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SYNTHESIS AND CHARACTERIZATION OF TETRA PEPTIDE AS A KEY INTERMEDIATE OF DOLASTATIN-15

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ABSTRACT

A tetra peptide containing two valine and two proline moieties is synthesized as a part of key intermediate and pharmacophore of Dolastatin – 15. Preparation of dimers of valine and proline followed sophisticated and high yield procedures. The coupling of dimmers was done for a tetra peptide. All the molecules were subjected for FTIR, ^{13}C & ^{1}H NMR, Mass spectroscopic techniques.

Keywords: Dolastatin, Cytotoxic, Cancer, Peptide synthesis

INTRODUCTION:

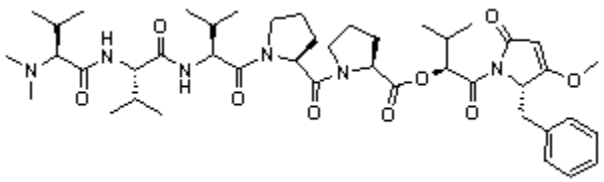
Cancer is medically known as a malignant neoplasm, is a broad group of various diseases, all involving unregulated cell growth. In cancer, cells divide and grow uncontrollably. Forming malignant tumors and invade nearby parts of the body. The cancer may also spread to more distant parts of the body through the lymphatic and blood stream. There are over 200 different known cancers afflict humans [1].

Many management options for cancer with the primary one including surgery, chemotherapy, radiation therapy and palliative cure. Among which chemotherapy is popularly useful in a number of different cancer types. Potent cytotoxic agents have a well established role in the treatment of cancer. Many of the anticancer agents currently in use are of natural origin (i.e. vinca alkaloids, taxanes, anthracyclins), derived from terrestrial plants, microorganisms and from marine sources are natural product derivatives. The relevance of the sea as a source to discover novel anti cancer compounds was validated by the discovery, development and marketing approval of ARA-C (1-beta-arabinofuranosyl cytosine). The available results clearly anticipated the potential of the marine eco system in cancer therapy [2, 3].

Dolastatins are natural peptides isolated from natural origin. Over the past three decades considerable effort has been dedicated to the search for biological constituents of marine organisms with anti-neoplastic activity. A 20 years pursuit of the cell growth inhibitory and anti neoplastic constituents of the western Indian ocean (Mauritius) seahare Dolabella auricularia, a marine shell like mollusk of the family aplyidae has resulted in the discovery of 15 structurally unique peptides, cyclo peptides and depsi peptide type substances designated Dolastatin 1-15. Among the dolastatin series dolastatin 10 and dolastatin 15 are more important for their activity towards malignancy. The natural cytotoxic compounds dolastatin 10 and dolastatin 15 exhibit greater similarities in structure and in their biological activities [4,5,6].

The extremely low yields of dolastatins and other metabolites obtained from D.auricularia leads to the laboratical synthesis of these lead molecules to improve the yield[7]. Congeners of these natural products are synthesized to improve the therapeutic profile as well as to decrease the toxicities of the existing molecules and essentially be in economical manner.

Dolastatin 15 is a natural depsi peptide with unusual amino acids derived from sea hare Dolabella auricularia. Showing anti-proliferative action against different solid tumors[8].



