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Syntheses of New Conformationally Constrained *S*-[2-[(1-Iminoethyl)amino] ethyl]homocysteine Derivatives as Potential Nitric Oxide Synthase Inhibitors

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ABSTRACT: *The efficient syntheses of two new types of conformationally constrained S-[2-[(1-iminoethyl) amino]ethyl]homocysteine derivatives, 1-amino-3-[2[(1-iminoethyl)amino]ethylthio]cyclobutane carboxylic acid (***5***) and (4S)-4-[[2-[(1-Iminoethyl)amino] ethyl]thio]-L-proline (***6***), are reported. These molecules represent the first attempts to probe conformational constraint near the α-amino acid moiety of known homocysteine-based inhibitors of nitric oxide synthase. Targets* **5** *and* **6** *were evaluated as potential inhibitors of the three human isoforms of nitric oxide synthase.* © 2002 John Wiley & Sons, Inc. Heteroatom Chem 13:77–83, 2002; DOI 10.1002/hc.1109

INTRODUCTION

Nitric oxide (NO) is an endogenously produced free radical that plays an important role in many physiological processes and has become one of the most widely studied substances in biomedical research [1,2]. Nitric oxide is produced by the conversion of L-arginine (**1**) to L-citrulline by a five-electron oxidation reaction catalyzed by the heme-containing enzyme, nitric oxide synthase (NOS) [3]. To date, three distinct human isoforms of NOS have been identified [4]. Two human constitutive isoforms, which require Ca^{2+} and calmodulin for activity, include the neuronal (hncNOS) and endothelial (hecNOS) isoforms, which are found mainly in the brain and the vascular endothelium, respectively. These constitutive isoforms produce small amounts of NO. Nitric oxide produced by hncNOS appears to act as a neurotransmitter, while NO produced by hecNOS helps regulate blood pressure and platelet aggregation [5–7]. In contrast, the inducible human isoform (hiNOS) is found in activated macrophages and is not activated by Ca^{2+} or calmodulin. Its major function is in inflammatory process and host defense, and the overproduction of much larger quantities of NO by hiNOS is implicated in multiple disease states [8–10]. Therefore, hiNOS represents an attractive therapeutic target, and several approaches for selective inhibitors have been identified for the potential treatment of these diseases [11–13].

Since L-arginine is the natural substrate of NOS and the NOS reaction takes place at the N^G-guanidino group, several extended arginine derivatives

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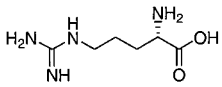
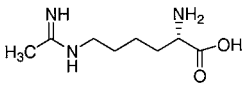
containing amidine replacements for the guanidinium moiety have recently been identified as potent and selective NOS inhibitors. Compared to **1** [14], the lysine derivative L-N^6-(1-iminoethyl)lysine (L-NIL)(**2**) contains an extra carbon atom between the α-amino acid moiety and the amidine group. L-NIL represents a low micromolar inhibitor of murine [15] and human [16] iNOS that also exhibits significant selectivity versus the respective constitutive isoforms. Similarly, the sulfur-linked analogs **3** and **4** have also recently been identified as potent and selective iNOS inhibitors [17]. Interestingly, in comparing **4** with either **3** or **2**, further extension of the distance between the amidine and α-amino acid functionalities appears to significantly enhance the selectivity of the homocysteine inhibitor **4** without detracting from its iNOS potency.



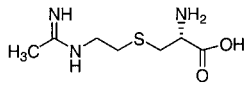
1

iNOS: K_m = 9.5 μM
eNOS: K_m = 1.1 μM
nNOS: K_m = 2.7 μM



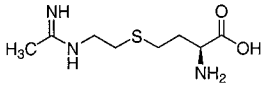
2

hiNOS: IC_{50} = 4.6 μM
hecNOS: IC_{50} = 138μM
hncNOS: IC_{50} = 61 μM



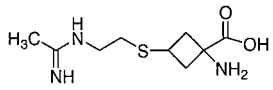
3

hiNOS: IC_{50} = 0.7 μM
hecNOS: IC_{50} = 40 μM
hncNOS: IC_{50} = 13 μM



4

hiNOS: IC_{50} = 1.4 μM
hecNOS: IC_{50} = 466 μM
hncNOS: IC_{50} = 145 μM



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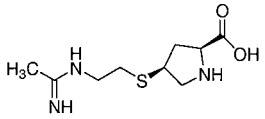


