Incorporation of the α-Mercapto Acid Unit into Peptides

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Dedicated to Prof. Dr. G.-V. Röschenthaler on the occasion of his 60th birthday

Abstract: Incorporation of α-mercapto acid units into the backbone of potential drug candidates improves the metal ion complexing properties. So far, only the methodology for the N-terminal incorporation of mercapto acid units into peptides has been described. Now, we report on a modified strategy for the incorporation of the α-mercapto acid unit into any position of a peptide backbone starting from hexafluoroacetone-protected thiomalic acid. Concomitantly with the incorporation of the α-mercapto unit the direction of the peptide chain is inverted.

Key words: hexafluoroacetone, thiomalic acid, dielectrophiles, thiols, acylations, peptides

A growing number of reports focus on peptidomimetics built from two or more different types of monomers. Consequently, the development of improved methodology for synthesis and incorporation of new types of monomers for peptide modification is of current interest. Surprisingly, mercapto acid derivatives have been virtually neglected as building blocks for peptide modification, although some applications in medicinal chemistry are described.

A β-mercapto acid is a subunit of Captopril, a reliable drug for treating hypertension. α-Mercapto subunits are present in Omapatrilat and Gemopatrilat; both are vasopeptidase inhibitors which are currently under clinical evaluation (Scheme 1). α-Mercapto acid amides are useful subunits in metal ion complexing agents and often exhibit strong inhibitory effects on metal-containing enzymes (metallozymes). Inhibition of matrix metalloproteases has been postulated as a possible therapeutic approach to a number of disease states. Some of the zinc-containing angiotensine converting enzymes (ACE) possess a mercaptoacetyl substructure.

The synthetic repertoire for incorporation of α-mercapto acid derivatives into peptides is limited. Conventional multistep syntheses via acylation of amino acid derivatives by carboxy-activated S-protected mercapto acid derivatives have been described. A more concise approach in which mercapto group protection and carboxy group activation as well as amide bond formation and deblocking of the mercapto group are achieved in one step, involves a heterocyclic intermediate, (Scheme 2). The disadvantage of this strategy is, that the mercapto acid unit can be incorporated only into N-terminal position of a peptide. However, this drawback can be overcome by modifying the above discussed ‘1,3-oxathiolan-5-one route’, by using multifunctional mercapto acids like thiomalic acid for the construction of a trifunctional, orthogonal protected monomer.

Scheme 1

Scheme 2
low monitoring of transformations of compound 3 (Scheme 3).

Scheme 3

A carboxy group present in the side-chain remains unaffected and can be transformed separately after activation. On heating 2 in the presence of an excess of thionyl chloride the corresponding β-acid chloride 3 is formed in high yield (86%). Compound 3 is a member of a preparative useful new class of electrophiles, with two centers of different reactivity. Under carefully controlled conditions two consecutive acylation processes can be achieved in a site-selective way. In the first step the acid chloride 3 reacts with equimolar amounts of N-nucleophiles, like primary, secondary amines and amino acid derivatives in the presence of a tertiary base. Surprisingly, lactams and diketopiperazines can be N-acylated or N,N-diacylated with equimolar amounts of 3 in excellent yields (87–100%) in boiling toluene without the need of a tertiary base (Scheme 4) while the lactone moiety acts as a protective group. Compounds 4–11 are stable at room temperature when stored on exclusion of water.

Scheme 4

In a consecutive step the lactone moiety reacts as an activated ester. By this means, amino acid derivatives 11–14 were obtained (Scheme 5).

Scheme 5

The above-described synthetic sequences are well suited for the generation of libraries of peptidomimetics equipped with mercapto groups in any position of the backbone. Remarkably, with the incorporation of the α-mercaptop unit the direction of the peptide chain is inverted. On further applications of the mercapto modified peptidomimetics like S-glycosylation, introduction of lipophilic anchors and disulfide formation we will report elsewhere. The present reaction may therefore offer considerable potential for the construction of low molecular weight libraries of metal complexing peptidomimetics.

