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Transformation of α-Hydroxymethylamino Acids into α-Mercaptomethylamino Acids (α-Alkylcysteines)* [1]

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N-Boc-α-alkylserines (α-hydroxymethylamino acids) undergo a cyclization reaction without racemization to N-Boc-α-alkylserine-β-lactones in 90–98% yield using Mitsunobu conditions (Ph_3P, diethyl azodicarboxylate). Treatment of the corresponding β-lactones with sulphur nucleophiles (thiolacetic acid or 4-methoxybenzylmercaptan) gives S-protected N-Boc-α-alkylcysteines (α-mercaptomethylamino acids) in high yield. These chimeric α,α-disubstituted amino acids, being incorporated into peptide chain, are able to close the cyclic structure as a disulfide bond.

Key words: α-mercaptomethylamino acids, α-hydroxymethylamino acids, α,α-disubstituted amino acids, Mitsunobu reaction

Linear peptides are highly flexible molecules, that can adopt a multitude of conformations in solution, while only a few are responsible for their biological activity. It has been known, that conformational rigidity is an essential requirement to increase potency, selectivity, bioavailability, and enhance the resistance toward proteolytic enzymes. The incorporation of α,α-disubstituted amino acids, as well as short-, medium- or long-range cyclization have been shown to restrict conformational flexibility of bioactive peptides. Cyclizations *via* side chains have produced several potent analogues of opioid peptides, bradykinin, and LHRH. Two approaches, that are employed to construct cylic analogs, involve (i) cyclization *via* C- and N-termini, the so-called "backbone-to-backbone" cyclization and (ii) the cyclizations using amino acid side chains [2]. The second method involves the formation of disulfide bridges between cysteines and lactam bridges between glutamic acid/lysine. A way to introduce these types of restrictions removes the respective side chains from bioactive peptides, which may in themselves, be important for receptor affinity.

α-Mercaptomethylamino acids (α-substituted cysteines) are chimeric analogues of proteinoceous amino acids with functionalized side chains, that provide a cyclization functionality, while preserving the side chain. Incorporation of α-substituted

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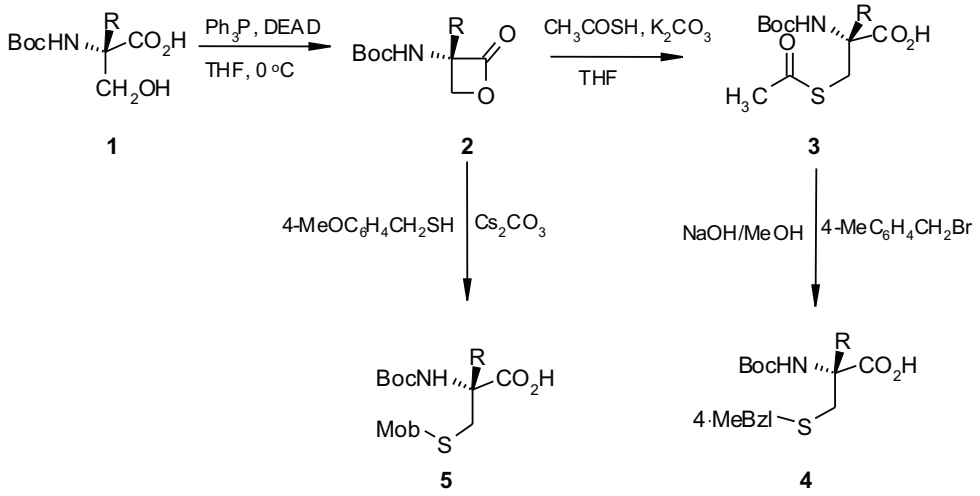
* ***Abbreviations:*** AcSH, thiolacetic acid; Boc, *tert*-butoxycarbonyl; Bzl(Me), 4-methylbenzyl; DEAD, diethyl azodicarboxylate; DIAD, diisopropyl azodicarboxylate; HmLeu, α-hydroxymethylleucine; HmPhe, α-hydroxymethylphenylalanine; HmVal, α-hydroxymethylvaline; LHRH, Luteinizing hormone releasing hormone; MeCys, α-methylcysteine; MmLeu, α-mercaptomethylleucine; MmPhe, α-mercaptomethylphenylalanine; MmVal, α-mercaptomethylvaline; MeSer, α-methylserine, α-hydroxymethylalanine; Mob, 4-methoxybenzyl.

cysteines into peptide sequences may present excellent tools to construct cyclic analogues of biologically active peptides with preserved side chains. In addition, the α-methylcysteine is an important building block for desferrithiocin [3] and a family of natural products isolated from blue green-algae *e.g.* tantazoles, mirabazoles [4] and thiangazole [5]. Because of the labile nature of the sulfhydryl group, very few routes have been successfully applied for the asymmetric synthesis of α-alkylcysteines [6–8].

