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ACIDIC COUPLING AND AMINOLYTIC TFA CLEAVAGE APPROACHES IN A NEW SYNTHESIS OF AN L- m-SARCOLYSIN CONTAINING ANTITUMOR TRIPEPTIDE ESTER.

Imre Weisz, John Roboz* and J. George Bekesi

Division of Neoplastic Diseases, Department of Medicine, The Mount Sinai School of Medicine

One Gustave Levy Place, New York, NY, 10029, U.S.A.

Abstract. L-prolyl-L-m-[bis(chloroethyl)amino]-phenylalanyl-L-norvaline ethyl ester hydrochloride, **4** (and its ^{3}H and ^{14}C doubly-labeled version) was synthesized starting with reacting unprotected L-m-sarcolysin, **1**, with TFA-Pro-Cl in an acidic system to yield TFA-Pro-L-m-sarcolysin, **2**, which was transformed to the TFA-tripeptide ethyl ester, **3**. Selective aminolytic cleavage of the TFA group with butylamine in abs. ethanol, followed by neutralization with HCl gave **4**.

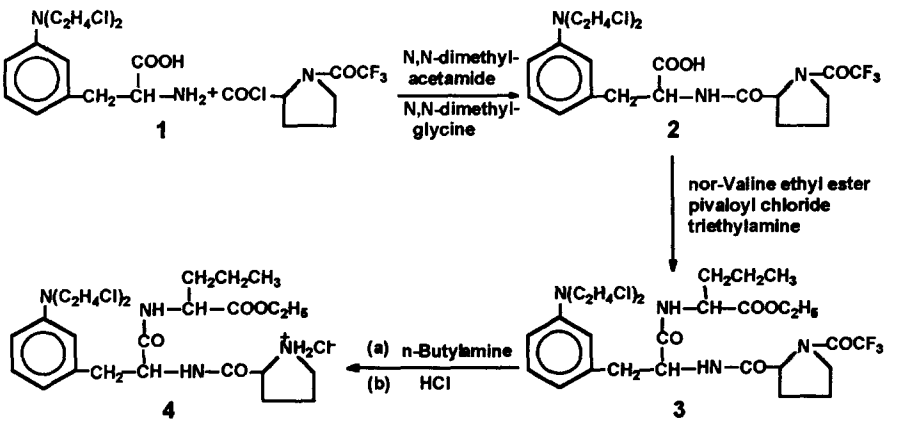
The demonstrated antineoplastic activity of several synthetic oligopeptides from which L-m-sarcolysin, L-3-[*bis*(2-chloroethyl)amino]-L-phenylalanine is released both *in vitro* and *in vivo*[1-3] has prompted us to explore alternative approaches to the synthesis of **4**, to circumvent experimental difficulties encountered with the technique described in an earlier patent.[4] The new synthesis involves two novel approaches to conventionally used steps in peptide synthesis. First, instead of reacting *N*-protected amino acid chlorides with carboxyl-protected amino acid bases or amino acids in alkaline solution,[5,6] we have acylated L-m-sarcolysin, L-*m*-[*bis*(2-chloroethyl)amino]-L-phenylalanine, **1**, with *N*-TFA-L-prolyl chloride in an acidic medium (preparation of **2**, Scheme 1). This decreases the chances of racemization and eliminates the traditional deprotection step of the carboxyl function. Second, instead of the generally used deprotection of TFA-peptide esters by alkaline hydrolysis,[7] we have used butylamine in a water-free system for the aminolytic *N*-deprotection of the TFA moiety (preparation of **4**). The main advantage is the virtual elimination of the well-known problems of hydrolysis of nitrogen mustards in the presence of aqueous alkaline systems (other advantages discussed later).

The synthesis of the tripeptide ester started with reacting L-m-sarcolysin[8] and TFA-L-prolyl chloride in *N,N*-dimethylacetamide (*N,N*-dimethyliformamide cannot be used because it reacts with acid chlorides at the temperature employed) in the presence of *N-N*-dimethylglycine, without any added basic reagent, to form the TFA-dipeptide, trifluoroacetyl-L-prolyl-L-m-sarcolysin, **2**, with a yield of >60%. Selective *N*-acylation of amino acids or amine HCl salts by acid chlorides in dimethylacetamide in an acidic system was reported earlier[9], however, this is the first report on the use of this approach to peptide synthesis. The absence of a reaction between the trifluoroacetyl-prolyl chloride and the carboxylic groups of either **1** or the added *N,N*-dimethylglycine made it possible to use the latter as an HCl scavanger in the preparation of **2**.