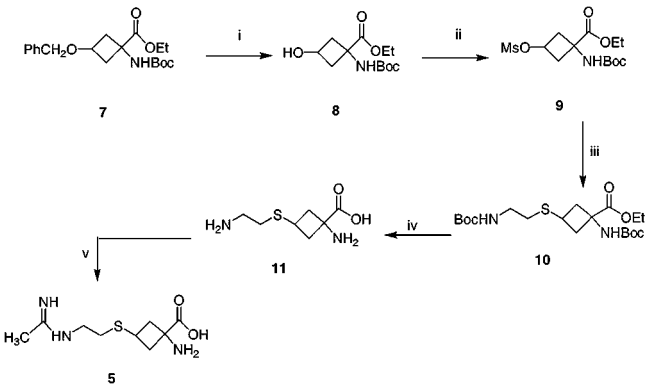
6

Modification of an enzyme's substrate is a well-tested strategy for the discovery of potent enzyme inhibitors. The introduction of conformational constraining elements has been a particularly effective approach to convert modest micromolar substrate-based inhibitors into dramatically more potent inhibitors. As recently reported, several approaches have been explored to improve the potency of arginine-based NOS inhibitors [14]. These efforts introduced aromatic rings or an unsaturated C=C as constraining elements either near the guanidinium center or within the arginine carbon backbone, and the resulting compounds failed to exhibit dramatically improved potency as arginine-based inhibitors [14]. To date there have been no reports on attempts to introduce constraining elements near or within the α-amino acid functionality in these systems. Molecular modeling studies and examination of the X-ray crystal structure of arginine complexed with the iNOS oxygenase domain indicated that the recognition subsite for the α-amino acid portion of the substrate has sufficient room to accommodate these changes [18].

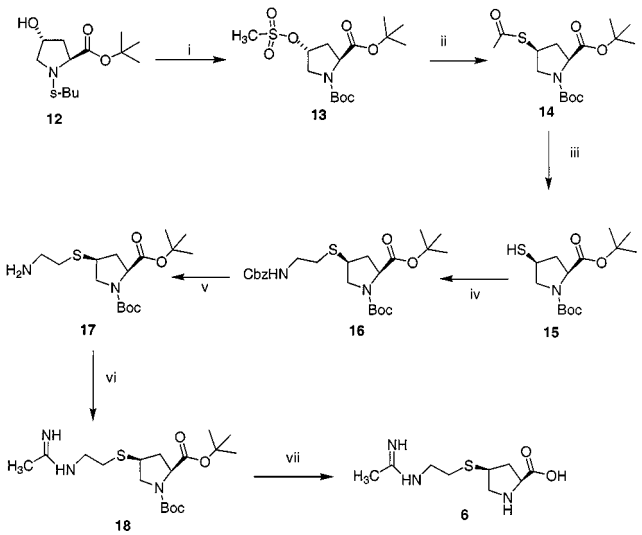
The enhanced potency and selectivity exhibited by the extended homocysteine inhibitor **4** appeared to be an attractive starting point to test conformational constraining elements near the α-amino acid moiety in **4**. Consequently, targets **5** and **6** were designed and selected to probe the introduction of small rings near or within the α-amino acid functional group in **4**. Moreover, the additional flexibility and extended chain length provided by the carbon–sulfur bonds in this system suggested that the enzyme-bound conformation for **4** must be significantly different from that for **1**. In addition, the ability to incorporate a heteroatom linking group greatly simplified the synthetic sequences needed to make such targets. Molecular modeling studies were unable to predict which of the geometric isomers of **5** would be the preferred inhibitor and so this system was initially prepared as a mixture of isomers to test this approach. In contrast, several chiral proline-based NOS inhibitors have been identified containing carbon linkers to the guanidinium moiety [19]. Target **6** provided a unique opportunity to build on these results and examine the sulfur-linked proline-containing amidine analog in a chiral context. In this paper, we describe the syntheses of the two new constrained homocysteine analogs **5** and **6** as well as their evaluation as potential inhibitors of the three human NOS isoforms.

