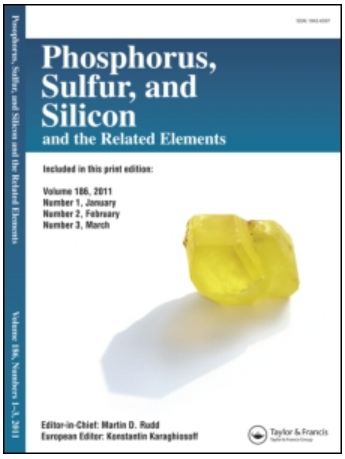

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A Synthetic Pathway to Cys-Xxx-Cys (N_2S_2) Analogue Ligands: An Improved Synthesis of $HSCH_2CH_2C(O)NHCH_2C(O)NHCH_2CH_2SH$

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Herein, two improved synthetic pathways to biologically relevant Cys-Xxx-Cys analogue ligands used in conjunction with metals as metalloenzyme models (Ni for carbon monoxide dehydrogenase/acetyl-CoA synthase A-cluster; Co and Fe for nitrile hydratase) are reported.

Keywords Carboxamido ligand; metal thiolate; peptide synthesis; synthetic analogue

INTRODUCTION

Over the past decade, examples of backbone amide nitrogen bonds to transition metal centers have been observed in metalloenzymes. While this is not a common binding mode, there exists three known cases where such binding occurs: (1) the A-Cluster in carbon monoxide dehydrogenase/acetyl-CoA synthase (CODH/ACS),[1] (2) the P-Cluster in nitrogenase,[2] and (3) the catalytic core of Fe(III) and Co(III) nitrile hydratase (NHase).[3] To gain a better understanding of these

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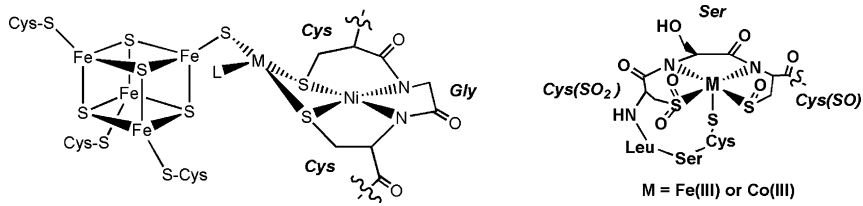
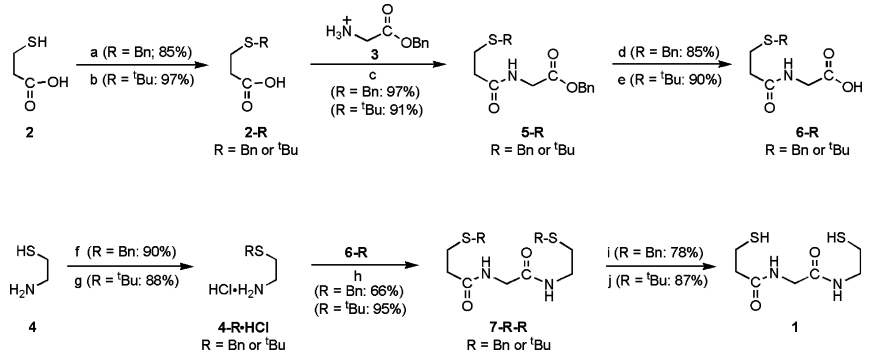


FIGURE 1 Depictions of the active sites of CODH/ACS (left) and NHase (right) that contain the Cys-Xxx-Cys peptide fragment coordinated to the metal center through the cysteine sulfurs and two backbone amide nitrogens.

biological systems, groups have used the synthetic analogue approach to synthesize active models of the catalytic centers of these metalloenzymes. In the case of CODH/ACS and the Fe- and Co-NHase, the catalytic center is bound by a Cys-Xxx-Cys fragment in a tetradentate plane from the two cysteine thiolates and the backbone amide nitrogen of the central amino acid (Xxx: Gly for CODH/ACS; Ser for NHase) and the adjacent cysteine (Figure 1). As such, synthetic routes to Cys-Xxx-Cys analogues are important. Herein we report an improved synthetic approach to obtain the generic Cys-Xxx-Cys analogue $HSCH_2CH_2C(O)NHCH_2C(O)NHCH_2CH_2SH$ (**1**) that can also be used to obtain other modified analogues of the Cys-Xxx-Cys form.

