)-N-Boc-trans-3-hydroxy-4-(4-phenoxybenzenesulfonamido)-pyrrolidine (**25**).* A mixture of *N*-Boc-3-pyrroline oxide (**3**) (0.925 g, 5.0 mmol), NaN_3 (0.65 g, 10 mmol), and NH_4Cl (5.0 mmol) in

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MeOH−H_2O (6:1, 16 mL) was stirred at 65 °C overnight. The reaction mixture was concentrated under reduced pressure, and the residue was extracted thoroughly with ethyl acetate. The combined ethyl acetate extract was washed with water and saturated brine, dried over anhydrous Na_2SO_4, and evaporated. The crude (±)-*N*-Boc-*trans*-3-hydroxy-4-azidopyrrolidine (**24**) was purified by recrystallization from ether−hexane: mp 34−36 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 4.16 (m, 1H), 3.95 (m, 1H), 3.62 (m, 1H), 3.51 (m, 1H), 3.35 (m, 2H), 1.47 (s, 9H).

To a stirred solution of the azido alcohol **24** (1.02 g, 4.45 mmol) in THF−H_2O (10:1, 10 mL) was added Ph_3P (1.28 g, 4.88 mmol), and the mixture was stirred at room temperature for 2 h and at 65 °C for 2 h. The solution was cooled with stirring to 0 °C. Et_3N (0.74 mL, 5.34 mmol) was added. Then a solution of 4-phenoxybenzenesulfonyl chloride (1.04 g, 4.45 mmol) in THF (5 mL) was added dropwise. The reaction mixture was warmed to room temperature and was stirred for 3 h. The mixture was concentrated under reduced pressure. Ethyl acetate was added to the residue, and the organic layer was washed with 10% citric acid and saturated aqueous $NaHCO_3$. Then it was dried over Na_2SO_4 and evaporated. The residue was purified by flash chromatography (50% ethyl acetate in hexane) to give (±)-*N*-Boc-*trans*-3-hydroxy-4-(4-phenoxybenzenesulfonamido)pyrrolidine (**25**) as a solid (1.52 g, 79%): mp 98−99 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 7.85 (d, J = 8, 2H), 7.45 (m, 2H), 7.24 (t, J = 7, 1H), 7.10 (m, 4H), 4.09 (m, 1H), 3.95 (m, 1H), 3.70 (m, 2H), 3.40 (m, 2H), 1.42 (s, 9H); MS, ESI m/e 434.1 (M^+); HRMS (ESI) calculated for $C_{21}H_{26}N_2O_6SNa^+$ 457.1391, found 457.1391.

*(±)-N-Boc-trans-3-tert-butylmercapto-4-(4-phenoxybenzenesulfonamido)pyrrolidine (**27**).* To a stirred solution of Ph_3P (0.88 g, 3.35 mmol) and (±)-*N*-Boc-*trans*-3-hydroxy-4-(4-phenoxybenzenesulfonamido)pyrrolidine (**25**) (1.316 g, 3.04 mmol) in THF (20 mL) at 0 °C was added DEAD (0.58 mL, 3.33 mmol) dropwise. The reaction mixture was stirred at room temperature overnight. Then it was evaporated under reduced pressure. The residue was purified by flash chromatography (30% ethyl acetate in hexane) to give the *N*-(4-phenoxybenzenesulfonyl)aziridine **26** as a solid (1.23 g, 98%): mp 136−138 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88 (m, 2H), 7.43 (m, 2H), 7.22 (m, 1H), 7.10 (m, 4H), 3.72 (m, 2H), 3.59 (m, 1H), 3.47 (m, 1H), 3.38 (m, 2H), 1.42 (s, 9H); MS, ESI m/e 416.1 (M^+); HRMS (ESI) calculated for $C_{21}H_{24}N_2O_5SNa^+$ 439.1290, found 439.1290.

To a stirred solution of the aziridine **26** (416 mg, 1.0 mmol) and *tert*-butyl mercaptan (0.15 mL, 1.2 mmol) in anhydrous MeOH (5 mL) at 0 °C was added *t*-BuOK (0.2 mmol). The mixture was stirred at room temperature for 4 h. Then it was evaporated under reduced pressure. Ethyl acetate was added to the residue, and the solution was extracted with water and saturated brine. Then it was dried over Na_2SO_4 and evaporated. The residue was purified by flash chromatography (20% ethyl acetate in hexane) to give (±)-*N*-Boc-*trans*-3-*tert*-butylmercapto-4-(4-phenoxybenzenesulfonamido)-pyrrolidine (**27**) as a solid (506 mg, 98%): mp 85−86 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 7.85 (d, J = 7, 2H), 7.45 (t, J = 7, 2H), 7.25 (t, J = 7, 1H), 7.09 (d, J = 7, 4H), 3.81 (m, 2H), 3.58 (m, 1H), 3.47 (m, 1H), 3.22 (m,1H), 3.09 (m, 1H), 1.45 (s, 9H), 1.29 (s, 9H); MS, ESI m/e 506.2 (M^+); HRMS (ESI) calculated for $C_{25}H_{34}N_2O_5S_2Na^+$ 529.1796, found 529.1795.