CHEMISTRY

Target **5** was prepared from the known benzyl ether **7** [20,21]. Hydrogenation of **7** (see Scheme 1) in methanol at 53 psi decreased the reaction time to produce alcohol **8** in 93% isolated yield. Mesylation of the alcohol **8**, with only pyridine alone or in combination with 4-dimethylaminopyridine (DMAP) as the base, was very sluggish. However, addition of a catalytic amount of DMAP to a mixture of pyridine and diisopropylethylamine greatly facilitated the reaction, which was now finished within 3 h to produce the desired mesylate **9** in 65% yield. The resulting mesylate was displaced using the anion of *tert*-butyl *N*-(2-mercaptoethyl)carbamate generated from solid sodium hydride in 1-methyl-2-pyrrolidinone (NMP) to give the thioether **10** in 30% yield. Efficient purging of the solvent for this reaction with nitrogen for 30 min prior to the reaction was necessary to minimize the amount of unreactive *tert*-butyl *N*-(2-mercaptoethyl)carbamate disulfide that formed



SCHEME 1 Reaction conditions: (i) H_2, 53 psi, 10% Pd/C, MeOH; (ii) MsCl, pyridine, *N,N*-disopropyl ethyl amine, CH_2Cl_2, r.t.; (iii) $BocNHCH_2CH_2SH$, NaH, NMP, N_2, r.t.; (iv) 6 N HCl, reflux; (v) ethyl acetimidate HCl, NaOH, pH 10, r.t.



SCHEME 2 Reaction conditions: (i) methanesulfonyl chloride, pyridine; (ii) potassium thioacetate, DMF; (iii) NH_3 (g), MeOH; (iv) (a) sodium hydride, DMF; (b) $PhCH_2O$ $CONHCH_2CH_2Br$; (v) 10% Pd/C, EtOH; (vi) 1-(2-naphthylthio)ethanimine hydrobromide, HOAC/EtOH; (vii) 4 N HCl in dioxane.

under these conditions. Formation of product **10** was troublesome since the disulfide of *tert*-butyl *N*-(2-mercaptoethyl)carbamate formed much faster than the reaction between the thiolate anion and the mesylate. Different solvents and base conditions were explored without success to improve this reaction. The desired aminoethyl thioether (**11**) was obtained in 40% yield after heating compound **10** to 80°C in 6 N HCl overnight and then at 110°C for 3 h. Analytically pure acetamidine target **5** was obtained as a hygroscopic hydrochloride salt in 58% yield by reaction of the intermediate amine **11** with ethyl acetimidate hydrochloride in aqueous sodium hydroxide solution at room temperature under careful pH control (pH 10). Purification of 5 was accomplished using ion-exchange resin after reacidification with aqueous HCl.

Synthesis of the chiral proline target **6** is outlined in Scheme 2. This synthesis began with the mesylation of (4*R*)-1-(*tert*-butyloxycabonyl)-4-(hydroxy) proline *tert*-butyl ester (**12**) in pyridine to give the 4*R*-mesylate (**13**), using literature methods [19]. The thioacetate moiety was introduced by replacing the mesylate with potassium thioacetate, leading to inversion at the C-4 center to give the protected 4*S*-thioacetate product **14**. The 4*R*-thiol (**15**) was obtained by hydrolysis of thioacetate **14** with methanolic ammonia, using literature methods [22]. The thiolate anion of **15** was then generated using solid sodium hydride. Removal of the thiol proton should be carried out cautiously since an extreme exotherm accompanies addition of the solid sodium hydride. Subsequent alkylation with benzyl *N*-(2-bromomethyl)carbamate afforded low yields of thioether **16** following purification by flash chromatography. Removal of the amino benzyl carbamate amino protecting group by hydrogenolysis was sluggish initially when 10% catalyst was added to the reaction. This was possibly due to initial sulfide linkage poisoning of the catalyst. Reaction was much faster when 50% catalyst and 20% HOAc were added. The compound **17** was obtained as an oil. The acetamidine moiety was introduced using 1-(2-naphthylthio)ethanimine hydrobromide in ethanol to give the acetamidine **18**, which is obtained cleanly as a hygroscopic oil, following partitioning of the concentrated crude reaction mixture between water and ether. The organic soluble reactants are cleanly removed, affording the desired product in the aqueous phase as the acetamidine hydrobromide salt. Final deprotection of **18** using 4 N HCl in dioxane afforded **6** as a hygroscopic hydrochloride salt that, upon isolation, retained a small amount of occluded dioxane.