RESULTS AND DISCUSSION

Rao and colleagues recently reported[4] the synthesis of **1** and its use as a synthetic analogue ligand for the nickel binding domain of the A-Cluster of CODH/ADS. The synthetic scheme was based on an earlier report for a similar ligand that entailed 7 steps and an overall 22% yield. The resultant product was described as an ether or water solvate based on elemental analysis. Our group's efforts to develop synthetic analogues resulted in the design of a number of Cys-Xxx-Cys type analogues that included the ligand reported by Rao and colleagues. Here we report an improved synthesis of **1** that reduces the number of synthetic steps by one (from 7 to 6) and increases the overall yield depending on the protecting groups utilized (*S*-benzyl = 33%; *S*-*tert*-butyl = 57%). Scheme 1 depicts the synthetic pathway used starting from either benzyl or *tert*-butyl protected 3-mercaptopropionic acid (**2**). The peptide couple of **2-R** (R = Bn or ^{t}Bu) with **3** utilized HBTU as a coupling reagent and DMF as a solvent. The products **5-R** (R = Bn or ^{t}Bu) were obtained in high yields, and their subsequent benzyl ester cleavage produced **6-R** (R = Bn or ^{t}Bu). When using the *tert*-butyl protected pathway, the



SCHEME 1 (a) BrBn, NaOH, EtOH. (b) H_2SO_4, ^{t}BuOH, 0°C to 25°C, 3 h. (c) HBTU, DIEPA, DMF. (d) $AlCl_3$, EtSH. (e) K_2CO_3, MeOH:H_2O, reflux, 16 h. (f) BrBn, K_2CO_3, EtOH. (g) HCl, ^{t}BuOH, reflux, 16 h. (h) HBTU, DIPEA, DMF. (i) NH_3, Na, THF, −78°C. (j) i. $Hg(OAc)_2$, TFA; ii. H_2S, MeCN.

use of K_2CO_3 to deprotect the carboxylic acid resulted in a high yield of product. However, under the same conditions, the benzyl ester was not cleaved from **5-Bn** owing to its insolubility in the reaction media. In order to dissolve **5-Bn**, a ratio of greater than 1:1 methanol:water was required. As reported earlier,[5] when the methanol:water ratio is greater than 70:30, reaction does not proceed cleanly. The reaction of **5-Bn** with $AlCl_3$ in ethanethiol was successful in cleaving the benzyl ester group providing an overall high yield. Once **6-Bn** and **6-^{t}Bu** were obtained, they were coupled under similar conditions used to make **5-R**. **6-R** was coupled with its corresponding protected thioamine **4-R**, where the same protecting groups were used (e.g., **6-Bn** + **4-Bn**), resulting in the di-*S*-benzyl or di-*S-tert*-butyl protected ligands **7** (**7-Bn-Bn** and **7-^{t}Bu-^{t}Bu**, respectively). The desired analogue **1** was obtained by either the dissolving metal reduction (from **7-Bn-Bn**) or the mercuric acetate in trifluoroacetic acid (from **7-^{t}Bu-^{t}Bu**) protocols. In each case, **1** was obtained as an off-white solid in good to excellent yields. This synthetic pathway is currently being used in our laboratory to synthesize a variety of N_2S_2 ligands by varying the amino acid (**3**) core.

EXPERIMENTAL

General

All materials were purchased from suppliers and used without further purification unless stated otherwise. All reactions were performed using standard Schlenk techniques unless stated otherwise. ^{1}H NMR data

were obtained on a Varian Mercury 300 MHz or INOVA 500 MHz NMR spectrometer. Chemical shifts for protons are referenced to tetramethylsilane (TMS) at 0.00 ppm. *S*-benzyl-2-aminoethanethiol (**4-Bn**) was synthesized using a literature procedure in 90% yield[6] and was converted to the HCl salt for prolonged storage.