Solvents were purified and dried prior to use. Reagents were used as purchased. Mps were determined on a Boetius heating table. MS were recorded on a VG-250 (Masslab) EI spectrometer (70 eV) or by a VG ZAB-HSQ FAB spectrometer. 1H (200 MHz or 300 MHz), 13C (50 MHz or 75 MHz) and 19F NMR (188 MHz or 282 MHz) spectra were recorded on a Varian Gemini 2000 or a Varian Gemini 300 spectrometer. TMS was used as reference standard for 1H and 13C NMR spectra (internal), and TFA for 19F NMR spectra (external). Flash chromatography was performed by using silica gel (32–63 m, ICN Biomedicals) with solvents systems given in the text.

2,2-Bis(trifluoromethyl)-5-oxo-1,3-oxathiolan-4-yl]-acetyl Chloride (3)

Compound 2 (25.0 g, 83.8 mmol) was heated with freshly distilled thionyl chloride (20 mL) under reflux for 6 h. After removal of the excess of thionyl chloride, the remaining liquid was distilled off in vacuo.

Yield: 88% (24.4 g); slightly yellow liquid; mp ca. 15 °C; bp 80 °C/4.5 torr.

IR (film): 1800 (br), 1385 cm–1.

1H NMR (CDCl3): δ = 3.47 (dd, J = 19.0 Hz, J = 10.0 Hz, 1 H), 3.86 (dd, J = 19.0 Hz, J = 4.0 Hz, 1 H), 4.55 (dd, J = 10.0 Hz, J = 4.0 Hz, 1 H).

13C NMR (CDCl3): δ = 41.6, 49.8, 83.9 (sept, J = 35 Hz), 120.9 (q, J = 286 Hz), 121.4 (q, J = 285 Hz), 169.1, 171.5.
A solution of 3 (3.17 g, 10.0 mmol) in anhyd EtO (25 mL) was added a solution of dibenzylamine (1.97 g, 10.0 mmol) and valerolactame (0.5 g, 5.0 mmol) was heated in toluene (20 mL) under reflux. The reaction mixture was stirred for 1 h keeping the temperature below –20 °C. After the reaction was complete, the solvent was removed in vacuo, EtOAc and sat. NaCl were added, and the layers were separated. The organic phase was washed with citric acid (10% solution, 2 × 10 mL) and water (5 × 10 mL) and dried (MgSO4). The solvent was removed in vacuo and the residue was purified by column chromatography (CH2Cl2).

Yield: 87% (1.65 g); mp 95 °C.

IR (KBr): 1680, 1645, 1610, 1505 cm–1.

1H NMR (CDCl3): δ = 1.85–1.91 (m, 4 H), 2.58–2.62 (m, 2 H), 3.50 (dd, J = 19.0 Hz, J = 11.0 Hz, 1 H), 3.74–3.78 (m, 2 H), 4.00 (dd, J = 19.0 Hz, J = 3.0 Hz, 1 H), 4.50 (dd, J = 11.0 Hz, J = 3.0 Hz, 1 H).

13C NMR (CDCl3): δ = 19.9, 22.1, 34.6, 42.0, 44.5, 44.8, 83.4 (sept, J = 35 Hz), 120.8 (q, J = 283 Hz), 121.3 (q, J = 284 Hz), 170.4, 172.5, 173.2.

19F NMR (DMSO-d6): δ = 0.78 (q, J = 9.0 Hz, 3 F), 1.54 (q, J = 9.0 Hz, 3 F).


A solution of 3 (3.1 g, 10.0 mmol) and glycine anhydride (0.57 g, 5.0 mmol) in toluene (50 mL) was refluxed for 3 d. The solvent was removed under reduced pressure to give product 7.

Yield: 100% (3.37 g); mp 208 °C; mixture of diastereomers in ca. 1:1 ratio.

IR (KBr): 1810, 1720 cm–1.

1H NMR (DMSO-d6): δ = 3.45, 3.46 (dd, J = 19.0 Hz, J = 9.5 Hz, 2 H), 3.97, 3.98 (dd, J = 19.0 Hz, J = 2.7 Hz, 2 H), 4.61–4.63 (m, 4 H), 5.18–5.21 (m, 2 H).