Dolastatin 15

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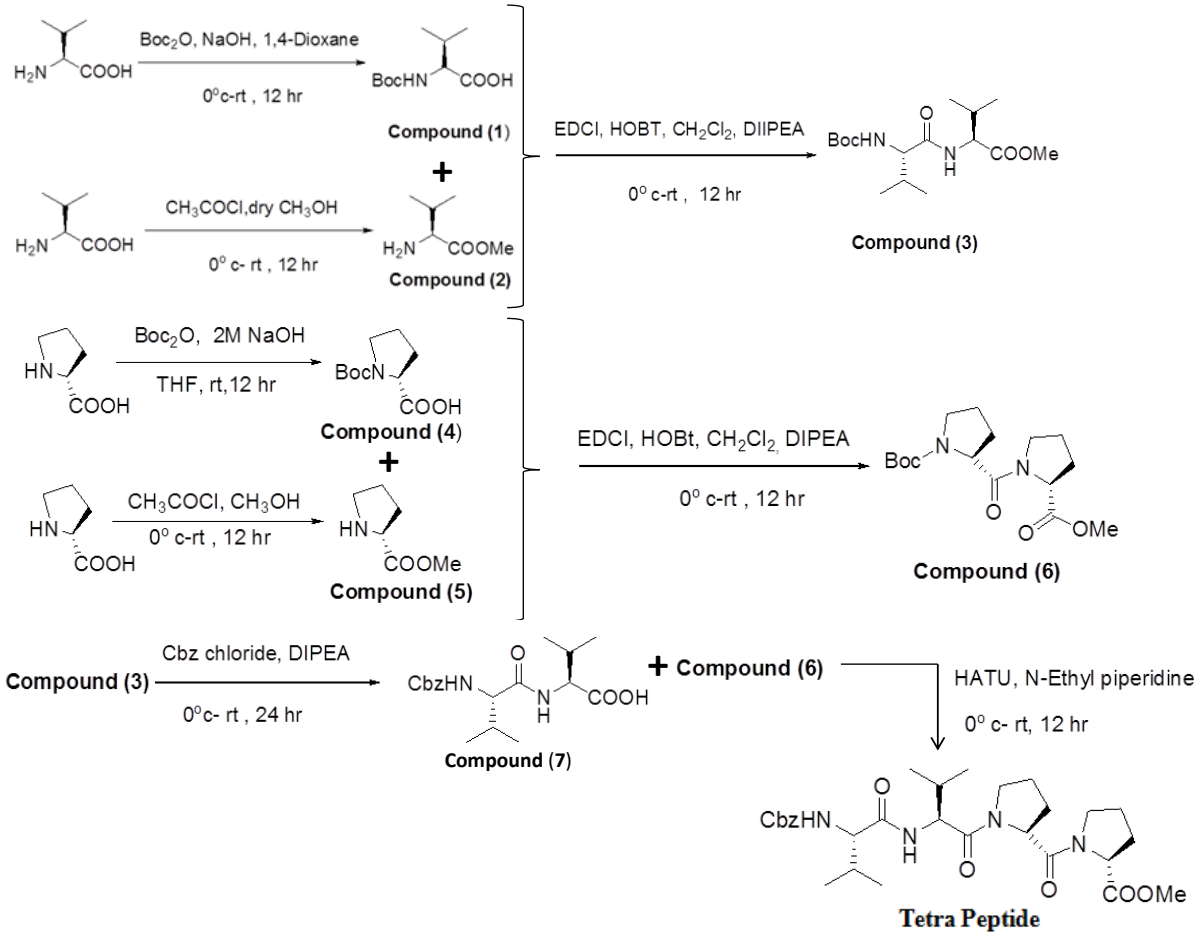
METHODOLOGY:

Synthetic procedure for the target compound:



RESULTS AND DISCUSSION:

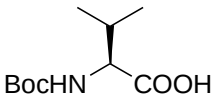
Synthesis:

A number of synthetic procedures were reported to synthesize Peptide molecules. Structurally Dolasatin-15 is a pentapeptide, in which all chiral centers are in *S* form in solution phase. Dolastatin 15 made up of mainly residues of valine, and proline amino acids. Basic skeleton of the drug is tetra peptide which is a coupled product of proline di peptide and valine di peptide. Tetra peptide is essential for showing the anti-proliferative action. The synthetic work was started with synthesis of dimmers from monomers (valine and proline). The coupling of amino acids is a complex process. Here, monomers were primarily protected by group protection phenomenon, carboxylic and amine groups were protected by substituting with acyl group and Tert-butyl oxycarbonyl(Boc)/ benzyl chloro formate(Cbz) group respectively(1,2,4 and 5). The coupling of monomers were done using a coupling reagent to give dimmers(3 and 6), resulted in a tetra peptide upon deprotection and coupling.