The Synthesis of 2-Bn

This reaction was performed in air. A 1 M NaOH solution (130 mL) was added to a 500-mL round-bottom flask along with ethanol (130 mL). 3-mercaptopropionic acid (**2**; 4.93 mL, 56.5 mmol) was subsequently added dropwise via a syringe to the reaction flask. The solution was cooled to 0°C and stirred for 5 min before benzyl bromide (7.06 mL, 59.4 mmol) was added dropwise via a syringe. After 10 min, the solution was allowed to warm to r.t. and stirred for a further 3 h. A 1 M HCl solution was added to the reaction flask to adjust the pH to 1, and the resulting solution was transferred to a 500-mL separatory funnel, and extracted with CH_2Cl_2 (5 × 30 mL). A 1 M NaOH solution was added to the combined organic phase to adjust the pH to 12. The solution was extracted with CH_2Cl_2 (2 × 30 mL). The aqueous layer was acidified to pH = 1 with 1 M HCl and extracted with CH_2Cl_2 (4 × 20 mL). The CH_2Cl_2 layer was dried over magnesium sulfate, filtered, and concentrated yielding **2-Bn** as a white solid (9.43 g, 48.1 mmol, 85.0% yield). ^{1}H NMR ($CDCl_3$, 500 MHz): δ2.61 (t, 2H, C*H*$_2$CH$_2$SCH$_2$Ar), 2.69 (t, 2H, CH$_2$C*H*$_2$SCH$_2$Ar), 3.75 (s, 2H, CH$_2$CH$_2$SC*H*$_2$Ar), 7.24–7.34 (m, 5H, Ar*H*). Anal. calcd. for $C_{10}H_{12}O_2S$: C, 61.20; H, 6.16. Found: C, 61.46; H, 6.17.

The Synthesis of 5-Bn

3-Benzylsulfanyl-propionic acid (**2-Bn**; 4.00 g, 20.4 mmol) was added to a 100-mL round-bottom flask equipped with a stirbar, followed by the addition of NH_3-Gly-OBnz· tosylate (**3**; 6.88 g, 20.4 mmol). Dry DMF (25 mL) was added to the flask, forming a light yellow solution, followed by the addition of diisopropylethylamine (10.65 mL, 61.13 mmol). HBTU (8.50 g, 22.4 mmol) was added to a separate 50-mL round-bottom flask equipped with a stirbar along with dry DMF (25 mL). The HBTU solution was added via cannula to the reaction flask. The solution was stirred for 16 h at r.t. under a nitrogen atmosphere. The solution was transferred to a 500-mL separatory funnel, and the organic phase was washed with 1 M HCl (5 × 20 mL), saturated sodium bicarbonate solution (5 × 20 mL), and deionized water (5 × 20 mL). The organic phase was dried over magnesium sulfate, filtered, and concentrated, affording

5-Bn as a peach solid (6.81 g, 19.8 mmol, 97.0% yield). ^{1}H NMR ($CDCl_3$, 500 MHz): δ2.42 (t, 2H, C*H*$_2$CH$_2$SCH$_2$Ar), 2.72 (t, 2H, CH$_2$C*H*$_2$SCH$_2$Ar), 3.72 (s, 2H, CH$_2$CH$_2$SC*H*$_2$Ar), 4.06 (d, 2H, CH$_2$CONHC*H*$_2$CO), 5.18 (s, 2H, CH$_2$CONHCH$_2$COOC*H*$_2$Ar), 6.19 (br t, 1H, CH$_2$CON*H*CH$_2$), 7.22–7.84 (m, 10H, Ar*H*). Anal. calcd. for $C_{19}H_{21}NO_3S$: C, 66.45; H, 6.16; N, 4.08. Found: C, 66.47; H, 6.42; N, 4.41.