13C NMR (DMSO-d6): δ = 41.1, 41.1, 41.7, 41.7, 47.2, 47.2, 82.3, 82.3 (sept, J = 35 Hz), 120.8, 120.8 (q, J = 283 Hz), 121.3, 121.3 (q, J = 284 Hz), 166.5, 166.5, 169.9, 169.9, 170.6, 170.6.

19F NMR (DMSO-d6): δ = 1.28, 1.28 (q, J = 9.3 Hz, 6 F), 2.36, 2.36 (q, J = 9.3 Hz, 6 F).


To a solution of (4) (3.17 g, 10.0 mmol) in anhyd CH2Cl2 (25 mL) was added a solution of dibenzylamine (1.97 g, 10.0 mmol) and N-ethylisopropylamine (1.29 g, 10.0 mmol) in anhyd Et2O (25 mL) at –78 °C, then NH3 (gas) (0.34 g, 20.0 mmol) was added. After warming to r.t., the precipitate was filtered off and the organic layer was evaporated to dryness. The residue was dissolved in CH2Cl2 (100 mL), washed with water (3 × 100 mL) and dried (MgSO4). The solvent was removed in vacuo and the residue was recrystallized from CHCl3.

Yield: 52% (1.55 g); mp 78 °C.

IR (film): 1825, 1715, 1670 cm–1.
N-[2-Bis(trifluoromethyl)-5-oxo-1,3-oxathiolan-4-yl]-acetyl-tyrosine tert-Butyl Ester (8b)

Yield: 76% (3.45 g); mp 55 °C; mixture of diastereomers in ca. 1:1 ratio.

IR (KBr): 3000, 1810, 1735, 1645 cm⁻¹.

1H NMR (CDCl₃): δ = 4.16 (s, 4.5 H), 1.47 (s, 4.5 H), 1.90–2.23 (m, 3 H), 2.19–2.23 (m, 0.5 H), 2.16–2.27 (m, 0.5 H), 2.69 (dd, J = 16.5 Hz, J = 11.5 Hz, 0.5 H), 2.81 (dd, J = 17.0 Hz, J = 11.5 Hz, 0.5 H), 3.28 (dd, J = 16.5 Hz, J = 3 Hz, 0.5 H), 3.42 (dd, J = 17.0 Hz, J = 3 Hz, 0.5 H), 3.43–3.48 (m, 1 H), 3.58–3.61 (m, 1 H), 4.23 (dd, J = 8.0 Hz, J = 3.0 Hz, 0.5 H), 4.40 (dd, J = 8.5 Hz, J = 3.0 Hz, 0.5 H), 4.53 (dd, J = 11.5 Hz, J = 3.0 Hz, 0.5 H), 4.55 (dd, J = 11.5 Hz, J = 3.0 Hz, 0.5 H).

13C NMR (CDCl₃): δ = 122.4 (q, J = 285 Hz), 121.5, 121.5 (q, J = 285 Hz), 167.0, 167.4, 170.5, 170.9, 171.1, 171.2.

MS (EI): m/z (%) = 543 [M⁺, 102 [CH₃O₂CCH(CH₃)NH]⁺, 87 [CH₃O₂C-CH₂CH₃]+, 69 [CF₃]+, 57 [CH₃C]+].


N-[2-Bis(trifluoromethyl)-5-oxo-1,3-oxathiolan-4-yl]-acetyl-tyrosine tert-Butyl Ester (8c)

Yield: 69% (2.93 g); oil; mixture of conformers in ca. ratio 2:1.

IR (film): 1815, 1735, 1650 cm⁻¹.