To investigate the possibility of racemization, the reaction was also carried out with DL-m-sarcolysin. The epimers (equal amounts formed) were separated (R_f=0.45 and R_f=0.54) on a silica gel TLC plate (Uniplate, Analtech, Newark, DE) developed in 2% AcOH in EtAc. Compound **2** gave only one major spot (R_f=0.45); the quantity of the other epimer was estimated <5%. This is in accordance with reported findings and opinions concerning the lack of significant racemization in coupling reactions with protected amino acid chlorides. [10]

In the next step, **2** was coupled with norvaline ethyl ester to yield the TFA-tripeptide ester, *N*-trifluoroacetyl-L-prolyl-L-m-[*bis*(2-chloroethyl)amino]-phenylalanyl-L-norvaline ethyl ester, **3**. The final steps in obtaining **4** were *N*-deprotection with n-butylamine and neutralization with HCl. We have used butylamine in absolute ethanol (water-free system) for the aminolysis (*N*-deprotection) of the TFA moiety of the trifluoroacetyl peptide ester (preparation of **4**). To investigate potentially deleterious effects, including epimerization, of the relatively long reaction time (see Experimental), we have also synthesized **4** using dilute alkaline (0.1 N NaOH in acetone-water, 8:2 v/v) to hydrolyze **3** to form the unprotected tripeptide, followed by esterification with EtOH-HCl. The melting points and optical rotations of 4 were nearly identical using either synthetic methods, and no epimers or impurities in excess of approximately 5% were detected using TLC and HPLC.

Advantages of this approach include: (i) Virtual elimination of the well-known problems of hydrolysis of nitrogen mustards in alkaline aqueous systems; (ii) Excess amounts of butylamine could be removed with ease, in contrast to higher boiling amines or 4-(aminomethyl)piperidine which have been used for the non-aminolytic deprotection of Fmoc peptide esters;[10] (iii) Peptides could be separated easily from the byproduct of aminolysis, TFA-butylamine, because of its excellent solubility in virtually any organic solvent. It is noted that secondary amines provide even more selectivity, i.e., very slow reaction with the ester in **3**; however, the rate of TFA-aminolysis is also reduced significantly. The less than 100% selectivity of butylamine was the reason for the appearance of some (about 20%) L-prolyl-L-m-[*bis*(2-chloroethyl) amino]-L-phenylalanyl-L-norvalyl-butylamide (confirmed by mass spectrometry) which required repeated recrystallizations of **4**.



Scheme 1.

Experimental. All commercial chemicals were purchased from Aldrich Chemical Co., Milwaukee, WI and used without purification. L-*N*-acetyl-(m-bis-hydroxyethylamino)-phenylalanine methyl ester, [^{3}H], which was specifically labeled with tritium on the benzyl group (specific activity 5 Ci/mmole, radiochemical purity 99.7%), and L-proline, [^{14}C(U0] (specific activity 246 mCi/mmole, radiochemical purity 98.2%) were purchased from Moravek Biochemicals, Inc., Brea, CA.

Trifluoroacetyl-L-prolyl-m-[bis(2-chloroethyl)amino]-L-phenylalanine, **2**. A stirred mixture of 1525 mg (5 mmole) of L-m-sarcolysin, 721 mg (7 mmole) *N,N*-dimethylglycine and 1377 mg (6 mmole) of L-*N*-trifluoroacetyl-prolyl chloride were reacted in 15 mL *N,N*-dimethylacetamide in a nitrogen atmosphere, first at room temperature for 15 min, followed by slowly increasing the temperature to 80-90 °C in 1 h. After cooling the reaction mixture, dil. HCl was added and the mixture was extracted with ethyl acetate, the solution was washed with water, dried and evaporated. The the final product was crystallized from the oily residue using ether-hexane. Yield: 1600 mg (64%). M.P.: 76-78 °C. $[\alpha]_D^{25}$=+18.5, c=2 in MeOH. When the reaction was carried out at room temperature for 3 h, the same product was obtained with a few percent smaller yield.