*(±)-trans-3-Mercapto-4-(4-phenoxybenzenesulfonamido)-pyrrolidine Hydrochloride (**29a**).* To a solution of (±)-*N*-Boc-*trans*-3-*tert*-butylmercapto-4-(4-phenoxybenzenesulfonamido)pyrrolidine (**27**) (117 mg, 0.226 mmol) in AcOH (1 mL) was added 2-nitrobenzenesulfenyl chloride (46.0 mg, 0.243 mmol), and the mixture was stirred at room temperature for 2 h. The AcOH was evaporated under vacuum, and to the residue were added THF (4 mL), 1 N NaOH (0.25 mL), and TCEP (67 mg, 0.234 mmol). The mixture was stirred at room temperature under argon for 3 h. Then it was condensed under vacuum. To the residue was added ethyl acetate, and it was dried over Na_2SO_4 and filtered. The solvent was evaporated and the residue was purified by flash chromatography (ethyl acetate) to give (±)-*N*-Boc-*trans*-3-mercapto-4-(4-phenoxybenzenesulfonamido)-pyrrolidine as a white solid (82 mg, 81%): mp 78−79 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 7.88 (m, 2H), 7.45 (m, 2H), 7.25 (t, J = 7, 1H), 7.10 (m, 4H), 3.74 (m, 1H), 3.55 (m, 2H), 3.17 (m, 2H), 3.00 (m, 1H), 1.44 (s, 9H); MS (ESI) m/e 450.2 (M^+); HRMS (ESI) calculated for $C_{21}H_{26}N_2O_5S_2Na^+$ 473.1172, found 473.1170.

To a solution of 2.5 M HCl in AcOH (1 mL) was added (±)-*N*-Boc-*trans*-3-mercapto-4-(4-phenoxybenzenesulfonamido)pyrrolidine (45 mg, 0.10 mmol), and the mixture was stirred at room temperature for 3 h. Liophylization of the solution gave the product **29a** as a white powder (38.7 mg, 100%): ^{1}H NMR (300 MHz, CD_3OD) δ 7.87 (d, J = 12, 2H), 7.44 (m, 2H), 7.24 (t, J = 7, 1H), 7.12 (m, 4H), 3.72 (m, 1H), 3.62 (m, 2H), 3.37 (m, 1H), 3.19 (m, 2H); MS (ESI) m/e 351.1 (MH^+); HRMS (ESI) calculated for $C_{16}H_{19}N_2O_3S_2^+$ 351.0837, found 351.0837.

*(±)-N-Carbamoyl-trans-3-mercapto-4-(4-phenoxybenzenesulfonamido)pyrrolidine (**29b**).* To a stirred solution of 1.5 M HCl in ethyl acetate (5 mL) was added (±)-*N*-Boc-*trans*-3-*tert*-butylmercapto-4-(4-phenoxybenzenesulfonamido)-pyrrolidine (**27**) (155 mg, 0.3 mmol), and stirring was continued at room temperature until no starting material was detected by TLC (50% ethyl acetate in hexane). The solvent was evaporated under reduced pressure, and the residue was dissolved in CH_2Cl_2. The solution was cooled to 0 °C, and Et_3N (46 μL, 33 mmol) and *N*-trimethylsilyl isocyanate (TMSNCO) (100 μL, 0.74 mmol) were added successively. The solution was stirred at room temperature overnight. The reaction mixture was extracted with water and saturated brine and was dried over Na_2SO_4 and filtered. The solvent was evaporated and the residue was purified by flash chromatography (5% MeOH in ethyl acetate) to give (±)-*N*-carbamoyl-*trans*-3-*tert*-butylmercapto-4-(4-phenoxybenzenesulfonamido)pyrrolidine (130 mg, 96%): mp 132−133 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 7.85 (d, J = 9, 2H), 7.44 (t, J = 8, 2H), 7.24 (t, J = 8, 1H), 7.10 (m, 2H), 7.09 (d, J = 9, 2H), 3.86 (m, 1H), 3.59 (m, 2H), 3.27 (m, 2H), 3.15 (m,1H), 1.29 (s, 9H); MS (ESI) m/e 449.2 (M^+); HRMS (ESI) calculated for $C_{21}H_{27}N_3O_4S_2Na^+$ 472.1322, found 472.1326.

To a solution of (±)-*N*-carbamoyl-*trans*-3-*tert*-butylmercapto-4-(4-phenoxybenzenesulfonamido)pyrrolidine (110 mg, 0.245 mmol) in AcOH (1 mL) was added 2-nitrobenzenesulfenyl chloride (51 mg, 0.269 mmol), and the mixture was stirred at room temperature for 2 h. The AcOH was evaporated under vacuum, and to the residue were added THF (4 mL), 1 N NaOH (0.3 mL), and TCEP (77 mg, 0.269 mmol) with stirring under argon. Stirring under argon was continued for 3 h at room temperature. Then the mixture was concentrated under vacuum. The residue was dissolved in ethyl acetate, and the solution was dried over Na_2SO_4 and filtered. The solvent was evaporated and the residue was purified by flash chromatography (5% MeOH in ethyl acetate) to give the mercaptan **29b** as a white solid (89 mg, 96%): mp 170−171 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 7.87 (m, 2H), 7.44 (m, 2H), 7.24 (m, 1H), 7.10 (m, 4H), 3.78 (m, 1H), 3.68 (m, 1H), 3.59 (m, 1H), 3.20 (m, 2H), 3.08 (m, 1H); MS (ESI) m/e 393.1 (M^+); HRMS (ESI) calculated for $C_{17}H_{19}N_3O_4S_2Na^+$ 416.0701, found 416.0699.