The Synthesis of 6-Bn

$AlCl_3$ (2.575 g, 19.24 mmol) was added to a 300-mL round-bottom flask under nitrogen atmosphere and dissolved in ethanethiol (17 mL) at 0°C. **5-Bn** (2.438 g, 7.100 mmol) was added to the solution, and the solution immediately turned bright yellow. The solution was stirred at 0°C for 30 min and for a further 2.5 h at r.t. Deionized water (40 mL) and 4 M HCl (40 mL) were added to the solution slowly and stirred until the $AlCl_3$ stopped reacting and the solution had a pH = 1. Note: After the addition of water, a white precipitate formed, added acid dissolved the precipitate. The solution was transferred to a 250-mL separatory funnel, and the aqueous layer was extracted with CH_2Cl_2 (6 × 20 mL). The combined organic phases were subsequently washed with brine (3 × 20 mL) then dried over magnesium sulfate. The solution was filtered and concentrated yielding a residue that was treated with hexanes (3 × 20 mL) and filtered. The product **6-Bn** was collected as a white solid (1.529 g, 6.035 mmol, 85.00% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ2.45 (t, 2H, C*H*$_2$CH$_2$SCH$_2$Ar), 2.74 (t, 2H, CH$_2$C*H*$_2$SCH$_2$Ar), 3.74 (s, 2H, CH$_2$CH$_2$SC*H*$_2$Ar), 4.03 (d, 2H, NHC*H*$_2$COOH), 6.21 (br t, 1H, N*H*CH$_2$COOH), 7.20–7.35 (m, 5H, Ar*H*). Anal. calcd. for $C_{12}H_{15}NO_3S$: C, 56.90; H, 5.97; N, 5.53. Found: C, 56.98; H, 6.12; N, 5.65.

The Synthesis of 7-Bn-Bn

6-Bn (0.3010 g, 1.188 mmol) was added to a 50-mL round-bottom flask equipped with a stirbar, followed by the addition of the **4-Bn · HCl** (0.1759 g, 0.8634 mmol) under a nitrogen atmosphere. Dry DMF (25 mL) was added followed by the addition of dry diisopropylethylamine (450 μL, 2.58 mmol) via a syringe. HBTU (0.3620 g, 0.9545 mmol) was dissolved in DMF (10 mL) in a separate 25-mL round-bottom flask equipped with a stirbar under a nitrogen atmosphere. The HBTU solution was added to the reaction flask via cannula, and the solution was stirred for 15 h at r.t. The solution was transferred to a 250-mL separatory funnel, and the organic phase was washed with a 1 M HCl (5 × 10 mL), saturated sodium bicarbonate solution (5 × 10 mL),

and deionized water (5 × 10 mL). The organic phase was dried over magnesium sulfate for 16 h, filtered, and concentrated, affording **7-Bn-Bn** as a cream colored solid (0.2334 g, 0.5698 mmol, 66.00%). ^{1}H NMR (500 MHz, $CDCl_3$): δ2.40 (t, 2H, $COCH_2CH_2S$), 2.57 (t, 2H, $NHCH_2CH_2S$), 2.77 (t, 2H, $COCH_2CH_2S$), 3.40 (q, 2H, $NHCH_2CH_2S$), 3.71 (s, 2H, SCH_2Ar), 3.73 (s, 2H, SCH_2Ar), 3.86 (d, 2H, $CONHCH_2CO$), 6.05 (br t, 1H, $NHCH_2CO$), 6.33 (br t, 1H, $NHCH_2CH_2S$), 7.24–7.36 (m, 10H, Ar*H*). Anal. calcd. for $C_{21}H_{26}N_2O_2S_2$: C, 62.65; H, 6.51; N, 6.96. Found: C, 62.92; H, 6.85; N, 6.75.