N-trifluoroacetyl-L-prolyl-L-m-sarcolysil-L-norvaline ethyl ester, **3**. 1.1 g (2.2 mmole) trifluoroacetyl-L-prolyl-sarcolysine, **2**, 0.27 mL (2.2 mmole) trimethylacetyl chloride and 0.3 mL (2.2 mmole) triethylamine were reacted in 20 mL chloroform at 5 °C for 30 min. Next, 0.6 g (4.13 mmole) L-norvaline ethylester in 10 mL chloroform and 0.3 mL triethylamine were added and the mixture was permitted to react at the same temperature for 20 min, followed by continuing the reaction for 20 more minutes after increasing the temperature to 20 °C. The resulting solution was diluted with chloroform, and washed with diluted HCl, water, potassium carbonate solution, water again, followed by evaporation. The compound **3** was obtained as a crystalline substance which was triturated with ether and filtered. Yield: 1.04 g (72%). M.P.: 178-180 °C. $[\alpha]_D^{25}$=-39.80, c=1 in MeOH.

L-prolyl-L-m-[bis(2-chloroethyl)amino]-phenylalanyl-L-norvaline ethyl ester hydrochloride, **4**. 625 mg (1 mmole) *N*-trifluoroacetyl-L-prolyl-L-m-sarcolysil-L-norvaline ethyl ester was stirred in a solution of 10 mL n-butylamine, 25 mL ethanol and 7 mL ethyl acetate. After a 20 h reaction at room temperature (20 h was selected for convenience when it was established that >8 h was needed), the solution was evaporated. The remaining viscous product was dissolved in ethanol, neutralized with HCl and precipitated with the addition of ether and repeatedly recrystallized from ethanol. Yield: 339 mg (60%). M.P.:=179-182 °C. $[\alpha]_D^{25}$=-17.0, c=1 in methanol.

Doubly-labeled tripeptide. The labeled tripeptide ethyl ester hydrochloride was prepared the same way as the unlabeled compound using tritium-labeled **1** and ^{14}C-labeled-TFA-prolyl chloride. The latter was prepared by a conventional technique.[7] The radioactivity of the final product was: 31,050 dpm/pmole tritium (on the sarcolysin moiety) and 9,170 dpm/pmole ^{14}C (on the proline moiety), determined by liquid scintillation counting (Model Tri Carb, Beckman Instruments).

Characterization by NMR. δ: 0.927 (tr 3 H, Me of propyl), 1.266 (tr, 3 H, Me of Et ester), 1.374 (sextet, 2 H, CH_2 of propyl), 1.69 and 1.76 (m each, 1 H each, ß-CH_2 of propyl) 1.987 and 2.41 (m each, 3 H and 1 H respectively, hydrogens in the proline part), 2.875, 3.11, 3.12 and 3.27 (dd or m each, α'-hydrogen in the proline part and benzyl hydrogens, 3.68 and 3.72 [dd each, 4 H each in the N-(ß-chloroethyl) groups], 4.15 (m, 2 H, CH_2 of Et ester), 4.22, 4.406 and 4.71 (m each, 1 H each, chiral hydrogens), 6.54, 6.66 and 7.14 (4 H, in the aromatic ring), 7.4 and 7.98 (m and d respectively, 1 H each, amide hydrogens), 8.52 and 8.83 ppm (d each, 1 H each, NH_2 in the proline part).

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, Altrincham, UK). When unwanted reaction products and/or impurities were found, synthetic methods and/or purification techniques were modified or repeated until the desired intermediates or products were obtained at >95% purity. In addition to molecular weight and chlorine isotope ratio determinations, peptide sequencing of the final product was also performed using collision-activated decomposition (MS/MS). The conventional electrospray mass spectrum (Fig. 1a) is excellent for identification using the protonated molecule, however, it provides little information concerning structure. Structurally significant fragments were obtained using increased cone voltage in the ion source (Fig. 1b) and using collision-induced decomposition in the MS/MS mode (Fig. 1c). Figure 1d shows the structural significance of the most important (and intense) fragments.