*(±)-N-Carbamoyl-trans-3-mercapto-4-(N-methyl-4-phenoxybenzenesulfonamido)pyrrolidine (**29c**).* To a solution of (±)-*N*-Boc-*trans*-3-*tert*-butylmercapto-4-(4-phenoxybenzenesulfonamido)pyrrolidine (**27**) (236 mg, 0.466 mmol) in DMF (2 mL) at 0 °C was added *t*-BuOK (0.69 mmol). The mixture was stirred at 0 °C for 10 min. Then MeI (0.69 mmol) was added. The solution was stirred at 0 °C until TLC analysis showed no starting material remained. Ethyl acetate (20 mL) was added, and the solution was extracted with water, then was dried over Na_2SO_4 and filtered. The solvent was evaporated and the residue was purified by flash chromatography (ethyl acetate) to give (±)-*N*-Boc-*trans*-3-*tert*-butylmercapto-4-(*N*-methyl-4-phenoxybenzenesulfonamido)-pyrrolidine (**28**, R = CH_3) as a white powder (240 mg, 99%): ^{1}H NMR (300 MHz, CD_3OD) δ 7.84 (d, J = 8, 2H), 7.44 (t, J = 8, 2H), 7.24 (t, J = 8, 1H), 7.10 (m, 2H), 7.09 (d, J = 9, 2H), 4.22 (m, 1H), 3.90 (m, 1H), 3.37 (m, 2H), 3.08 (m, 2H), 2.86 (s, 3H), 1.44 (s, 9H), 1.28 (s, 9H); MS (ESI) m/e 520.2 (M^+); HRMS (ESI) calculated for $C_{26}H_{36}N_2O_5S_2Na^+$ 543.1950, found 543.1950.

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To a stirred solution of 1.5 M HCl in ethyl acetate (5 mL) was added (±)-*N*-Boc-*trans*-3-*tert*-butylmercapto-4-(*N*-methyl-4-phenoxybenzenesulfonamido)pyrrolidine (**28**, R = CH_3) (130 mg, 0.25 mmol), and stirring was continued at room temperature until no starting material was detected by TLC. The solvent was evaporated under reduced pressure, and the residue was dissolved in CH_2Cl_2. The solution was cooled to 0 °C, and Et_3N (46 μL, 0.33 mmol) and TMSNCO (100 μL, 0.74 mmol) were added successively. The solution was stirred at room temperature overnight. The reaction mixture was extracted with water and saturated brine and was dried over Na_2SO_4 and filtered. The solvent was evaporated and the residue was purified by flash chromatography (5% MeOH in ethyl acetate) to give (±)-*N*-carbamoyl-*trans*-3-*tert*-butylmercapto-4-(*N*-methyl-4-phenoxybenzenesulfonamido)pyrrolidine as a white powder (110 mg, 95%): ^{1}H NMR (300 MHz, CD_3OD) δ 7.85 (m, 2H), 7.42 (m, 2H), 7.22 (m, 1H), 7.10 (m, 4H), 4.24 (m, 1H), 3.96 (m, 1H), 3.42 (m, 2H), 3.08 (m, 2H), 2.87 (s, 3H), 1.28 (s, 9H); MS (ESI) *m*/*e* 463.2 (M^+); HRMS (ESI) calculated for $C_{22}H_{29}N_3O_4S_2Na^+$ 486.1479, found 486.1481.

To a solution of (±)-*N*-carbamoyl-*trans*-3-*tert*-butylmercapto-4-(*N*-methyl-4-phenoxybenzenesulfonamido)pyrrolidine (90 mg, 0.194 mmol) in AcOH (1 mL) was added 2-nitrobenzenesulfenyl chloride (38.6 mg, 0.204 mmol), and the mixture was stirred at room temperature for 2 h. The AcOH was evaporated under vacuum, and to the residue were added THF (4 mL), 1 N NaOH (0.25 mL), and TCEP (67 mg, 0.234 mmol). The mixture was stirred at room temperature under argon for 3 h. Then it was condensed under vacuum. To the residue was added ethyl acetate, and it was dried over Na_2SO_4. The solvent was evaporated and the residue was purified by flash chromatography (5% MeOH in ethyl acetate) to give the mercaptan **29c** as a white powder (75.1 mg, 95%): ^{1}H NMR (300 MHz, CD_3OD) δ 7.87 (m, 2H), 7.44 (m, 2H), 7.24 (m, 1H), 7.10 (m, 4H), 4.32 (q, *J* = 9, 1H), 3.83 (dd, *J* = 10, 8 Hz, 1H), 3.42 (m, 2H), 3.13 (q, *J* = 10, 2H), 2.83 (s, 3H); MS (ESI) *m*/*e* 407.2 (M^+); HRMS (ESI) calculated for $C_{22}H_{29}N_3O_4S_2Na^+$ 430.0864, found 430.0867.

*(±)-N-(2-Phthalimidoethylaminocarbonyl)-trans-3-mercapto-4-(4-phenoxybenzenesulfonamido)pyrrolidine (**29d**).* To a stirred solution of 1.5 M HCl in ethyl acetate (3 mL) was added (±)-*N*-Boc-*trans*-3-*tert*-butylmercapto-4-(4-phenoxybenzenesulfonamido)-pyrrolidine (**27**) (77.5 mg, 0.15 mmol), and stirring was continued at room temperature until no starting material was detected by TLC (50% ethyl acetate in hexane). The solvent was evaporated under reduced pressure, and the residue was dissolved in CH_2Cl_2 (3 mL). The solution was cooled to −10 °C, and diisopropylethylamine (63 μL, 0.36 mmol) and triphosgene (14.9 mg, 0.05 mmol) in CH_2Cl_2 (3 mL) were added. The mixture was stirred at −10 °C for 20 min. Then diisopropylethylamine (63 μL, 0.36 mmol) and 2-phthalimidoethylamine hydrochloride (34 mg, 0.15 mmol) were added successively. The solution was warmed to room temperature and was stirred for 4 h. The reaction mixture was extracted with water, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by flash chromatography (5% MeOH in ethyl acetate) to give (±)-*N*-(2-phthalimidoethylaminocarbonyl)-*trans*-3-*tert*-butylmercapto-4-(4-phenoxybenzenesulfonamido)pyrrolidine as a white solid (79 mg, 84%): mp 105−106 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92 (m, 2H), 7.85 (m, 2H), 7.72 (m, 2H), 7.42 (m, 2H), 7.24 (m, 1H), 7.10 (m, 4H), 5.62 (d, *J* = 9, 1H), 4.62 (m, 1H), 3.98−3.70 (m, 3H), 3.62 (m, 1H), 3.48 (m, 3H), 3.24 (m, 2H), 3.14 (m, 1H), 1.32 (s, 9H); MS (ESI) *m*/*e* 622.2 (M^+); HRMS (ESI) calculated for $C_{31}H_{34}N_4O_6S_2Na^+$ 645.1812, found 645.1814.

