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Synthesis of protected ω-mercapto amino acids: precursors for incorporation of elongated cysteines into peptides

Joseph T. Lundquist IV,[a] Erika E. Büllesbach[b] and Thomas A. Dix[a,*]

[a]*Department of Pharmaceutical Sciences, Medical University of South Carolina, Charleston, South Carolina 29425, USA*
[b]*Department of Biochemistry, Medical University of South Carolina, Charleston, South Carolina 29425, USA*

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Abstract

A series of protected ω-mercapto amino acids with side-chain lengths ranging from 3–5 methylene units has been synthesized via nucleophilic substitution of ω-bromo-α-azido acids by 4-methoxy-α-toluenethiol followed by reduction of the azido functionality with $SnCl_2$. These enantiomerically pure protected cysteine analogues can be used to optimize the length of disulfide connections in cyclically constrained peptide pharmacophores.

1. Introduction

There is considerable interest in producing small peptides and peptidomimetics with the structural conformation of the active binding pharmacophore of much larger peptides or proteins. These small active moieties offer potential advantages of improved binding potency and selectivity, chemical and biological stability, and pharmacokinetic characteristics when compared to their more complex molecules of origin. A popular strategy has been to cyclize the essential functional elements via a disulfide linker which can lock in the necessary three-dimensional structure for binding and biological activity as evidenced in somatostatin mimetics.[1] A pair of sulfhydryl containing amino acids placed in the proper position of the peptide can stabilize a β-turn,[1] one turn of an α-helix,[2] or two turns of an α-helix.[3] To establish these motifs, the variety of natural thiol-containing amino acids, cysteine and homocysteine, is insufficient: i.e. D-enantiomers and extended side-chains are required for optimization of the disulfide connection and structure stabilization. Thus, we are interested in strategically protected ω-mercapto amino acids (Fig. 1) for incorporation into relevant peptides.

D- and L-2-Amino-6-mercaptohexanoic acids have been prepared previously[3] by converting the ω-amino group of lysine to a pyridinium salt which is then displaced by a thiol, leaving S-protected D- or L-

* Corresponding author. Tel: (843)-953-6614; fax: (843)-953-6615; e-mail: dixta@musc.edu

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3a: n = 3
3b: n = 4
3c: n = 5

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Fig. 1. Protected ω-mercapto amino acids

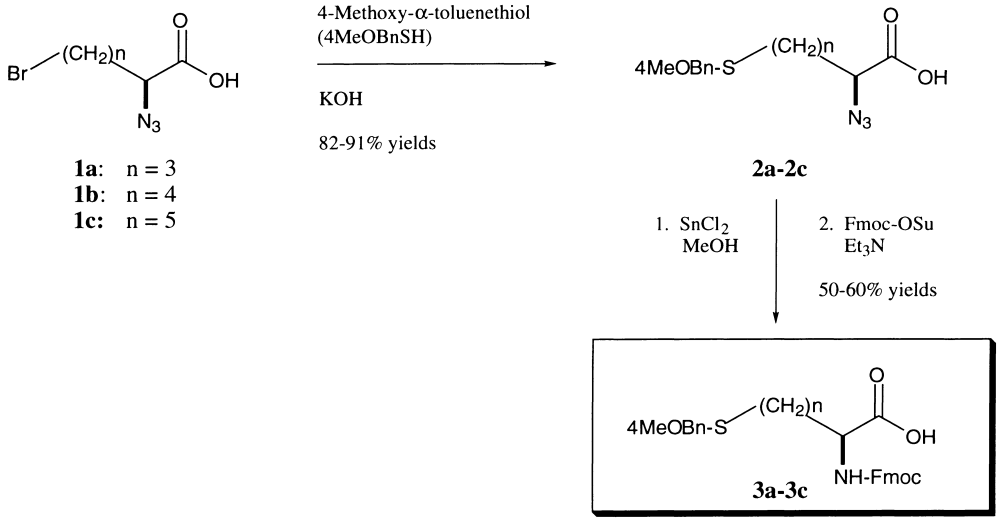
2-amino-6-mercaptohexanoic acids.[3,4] Disadvantages of this chemistry are the use of a large chromogenic leaving group, high excess of reagent, harsh reaction conditions, and subsequently low overall yields. Although identical chemistry could be used to generate D- and L-2-amino-5-mercaptovaleric acids using the corresponding ornithine analogue, other ω-mercapto-α-amino acids are not accessible via this route. In this paper, we describe a straightforward scheme which may be used to produce ω-sulfur containing α-amino acids of various side-chain lengths in good yields.

