SYNTHESIS OF NOVEL (±)-*CIS-EXO*-NORBORNANE AMINO ACID CONTAINING CYCLIC HEXAPEPTIDE: ANALOGUE OF DOLASTATIN 16

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ABSTRACT

Novel cyclohexapeptide has been synthesized by replacing the unusual amino acids (dolaphenvaline and dolaphenleuine) of dolastatin 16 with (±)-*cis*-*exo*-norbornane and phenyl alanine amino acids. Novel cyclic hexapeptide alongwith dimers, tetramers composed of alternating L-proline subunin have been synthesized in solution phase and well characterized.

KEYWORDS

Dolastatin 16, *exo*-norbornane amino acid, peptide synthesis, cyclic hexapeptide.

INTRODUCTION

Peptides are the largest chemical group prevalent in nature. Among them, cyclic peptides are of great interest in both chemistry and biology.[1-3] The outstanding biological and biochemical diversity of marine environment serves as a source of inspiration for chemists and pharmacologists. Marine organisms are one of the rich sources for natural cyclic peptides and many compounds derived from these organisms have generated interest in structure elucidation and synthesis. The secondary metabolites produced by diverse marine organisms are crucial leads for the development of array of pharmaceuticals of diverse biological activities such as antibacterial activity, immunosuppressive activity, and anti-tumor activity, and so on.[4-8] NW-G06 isolated from Streptomyces alboflavus 313, Microsclerodermins J isolated from the deep-water sponge *Microscleroderma herdmani*, Dolastatin 16, Sclerotides A, B isolated from the marine-derived halotolerant *Aspergillus sclerotiorum* PT06-1 are some of the novel cyclic hexapeptides exhibiting antibacterial, antifungal, anticancer and both antifungal, antibacterial activities respectively.[9-15]

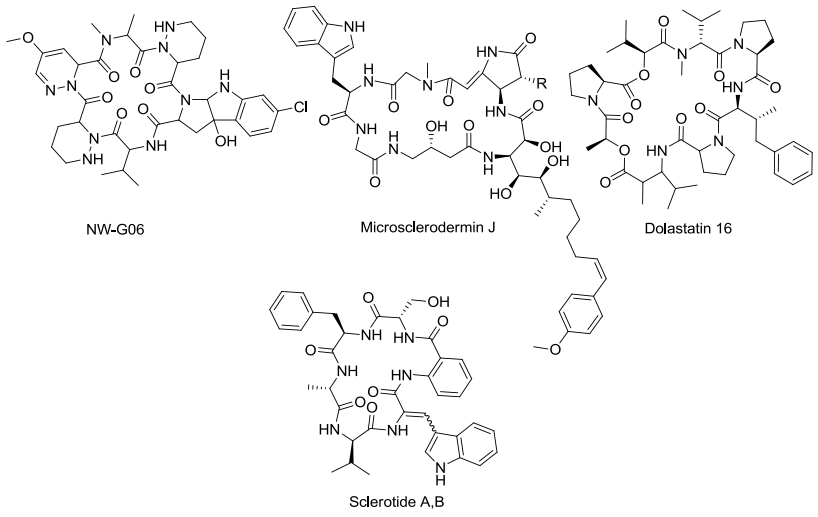


Figure 1: Representative structures of cyclic hexapeptides.

Among diverse cyclic peptide we are interested to study dolastatins, in particular dolastatin 16. Dolastatin 16 especially proved to be an exceptionally potent inhibitor of cancer cell growth and a candidate for further development. Structurally, dolastatin 16 is a cyclodepsipeptide containing two new amino acids, dolamethyleuine, a β-amino acid, and dolaphenvaline. In this study, we have synthesized the novel

cyclodepsipeptide by replacing these unusual aminoacids with (±) *cis- exo*-norbornane amino acid along with newer dimer, tetramer and hexamer.

MATERIALS AND METHODS:

Mass spectra are recorded on a Quattro-LC, (ESI). ^{1}H spectra are recorded on Gemini 200, Avance 300, Inova 400, Inova 500, and Bruker 600 MHz spectrometers using tetramethyl silane (TMS) as the internal standard. Chemical shifts are reported in parts per million (ppm) downfield from tetramethyl silane. Spin multiplicities are described as s (singlet), brs (broad singlet), d (doublet), dd (double doublet), t (triplet), q (quartet), and or m (multiplet). Coupling constants are reported in Hertz (Hz). Analytical thin layer chromatography (TLC) is performed on MERCK precoated silica gel 60- F254 (0.5-mm) glass plates. Visualization of the spots on TLC plates is achieved either by exposure to iodine vapour or UV light or by dipping the plates into β-naphthol or ethanolic ninhydrin solution and heating the plates to 120 ^{0}C. Column chromatography is performed using silica gel 60-120. Moisture sensitive reactions are carried out using standard syringe septum techniques and under inert atmosphere of nitrogen. All solvents and reagents are purified by standard techniques. All evaporation of solvents is carried out under reduced pressure on Laborota-4000 rotary evaporator below 45 ^{0}C.