To a solution of (±)-*N*-(2-phthalimidoethylaminocarbonyl)-*trans*-3-*tert*-butylmercapto-4-(4-phenoxybenzenesulfonamido)pyrrolidine (62.3 mg, 0.10 mmol) in AcOH (1 mL) was added 2-nitrobenzenesulfenyl chloride (21 mg, 0.11 mmol), and the mixture was stirred at room temperature for 2 h. The AcOH was evaporated under vacuum, and to the residue were added THF (2 mL), 1 N NaOH (0.2 mL), and TCEP (34 mg, 0.12 mmol). The mixture was stirred at room temperature under argon for 3 h. Then it was concentrated under vacuum. To the residue was added ethyl acetate, and the solution was dried over Na_2SO_4 and filtered. The solvent was evaporated and the residue was purified by flash chromatography (ethyl acetate) to give the mercaptan **29d** as a white solid (48 mg, 87%): mp 140−141.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.93−7.80 (m, 4H), 7.73 (m, 2H), 7.41 (m, 2H), 7.23 (t, *J* = 8, 1H), 7.15−7.00 (m, 4H), 5.73 (br, 1H), 4.73 (m, 1H), 3.87 (m, 2H), 3.71 (m, 2H), 3.55 (m, 1H), 3.47 (m, 2H), 3.37 (m, 1H), 3.24 (m, 1H), 3.13 (m, 1H), 1.74 (d, *J* = 7, 1H); MS (ESI) *m*/*e* 566.2 (M^+); HRMS (ESI) calculated for $C_{27}H_{26}N_4O_6S_2Na^+$ 589.1191, found 589.1190.

*(±)-N-(6-Acetamidohexanoyl)-trans-3-mercapto-4-(4-phenoxybenzenesulfonamido)pyrrolidine (**29e**) and (±)-N-(6-Aminohexanoyl)-trans-3-mercapto-4-(4-phenoxybenzenesulfonamido)pyrrolidine Hydrochloride (**29f**).* To a stirred solution of 1.5 M HCl in ethyl acetate (1 mL) was added (±)-*N*-Boc-*trans*-3-*tert*-butylmercapto-4-(4-phenoxybenzenesulfonamido)pyrrolidine (**27**) (200 mg, 0.39 mmol), and stirring was continued at room temperature until no starting material was detected by TLC (50% ethyl acetate in hexane). The solvent was evaporated under reduced pressure, and the residue was dissolved in CH_2Cl_2. The solution was cooled to 0 °C, and Et_3N (60 μL, 0.43 mmol), HOBt·H_2O (54 mg, 0.40 mmol), 6-(*tert*-butoxycarbonylamino)hexanoic acid (90 mg, 0.39 mmol), and DCC (80 mg, 0.39 mmol) were added. The mixture was stirred at room temperature overnight. The solvent was evaporated under reduced pressure, and the residue was dissolved in ethyl acetate (10 mL). The organic layer was washed with 10% citric acid and saturated aqueous $NaHCO_3$. Then it was dried over Na_2SO_4 and evaporated. The residue was purified by flash chromatography (50% ethyl acetate in hexane) to give (±)-*N*-(6-(*tert*-butoxycarbonylamino)hexanoyl)-*trans*-3-*tert*-butylmercapto-4-(4-phenoxybenzenesulfonamido)pyrrolidine (201 mg, 84%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.82 (dd, *J* = 9, 3, 2H), 7.43 (t, *J* = 8, 2H), 7.24 (t, *J* = 7, 1H), 7.08 (m, 4H), 5.13 (br m, 1H, NH), 4.57 (br s, 1H, NH), 3.72−4.10 (m, 2H), 3.00−3.45 (m, 6H), 2.21 (t, *J* = 7, 2H), 1.64 (m, 2H), 1.48 (m, 2H), 1.44 (s, 9H), 1.36 and 1.30 (s, 9H), 1.26 (m, 2H); HRMS (ESI) calculated for $C_{31}H_{45}N_3O_6S_2Na^+$ 642.2642, found 642.2654.

To a solution of (±)-*N*-(6-(*tert*-butoxycarbonylamino)hexanoyl)-*trans*-3-*tert*-butylmercapto-4-(4-phenoxybenzenesulfonamido)-pyrrolidine (130 mg, 0.21 mmol) in AcOH−CH_2Cl_2 (1:1, 1 mL) was added 2-nitrobenzenesulfenyl chloride (42 mg, 0.22 mmol), and the mixture was stirred at room temperature for 2 h. The solvents were evaporated under vacuum and the residue was purified by flash chromatography (50% ethyl acetate in hexane) to give (±)-*N*-(6-(*tert*-butoxycarbonylamino)hexanoyl)-*trans*-3-(2-nitrobenzenedisulfido)-4-(4-phenoxybenzenesulfonamido)pyrrolidine: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.28 (m, 1H), 8.22 and 8.11 (d, *J* = 8, 1H), 7.75 (m, 3H), 7.42 (m, 3H), 7.24 (m, 1H), 7.10 (m, 2H), 7.02 (m, 2H), 5.40 and 5.12 (br s, 1H, NH), 4.57 (br s, 1H, NH), 3.75−4.00 (m, 3H), 3.21−3.53 (m, 3H), 2.95−3.14 (m, 2H), 2.19 (m, 2H), 1.61 (m, 2H), 1.47 (m, 2H), 1.43 (s, 9H), 1.33 (m, 2H).