Experimental section

All moisture sensitive reactions were performed under a nitrogen atmosphere using dried glass wares. Solvents were dried over standard drying agents and freshly distilled prior to use. Organic solutions were dried over anhydrous Na2SO4 and concentrated below 40 0C in vacuo. NMR spectra were recorded on Varian Gemini FT-200 MHz, Unity-400 MHz (21 0C) and Inova-500 MHz (30° c) spectrometers, with 7–10 mM solutions in appropriate solvents using TMS as the internal standard.13C NMR spectra were recorded with complete proton decoupling. Mass spectra were recorded on CEC-21-11013 or Finnigan Mat 1210 double focusing mass spectrometers operating at a direct inlet system, and FAB MS were measured using VG AUTOSPEC mass spectrometers at 5 or 7 K resolution, using per-fluoro kerosene as an internal reference.

Synthesis of (*S*)-*2*-(Tert-butoxycarbonylamino)-3-methylbutanoicacid:



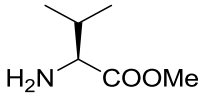
Compound (1)

L-Valine (5 g, 42.5 mmol) was combined with 1, 4-dioxane (30 ml) and H_2O (30 ml) and the resulting slurry was cooled to 0 °C. A solution of NaOH (3N, 60 ml) was added followed by molten Boc_2O (11.2 ml, 47 mmol). The cooling bath was removed and the reaction mixture was stirred at 25° c for over night. The reaction mixture was diluted with H_2O (10 ml) and washed with

pentane (30 ml, 3 times). The aqueous layer was cautiously acidified with 1M HCl, and the mixture was then extracted with EtOAc (500 ml). The extracts were combined, washed with H_2O, saturated sodium chloride and then dried (Na_2SO_4). Filtration and concentration of the filtrate under reduced pressure resulted in *N*-Boc-*L*-Valine (80%) as color less oil.

R_f	**:**	0.5 in (10% methanol in chloroform)
Yield	**:**	80%
^{1}H NMR (300 MHz, $CDCl_3$)	**:**	δ 5.0 (d, 1H, *J*= 8.9 Hz), 4.25 (m, 1 H), 2.20(m, 1H), 1.45 (s, 9H), 5.06 (d, 1H, *J*= 6.46 Hz), 5.1 (d, 1H, *J*= 7.18 Hz)
^{13}C NMR (75 MHz, $CDCl_3$)	**:**	172.1, 171.6, 155.8, 79.8, 59.9, 57.0, 52.0, 31.0, 30.6, 28.2, 19.3,
ESI-MS	**:**	240 **$(M+Na)^+$**
IR (neat)	**:**	ν_{max}3435, 2883, 1709 cm^{-1}

Synthesis of(*S*) Methyl2-amino-3-butanoate:

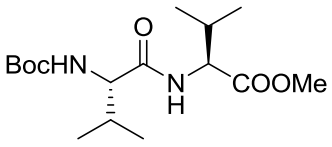


Compound (**2**)

L-proline (5g, 42.6 mol) was combined with acetyl chloride (18.2ml, 255.6 mol) in 120 ml of dry methanol and reaction mixture was cooled to 0^o c. Then reaction mixture was stirred at room temperature for over night, followed by evaporation of methanol gave the 90% colorless solid as product.