RESULTS AND DISCUSSION

The pure targets **5** and **6** were evaluated as potential inhibitors of the three human NOS isoforms using previously described assays [23]. Unfortunately, none of these compounds displayed significant activity against any of the enzymes tested under conditions where 2 was used as a positive control (Table 1).

The incorporation of conformational constraining elements near or within the α-amino acid portion of the homocysteine inhibitor **4** significantly decreases the potency of inhibitors **5** and **6** for the three human NOS isoforms. These results suggest that adding steric bulk at the α-amino acid recognition site of these homocysteine systems prevents

TABLE 1 Inhibitory Properties of Conformationally Constrained *S*-[2-[(1-iminoethyl)amino]ethyl]homocysteine Analogs Versus Three Human NOS Enzymes

	IC_{50} (μM)		
Compound	*hiNOS*	*hecNOS*	*hncNOS*
2[a]	4.6	138	61
3[b]	0.7	40	13
4[b]	1.4	466	145
5	>200	>5000	>2000
6	>400	>400	>400

[a]See Ref. [16,23].
[b]See Ref. [17].

these inhibitors from adopting the correct binding orientation to fit within the enzyme substrate pocket. These data further confirmed those results previously observed with conformationally constrained analogs of L-arginine, containing constraining elements along the carbon backbone or near the guanidinium group [14].

EXPERIMENTAL

^{1}H NMR spectra were recorded on either a Varian Unity Plus 300 (300 MHz) or a Varian Unity Inova 400 (400 MHz) spectrometer. All proton chemical shifts were recorded in ppm (δ) relative to trimethylsilane (TMS). Flash column chromatography was performed using either fresh silica gel (200–400 mesh) or on a Biotage Flash 40 or Flash 12 system, which uses prepacked silica cartridge. Resin purifications were performed using Dowex^R 50WX4-100 ion-exchange resin. Mass spectra were obtained on a HP series 1100 MSD instrument, and high-resolution mass spectra were obtained with a PerSeptive Biosystems Mariner TOF. All solvents and reagents were purchased from Sigma-Aldrich.

*Preparation of Ethyl 1-[[(tert-Butyloxy)carbonyl] amino]-3-hydroxycyclobutane-1-carboxylate (**8**)*

To a solution of benzyl ether [20,21] **7** (4.5 g, 12.9 mmol) in MeOH (30 ml) at room temperature under nitrogen was added 10% Pd-complex (Pd/C) (1 g). The mixture was attached to a hydrogenation apparatus and hydrogenated at 53 psi for 24 h, at which time LCMS analysis (LCMS: $m/z = 282.2$ $[M + Na]^+$) indicated complete reaction and no starting material remained. The Pd/C was removed by filtration through a celite pad, and the pad was washed with MeOH. The methanolic filtrates were combined, the solvent was removed, and the product was dried in vacuo to give 3.1 g (93%) of the desired alcohol as a white solid, 107.4–109.4°C. ^{1}H NMR (CD_3OD) δ 1.23 (t, 3H), 1.41 (s, 9H), 2.08 (m, 1H), 2.44 (m, 2H), 2.86 (m, 1H), 4.14 (q, 2H), 4.27 (m, 1H). HRMS calcd. for $C_{11}H_{21}NO_5$: $m/z = 282.1317$ $[M + Na]^+$, found: 282.1298.

*Preparation of Ethyl 1-[[(tert-Butyloxy)carbonyl] amino]-3-(methanesulfonyloxy)cyclobutane-1-carboxylate (**9**)*

To a methylene chloride (30 ml) solution of compound **8** (1.50 g, 5.8 mmol), containing 4-(dimethylamino)pyridine (catalytic amount), and diisopropylethylamine (2 ml, 11.6 mmol) was added methanesulfonyl chloride (0.67 ml, 8.7 mmol) dropwise at room temperature. The reaction mixture was stirred at room temperature for 3 h, at which time LCMS analysis (LCMS: $m/z = 360.10$ $[M + Na]^+$) indicated that the reaction had proceeded to completion. The reaction was quenched with water and the mixture was extracted with CH_2Cl_2 three times. The combined extracts were washed with brine, dried ($MgSO_4$), filtered, and evaporated to give 1.26 g (64.5%) of the desired mesylate as an off-white solid (m.p. 90.4–91.3°C), which was taken forward crude without further purification. ^{1}H NMR ($CDCL_3$) δ 1.28 (t, 3H), 1.43 (s, 9H), 2.73 (m, 2H), 2.92 (m, 1H), 3.00 (s, 3H), 3.03 (m, 1H), 4.22 (q, 2H), 5.17 (m, 1H), 5.17 (b, 1H). HRMS calcd. for $C_{13}H_{23}NO_7S$: $m/z = 360.1093$ $[M + Na]^+$, found: 360.1091.