A solution of (±)-*N*-(6-(*tert*-butoxycarbonylamino)hexanoyl)-*trans*-3-(2-nitrobenzenedisulfido)-4-(4-phenoxybenzenesulfonamido)-pyrrolidine (78 mg, 0.11 mmol) in trifluoroacetic acid (1 mL) was stirred at room temperature for 1 h. The trifluoroacetic acid was evaporated under vacuum, and the residue was dissolved in CH_2Cl_2 (1 mL). The solution was cooled to 0 °C, and Et_3N (34 μL, 0.244 mmol) and acetyl chloride (8.6 μL, 0.12 mmol) were added. The mixture was stirred for 2 h. The solution was evaporated under vacuum. The residue was dissolved in ethyl acetate (5 mL), washed with water, dried over Na_2SO_4, and the solvent was evaporated. The residue was purified by flash chromatography (100% ethyl acetate) to give (±)-*N*-(6-acetamidohexanoyl)-*trans*-3-(2-nitrobenzenedisulfido)-4-(4-phenoxybenzenesulfonamido)pyrrolidine (68 mg, 94%): ^{1}H NMR (300 MHz, CD_3OD) δ 8.20−8.33 (m, 2H), 7.76 (m, 3H), 7.47 (m, 3H), 7.25 (t, *J* = 8, 1H), 7.10 (d, *J* = 9, 2H), 6.99 (m, 2H), 3.92 (m, 2H), 3.68−3.86 (m, 2H), 3.52 (m, 1H), 3.39 (m, 1H), 3.14 (m, 2H), 2.24 (m, 2H), 1.93 and 1.92 (s, 3H), 1.54 (m, 4H), 1.34 (m, 2H). To a solution of the 2-nitrobenzene disulfide (10 mg, 0.015 mmol) in THF−H_2O (4:1, 1 mL) were added TCEP (5 mg, 0.017 mmol) and 1 N NaOH (20 μL). The mixture was stirred until no starting material was detected by TLC (100% ethyl acetate). The solvent was

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evaporated under reduced pressure and the residue was purified by short flash column chromatography (100% ethyl acetate) to give the mercaptan **29e** (7 mg, 91%): ^{1}H NMR (300 MHz, CD_3OD) δ 7.88 (m, 2H), 7.45 (t, J = 8, 2H), 7.25 (t, J = 8, 1H), 7.10 (m, 4H), 3.81−3.98 (m, 2H), 3.53−3.72 (m, 2H), 3.05−3.40 (m, 4H), 2.26 (m, 2H), 1.92 (s, 3H), 1.46−1.65 (m, 4H), 1.30−1.41 (m, 2H); HRMS (ESI) calculated for $C_{24}H_{32}N_3O_5S_2^+$ 506.1778, found 506.1786.

To a solution of (±)-*N*-(6-(*tert*-butoxycarbonylamino)hexanoyl)-*trans*-3-(2-nitrobenzenedisulfido)-4-(4-phenoxybenzenesulfonamido)-pyrrolidine (50 mg, 0.7 mmol) in THF−H_2O (4:1, 1 mL) were added TCEP (23 mg, 0.8 mmol) and 1 N NaOH (100 μL). The mixture was stirred until no starting material was detected by TLC (50% ethyl acetate in hexane). The solution was evaporated under reduced pressure, and the residue was purified by short flash column chromatography (50% ethyl acetate in hexane) to give the thiol. The latter was dissolved in 2 M HCl in acetic acid (1 mL), and the mixture was stirred at room temperature for 1 h. Liophilization of the solution gave the mercaptan **29f** as a white hygroscopic solid (33 mg, 94%): ^{1}H NMR (300 MHz, CD_3OD) δ 7.88 (m, 2H), 7.45 (t, J = 8, 2H), 7.25 (t, J = 8, 1H), 7.10 (m, 4H), 3.84−3.97 (m, 2H), 3.71 (dd, J = 12, 7, 1H), 3.52−3.64 (m, 2H), 3.06−3.18 (m, 1H), 2.93 (br m, 2H), 2.31 (q, J = 7, 2H), 1.66 (br m, 4H), 1.42 (br m, 2H); HRMS (ESI) calculated for $C_{22}H_{30}N_3O_4S_2^+$ 464.1672, found 464.1672.