1H NMR (CDCl₃): δ = 1.40, 1.43 (s, 9 H), 2.81 (dd, J = 17.0 Hz, J = 11.5 Hz, 0.66 H), 2.70 (dd, J = 16.5 Hz, J = 11.5 Hz, 0.33 H), 3.00, 2.93 (s, 3 H), 3.42 (dd, J = 17.0 Hz, J = 3.0 Hz, 0.66 H), 3.26 (dd, J = 16.5 Hz, J = 3.0 Hz, 0.33 H), 3.36 (dd, J = 17.0 Hz, 0.66 Hz), 3.38 (s, 0.66 H), 4.07 (dd, J = 17.0 Hz, 0.66 Hz), 4.45, 4.18 (dd, J = 11.5 Hz, J = 3.0 Hz, 1.0 H).

13C NMR (CDCl₃): δ = 122.4, 28.2, 28.1, 36.5, 35.4, 39.1, 38.9, 42.7, 42.9, 50.6, 52.4, 82.5, 83.6, 84.6 (sept, J = 35 Hz), 121.4 (q, J = 285 Hz), 122.4 (q, J = 285 Hz), 167.7, 167.2, 169.2, 169.2, 171.1, 171.1.

MS (EI): m/z (%) = 543 [M⁺, 102 [CH₃O₂CCH(CH₃)NH]⁺, 87 [CH₃O₂C-CH₂CH₃]+, 69 [CF₃]+, 57 [CH₃C]+].


2-Mercaptosuccinic Acid 1-(1-Phenylalanine Methyl Ester) 4-(2-Oxopiperidin)-12

Under a nitrogen atmosphere, to a solution of 9 (0.76 g, 2.0 mmol) in anhyd Et₂O (10 mL), was added dropwise a solution of L-phenylalanine methyl ester (0.54 g, 3.0 mmol) in anhyd Et₂O (5 mL). The reaction was stirred for 4 h at r.t., and then the precipitate was filtered off, dissolved in EtOAc, and the solvent was evaporated in vacuo.

Yield: 52% (0.41 g); mp 120 °C; mixture of diastereomers.

IR (KBr): 3350, 2970, 1740, 1700, 1650, 1545 cm⁻¹.

1H NMR (DMSO-d₆): δ = 7.10–7.13 (m, 2 H), 7.17–7.20 (m, 2 H), 2.10–2.17 (d, 5 H), 1.39 (d, J = 7.0 Hz, 1.5), 1.46 (s, 4.5 H), 1.46 (s, 4.5 H), 1.47 (s, 4.5 H), 1.47 (s, 4.5 H), 2.10–2.17 (m, 0.5 H), 2.10–2.17 (m, 0.5 H), 2.43 (d, J = 10.0 Hz, 0.5 H), 2.38 (d, J = 10.0 Hz, 0.5 H), 2.76 (dd, J = 15.0 Hz, 6.5 Hz, 0.75 Hz), 2.78 (dd, J = 15.0 Hz, 5.0 Hz, 0.25 Hz), 2.97 (dd, J = 15.0 Hz, 6.0 Hz, 0.75 Hz), 2.95 (dd, J = 15.0 Hz, 7.5 Hz, 0.25 Hz), 3.67–3.78 (m, 0.5 H), 4.26–4.45 (m, 1 H), 4.36–4.45 (m, 1 H), 6.37 (d, J = 8.7 Hz, 0.75 Hz) 0.63 (d, J = 9.0 Hz, 0.25 Hz), 7.15 (d, J = 6.5 Hz, 0.75 Hz), 7.05 (d, J = 7.0 Hz, 0.25 Hz).

13C NMR (CDCl₃): δ = 17.8, 17.9, 18.3, 18.5, 19.1, 19.1, 28.2, 28.2, 28.2, 28.2, 28.3, 31.5, 31.5, 38.9, 39.4, 42.4, 42.5, 49.5, 49.5, 57.8, 57.9, 82.3, 82.3, 169.8, 169.8, 169.9, 169.9, 170.1, 170.1, 171.2, 171.2, 171.2, 171.2, 171.4, 171.6, 171.6, 171.6, 171.6.

MS (ESI): m/z (%) = 1007 [2 × M + Na⁺], 986 [2 × M + H⁺], 493 [M + H⁺].

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References