*Preparation of Ethyl 1-[(tert-Butyloxy)carbonyl-amino-3-{2-[(tert-butoxy)carbonylamino]ethyl-thio}cyclobutane-1-carboxylate (**10**)*

Solid NaH (60% in mineral oil, 78 mg, 1.95 mmol) was suspended in dry 1-methyl-2-pyrrolidinome (10 ml), which was purged with nitrogen for 0.5 h at 0°C. Then *tert*-butyl *N*-(2-mercaptoethyl)carbamate (2.5 ml, 1.5 mmol) was added to the above solution dropwise at 0°C. The mixture was stirred at 0°C for 0.5 h. Then a solution of compound **9** (0.5 g, 1.5 mmol) in nitrogen-purged, dry NMP (10 ml) was added, and the resulting mixture was stirred at room temperature under nitrogen overnight, at which time LCMS analysis (LCMS: $m/z = 441.20$ $[M + Na]^+$) indicated product formation, although a small amount of starting material still remained. Most of the NMP was removed under high vacuum, and then water was added to the crude product mixture. The product was extracted into ether, concentrated and purified by Biotage chromatography eluting with ethyl acetate/hexane = 1/6 to give 190 mg (30%) of the desired pure thioether product as an off-white oil. ^{1}H NMR ($CDCl_3$) δ 1.23 (t, 3H), 1.45 (s, 9H), 1.47 (s, 9H), 2.68 (m, 2H), 3.00 (m, 2H), 3.30 (m, 2H), 3.5 (m, 2H), 4.13 (q, 2H), 4.25 (m, 1H), 4.93 (b, NH), 5.28

(b, NH). HRMS calcd. for $C_{19}H_{34}N_2O_6S$: m/z = 419.2216 $[M+H]^+$, found: 419.2239.

*Preparation of 1-Amino-3-(2-aminoethylthio)-cyclobutane Carboxylic Acid (**11**)*

A solution of compound **10** (180 mg, 0.4 mmol) in 20 ml of 6 N HCl was heated to 80°C for 18 h, and then at 110°C for 3 h, at which time LCMS analysis (LCMS: $m/z = 191.1$ $[M+H]^+$) indicated that the desired deprotected amine hydrochloride product had formed. The excess HCl and water were removed in vacuo to give 45 mg (43%) of the desired amine hydrochloride salt as an oil, which was taken forward crude without further purification. HRMS calcd. for $C_7H_{14}N_2O_2S$: $m/z = 191.0854$ $[M+H]^+$, found: 191.0828.

*Preparation of 1-Amino-3-[2[(1-iminoethyl) amino]ethylthio]cyclobutane Carboxylic Acid (**5**)*

To a solution of compound **11** (45 mg, 0.17 mmol) in 10 ml of water was added aqueous NaOH (2.5 N) dropwise until the pH was 10. Ethyl acetimidate (86 mg, 0.68 mmol) was added to the above solution in three portions at room temperature. The reaction mixture was stirred at room temperature overnight, at which time LCMS analysis (LCMS: $m/z = 232.20$ $[M+H]^+$) indicated that the desired product had formed. The reaction mixture was neutralized by adding 1 N HCl until pH was 4.5 and then loaded on prewashed Dowex 50WX4-100 ion-exchange resin and eluted with 0.5–1.0 N aqueous NH_4OH. Fractions containing the desired free amidine product were combined, and the aqueous NH_4OH was removed in vacuo. The resulting product was treated with HCl to form the corresponding HCl salt. After vacuum drying, 30 mg (58%) of the desired acetamidine hydrochloride product was obtained as a hygroscopic solid. ^{1}H NMR (D_2O) δ 2.29 (m, 1H), 2.60 (m, 1H), 2.75 (m, 2H), 2.85 (m, 2H), 2.92 (m, 1H), 3.02 (m, 1H), 3.08 (m, 3H), 3.61 (m, 1H). HRMS calcd. for $C_9H_{17}N_3O_2S$: $m/z = 232.1120$ $[M+H]^+$, found: 232.1141.