2. Results

The 2-(*S*)-azido-ω-bromo acids synthesized previously in this laboratory[5] were chosen as starting materials (Scheme 1) to produce ω-mercapto-containing amino acid analogues as follows: 2-(*S*)-azido-5-bromovaleric acid (**1a**) for amino acids possessing three methylene groups in the side-chain, 2-(*S*)-azido-6-bromohexanoic acid (**1b**) for analogues containing four methylene units, and 2-(*S*)-azido-7-bromoheptanoic acid (**1c**) for analogues containing five methylene units in the side-chain. These chiral building blocks initially are substituted at the ω-carbon by equimolar amounts of 4-methoxy-α-toluenethiol under basic conditions providing **2a**–**c**. The reactions occur at room temperature and are complete after 60 min. The azido group is reduced to the free amine with stannous chloride in methanol following the method of Maiti et al.[6] To provide monomers compatible with liquid- or automated solid-phase peptide synthesis, the amino group of each 2-amino-ω-mercapto acid was either 9-fluorenylmethoxycarbonyl (Fmoc) protected[7] yielding **3a**–**c** or the D-enantiomer of **3b** was *tert*-butoxycarbonyl (*t*-Boc) protected providing D-*N*α-(*tert*-butoxycarbonyl)-6-(4-methoxybenzylmercapto)hexanoic acid **4** (depicted in Fig. 1).

3. Discussion

Since a general route to produce a variety of sulfur containing amino acids does not exist, we designed and carried out the straightforward synthesis of a homologous series of protected 2-amino-ω-mercapto acids with variable side-chain lengths. The products, L-*N*α-(9-fluorenylmethoxycarbonyl)-5-(4-methoxybenzylmercapto)valeric acid (**3a**), L-*N*α-(9-fluorenylmethoxycarbonyl)-6-(4-methoxybenzylmercapto)hexanoic acid (**3b**), and L-*N*α-(9-fluorenylmethoxycarbonyl)-7-(4-methoxybenzylmercapto)heptanoic acid (**3c**), have been prepared from the appropriate 2-(*S*)-azido ω-bromo acid in 44%, 55%, and 53% overall yields, respectively. The *t*-Boc-protected enantiomer of **3b**, D-*N*α-(*tert*-butoxycarbonyl)-6-(4-methoxybenzylmercapto)hexanoic acid (**4**), was prepared following the same general route in 79% overall yield. The versatility of our route is that any amino protecting group can be incorporated at the end of the synthesis. Also advantageous is that the sulfur is introduced with a trifluoroacetic acid labile protecting group (4-methoxybenzyl) attached. The



Scheme 1. Synthesis of protected L-2-amino-ω-mercapto acids

conditions for this nucleophilic substitution are mild enough so that even sterically hindered thiols like triphenylmethanethiol might be considered for this reaction. The series of 2-amino-ω-mercapto acids offer the possibility to optimize the active binding motifs in many settings including simple one-turn cyclizations, two-turn α-helixes, and macromolecular disulfide optimizations. Additionally, the availability of a variety of alkylated thiols offer the potential to produce many additional products, such as non-natural methionine homologues and derivatives.