*(±)-N-(6-Aminohexanoyl)-trans-3-hydroxy-4-(4-phenoxybenzenesulfonamido)pyrrolidine Hydrochloride (**29g**).* To a stirred solution of 1.5 M HCl in ethyl acetate (3 mL) was added (±)-*N*-Boc-*trans*-3-hydroxy-4-(4-phenoxybenzenesulfonamido)-pyrrolidine (**25**) (430 mg, 0.99 mmol), and stirring was continued until no starting material was detected by TLC (50% ethyl acetate in hexane). The solvent was evaporated under reduced pressure, and the residue was dissolved in CH_2Cl_2. The solution was cooled to 0 °C, and Et_3N (153 μL, 1.1 mmol), HOBt·H_2O (135 mg, 1.0 mmol), 6-(*tert*-butoxycarbonylamino)hexanoic acid (231 mg, 1.0 mmol), and DCC (206 mg, 1.0 mmol) were added. The mixture was stirred at room temperature overnight. The solvent was evaporated under reduced pressure, and the residue was dissolved in ethyl acetate (10 mL). The organic layer was washed with 10% citric acid and saturated aqueous $NaHCO_3$. Then it was dried over Na_2SO_4 and evaporated. The residue was purified by flash chromatography (50% ethyl acetate in hexane) to give (±)-*N*-(6-(*tert*-butoxycarbonylamino)hexanoyl)-*trans*-3-hydroxy-4-(4-phenoxybenzenesulfonamido)pyrrolidine (400 mg, 74%). The latter was dissolved in 2 M HCl in acetic acid (3 mL), and the mixture was stirred at room temperature for 1 h. Liophylization of the solution gave (±)-*N*-(6-aminohexanoyl)-*trans*-3-hydroxy-4-(4-phenoxybenzenesulfonamido)pyrrolidine hydrochloride (**29g**) as a white hygroscopic solid (353 mg, 100%): ^{1}H NMR (300 MHz, CD_3OD) δ 7.86 (dd, J = 9, 3, 2H), 7.46 (t, J = 8, 2H), 7.25 (t, J = 8, 1H), 7.10 (m, 4H), 4.24 and 4.12 (m, 1H), 3.70−3.76 (m, 1H), 3.47−3.63 (m, 3H), 3.34−3.42 (m, 1H), 2.93 (br t, J = 7, 2H), 2.35 and 2.31 (t, J = 7, 2H), 1.67 (m, 4H), 1.44 (m, 2H); HRMS (ESI) calculated for $C_{22}H_{30}N_3O_5S^+$ 448.1906, found 448.1904.

Enzymatic Resolution of (±)-N-Boc-trans-3-hydroxy-4-azidopyrrolidine (Scheme 4). To a solution of (±)-*N*-Boc-*trans*-3-hydroxy-4-azidopyrrolidine (**24**) (2.28 g, 10 mmol) in *tert*-butyl methyl ether (50 mL) were added isopropenyl acetate (3 g, 3 mmol) and lipase Amano AK-20 (3 g), and the mixture was stirred at room temperature for 72 h. The mixture was filtered. The filtrate was evaporated under reduced pressure, and the product was separated by flash chromatography (25% ethyl acetate in hexane). First eluted was (3*R*,4*R*)-*trans*-*N*-Boc-3-acetoxy-4-azidopyrrolidine (**31**) (1.30 g): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.09 (br s, 1H), 4.04 (m, 1H), 3.57−3.74 (m, 2H), 3.34−3.55 (m, 2H), 2.09 (s, 3H), 1.47 (s, 9H); $[\alpha]_D^{20}$ −30.4° (*c* 1.0, $CHCl_3$).

Second to be eluted was (3*S*,4*S*)-*trans*-*N*-Boc-3-hydroxy-4-azidopyrrolidine (**30**) (1.07 g): ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.25 (br s, 1H), 3.93 (br s, 1H), 3.55−3.75 (m, 2H), 3.30−3.50 (m, 2H), 2.05 (br s, 1H), 1.46 (s, 9H); $[\alpha]_D^{20}$ +26.8° (*c* 0.5, $CHCl_3$).

To a stirred solution of (3*R*,4*R*)-*trans*-*N*-Boc-3-acetoxy-4-azidopyrrolidine (**31**) (1.0 g, 3.4 mmol) in THF−MeOH (3:1, 8 mL) at 0 °C was added 1 N LiOH (4 mL) dropwise. The reaction mixture was stirred at room temperature overnight. Then it was evaporated under reduced pressure. Ethyl acetate (20 mL) was added to the residue, and the solution was washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by flash column chromatography (50% ethyl acetate in hexane) to give (3*R*,4*R*)-*trans*-*N*-Boc-3-hydroxy-4-azidopyrrolidine (**32**): ^{1}H NMR (300 MHz, $CDCl_3$) identical to that of the (3*S*,4*S*) isomer; $[\alpha]_D^{20}$ −26.8° (*c* 1.0, $CHCl_3$).

A sample of the (3*S*,4*S*)-*trans*-*N*-Boc-3-hydroxy-4-azidopyrrolidine (**30**) (80 mg, 0.35 mmol) was dissolved in trifluoroacetic acid (1 mL) and was stirred until no starting material was detected on TLC (25% ethyl acetate in hexane). The solution was evaporated under reduced pressure, and the residue was dissolved in CH_2Cl_2 (2 mL). The solution was cooled to 0 °C. Et_3N (117 μL, 0.84 mmol) and Cbz-Cl (60 mg, 0.35 mmol) were added, and the resulting solution was stirred for 2 h at room temperature. The reaction mixture was washed with water, dried over Na_2SO_4, and evaporated. The residue was purified by flash chromatography (25% ethyl acetate in hexane) to give (3*S*,4*S*)-*trans*-*N*-Cbz-3-hydroxy-4-azidopyrrolidine (89 mg, 97%): $[\alpha]_D^{20}$ +16.1° (*c* 1.00, $CHCl_3$) (lit.:[64] $[\alpha]_D^{25}$ +14.3° (*c* 1.06, $CHCl_3$)).

