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NEW SYNTHESIS OF L-m-SARCOLYSIN AND TRITIATED SARCOLYSIN.

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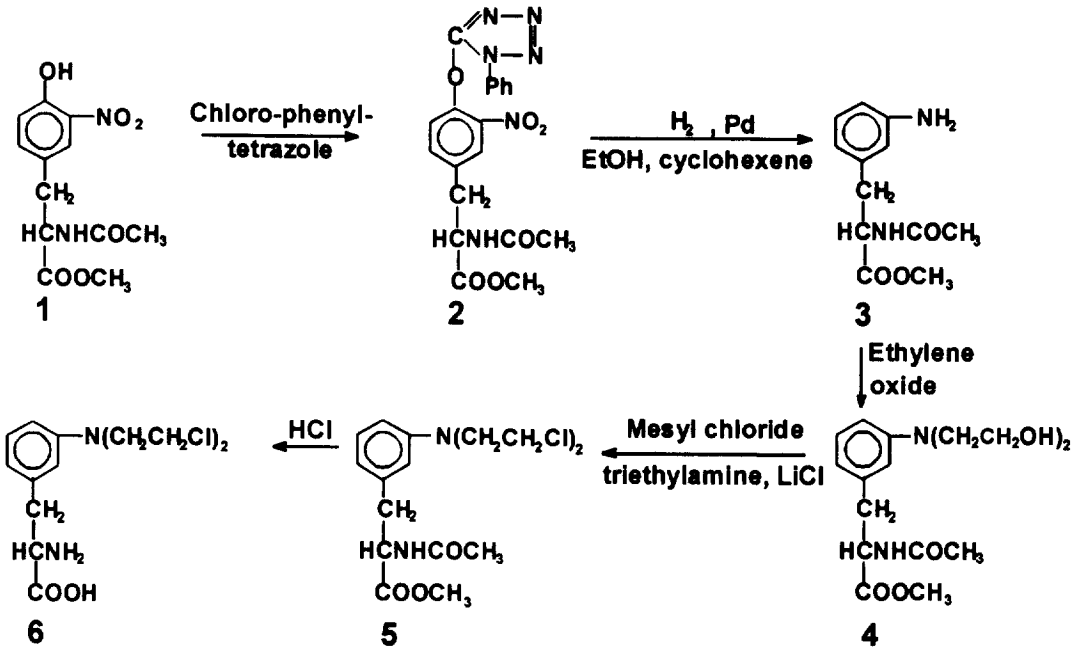
Abstract . L-m-sarcolysin, L-3-[bis(2-chloroethyl) amino]-L-phenylalanine was synthesized by converting 3-nitro-L-tyrosine to L-N-acetyl-3-aminophenyl-alanine Me-ester which was hydroxyethylated and converted into the N-mustard with mesyl chloride and LiCl; the title compound was obtained by hydrolysis of the protecting groups. The tritiated compound was specifically labeled on the benzyl group.

L-m-sarcolysin, L-3-[bis(2-chloroethyl) amino]-L-phenylalanine (**6**) is of current interest as a component of several newly synthesized antineoplastic oligopeptides from which it is released both *in vivo* and *in vitro*.[1-3] Previous methods of preparing racemic m-sarcolysin were based on the construction of m-substituted phenylalanine derivatives by the acylaminomalonic acid synthesis route.[4-6] The synthesis of optically active sarcolysines included a resolution step of the N-phtalyl amino acid derivative with brucine[7]. Our approach has two major advantages. First, our synthesis starts with commercially available 3-nitro-L-tyrosine which is converted to L-N-acetyl-3-nitrotyrosine methyl ester (**1**) using a well known method.[8] The choice of this starting material allows the bypass of both the malonic acid synthesis and the resolution steps, thus considerably shortening the duration and increases the efficiency of the synthesis. Second, the use of methanesulfonyl chloride and LiCl for the preparation of the N-mustard moiety is considered superior to the previously used $SOCl_2$ which inevitably results in tarry (gummy) products which are difficult to handle.[4-6] Electrospray and cone voltage induced fragmentation mass spectra are also included because, as far as we know, no such characterization has been published to date.