4. Experimental

^{1}H and ^{13}C NMR were obtained on a Varian Gemini 300 spectrometer. All solvents were reagent grade obtained from commercial suppliers and used without distillation. Reagents were obtained as follows: 4-methoxy-α-toluenethiol from Fluka, 9-fluorenylmethyl succinimidyl carbonate and di-*tert*-butyl pyrocarbonate from Acros Organics, and stannous chloride from Aldrich. Flash chromatography was performed using Whatman silica gel 60 (230–400 mesh) and monitored using Whatman UV_{254} thin layer chromatography plates. Analytical HPLC was used to monitor nucleophilic substitution reactions with the following system: the mobile phase consisted of 0.1% trifluoroacetic acid in water (solvent A) and 0.1% trifluoroacetic acid in 80% acetonitrile (solvent B). Analysis was performed on a Waters dual pump HPLC system in combination with a Bakerbond (C18, wide bore) column. Approximately 10 to 50 μg of azido acid was injected and separated at a flow rate of 1 mL/min. Linear gradients from 20% to 80% B over 30 min were used and the effluent detected by UV absorbance at 220 nm. Optical rotations were measured in 1 and 0.5 dm cells of 10 and 0.1 mL capacity, respectively, and are quoted in units of deg cm^{-3} g^{-1} dm^{-1}.

*4.1. Representative synthesis of 2-(S)-azido-7-(4-methoxybenzylmercapto)heptanoic acid (**2c**)*

To a solution of **1c** (310 mg, 1.24 mmol) in EtOH (9 mL) was added 4-methoxy-α-toluenethiol (purity 90%, 212 mg, 1.00 equiv.) followed by immediate addition of aqueous KOH (344 μL, 9.0 M KOH solution, 2.5 equiv., diluted to 3 mL with distilled H_2O). The reaction was allowed to stir at room

temperature and monitored by analytical HPLC. The reaction was complete after 1 h and concentrated under reduced pressure, diluted with 40 mL H_2O, and acidified to pH 2 by dropwise addition of 37% HCl. The crude product was extracted with ethyl acetate (3×), dried ($MgSO_4$), and concentrated. Purification on silica gel (elution with 5% MeOH in CH_2Cl_2, R_f=0.54) provided 353 mg of **2c** as a clear oil (88% yield). $[\alpha]_D^{22}=-15.6$ (*c*=1.0, methanol, *l*=1 dm). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.23 (d, *J*=8.6 Hz, 2H), 6.86 (d, *J*=8.7 Hz, 2H), 3.89 (dd, *J*=5.2, 8.5 Hz, 1H), 3.80 (s, 3H), 3.67 (s, 2H), 2.41 (t, *J*=6.9 Hz, 2H), 1.90–1.70 (m, 2H), 1.65–1.50 (m, 2H), 1.50–1.35 (m, 4H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 176.4, 158.6, 130.6, 129.9, 113.9, 61.7, 55.3, 35.7, 31.2, 31.1, 28.9, 28.2, 25.3.

*4.2. 2-(*S*)-Azido-5-(4-methoxybenzylmercapto)valeric acid (**2a**)*

$[\alpha]_D^{22}=-26.0$ (*c*=1.0, methanol, *l*=1 dm). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.23 (d, *J*=8.3 Hz, 2H), 6.86 (d, *J*=8.2 Hz, 2H), 3.89 (dd, *J*=5.0, 8.2 Hz, 1H), 3.79 (s, 3H), 3.67 (s, 2H), 2.44 (t, *J*=6.9 Hz, 2H), 2.00–1.80 (m, 2H), 1.80–1.60 (m, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 175.9, 158.6, 130.3, 130.0, 114.0, 61.4, 55.4, 35.5, 30.4, 30.3, 25.2.

*4.3. 2-(*S*)-Azido-6-(4-methoxybenzylmercapto)hexanoic acid (**2b**)*

$[\alpha]_D^{22}=-16.7$ (*c*=1.0, methanol, *l*=1 dm). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.23 (d, *J*=8.5 Hz, 2H), 6.85 (d, *J*=8.7 Hz, 2H), 3.89 (dd, *J*=5.2, 8.3 Hz, 1H), 3.80 (s, 3H), 3.67 (s, 2H), 2.41 (t, *J*=6.9 Hz, 2H), 1.90–1.70 (m, 2H), 1.65–1.45 (m, 4H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 176.1, 158.6, 130.4, 129.9, 114.0, 61.9, 55.3, 35.7, 30.9, 31.0, 28.6, 25.0.