*(3S,4S)-trans-3-Mercapto-4-(4-phenoxybenzenesulfonamido)-pyrrolidine Hydrochloride (**39a**).* A solution of (3*S*,4*S*)-*trans*-*N*-Boc-3-hydroxy-4-azidopyrrolidine (**30**) (850 mg, 3.72 mmol) and Et_3N (622 μL, 4.46 mmol) in CH_2Cl_2 (10 mL) was cooled with stirring to 0 °C, and methanesulfonyl chloride (331 μL, 4.28 mmol) was added dropwise. The solution was stirred at room temperature until no starting material was detected by TLC (25% ethyl acetate in hexane). The reaction mixture was washed with 10% aqueous citric acid and water, then was dried over Na_2SO_4 and evaporated. The residual crude methanesulfonate was dissolved in anhydrous DMF (20 mL). Potassium acetate (1.10 g, 11.2 mmol) was added, and the mixture was stirred at 105 °C under N_2 for 6 h. The reaction mixture was poured into ice−water and was extracted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by flash chromatography (25% ethyl acetate in hexane) to give (3*R*,4*S*)-*cis*-*N*-Boc-3-acetoxy-4-azidopyrrolidine (**33**) (860 mg, 86%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.31 (br m, 1H), 4.07 (m, 1H), 3.60−3.71 (m, 2H), 3.36−3.50 (m, 2H), 2.16 (s, 3H), 1.46 (s, 9H); $[\alpha]_D^{20}$ −34.5° (*c* 1.00, $CHCl_3$).

To a stirred solution of the (3*R*,4*S*)-azidoacetate **33** (560 mg, 2.07 mmol) in THF−MeOH (3:1, 8 mL) at 0 °C was added 1 N LiOH (3 mL) dropwise. The reaction mixture was stirred at room temperature overnight. Then it was evaporated under reduced pressure. Ethyl acetate (20 mL) was added to the residue, and the solution was washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by flash chromatography (50% ethyl acetate in hexane) to give (3*R*,4*S*)-*cis*-*N*-Boc-3-hydroxy-4-azidopyrrolidine (**34**) (460 mg, 97%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.35 (br m, 1H), 4.02 (br m, 1H), 3.25−3.72 (m, 4H), 2.16 (br s, 1H), 1.46 (s, 9H); $[\alpha]_D^{20}$ +32.3° (*c* 0.69, $CHCl_3$).

To a solution of the (3*R*,4*S*)-azido alcohol **34** (470 mg, 2.06 mmol) in THF−H_2O (10:1, 10 mL) was added Ph_3P (540 mg, 2.06 mmol), and the mixture was stirred at room temperature for 2 h and at 65 °C until no starting material was detected on TLC (25% ethyl acetate in hexane). The solution was cooled with stirring to 0 °C, and Et_3N (345 μL, 2.48 mmol) was added. Then a solution of 4-phenoxybenzenesulfonyl chloride (529 mg, 2.26 mmol) in THF (2 mL) was added dropwise. The reaction mixture was warmed to room temperature and was stirred for 3 h. The mixture was concentrated under reduced pressure. Ethyl acetate was added to the residue, and the organic layer was washed with 10% aqueous citric acid and saturated aqueous $NaHCO_3$. Then it was dried over Na_2SO_4 and evaporated. The residue was purified by flash chromatography (50% ethyl acetate in hexane) to give (3*R*,4*S*)-*cis*-*N*-Boc-3-hydroxy-4-(4-phenoxybenzenesulfonamido)-pyrrolidine (**35**) (670 mg, 75%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (d, J = 7, 2H), 7.42 (t, J = 8, 2H), 7.24 (t, J = 8, 1H), 7.06 (m, 4H), 5.14 (br s, 1H), 4.25 and 4.11 (br s, 1H), 3.76 (m, 1H), 3.55 (m, 1H), 3.43 (m, 2H), 3.07 (m, 1H), 2.36 and 2.23 (br s, 1H), 1.43 (s, 9H); $[\alpha]_D^{20}$ +15.6° (*c* 0.80, $CHCl_3$).

To a stirred solution of Ph_3P (525 mg, 2.0 mmol) in THF (3 mL) at 0 °C was added DEAD (315 μL, 2.0 mmol) dropwise. After 10 min, a solution of alcohol **35** (435 mg, 1.0 mmol) and thiolacetic acid (228

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μL, 2.29 mmol) in THF (3 mL) was added. The resulting reaction mixture was stirred at room temperature for 6 h. Then it was concentrated under reduced pressure. The residue was purified by flash chromatography (15% ethyl acetate in hexane) to give (3*S*,4*S*)-*trans*-*N*-Boc-3-acetylthio-4-(4-phenoxybenzenesulfonamido)-pyrrolidine (**36**) (400 mg, 81%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80 (d, J = 9, 2H), 7.42 (m, 2H), 7.23 (t, J = 8, 1H), 7.06 (m, 4H), 5.21 and 5.00 (br s, 1H, NH), 3.75 (m, 4H), 3.16 (m, 2H), 2.31 and 2.25 (br s, 3H), 1.44 (s, 9H); $[\alpha]_D^{20}$ +22.8° (c 0.62, $CHCl_3$).

To a stirred solution of the acetylthiopyrrolidine **36** (60 mg, 0.122 mmol) in MeOH (1 mL) under nitrogen was added 40% aqueous $MeNH_2$ (100 μL, 1.16 mmol), and the mixture was stirred for 10 min. The solution was concentrated under vacuum and the residue was purified by flash chromatography (50% ethyl acetate in hexane) to give (3*S*,4*S*)-*trans*-*N*-Boc-3-mercapto-4-(4-phenoxybenzenesulfonamido)-pyrrolidine (**37**) (54 mg, 98%) as an oil: ^{1}H NMR (300 MHz, CD_3OD) identical to that of the (±)-modification described earlier; $[\alpha]_D^{20}$ +39.2° (c 0.54, MeOH).