As shown in Scheme 1, the protected nitrotyrosine (**1**) was treated with 5-chloro-1-phenyl-1*H*-tetrazole to form (**2**) which, in turn, was boiled with palladium charcoal in ethanol-cyclohexene to yield the 3-amino-phenylalanine derivative (**3**). This step, originally developed for the hydrogenolytic elimination of the phenol OH groups[9] also resulted in the reduction of the m-nitro group. Next, **3** was hydroxyethylated with ethylene oxide to yield the bis(2-hydroxyethyl) derivative, L-N-acetyl-(m-bis-hydroxyethylamino)-phenylalanine methyl ester (**4**). The N-mustard (bis-chloroethylamino) moiety was prepared from **4** in a one-pot reaction with methanesulfonyl chloride and triethylamine in chloroform followed by a subsequent reaction with LiCl in hot methanol to yield N-acetyl-sarcolysine methyl ester (**5**). The final product **6** was obtained by hydrolysis with boiling HCl.

The tritium labeled compound was prepared as described above starting with L-N-acetyl-(m-bis-hydroxyethylamino)-phenylalanine methyl ester, [^{3}H], i.e., **4**, which was specifically labeled with tritium on the benzyl group.



Scheme 1.

Experimental. All commercial chemicals were purchased from Aldrich Chemical Co., Milwaukee, WI and used without further purification.

L-methyl N-acetyl-m-nitrotyrosinate-5(1-phenyl-1H-tetrazole)-ether (**2**). 14.1 g (0.05 mole) L-methyl N-acetyl-3-nitrotyrosinate, 10.44 g (0.058 mole) 5-chloro-1-phenyl-1H-tetrazole, and 7.14 g (0.085 mole) $NaHCO_3$ in 100 mL N,N-dimethylacetamide were reacted at 100 °C for one hour. The reaction mixture was poured on ice, the separated solid was filtered, dried, and recrystallized from benzene-hexane. Yield: 17.4 g (80%). M.P.=142-144 °C. $[\alpha]_D^{25}$=+5.0 (c=2 in MeOH).

L-Methyl 3-amino-N-acetyl-phenylalanate (**3**). A stirred suspension of 4.26 g (0.01 mole) **2** in 50 mL cyclohexane, 50 mL ethanol and 10 g 10% Pd charcoal was boiled for 3.5 h. The reaction mixture was filtered and the solvent evaporated. The residue was treated with diluted HCl and ethyl acetate. The acidic aqueous solution was saturated with K_2CO_3 and extracted several times with ethyl acetate. The thick brown oil remaining after evaporation of the ethyl acetate extractions was used without further purification.

L-methyl N-acetyl-3-[bis-(2-hydroxyethyl)amino]-phenylalanate (**4**). 4.7 g crude **3** dissolved in 15 mL acetic acid and 15 mL water was reacted with 15 mL ethylene oxide at room temperature for 14 h. The solution was diluted with 30 mL water and neutralized with $NaHCO_3$. The separated solid was filtered, dried, and recrystallized from acetone-ether. Yield: 4.95 g (73%). M.P.=81-83 °C. $[\alpha]_D^{25}$=+18.84 (c=2 in MeOH).

L-methyl N-acetyl-3-[bis-(2-chloroethyl)amino]-phenylalanate (**5**). 3.24 g (0.01 mole) **4**, 2 mL (0.026 mole) methanesulfonyl chloride, and 1.6 mL triethylamine were reacted in 200 mL dichloromethane at 20 °C for 30 min. After removing the solvent in vacuum, 15 g LiCl was added and the mixture was boiled in 50 mL methanol for 2 h. Next, the reaction mixture was evaporated, 100 mL water was added, followed by extraction with ethyl acetate. The oil remaining after the evaporation of the solvent was crystallized from hexane-ether. Yield: 2.96 g (82%). M.P.=95-98 °C. $[\alpha]_D^{25}$=+20.82 (c=2 in MeOH).