In our laboratory we have elaborated a procedure for the synthesis of racemic α-hydroxymethyl analogues of various amino acids [9,10]. These amino acids were resolved into the enantiomers by fractional crystallization of the diastereomeric salts of their N-benzoyl derivatives with different alkaloids. The absolute configurations of some α-hydroxymethylamino acids were determined by the chemical correlation with respective α-methylamino acids or X-ray analysis [11–13]. In the present paper we report the transformation of various α-hydroxymethyl-α-amino acids into their α-mercaptomethyl analogues (Scheme 1).



R = CH_3 (**a**); $CH(CH_3)_2$ (**b**) $CH_2C_6H_5$ (**c**); $CH_2CH(CH_3)_2$ (**d**).

Scheme 1. Transformation of α-hydroxymethylamino acids into α-mercaptomethylamino acids.

MATERIALS AND METHODS

Racemic α-hydroxymethylamino acids were synthesized by selective α-hydroxymethylation [9,10] and were resolved into enantiomers by fractional crystallization of the diastereomeric salts of their N-benzoyl derivatives with (–)-ephedrine, (–)-quinine or (–)-cinchonidine [11,14]. N-Boc-hydroxymethylamino acids **1** were obtained, according to the procedure described elsewhere [15].

HPLC analysis were carried out on a LDC analytical instrument using a Vydac C_{18} (0.46×25 cm) column, flow rate 1.0 ml/min, detection at 220 nm, and solvents (A) 0.05% trifluoroacetic acid (TFA) in water and (B) 0.038% TFA in acetonitrile/H_2O 90:10 in linear gradient application. Optical rotations were measured in a 1 dcm cell (1 ml) on a Horiba (Kyoto, Japan) high-speed automatic polarimeter at 589 nm.

^{1}H-NMR spectra were recorded on a Bruker 250 MHz (Bruker, Germany) spectrometer. Molecular weights of compounds were determined on Finningan MAT 95 (Finningan MAT GmbH, Bremen Germany) mass spectrometer.

N-Boc-α-alkylserine-β-lactones (2) (general procedure). To a solution of triphenylphoshine (524.6 mg, 2 mM) in dry THF, DEAD (313 μl, 2 mM) was added at 0°C. The mixture was stirred 20 min at 0°C, then a solution of Boc-α-hydroxymethylamino acid (2 mM) in dry THF (1 mL) was added. Stirring was continued for 1 hr at 0°C and then 16 hrs at room temperature. The THF was removed *in vacuo* and the crude product was flash chromatographed on silica gel 60 (230–400 mesh) [16] using ethyl acetate-n-hexane (1:1) as eluant, to give the N-Boc-α-alkylserine-β-lactone **2** in 90–98% yield.

N-Boc-S-Bzl(4-Me)-α-alkylcysteines (4) (general procedure). To a solution of **2** (1 mM) in dry THF (5 mL), containing 207 mg (1.5 mM) anhydrous K_2CO_3, 107 μL (1.5 mM) AcSH was added under stirring. After 16 hrs at room temperature, the mixture was concentrated under vacuum, dissolved in water, acidified with 1N $NaHSO_4$ and extracted with AcOEt. The organic phase was washed with brine, dried, and evaporated. To the resulting residue (**3**) dissolved in 2 mL MeOH and 2 mL 1N NaOH, α-bromo-p-xylene (190 mg, 1mM, ≥ 97%) was added and the mixture was stirred 24 hrs at room temperature. The solvent was removed *in vacuo,* and the remaining residue was acidified and the product was extracted with AcOEt. The organic layers were washed with brine, dried, and evaporated. The crude product was flash chromatographed on silica gel with $CHCl_3$/MeOH/AcOH (95:5:1) to afford **4** (85–90%).

Boc-(*S*)-MeCys(4-MeBzl) (*S*-4a). Yield 86%, ^{1}H NMR ($CDCl_3$) δ: 1.45 (s, 9H); 1.57 (s, 3H); 2.33 (s, 3H); 3.12 (s, 2H); 3.71 (s, 2H); 5.45 (bs, 1H); 7.21 (m, 4H), $[\alpha]_D^{20}$ = +24.8 (c = 1, MeOH), HR-MS (FAB) m/z 338.1466 (M-H), calcd. for $C_{17}H_{24}NSO_4$ 338.1426; HPLC purity 97%, t_R = 9.74 min., linear gradient 50–90%B, 25 min.

