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NEW α-AMINO ACIDS FOR PEPTIDES OF BIOLOGICAL INTEREST: 2-[2'-(METHYLTHIO)ETHYL]METHIONINE DERIVATIVES

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Abstract: Synthetic route to *N*- and *C*-protected derivatives of the new α,α-disubstituted achiral methionine analogue 2-[2'-(methylthio)ethyl]methionine is described starting from diethyl bis(2-methylthioethyl)malonate.

Continuous efforts are currently devoted to the chemical modification of bioactive natural peptides in order to improve potency and selectivity.[1] In this connection there is great interest in the design and synthesis of new α-amino acid residues which may provide specific conformational constraint and/or increased stability against enzymatic degradation. Incorporation of C-α,α-dialkyl substituted α-amino acids into peptide backbone represents one of the most effective strategies to confer specific three dimensional properties to peptides.[2]

In this paper we report synthesis and properties of the *N*-protected and *C*-protected derivatives **3a,b** and **4**, suitable for the incorporation of the new 2-[2'-(methylthio)ethyl]methionine residue (Dmt) into peptide backbone. This achiral α-amino acid, possessing two "methionine" side chains, is expected to combine the tendency of C-α,α-disubstituted residues to restrict the available range of peptide backbone conformations with the maintenance of the chemical and binding properties specific of the methionine side chain. It should be noted in this context

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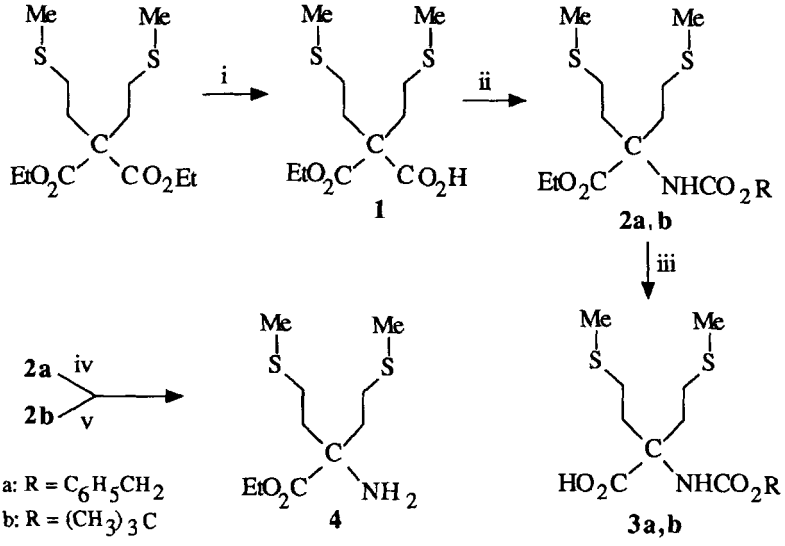
that methionine as well as being an essential nutritional amino acid and a constituent of proteins and bioactive peptides, plays a key role, as a methyl group donor, in biochemical processes of general significance.

Diethyl bis(2-methylthioethyl)malonate[3] (Scheme) has been selected as suitable starting material. This can be efficiently converted (70% yield) into the monoester **1** by treatment at room temperature with one equivalent of KOH in dry ethanol. The modified Curtius reaction[4] involving the use of diphenylphosphoryl azide (DPPA) and triethylamine (TEA) in the presence of an alcohol has been exploited for the one-pot conversion of the half-ester **1** to both the *N*-benzyloxycarbonyl and *N-tert*-butoxycarbonyl derivatives Z-Dmt-OEt (**2a**) and Boc-Dmt-OEt (**2b**). Due to the presence of sulfur atoms, usual catalytic (Pd)



Reagents: i) KOH/dry EtOH; ii) DPPA, TEA, ROH; iii) NaOH/aqueous MeOH; iv) HCO_2NH_4, Pd/C; v) $CF_3CO_2H/CHCl_3$

Scheme

hydrogenation was found ineffective to deprotect **2a**; high yields of the amino ester H-Dmt-OEt (**4**) can be obtained, however, by using ammonium formate catalytic (10% Pd/C) transfer hydrogenation.[5] The same amino ester **4** can be obtained by

treatment of Boc-Dmt-OEt (**2b**) at room temperature with CF_3COOH. Alkaline hydrolysis of **2a,b** can be performed at room temperature with NaOH in aqueous methanol to give the corresponding acids **3a,b**. As expected for α,α-dialkyl substituted α-amino esters, the hydrolysis rate is slow; five and six days are necessary to obtain a complete transformation of Z-Dmt-OEt (**2a**) and Boc-Dmt-OEt (**2b**), respectively.