L-m-sarcolysin (**6**). 3.6 g **5** was hydrolyzed by boiling with 40 mL conc. HCl for 100 min. The solution was cooled to 5 °C and the pH was adjusted to 5.0 with Na-hydrocarbonate. The separated solid was filtered and triurated with ethanol. Yield: 2.5 g (82%). M.P.=174-177 °C. $[\alpha]_D^{25}$=-20.6 (c=1 in MeOH).

Tritiated L-N-acetyl-[m-bis-(hydroxyethylamino)]-phenylalanine methyl ester. Specific tritiation of the benzyl group was performed by Moravek Biochemicals, Brea CA. Specific activity: 5 Ci/mmole, concentration: 2.0 mCi/mL; 129.8 ug/mL. Radiochemical purity was 99.7%, determined on silica gel medium, using chloroform-methanol-acetone-triethylamine, 90:3;6:1.

Characterization by NMR. ^{1}H NMR spectra were recorded with a Bruker AM 400 MHz spectrometer in $CDCl_3$-DMSO-[D_6] solution. δ:1.265 (d_7 1 H of NH_2), 2.9 (dd, 1 H of NH_2), 3.53 (m, 2 H, benzyl CH_2), 3.66 (m, 1 H, chiral H), 3.71-3.79 {m, 3 H of N,N-di(ß-chloroethyl)}, 6.56 and 6.68 (d each, 1 H each, 4'-, and 6'-aromatic hydrogens), 6.69 (s, 1 H, 1'-aromatic H), 7.16 (tr, 1 H, 5'-aromatic H), 11.2 (COOH) ppm.

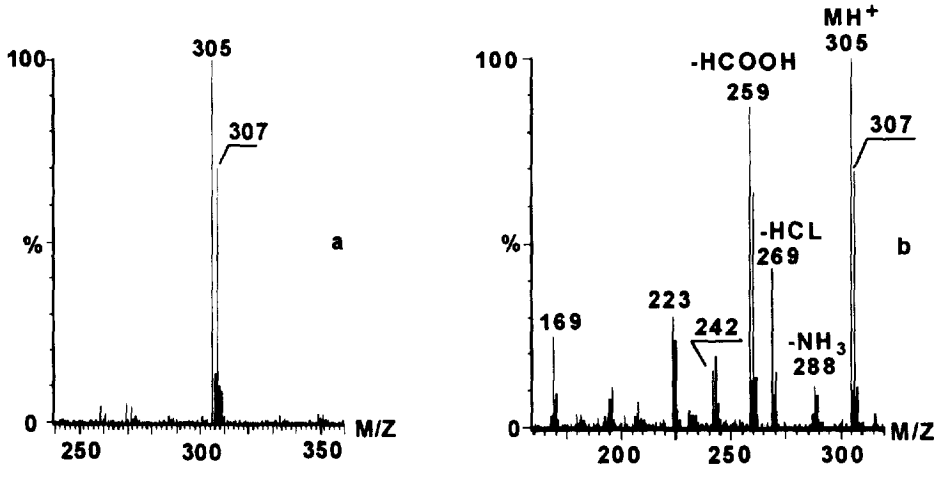


Figure 1.

Characterization by mass spectrometry. The identity of every intermediate product was confirmed using electron, fast atom bombardment, and/or electrospray mass spectrometry (Models Trio or Quattro triple quadrupole mass spectrometers, Fisons, Althricham, GB). The identity of the final product was confirmed by high resolution mass spectrometry in the electron ionization mode (Model ZAB-1A instrument, Fisons). The measured mass of 304.0701 was 4.4 mmu away from theoretical value corresponding to $C_{13}H_{18}N_2O_2Cl_2$. The chlorine isotopic ratios were as expected. The electrospray mass spectra (Fig. 1a), at 30 V cone voltage, revealed the protonated molecules (for the Cl isotopes) at high abundance. Increasing the cone voltage to 50 V (Fig. 1b) resulted, as expected, in considerable structurally revealing fragmentation. In addition to fragments identified in Figure 1b, the lower mass ions were also recognized as structurally relevant.

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