Boc-(*R*)-MeCys(4-MeBzl) (*R*-4a). Yield 86%, $[\alpha]_D^{20}$ = –24.3 (c = 1, MeOH).

Boc-(*S*)-MmVal(4-MeBzl) (*S*-4b). Yield 69%, ^{1}H NMR ($CDCl_3$) δ: 0.92 (d, 3H, J = 7.5 Hz); 0.95 (d, 3H, J = 7.5 Hz); 1.44 (s, 9H); 2.34 (s, 3H); 2.62 (m, 1H); 3.22, 3.30 (AB system, 2H, J = 15 Hz); 3.70 (s, 2H); 5.5 (bs, 1H); 6.49 (bs, 1H); 7.1 (m, 4H); $[\alpha]_D^{20}$ = –19.59 (c = 1, MeOH); HR-MS (FAB) m/z 366.1719 (M-H), calcd. for $C_{19}H_{28}NSO_4$ 366.1739; HPLC purity 95%, t_R = 11.33 min., linear gradient 50–90%B, 25 min.

Boc-(*R*)-MmVal(4-MeBzl) (*R*-4b). Yield 69%, $[\alpha]_D^{20}$ = +19.3 (c = 1, MeOH).

Boc-(*S*)-MmPhe(4-MeBzl) (*S*-4c). Yield 70%, ^{1}H NMR ($CDCl_3$) δ: 1.49 (s, 9H); 2.33 (s, 3H); 3.04, 3.33 (AB system, 2H, J = 14.0 Hz); 3.55, 3.59 AB system, 2H, J = 5.0 Hz); 3.72 (d, 2H, J = 3.0 Hz); 5.46 (bs,1H); 7.10–7.29 (m, 9H); $[\alpha]_D^{20}$ = +23.29 (c = 1, MeOH); HR-MS (FAB) m/z 414.1701 (M-H), calcd. for $C_{23}H_{28}NSO_4$ 414.1739; HPLC purity 96%, t_R = 15.93 min., linear gradient 50–90%B, 25 min.

Boc-(*R*)-MmPhe(4MeBzl) (*R*-4c). Yield 77%; $[\alpha]_D^{20}$ = –23.59 (c = 1, MeOH).

Boc-(*S*)-MmLeu(4-MeBzl) (S-4d). Yield 82%; ^{1}H NMR ($CDCl_3$) δ: (d, 3H, J = 6.3 Hz); 0.91 (d, 3H, J = 6.3 Hz); 1.29 (m, 1H); 1.44 (s, 9H); 1.58 (m, 2H); 2.31 (s, 3H); 3.62, 3.70 (system AB, 2H, J = 15.0 Hz); 5.75 (s, 1H); 7.17 (m, 4H); $[\alpha]_D^{20}$ = –9.25 (c = 1, MeOH); HR-MS (FAB) m/z 380.1893 (M-H), calcd. for $C_{20}H_{30}NSO_4$ 380.1896; HPLC purity 93%, t_R = 16.2 min., linear gradient 50–90%B, 25 min.

Boc-(*R*)-MmLeu(4-MeBzl) (R-4d). Yield 79%; $[\alpha]_D^{20}$ = +9.84 (c = 1, MeOH).

N-Boc-S-Bz(Mob)-α-alkylcysteines (5) (general procedure). To a solution of **2** (1 mM) in dry DMF (5 mL), containing 207 mg (1.5 mM) anhydrous Cs_2CO_3, 208.5 μL (1.5 mM) $CH_3OC_6H_4CH_2SH$ was added under stirring. After 16 hrs at room temperature, the mixture was concentrated under vacuum, dissolved in water, and acidified with 1N $NaHSO_4$. The product was extracted with AcOEt; the organic phase was washed with brine, dried, and evaporated. The residue was flash chromatographed on silica gel [15] with $CHCl_3$/MeOH/AcOH (95:5:1) to afford **4** (80–90% yield).