The Synthesis of the Cys-Xxx-Cys Analogue Ligand (1) From Benzyl Protected Thiols

Liquid NH_3 (50 mL) was condensed into a 250-mL flask cooled in an isopropyl alcohol/dry ice bath. Sodium metal (0.0400 g, 1.74 mmol) was added to the ammonia in small portions until the solution remained deep blue. The protected ligand **7-Bn-Bn** (0.1812 g, 0.4501 mmol) was dissolved in THF (10 mL) in a 50-mL round-bottom flask under a nitrogen atmosphere. The THF solution was slowly delivered by cannula into the ammonia solution. Excess sodium (0.080 g, 3.5 mmol) was used in order to maintain the blue color for 45 min. After an hour of stirring, the solution was quenched with ammonium chloride (2.00 g, 37.4 mmol). This mixture was stirred for 5 min, then the dry ice bath was removed, and the ammonia evaporated over 1–2 h with the aid of a flow of nitrogen gas. The residue was reverse extracted with THF (4 × 15 mL), and the THF filtrate concentrated under vacuum to yield **1** as a white solid (0.0781 g, 0.352 mmol, 78.1% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ1.40 (t, 1H, $NHCH_2CH_2SH$), 1.66 (t, 1H, $COCH_2CH_2SH$), 2.58 (t, 2H, $COCH_2CH_2SH$), 2.68 (td, 2H, $NHCH_2CH_2SH$), 2.85 (td, 2H, $COCH_2CH_2SH$), 3.47 (q, 2H, $NHCH_2CH_2SH$), 3.98 (d, 2H, $CONHCH_2CO$), 6.42 (br t, 1H, $CONHCH_2CO$), 6.61 (br t, 1H, $NHCH_2CH_2SH$). Anal. calcd. for $C_7H_{14}N_2O_2S_2$: C, 37.82; H, 6.35; N, 12.60. Found: C, 37.68; H, 6.80; N, 12.25.

The Synthesis of 2-^{t}Bu

This reaction was performed in air. Deionized water (100 mL) was added to a 500-mL round-bottom flask, followed by the dropwise addition of concentrated sulfuric acid (160 mL). The solution was stirred at 0°C in an ice/water bath for 5 min. *Tert*-Butanol (80.0 mL, 0.837 mol) was added dropwise, followed by the addition of 3-mercaptopropionic acid (8.20 mL, 0.941 mmol). This solution was stirred for 10 min at 0°C then warmed to r.t. and stirred for an additional 3 h. The

solution was quenched with ice (300 g) and transferred to a 1-L separatory funnel, and the aqueous phase was washed with Et_2O (4 × 50 mL). The aqueous phase was then made basic with 2 M NaOH so the pH = 12. The aqueous phase was washed with CH_2Cl_2 (3 × 50 mL) then reacidified to pH = 2 and extracted with CH_2Cl_2 (3 × 25 mL). The latter combined organic phases were washed with brine (2 × 50 mL) and deionized water (1 × 20 mL), dried over magnesium sulfate, filtered, and concentrated, affording a yellow liquid. On standing in air, the liquid crystallized into colorless crystals of **2-^{t}Bu** (14.8 g, 0.912 mol, 96.9% yield). In subsequent syntheses, seed crystals were added to the oil to promote crystallization. ^{1}H NMR ($CDCl_3$, 500 MHz): δ1.34 (s, 9H, $CH_2CH_2SC(CH_3)_3$), 2.64 (t, 2H, $CH_2CH_2SC(CH_3)_3$), 2.80 (t, 2H, $CH_2CH_2SC(CH_3)_3$). Anal. calcd. for $C_7H_{14}O_2S$: C, 62.83; H, 6.71. Found: C, 62.83; H, 6.76.

The Synthesis of 4-^{t}Bu•HCl

This reaction was performed in air. β-mercaptoethylamine·hydrochloride (15.20 g, 0.1337 mol) and *tert*-butanol (16.6 mL, 0.174 mol) were added to a 250-mL round-bottom flask. Two M HCl (50 mL) was added, which dissolved the salt and made the solution clear and colorless. The reaction mixture was refluxed overnight at 100°C. The reaction mixture was cooled to r.t. and concentrated revealing a white solid. The product was filtered, washed, and dried with acetone to remove any water. **4-^{t}Bu · HCl** was obtained as a white solid (19.88 g, 0.1171 mol, 87.58% yield). ^{1}H NMR (500 MHz, D_2O): δ1.19 (s, 9H, $CH_2CH_2SC(CH_3)_3$), 2.72 (t, 2H, $CH_2CH_2SC(CH_3)_3$), 3.04 (br t, 2H, $CH_2CH_2SC(CH_3)_3$). Anal. calcd. for $C_6H_{16}ClNS$: C, 42.46; H, 9.50. Found: C, 42.65; H, 9.86.