*Preparation of tert-Butyl (2S,4R)-1-[(tert-Butyl) oxycarbonyl]-4-(methylsulfonyloxy)pyrrolidine-2-carboxylate (**13**)* [19]

To a cold (0–5°C) solution of tert-butyl (2S,4R)-1-[(tert-butyl)oxycarbonyl]-4-hydroxypyrrolidine-2-carboxylate (**12**) (5.0 g, 17.0 mmol) in pyridine (35 ml) under nitrogen was added methanesulfonyl chloride (7.6 ml, 11.2 mmol). The resulting mixture was stirred at 0°C for 3 h, at which time TLC (1% methanol in methylene chloride) showed that no starting material remained. The resulting mixture was poured into ethyl acetate (300 ml) and washed with 1.0 M citric acid (2 × 50 ml) and water (2 × 50 ml). The organic layer was collected, dried (Na_2SO_4), and concentrated to give a dark oil containing some precipitate which was carried on crude without further purification, since the ^{1}H NMR spectrum of this material was identical to that reported in the literature [18]. ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 18H), 2.21 (m, 1H), 2.60 (m, 1H), 3.02 (s, 3H), 3.70 (dd, 1H), 3.81 (dd, 1H), 4.26 (m, 1H), 5.21 (s, 1H).

*Preparation of tert-Butyl (2S,4S)-4-Acetylthio-1-[(tert-Butyl)oxycarbonyl]pyrrolidine-2-carboxylate (**14**)*

Potassium thioacetate (1.6 g, 14.0 mmol) was added to a solution of **13** (3.0 g, 8.0 mmol) dissolved in 30 ml of dimethylformamide (DMF). The resulting mixture was heated at 65°C for 18 h, at which time LCMS analysis (LCMS: $m/z = 368.1$ $[M+Na]^+$) showed that no starting material remained, and the product was more than 80% pure. The dark solution was diluted with ether (20 ml) and washed with water (2 × 30 ml). The aqueous layer was extracted with ether (2 × 25 ml). The organic layers were combined, dried ($MgSO_4$), filtered, and evaporated to give a dark oil which was carried on crude without further purification. ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 18H), 1.90 (m, 1H), 2.29 (s, 3H), 2.60–2.78 (m, 1H), 3.28 (m, 1H), 3.92 (m, 2H), 4.20 (dt, 1H). HRMS calcd. for $C_{16}H_{27}NO_5S$: $m/z = 346.1688$ $[M+H]^+$, found: 346.1674.

*Preparation of tert-Butyl (2S,4S)-1-[(tert-Butyl) oxycarbonyl]-4-thiopyrrolidine-2-carboxylate (**15**)*

A solution of **14** (1.1 g, 3.1 mmol) in MeOH (10 ml) was added to a saturated solution of NH_3 (g) in MeOH (10 ml). The resulting mixture was stirred at room temperature for 3 h, at which time TLC analysis (25% EtOAc/hexane) indicated that no starting material remained. Concentration of this mixture gave 0.9 g (96%) of the desired thiol product as an oil. This crude thiol product was used in the next step without additional purification. ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 18H), 1.60 (bs, 2H), 1.90 (m, 1H), 2.75 (m, 1H), 3.32 (m, 1H), 4.01 (m, 1H), 4.20 (m, 1H). LCMS: $m/z = 326.1$ $[M+Na]^+$.