R_f	**:**	0.5 in (10% methanol in chloroform)
Yield	**:**	82%
^{1}H NMR (300 MHz, DMSO)	**:**	δ 8.9 (s, 2H), 3.9(s, 3H), 3.8(s, 1H), 1.19 (m, 7H)
ESI-MS	**:**	132 **$(M+H)^+$.**
IR (neat)	**:**	ν_{max} 3617, 3441, 1712 cm^{-1}

Synthesis of (*S*)-*2*-(*S*)-2-(Tert-butoxycarbonylamino)-3-methyl butanamido)-3-methyl butanoate:



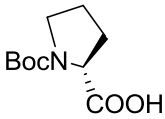
Compound (**3**)

To a magnetically stirred solution of N-Boc-*L*-valine (2g, 9.069 mmol) in dry CH_2Cl_2 (27ml) at 0^o c HOBt (1.83g, 13.6 mmol) was added. After 10 min EDCI (2.607g, 13.6mmol) was added. After 20 minutes methyl ester of valine (1.8g, 9 mmol) was added. Then DIPEA (4.74ml, 27.mmol) was added to make the pH basic. Reaction mixture was stirred under room temperature over night.

Reaction mixture was quenched with the 1N HCl and work up with ethyl acetate (200 ml) then with saturated Na_2HCO_3. Combined organic extracts were washed with water and saturated sodium chloride, dried (Na_2SO_4) and concentrated under vacuo. The crude sample was purified by column chromatography (30% ethyl acetate in petroleum ether) to obtain the above compound as a color less solid.

R_f	**:**	06 in (40% ethhylactete in petroleum ether)
Yield	**:**	74%
^{1}H NMR (300MHz, $CDCl_3$)	**:**	δ 6.310 (m, 1H), 4.963 (m, 1H), 4.513 (m, 1H), 3.821 (m, 1H), 3.727 (s, 3H), 2.14 (m, 2H), 1.43 (m, 1H), 0.92 (m, 1H).
^{13}C NMR (75MHz, $CDCl_3$)	**:**	191.5, 191.2, 99.1, 79.5 76.5, 71.5, 50.5, 50.1, 47.7, 38.6, 38.3, 37.4, 37.2.
ESI-MS	**:**	331 **$(M+H)^+$,** 353 **$(M+Na)^+$**
IR(neat)	**:**	ν_{max} 3618, 3321, 1751, 1683, 1248 cm^{-1}.

Synthesis of (*R*) -1-(Tert-butoxycarbonyl) pyrrolidine-2-carboxylicacid:

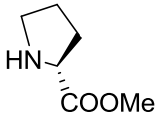


Compound (**4**)

L- proline (5g) was dissolved in 2M sodium hydroxide (65 ml) and tetra hydro furan (65 ml).Reaction mixture was cooled to 0^o c. Molten Boc anhydride (9.5 ml) was added to the reaction mixture. Then cooling bath was removed and the reaction mixture was stirred at 25^oc over night. Then THF was removed under vacuo and washed with diethyl ether. Recrystallization with hexane and ethyl acetate to obtain compound (4) as pure white solid.

R_f	**:**	0.5 in (5% acetic acid in ethyl acetate)
Yield	**:**	84%
^{1}H NMR (300 MHz, $CDCl_3$)	**:**	δ 4.36(m, 1H), 3.5 (m, 2H), 2.3 (m, 2H), 2.1 (s, 2H), 1.5 (m, 9H).
^{13}C NMR (75MHz, $CDCl_3$)	**:**	99.8, 78.3, 66.2, 65.8, 50.2, 48.6, 47.7, 43.6, 43.
ESI-MS	**:**	238 **$(M+Na)^+$.**
IR (neat)	**:**	ν_{max} 2972, 2928, 1460, 1164 cm^{-1}.

Synthesis of (*R*) –Methyl pyrrolidine-2-carboxylate:



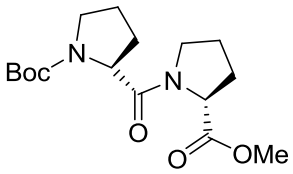
Compound (5)

L-proline (20 g) was dissolved in dry methanol (207.4 ml). Then the reaction mixture was cooled to 0^o c. Acetyl chloride (20.7 ml) was added to the reaction

mixture and cooling bath was removed and the reaction mixture was stirred at room temperature for 12 hours, then methanol was removed under vacuo and the reaction mixture was azeotrope With methanol (3times), benzene (3times), ether (3times) to obtain compound (5) as yellow color oily liquid.