Introduction of the new Dmt residue into bioactive peptides is now in progress in our laboratory.

Experimental

Melting points were determined with a Büchi oil bath apparatus and are uncorrected. IR spectra were recorded with a Perkin-Elmer 983 spectrophotometer in $CHCl_3$ solution. ^1H NMR spectra were measured with a Varian EM-390 spectrometer in $CDCl_3$ (tetramethylsilane as internal standard). Column chromatography was carried out using Merck silica gel 60 (230-400 mesh). Preparative-layer chromatography (PLC) was performed on Merck silica gel 60 F_{254} plates. Light petroleum refers to 40-60°C bp fraction. The drying agent was sodium sulfate. Analytical data: Servizio Microanalisi del CNR, Area della Ricerca di Roma, Montelibretti, Italy.

Monoethyl bis(2-methylthioethyl)malonate (1)

Diethyl bis(2-methylthioethyl)malonate[3] (1.13 g, 3.66 mmol) was dissolved in dry ethanol (2.4 mL). A solution of potassium hydroxide (0.205 g, 3.66 mmol) in dry ethanol (7.7 mL) was added at room temperature under stirring over a period of 1 h. After stirring for 40 h the solvent was evaporated under reduced pressure and the residue was partitioned between water and ether. The aqueous phase was acidified with 6N HCl and extracted with ethyl acetate. The organic layers were washed with water, dried and evaporated to give the title monoester **1** (0.716 g, 70% yield) as an homogeneous oil; IR: 2990, 2920, 1726, and 1265 cm^{-1}; ^1H NMR: δ 1.28 (3H, t, J = 7 Hz, OCH_2-$C\underline{H}_3$), 2.08 (6H, s, two SCH_3), 2.09-2.67 (8H, m, two CH_2-CH_2S), 4.27 (2H, q, J = 7 Hz, $OC\underline{H}_2$-CH_3), 9.34 (1H, s, COOH). The product was used without further purification.

N-Benzyloxycarbonyl-2-[2'-(methylthio)ethyl]methionine ethyl ester (2a)

A stirred mixture of **1** (0.334 g, 1.19 mmol), DPPA (0.344 g, 1.25 mmol) and TEA (0.129 g, 1.27 mmol), was refluxed in dry benzene (3.6 mL) for 3 h. After addition of benzyl alcohol (0.141 g, 1.31 mmol), the mixture was refluxed for 25 h. The solvent was evaporated under vacuum to give a residue which was dissolved in ethyl acetate. The organic solution was washed with cold 1N HCl, water, saturated aqueous $NaHCO_3$, and brine. After drying and evaporation , the residue was chromatographed on a silica column (1:50). Elution with *n*-hexane-dichloromethane (1:1) afforded pure *N*-benzyloxycarbonyl-2-[2'-(methylthio)ethyl] methionine ethyl ester (**2a**) (0.356 g, 77% yield), as an oil; IR: 3409, 2986, 2918, 1713, and 1500 cm^{-1}; ^1H NMR: δ 1.27 (3H, t, J = 7 Hz, OCH_2-$C\underline{H}_3$), 1.97 (6H, s, two SCH_3), 2.00-2.82 (8H, m, two CH_2-CH_2S), 4.25 (2H, q, J = 7 Hz, $OC\underline{H}_2$-CH_3), 5.09 (2H, s, O-$C\underline{H}_2$-Ph), 5.97 (1H, s, $\underline{N}H$), 7.37 (5H, s, aromatic).

N-*tert*-Butoxycarbonyl-2-[2'-(methylthio)ethyl]methionine ethyl ester (2b)

The title Boc-derivative **2b** was prepared as described for the Z-analogue **2a** using an excess of *tert*-butyl alcohol (3 mL *per* mmol of **1**) and refluxing for two days. Usual work up gave a residue which was chromatographed on a silica column (1:40), eluting with *n*-hexane-dichloromethane (1:9). Further purification by PLC [dichloromethane-ethyl acetate(99:1) as eluant] of nearly homogeneous fractions afforded pure *N*-protected ethyl ester **2b** in 38% yield, as an oil; IR: 3418, 2981, 2918, 1711, and 1493 cm^{-1}; H^1 NMR: δ 1.28 (3H, t, J = 7 Hz, OCH_2-$C\underline{H}_3$), 1.42 (9H, s, $C(CH_3)_3$), 2.05 (6H, s, two SCH_3), 2.19-2.83 (8H, m, two CH_2-CH_2S), 4.24 (2H, q, J = 7 Hz, $OC\underline{H}_2$-CH_3), 5.67 (1H, s, NH).