The Synthesis of 5-^{t}Bu

Protected thiol **2-^{t}Bu** (4.01 g, 24.7 mmol) was added to a 100-mL round-bottom flask equipped with a stirbar, followed by the addition of NH_3-Gly-OBn· tosylate (**3**; 6.93 g, 20.5 mmol) under a nitrogen atmosphere. Dry DMF (25 mL) was added to the flask containing the protected thiol and protected glycine via a syringe, thereby forming a light yellow solution followed by the addition of dry diisopropylethylamine (10.7 mL, 61.6 mmol) via a syringe. HBTU (8.56 g, 22.6 mmol) was added to a separate 50-mL round-bottom flask, and dissolved in DMF (25 mL) under a nitrogen atmosphere. The HBTU solution was subsequently added via cannula to the reaction flask, and the reaction mixture was stirred overnight at r.t. The solution

was transferred to a 500-mL separatory funnel, and the organic phase was washed with a 1 M HCl solution (5 × 20 mL), saturated sodium bicarbonate solution (5 × 20 mL), and de-ionized water (2 × 10 mL). The organic layer was dried overnight with magnesium sulfate, filtered, and concentrated, affording viscous oil (5.76 g, 18.6 mmol, 90.7%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ1.33 (s, 9H, $CH_2CH_2SC(CH_3)_3$), 2.50 (t, 2H, $CH_2CH_2SC(CH_3)_3$), 2.83 (t, 2H, $CH_2CH_2SC(CH_3)_3$), 4.11 (d, 2H, $CONHCH_2CO$), 5.20 (s, 2H, CO_2CH_2Ar), 6.18 (br t, 1H, $CONHCH_2CO$), 7.33–7.40 (m, 5H, Ar*H*). Anal. calcd. for $C_{16}H_{23}NO_3S$: C, 62.11; H, 7.49; N, 4.53. Found: C, 61.91; H, 7.75; N, 4.58.

The Synthesis of 6-^{t}Bu

This reaction was performed in air. Protected ester **5-^{t}Bu** (4.67 g, 15.1 mmol) and potassium carbonate (4.18 g, 30.3 mmol) were added to a 300-mL round-bottom flask with methanol (75 mL) and deionized water (75 mL). The solution was stirred for 10 min, and then additional potassium carbonate (2.79 g, 20.2 mmol) was added. Additional methanol (30 mL) was added to dissolve all solids. Note: the reaction did not proceed when less than 30% (v/v) water in ethanol was used. The solution was refluxed for 16 h and then transferred to a separatory funnel. The reaction mixture was extracted with CH_2Cl_2 (3 × 25 mL). The aqueous phase was made acidic (pH = 1) by the addition of 4 M HCl (30 mL), and then the solvent was removed under vacuum to yield an oil-solid residue. The residue was extracted with Et_2O (3 × 50 mL), and the Et_2O was removed under vacuum to yield **6-^{t}Bu** as a white solid (2.98 g, 13.6 mmol, 90.1%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.33 (s, 9H, $CH_2CH_2SC(CH_3)_3$), 2.50 (t, 2H, $CH_2CH_2SC(CH_3)_3$), 2.80 (t, 2H, $CH_2CH_2SC(CH_3)_3$), 4.10 (d, 2H, $CONHCH_2CO$), 6.40 (br t, 1H, $CONHCH_2CO$). Anal. calcd for $C_9H_{17}NO_3S$: C, 49.29; H, 7.81; N, 6.39. Found: C, 48.95; H, 8.04; N, 6.19.