4.4. Representative synthesis of L-N*α-(9-fluorenylmethoxycarbonyl)-7-(4-methoxybenzylmercapto)-heptanoic acid (**3c**)*

The $SnCl_2$ reduction follows the method of Maiti et al.[6] for aliphatic amine production, except that the workup is modified to accommodate the amino acid product. 2-(*S*)-Azido-7-(4-methoxybenzylmercapto)heptanoic acid (**2c**) (353 mg, 1.09 mmol), dissolved in 1 mL MeOH, is added dropwise to $SnCl_2$ (414 mg, 2 equiv.) suspended in MeOH (1 mL). The reaction was followed by monitoring the disappearance of **2c** using the TLC system described in Section 4.1 and was complete in 1 h at which time MeOH was removed *in vacuo*. The solid was diluted with a 50:50 mixture of H_2O:dioxane and brought to pH 11 by addition of KOH solution. The inorganic precipitate was filtered (Whatman 0.45 μm nylon membrane filter) and washed once with the pH 11 mixture. If necessary, the amino acid can be purified by reverse phase HPLC at this point, to further remove the inorganic material. The solution containing the amino acid was concentrated under reduced pressure to 2–3 mL. The pH was adjusted to 10–11 with HCl or triethylamine (TEA) as needed to facilitate protection of the amino group following a general procedure,[7] utilizing the dropwise addition of dioxane to aid solubility. 9-Fluorenylmethyl succinimidyl carbonate (441 mg, 1.31 mmol) was solubilized in acetonitrile (2 mL) and added in one portion. The pH was maintained between 9 and 9.5 by dropwise TEA addition. After 1 h, the reaction was concentrated *in vacuo*, diluted with H_2O (75 ml), acidified to pH 2 with HCl, and extracted with ethyl acetate (3×), which was dried ($MgSO_4$) and concentrated. Purification on silica gel (elution with 5% MeOH in CH_2Cl_2, R_f=0.38) provided 340 mg of **3c** as a clear oil (60% yield). $[\alpha]_D^{22}=-3.3$ (*c*=2.5, methanol, *l*=0.5 dm). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80–7.25 (m, 8H), 7.20 (d, *J*=8.7 Hz, 2H), 6.82 (d, *J*=8.7 Hz, 2H), 5.27 (d, *J*=8.4 Hz, 1H, N–*H*), 4.25–4.35 (m, 3H), 4.22 (t, *J*=6.9 Hz, 1H), 3.77 (s, 3H), 3.64 (s, 2H), 2.37 (t, *J*=6.7 Hz, 2H), 1.90–1.25 (m, 8H); ^{13}C NMR (75.5 MHz,

$CDCl_3$) δ 177.1, 158.6, 156.2, 143.7, 141.4, 130.6, 129.9, 127.8, 127.1, 125.1, 120.1, 114.0, 67.1, 55.3, 53.7, 47.2, 35.7, 32.2, 31.1, 28.9, 28.3, 24.9.

4.5. L-N*α-(9-Fluorenylmethoxycarbonyl)-7-(4-methoxybenzylmercapto)valeric acid (**3a**)*

$[\alpha]_D^{22}=-5.8$ (*c*=2.5, methanol, *l*=0.5 dm). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80–7.25 (m, 8H), 7.22 (d, *J*=8.5 Hz, 2H), 6.84 (d, *J*=8.5 Hz, 2H), 5.40 (d, *J*=8.2 Hz, 1H, N–*H*), 4.55–4.40 (m, 3H), 4.23 (t, *J*=6.8 Hz, 1H), 3.78 (s, 3H), 3.65 (s, 2H), 2.50–2.30 (m, 2H), 2.10–1.50 (m, 4H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 176.6, 158.6, 156.1, 143.7, 141.4, 130.3, 129.9, 127.8, 127.2, 125.1, 120.1, 114.0, 67.2, 55.3, 53.4, 47.2, 35.7, 31.4, 30.7, 24.9.

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