To a solution of 2.0 M HCl in AcOH (1 mL) was added (3*S*,4*S*)-*trans*-*N*-Boc-3-mercapto-4-(4-phenoxybenzenesulfonamido)-pyrrolidine (**37**) (45 mg, 0.10 mmol), and the mixture was stirred at room temperature for 1 h. Liophilization of the reaction mixture gave the amine hydrochloride **39a** as a white hygroscopic solid (37 mg, 100%): ^{1}H NMR (300 MHz, CD_3OD) identical to that of the racemic inhibitor **29a**; $[\alpha]_D^{20}$ +21.9° (c 0.7, MeOH); HRMS (ESI) calculated for $C_{16}H_{19}N_2O_3S_2^+$ 351.0832, found 351.0824.

*(3R,4R)-3-Mercapto-4-(4-phenoxybenzenesulfonamido)-pyrrolidine Hydrochloride (**39b**).* By the procedures used for the 3*S*,4*S* enantiomer, (3*R*,4*R*)-*trans*-*N*-Boc-3-hydroxy-4-azidopyrrolidine was converted to the title inhibitor **39b** as a white hygroscopic solid: ^{1}H NMR (300 MHz, CD_3OD) identical to those of **29a** and **39a**; $[\alpha]_D^{20}$ −21.0° (c 1.0, MeOH); HRMS (ESI) calculated for $C_{16}H_{19}N_2O_3S_2^+$ 351.0832, found 351.0820.

Enzyme Kinetics. Enzymatic assays to characterize inhibitor potency were performed as previously described.[17,65] Assays were consistently carried out at 25 °C in 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer, pH 7.5, with 10 mM $CaCl_2$, 200 mM NaCl, and 0.01% (w/v) Brij-35. The reducing agent TCEP was also included at 5 μM to maintain the fully reduced form of the MMPIs. Briefly, 10 μL of varying concentrations of MMPIs dissolved in DMSO, 176 μL of HEPES buffer, and 10 μL of enzyme stock solution were mixed and incubated for 30 min. The assay was started by addition of 4 μL of a 1 μM solution of the synthetic substrate Mca-PLGLDpa-AR-NH_2 (M-1895, Bachem)[66] in 50% DMSO, and the release of product was monitored by fluorescence (λ_{ex} = 328 nm and λ_{em} = 393 nm) with a PerkinElmer luminescence spectrophotometer LS50B (FL WinLab, version 3.0) connected to a temperature-controlled water bath. Enzyme concentrations ranged from 0.2 to 7 nM during the assays.

Stability Assays. To assess the relative stability of the thiol inhibitors over time, the inhibitory potencies of these compounds were assessed at varying time points. The compounds were initially dissolved in DMSO and diluted in cell culture medium (containing either 20%, 10%, or 0% fetal bovine serum) to a concentration approximately 10 000 times their IC_{50} value. They were then incubated in a humidified environment at 37 °C and 5% CO_2 for varying periods up to 72 h. After the initial incubation period, 10 μL of the inhibitor solution was further incubated with 176 μL of HEPES buffer and 10 μL of MMP-9 enzyme (final concentration of ∼1 nM) for 30 min prior to initiation of the assay. The relative rates (v_i/v_o) for each compound at its various time points were obtained in triplicate as previously described and plotted versus time to indicate their corresponding stability.

Cell Culture and Compound Toxicity. Low passage hMSCs were routintely cultured in α-modified minimum essential medium (αMEM) supplemented with L-glutamine, penicillin, streptomycin, and 20% fetal bovine serum. Prior to the initial seeding of the cells at low density (∼60 cells/cm^2), cells were plated for 24 h of recovery and maintained at 37 °C in a humidified atmosphere containing 5% CO_2. The addition of fresh culture medium was provided every 3 days until a confluency of 75% was reached. At this point varying dosages of inhibitor (maintaining a constant final DMSO concentration) were used to supplement fresh medium and added to the cells for a period of 24 h. The logarithmic panel of inhibitor concentrations ranged from 100 to 1 μM. At the end of the treatment period the inhibitor-conditioned medium was removed and the cells were trypsinized (0.25% trypsin, Hyclone) after being washed with phosphate-buffered saline (pH 7.4).

To quantify the levels of toxicity these compounds elicited on hMSC growth, cells were pelleted by centrifugation at 450*g* and resuspended in 1 mL of medium. An aliquot of the suspension was then diluted 1:1 with a 0.4% (w/v) trypan blue solution and counted with a hemocytometer. Determined by normalization of the number of cells counted for each treatment against its respective control, the relative groups were plotted with error bars representing the standard error in the mean (mean ± SEM). Each of the treatments and hemocytometer counts were performed in triplicate, and compounds at a particular concentration killing more than 20% of the cells were deemed cytotoxic.

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Author Contributions

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Y.J. performed compound synthesis and characterization. M.D.R. was responsible for cell culture, cytotoxicity, and stability results. D.B.B., Q.C., and M.H.C. determined inhibition constants and IC_{50} values. M.A.S. and Q.-X.A.S. conceived the study and experimental designs, and Y.J., M.D.R., M.A.S., and Q.-X.A.S. wrote the manuscript.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

DEAD, diethyl azodicarboxylate; ECM, extracellular matrix; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; hMSC, human mesenchymal stem cell; MMPI, matrix metalloproteinase inhibitor; TCEP, tris(2-carboxyethyl)phosphine; TIMP, tissue inhibitor of metalloproteinase; TMSNCO, trimethylsilyl isocyanate; ZBG, zinc-binding group

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