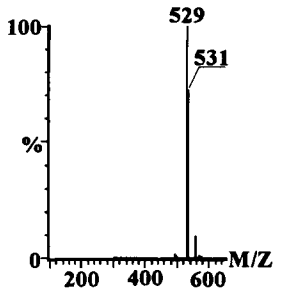


Fig. 1a.

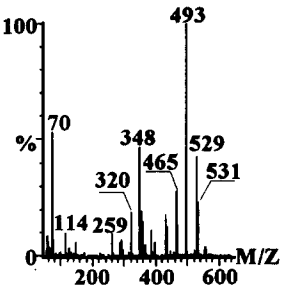


Fig. 1b.

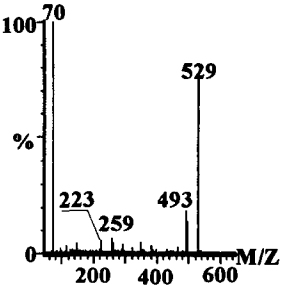


Fig. 1c.

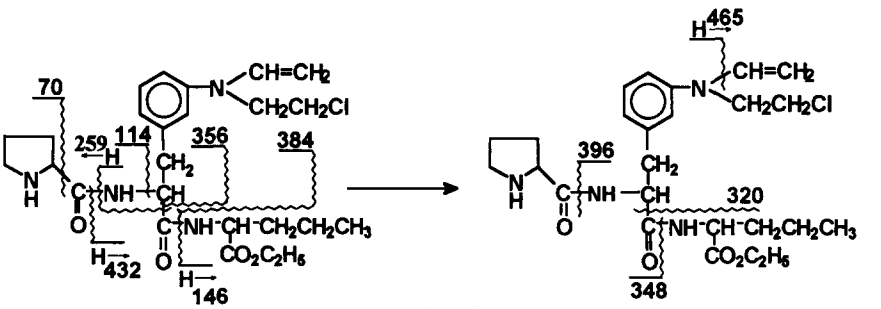


Fig. 1d.

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References

1. Yagi, M. J.; Bekesi, J. G.; Daniel, M. D.; Holland, J. F.; deBarbieri, A. *Cancer Chemother. Pharmacol.* **1984**, *12*, 70.
2. Hansson, J.; Lewensohn, R.; Ringborg, U. *Anticancer Res.* **1991**, *11*, 1725.
3. Ehrsson, H.; Lewensohn, R.; Wallin, I.; Hellstrom, M.; Merlini, G.; Johansson, N. *Cancer Chemother. Pharmacol.* **1993**, *31*, 265.
4. deBarbieri, A. Patent (Offenlegungsschrift) # 2128623, Deutsches Patentamt, June 9, 1973.
5. Jones, J. *The Chemical Synthesis of Peptides*, Claredon Press, Oxford, 1991, p. 42.
6. Muller, E. *Methoden der Organischen* Chemie (Houben-Weyl), Band XV (Synthese von Peptiden), Teil II, G. Thieme Verlag, Stuttgart, 1974, p. 355.
7. *ibid*, Teil I, p. 171.
8. The key intermediate, L-m-sarcolysin, L-3-[bis(2-chloroethyl)amino]-L-phenlylalanine, **1**, was prepared by converting commercially available 3-nitro-L-tyrosine after esterification and *N*-acetylation to its phenyl-tetrazole-ether. The catalytic reduction of this compound led to L-*N*-acetyl-3-aminophenyl-alanine methyl ester which was next hydroxyethylated and converted into the *N*-. mustard with mesyl chloride and LiCl; m-sarcolysin was obtained by hydrolysis of the protecting groups.
9. Weisz, I.; Otvos, L.; *Arch. Pharm. (Weinheim, Germany)*, **1985**, *318*, 766
10. Carpino, L.; Cohen, B.; Stephens, K.; Sadat-Aalae, Y.; Tien, J.; Langridge, D. J. *Org. Chem.* **1986**, *51*, 3732.

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