The Synthesis of 7-^{t}Bu-^{t}Bu

Deprotected ester **6-^{t}Bu** (11.1 g, 50.6 mmol) was added to a 500-mL round-bottom flask equipped with a stirbar, followed by the addition of the **4-^{t}Bu·HCl** (8.59 g, 50.6 mmol) under nitrogen atmosphere. DMF (50 mL) was added to the flask followed by the addition of diisopropylethylamine (780 μL, 4.49 mmol) via a syringe yielding a yellow colored solution. HBTU (21.1 g, 55.7 mmol) was added to a separate 250-mL round-bottom flask and dissolved in DMF (80 mL) under-nitrogen atmosphere. The HBTU solution was added via cannula to the reaction flask, and the pale gold-colored solution was allowed to react overnight at r.t. The solution was transferred to a 500-mL separatory

funnel, and the organic phase was washed with a 1 M HCl solution (5 × 50 mL), and saturated sodium bicarbonate solution (5 × 50 mL), brine (3 × 50 mL), and deionized water (2 × 50 mL). The organic phase was dried overnight with magnesium sulfate, filtered, and concentrated, affording **7-^{t}Bu-^{t}Bu** as a viscous oil (16.08 g, 48.07 mmol, 95.0%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ1.33 (s, 9H, $NHCH_2CH_2SC(CH_3)_3$), 1.34 (s, 9H, $COCH_2CH_2SC(CH_3)_3$), 2.51 (t, 2H, $COCH_2CH_2SC(CH_3)_3$), 2.69 (t, 2H, $NHCH_2CH_2SC(CH_3)_3$), 2.86 (t, 2H, $COCH_2CH_2SC(CH_3)_3$), 3.46 (q, 2H, $NHCH_2CH_2SC(CH_3)_3$), 3.95 (d, 2H, $CONHCH_2CO$), 6.30 (br t, 1H, $CONHCH_2CO$), 6.42 (br t, 1H, $NHCH_2CH_2SC(CH_3)_3$). Anal. calcd. for $C_{15}H_{30}N_2O_2S_2$: C, 53.85; H, 9.04; N, 8.37. Found: C, 53.45; H, 9.46; N, 8.79.

The Synthesis of the Cys-Xxx-Cys Analogue Ligand (1) From *tert*-Butyl Protected Thiols

The protected ligand **7-^{t}Bu-^{t}Bu** (4.00 g, 12.0 mmol) was added to a 100-mL round-bottom side-arm flask under nitrogen. Trifluoroacetic acid (25 mL) and anisole (0.1 mL) were added, and the flask was cooled to 0°C. Mercuric acetate (8.01 g, 25.1 mmol) was added as a solid to the solution, which turned dark yellow on addition. The mixture was allowed to stir at 0°C for 1 h, after which time, the solution was burgundy colored. The solvent was removed under vacuum, and the residue was redissolved in acetonitrile (25 mL). The solution was then bubbled with hydrogen sulfide gas for 30 min, ensuring all mercury had reacted, yielding a black precipitate. The solution was filtered through a Celite column (diam. × height = 1.5″ × 2″; well packed) and washed with acetonitrile (50 mL), followed by THF (300 mL). The clear, yellow filtrate was concentrated, yielding an off-white paste. The residue was washed and filtered with Et_2O (4 × 20 mL) to yield **8** as a white solid (2.32 g, 10.4 mmol, 86.7%). Analysis indicated that the product **1** obtained was identical to that described for the synthesis of **1** from **7-Bn-Bn**.

CONCLUSIONS

The synthesis of analogue biologically relevant peptide fragments is of great importance owing to the increased understanding of native enzymes using the synthetic analogue approach. As such, new and/or better methods to synthesize key binding fragments are of continued interest to the synthetic community. The reported new synthetic pathway incorporates two different thiol-protecting groups and could thus be suitably used to design modified Cys-Xxx-Cys ligands that may be sensitive to the cleavage method of one of the protecting groups. Overall,

this strategy will allow for the development of many new analogue ligands in good to excellent yields.

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