Boc-(*R*)-MmVal(Mob) (*R*-5b). Yield 88%; ^{1}H NMR ($CDCl_3$) δ: 0.92 (d, 3H, J = 6.5 Hz); 0.95 (d, 3H, J = 6.5Hz); 1.45 (s, 9H); 2.65 (m, 1H); 3.21, 3.29 (AB system, 2H, J = 14.0 Hz); 3.69 (s, 2H); 3.79 (s, 3H); 5.52 (bs,1H); 6.39 (m,1H); 6.86 (m, 2H); 7.19 (m,2H); $[\alpha]_D^{20}$ = +18.9 (c = 1, MeOH); HR-MS (FAB) m/z 382.1833 (M-H), calcd. for $C_{19}H_{28}NSO_5$ 392.1688; HPLC purity 93%, t_R = 10.55 min., linear gradient 50–90%B, 25 min.

Boc-(*S*)-MmVal(Mob) (*S*-5b). Yield 90%; $[\alpha]_D^{20} = -18.50$ (c = 1, MeOH).

Boc-(*R*)-MmPhe(Mob) (*R*-5c). Yield 80%; ^{1}H NMR ($CDCl_3$) δ: 1.46 (s, 9H); 2.98 (ds, 2H); 3.07, 3.28 (AB system, 2H, J = 12.5 Hz); 3.59 (m, 2H); 3.79 (s, 3H); 5.48 (bs, 1H); 6.38 (m, 2H); 7.12 (m, 2H); 7.23 (m, 5H); 8.02 (s, 1H); $[\alpha]_D^{20} = -28.75$ (c = 1, MeOH); HR-MS (FAB) m/z 430.1653 (M-H), calcd. for $C_{23}H_{28}NSO_5$ 430.1688; HPLC purity 96%, t_R = 13.43 min., linear gradient 50–90%B, 25 min.

Boc-(*S*)-MmPhe(Mob) (*S*-5c). Yield 80%; $[\alpha]_D^{20} = +28.60$ (c = 1, MeOH).

Boc-(*R*)-MmLeu(Mob) (*S*-5d). Yield 81%; ^{1}H NMR ($CDCl_3$) δ: 0.84 (d, 3H, J = 6.50 Hz), 0.92 (d, 3H; J = 6.50 Hz); 1.25 (m, 1H); 1.44 (s, 9H); 1.62 (m, 2H); 3.63–3.71 (AB system, 2H, J = 13.2 Hz); 3.78 (s, 3H); 3.80 (s, 2H); 6.83, 7.21 (system AA`XX`, 4H, J = 8.4 Hz); $[\alpha]_D^{20} = +13.4$ (c = 1, MeOH); HR-MS (FAB) m/z 396.1849 (M-H), calcd. for $C_{20}H_{30}NSO_5$ 396.1845, HPLC purity 93%, t_R = 13.81 min., linear gradient 50–90%B, 25 min.

Boc-(*S*)-MmLeu(Mob) (*R*-5d). Yield 79%; $[\alpha]_D^{20} = -13.1$ (c = 1, MeOH).

RESULTS AND DISCUSSION

Easily available Boc-α-hydroxymethylamino acids were found to be useful substrates for the synthesis of S protected Boc-α-mercaptomethylamino acids. Boc-α-hydroxymethylamino acids undergo the cyclization reaction to N-Boc-α-alkylserine-β-lactones in 90–98% yield under Mitsunobu conditions. Although the efficiency of the Mitsunobu reaction does not depend on the alkyl group of azodicarboxylate and ethyl or isopropyl azodicarboxylates can be used interchangeably [17], attempt to use commercially available DIAD was inefficient. Only N-Boc-α-methylserine-β-lactone was obtained in reasonable yield (60%). Instead the application of DEAD for sterically hindered α-hydroxymethylamino acids, such as β-branched (Val) or γ-branched (Leu, Phe) provides β-lactones in excellent yields. DEAD is easily accessible in two step reaction [18]. Ring opening of this β-lactones with 4-thiolacetic acid or methoxybenzylmercaptan [19] resulted in the formation of S-protected Boc-mercaptomethylamino acids.

Attempted simple nucleophilic substitution of methylserine derivatives with cesium thiolcarboxylates as nucleophiles was unsuccessful. No leaving group (OTs, OMs or OTf) could be displaced with thiolacetate or thiolbenzoate anions to give methylcysteine derivative. The steric hindrance of hydroxyl group of α-hydroxymethylamino acid can be comparable with that of the neopentyl position.

Described S-protected Boc-α-mercaptomethylamino acids, α-chimeras combining side chains Ala/Cys, Phe/Cys, Val/Cys and Leu/Cys are orthogonally protected, building block with free carboxylic acid groups, suitable for peptide synthesis.

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