N-Benzyloxycarbonyl-2-[2'-(methylthio)ethyl]methionine (3a)

The ethyl ester **2a** (0.158 g, 0.41 mmol) was dissolved in methanolic 4% sodium hydroxide (0.9 mL) and stirred at room temperature for 5 days. After evaporation of methanol under reduced pressure, water and ether were added. The aqueous phase was acidified with 6N HCl (until pH reached 3), and extracted with

ethyl acetate. The organic layers were washed with brine, dried and evaporated to give pure title compound **3a** (0.138 g, 94%) as an oil; IR: 3411, 2920, 1711, and 1501 cm^{-1}; ^1H NMR: δ 1.97 (6H, s, two SCH_3), 2.08-2.77 (8H, m, two CH_2-CH_2S), 5.09 (2H, s, O-C$\underline{H}_2$-Ph), 6.00 (1H, br s, NH), 7.35 (5H, s, aromatic), 9.27 (1H, s, COOH).

Dicyclohexylammonium salt of **3a** crystallized by adding dicyclohexylamine to a dry ethereal solution of the crude acid followed by addition of light petroleum. The salt was recrystallized from CH_2Cl_2-light petroleum; mp 158-158.5 °C.
Anal. Calcd. for $C_{28}H_{46}N_2O_4S_2$: C, 62.41; H, 8.61; N, 5.12. Found: C, 62.20; H, 8.77; N, 5.03.

N-tert-butoxycarbonyl-2-[2'-(methylthio)ethyl]methionine (3b)

Alkaline hydrolysis of ester **2b** was performed as described for **2a**. After stirring for 6 days and usual work up, the title acid **3b** was obtained in 93% yield as an oil; IR: 3417, 2979, 2919, 1703, and 1493 cm^{-1}; H^1 NMR: δ 1.44 (9H, s, $C(CH_3)_3$), 2.07 (6H, s, two SCH_3), 2.13-2.77 (8H, m, two CH_2-CH_2S), 5.5-7.2 (1H, broad signal, NH), 10.93 (1H, s, COOH).

2-[2'-(Methylthio)ethyl]methionine ethyl ester (4)

From 2a

To a solution of *N*-protected ethyl ester **2a** (0.180 g, 0.47 mmol) and HCO_2NH_4 (0.044 g, 0.70 mmol) in *N,N'*-dimethylformamide (4.7 mL) and water (0.81 mL), 10% Pd/C (0.18 g) was added. The solution was slightly shaken at room temperature and additional portions of HCO_2NH_4 (0.044 and 0.022 g) and catalyst (0.18 and 0.09 g) were added after 15 and 45 min from beginning. The mixture was stirred for 70 min and then the catalyst was removed by filtration. The solvent was evaporated under reduced pressure and the residue was partitioned between 2N HCl and ether. The aqueous phase was alkalinized with 12.5% NH_4OH, and extracted with ethyl acetate.

The organic layers were washed with brine, dried and evaporated. PLC (ethyl acetate as eluant) allowed the separation of pure amino ester **4** (0.074 g, 68%), as an oil; IR: 3380, 2982, 2919, and 1722 cm^{-1}; NMR: δ 1.26 (3H, t, J = 7 Hz, OCH_2-C$\underline{H}_3$), 1.68 (2H, s, NH_2), 2.05 (6H, s, two SCH_3), 1.73-2.63 (8H, m, two CH_2-CH_2S), 4.20 (2H, q, J = 7 Hz, OC$\underline{H}_2$-CH_3).

*From **2b***

The Boc-ester **2b** (0.18 g, 0.512 mmol) was dissolved in a mixture of trifluoroacetic acid and dry chloroform [(1:1), 0.6 mL] and stirred at room temperature for 4 h. The organic solvent was removed under reduced pressure and the residue, dissolved in water, was alkalinized with 12.5% NH_4OH, and extracted with ethyl acetate. The organic layers were washed with brine, dried and evaporated to give the title ethyl ester **4** (0.095 g, 74%)

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