R_f	**:**	0.3 (10% methanol in chloroform):
Yield	**:**	73%
^{1}H NMR (300 MHz, $CDCl_3$)	**:**	δ 4.15 (m, 1H), 3.8 (s, 3H), 3.31 (m, 1H), 2.3 (m, 2H); 2.2 (m, 2H), 1.944 (m, 2H).
ESI-MS	**:**	130 **$(M+H)^+$.**
IR (neat)	**:**	ν_{max} 3413, 1743, 1238 cm^{-1}.

Synthesis of (*R*)-Tert-butyl-2-((*R*) -2-(methoxy carbonyl) pyrrolidine-1-carbonyl) pyrrolidine-1-carboxylate:

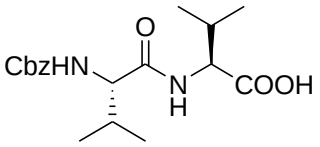


Compound (**6**)

To a magnetically stirred solution of N-Boc-*L*-proline (5g, 23.22mmol), in dry CH_2Cl_2 (65ml) at 0^o c HOBt (4.7g, 34.83mmol) was added. After 10 minutes EDCI (6.676g, 34.83mmol) was added. After 20 minutes methyl ester of proline (6g, 46.457mmol) was added. Then DIPEA (12.14, 27.69.66mmol) was added to make the reaction mixture pH as basic. Reaction mixture was stirred under room temperature overnight. Then the reaction mixture was quenched with the 1N HCl and extracted with ethyl acetate (250ml) then with saturated Na_2HCO_3.Combined organic extracts were washed with water and saturated sodium chloride, dried (Na_2SO_4) and concentrated under vacuo. The crude sample was purified by column chromatography (30%ethyl acetate in petroleum ether) to obtain the compound 6 as a oily liquid.

R_f	**:**	0.4 (3% methanol in chloroform)
Yield	**:**	75%
^{1}H NMR (300 MHz, $CDCl_3$)	**:**	δ 4.44 (m, 2H), 3.71 (m, 1H), 3.6 (s, 1H), 3.26 (m, 1H), 2.876 (m, 1H), 2.78 (s, 1H), 2.01 (m, 10H), 1.34 (s, 1H).
^{13}C NMR (75 MHz, $CDCl_3$)	**:**	172.5, 154.6, 128.6, 124.9, 109.2, 79.1, 58.5, 51.9, 46.6, 29.7, 28.4, 28.2, 27.6, 24.7, 23.8.
ESI-MS	**:**	327 **$(M+H)^+$,** 349 **$(M+Na)^+$.**
IR (neat)	**:**	ν_{max} 1744, 1693, 1322, 1160 cm^{-1}.

Synthesis of (*S*) -3-methyl-2-((*S*)-3-methyl-2-(2-oxo-2-phenylethylidine amino) butanamido) butanoic acid:



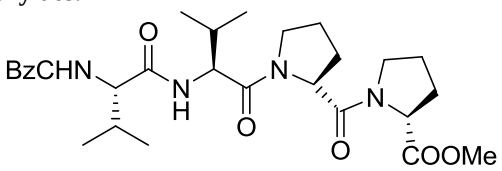
Compound (**7**)

Valine di peptide (0.5 g) was treated with TFA (4.5 ml) in dry DCM. Reaction mixture was stirred at 0^o c to room temperature for 30 min. Then reaction mixture was azeotroped with dry DCM and dried under vacuo to remove the Boc from the valine di peptide to obtain free amine group of valine dimer.