*Preparation of tert-Butyl (2S,4S)-1-[(tert-Butyl) oxycarbonyl]-4-{2-[(phenylmethoxy)carbonyl-amino]ethylthio}pyrrolidine-2-carboxylate (**16**)*

Solid sodium hydride (60% in mineral oil, 142 mg, 3.56 mmol) was slowly added to a stirring solution of **15** (0.9 g, 3.0 mmol) in DMF (10 ml) at room

temperature. After 5 min, benzyl *N*-(2-bromoethyl) carbamate (774 mg, 3.0 mmol) was added. The resulting mixture was stirred for 1 h, at which time TLC analysis (25% EtOAc/hexane) indicated that the desired product formed. The solution was extracted with EtOAc (2 × 10 ml). The organic layers were combined, dried (Na_2SO_4), and concentrated. The resulting oil was purified by silica gel chromatography eluting with 25% EtOAc in hexanes to give 410 mg (28%) of the desired thioether product as a clear oil. ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 18H), 1.80 (s, 1H), 1.90 (m, 1H), 2.60–2.78 (m, 2H), 3.21 (m, 1H), 3.40 (m, 2H), 4.01 (s, 1H), 4.20 (dt, 1H), 7.38 (s, 5H). LCMS: $m/z = 503.2$ $[M + Na]^+$.

*Preparation of tert-Butyl (2S,4S)-4-(2-Aminoethylthio)-1-[(tert-Butyl)oxycarbonyl]pyrrolidine-2-carboxylate (**17**)*

Intermediate **16** (410 mg, 850 mmol) was dissolved in EtOH (5 ml) at room temperature under nitrogen. Then 10% Pd/C (50 mg, 0.05 mmol) was added, the mixture was placed under a hydrogenation apparatus, and then hydrogenated at 45 psi for 26 h. The Pd/C catalyst was removed by filtration through a celite plug, and the filtrate was concentrated to an oil. Subsequent LCMS analysis showed that some unreacted starting material remained. The oil was redissolved in 20% HOAc/EtOH (5 ml) and resubjected to hydrogenation at 45 psi for 3 h in the presence of 10% Pd/C (200 mg, 0.2 mmol). After filtration through a celite plug, the filtrate was concentrated to give 204 mg (69%) of the desired free amine product as a clear oil. ^{1}H NMR ($CDCL_3$) δ 1.45 (s, 18H), 1.80 (bs, 2H), 2.60 (m, 1H), 2.81 (m, 1H), 3.21 (m, 2H), 4.01 (m, 1H), 4.20 (m, 1H). HRMS calcd. for $C_{16}H_{30}N_2O_4S$: $m/z = 347.2005$ $[M + H]^+$, found: 347.2010.

*Preparation of tert-Butyl (2S,4S)-1-[(tert-Butyl) oxycarbonyl]-4-{2-[(iminoethyl)amino] ethylthio}pyrrolidine-2-carboxylate (**18**)*

To a cold (0–5°C) solution of **17** (240 mg, 415 mmol) in EtOH (6 ml) was added 1-(2-naphthylthio)ethanimine hydrobromide (120 mg, 415 mmol). After 25 min, the solvent was removed in vacuo, and the product was partitioned between ether (3 ml) and water (3 ml). The aqueous layer was removed and concentrated to afford 102 mg (64%) of the desired acetamidine hydrobromide salt as a hygroscopic white solid. ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 18H), 1.70–1.81 (m, 1H), 2.15 (s, 3H), 2.66 (m, 1H), 2.77 (m, 2H), 3.11 (m, 1H), 3.32–3.39 (m, 3H), 3.82 (m, 1H), 4.13 (m, 1H). HRMS calcd. for $C_{18}H_{33}N_3O_4S$: $m/z = 388.2270$ $[M + H]^+$, found: 388.2310.

*Preparation of (4S)-4-[[2-[(1-Iminoethyl) amino]ethyl]thio]-L-proline (**6**)*

A solution of **18** (890 mg, 2.3 mmol) was stirred in 4 N HCl (6 ml, 1.5 mmol) in dioxane at room temperature for 18 h. The solvent was removed in vacuo followed by ether and methanol co-evaporations to afford 190 mg (30%) of the desired fully deprotected product as a hygroscopic foamy-yellow solid. ^{1}H NMR ($CDCL_3$) δ 2.20 (m, 1H), 2.23 (s, 3H), 2.81 (t, 1H), 3.57–3.83 (m, 8H), 4.56 (t, 1H). HRMS calcd. for $C_9H_{17}N_3O_2S$: $m/z = 232.1120$ $[M + H]^+$, found: 232.1093. Anal. calcd. for $C_9H_{17}N_3O_2S{\cdot}3.6\ HCl{\cdot}0.3\ H_2O{\cdot}0.6\ C_4H_8O_2$: C, 32.37; H, 6.22; N, 9.69. Found: C, 32.54; H, 6.23; N, 9.98.

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