Valine di peptide (0.5 g) was dissolved in CH_2Cl_2 (8ml).To the dissolved compound dry DIPEA (1.2 ml) was added. To the reaction mixture Cbz chloride (0.687 ml) was added After 15 minutes basicity was checked, and the reaction mixture was stirred at room temperature for 2-3 hours.Then it was quenched with the saturated $NaHCO_3$ and extracted with ethyl acetate (75ml), and washed with water then with saturated NaCl solution and dried (Na_2SO_4), under vacuo to obtain compound (8) as colorless solid.

R_f	**:**	0.5 (50% ethyl acetate in petroleum ether)
Yield	**:**	78%
^{1}H NMR (300 MHz, $CDCl_3$)	**:**	δ 7.3 (s, 5H), 6.43 (d, 2H, *J* = 7.4 Hz), 5.426 (d, 1H, *J* = 3.1 Hz), 5.12(s, 2H), 4.54 (m, 1H), 4.1 (t, 1H, *J* = 7.12 Hz), 3.73 (s, 3H), 2.15 (m, 2H), 0.94 (m, 12H).
ESI-MS	**:**	365 **$(M+Na)^+$**
IR (neat)	**:**	ν_{max} 3310, 3064, 1659, 1529, 1281, 1211cm^{-1}.

Synthesis of (*R*) –methyl1-((*R*)-1-(*S*)-3-methyl-2-((*S*)-3-methyl- 2-(2- oxo 2-phenylidineamino) butanamido) butanoyl) pyrrolidine-2-carbonyl)-pyrrolidine-2-carboxylate:



Tetra peptide

Methyl ester of valine di peptide (0.151mg, 0.45mmol) was dissolved in the mixture of THF:H_2O:CH_3OH (3:1:1).Reaction mixture was cooled to 0^oc.Then LiOH (0.035g, 1.37 mmol) was added to the reaction mixture. Cooling bath was removed and reaction was stirred for 30 minutes to 1 hour at room temperature. Reaction mixture was quenched with the 1NHCl to pH2(acidic) and workup with ethyl acetate (50ml)and then with $NaHCO_3$,and washed with water, saturated sodium chloride solution, and organic layer was collected dried(Na_2SO_4)and evaporated under vacuo to get the deprotected valine dimer (acid). Proline di peptide (100mg) was dissolved in CH_2Cl_2 (2.5ml) and the reaction mixture was cooled to 0^oc.Then after 5 minutes TFA (0.7ml) was added. Cooling bath was removed and

reaction mixture was stirred at room temperature for 30 minutes.Then reaction mixtures was azeotrope with the CH_2Cl_2 to remove TFA and dried under vacuo to get the deprotected free amine group of proline dimer. Deprotected valine dimer and Deprotected proline dimer was dissolved in aceto nitrile (2ml) separately. Deprotected proline dimer was added to the Deprotected valine dimer at room temperature. Then the reaction mixture was cooled to 0^oc and HATU (0.139 g, 0.37 mmoles) was added. Then N-ethyl piperidine (0.1 ml, 0.76 mmol) was added. Reaction mixture was stirred at room temperature for overnight. Then reaction mixture was quenched with the NH_4Cl, and extracted with ethyl acetate and washed with water, saturated NaCl solution.Then organic layer was collected and it was dried (Na_2SO_4), under vacuum. Crude compound was purified by column chromatography (3% methanol in chloroform) to obtain **Tetra Peptide** as colorless oily liquid.

R_f	**:**	0.5 (10%methanol in chloroform):
ESI-MS	**:**	559 **$(M+H)^+$,** 581 **$(M+Na)^+$**
IR (neat)	**:**	**ν_{max}** 2932, 2857, 1729**,** 1465, 1383, 1248, 1172 cm^{-1}.

CONCLUSION

In this study the tetra peptide was synthesized by coupling of Valine dimer and Proline dimer which are required for synthesis of target molecule. Tetra peptide is the major fragment of the Dolastatin 15 analogue. Elongation of the C- terminus and N- terminus with the desired molecules to yield the total analogue. Further studies should be carried for synthesis of target molecule, Dolastatin.

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