RESULTS AND DISCUSSION:

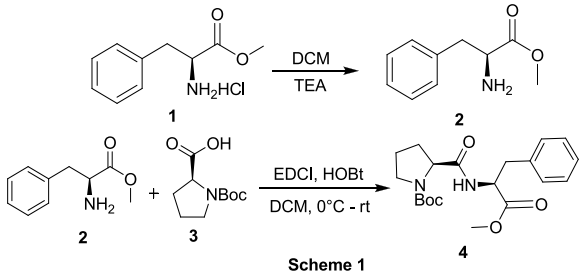
CHEMISTRY:

Norbornadiene is a strained bicyclic compound used extensively in mechanistic organic chemistry and energy chemistry. However, this scaffold is not well utilized by bioorganic chemist. Norbornadiene was converted in to strained unusual amino acid and oligomerized to obtain hetero-oligomers with other unusual amino acids.[16,17]

The unusual norbornane amino acid was synthesized as reported in the literature using commercially available norbornene.[16-20]

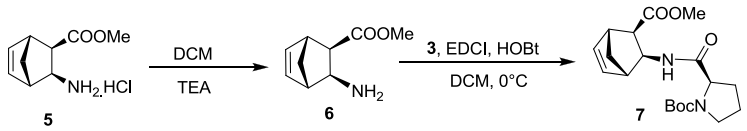
The synthesis was performed in two pathways: (i) synthesis of dimers and tetramer (ii) synthesis of hexamer followed by Yamaguchi macrocyclization.[21-23]

(*S*)-Phenylalanine amine salt **(1)** in DCM solution is neutralized with TEA at 0°C to get (*S*)-methyl 2-amino-3-phenyl propionate **(2)**. Dimer **4** is synthesized by a coupling reaction between L-phenylalanine ester **2** and Boc protected L-proline acid **(3)** using EDCI, HOBt in dry DCM as solvent at 0°C for 24h to obtain 78% yield (**Scheme 1**). The ^{1}H NMR spectrum of **4** showed the aromatic protons resonated at δ 7.35-7.23and 7.16-7.09. The resonance corresponding to -OCH_3 of ester group appeared at δ 3.75 as a singlet, *tert*-butyloxy protons of Boc group resonated as a singlet at δ 1.46. ^{13}C NMR spectrum displayed two signals at δ 171.6 and171.2 corresponding to carbonyl carbon confirmed the formation of dipeptide **4** and ESI-MS also showed a peak at 377 $[M+H]^{+}$.



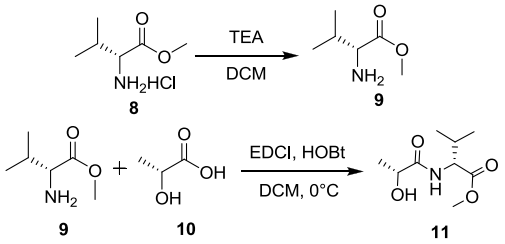
Scheme 1

(±)-*Cis-exo*-norbornane amine salt (**5)** was prepared as reported in literature.[9] Amine salt **5** in DCM solution is neutralized with TEA at 0°C to get **6**. Dimer **7** is synthesized by a coupling reaction between Boc protected *L*-proline acid **(3)** and norbornadiene ester **(6)** using EDCI, HOBt in dry DCM as solvent at 0°C for 24 h to obtain 88% yield **(Scheme 2**). ^{1}H NMR of the dipeptide **7** revealed the presence of double bond protons at δ 6.28-6.17 as multiplet. Boc and ester OMe protons resonated at δ 1.45 and δ 3.72 as singlet respectively. ^{13}C NMR spectrum displayed three signals at δ 174.6, 172.6, and 155.3 corresponding to carbonyl carbon confirmed the formation of dipeptide **7**. ESI-MS also showed a peak at 365 $[M+H]^{+}$.



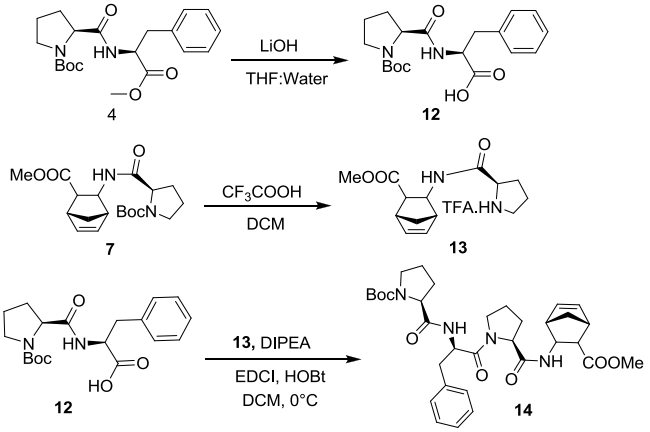
Scheme 2

L-valine amine salt **(8)** in DCM solution is neutralized with TEA at 0°C to get **9.** Dimer **13** is synthesized by a coupling reaction between *L*-valine ester **9** and lactic acid **10** using EDCI and HOBt in dry DCM as solvent at 0°C for 24h to obtain 68% yield (**Scheme 3**). The ^{1}H NMR spectrum of **11** showed the presence of –OH at δ 3.96 as broad singlet. Ester -OMe protons resonated at δ 3.74 as singlet. ^{13}C NMR spectrum displayed three signals at δ 175.1, 172.2 corresponding to carbonyl carbon and two signals at δ 20.9, 18.7 corresponding to isopropyl carbon. ESIMS also showed a peak at 204 $[M+H]^+$.



Scheme 3

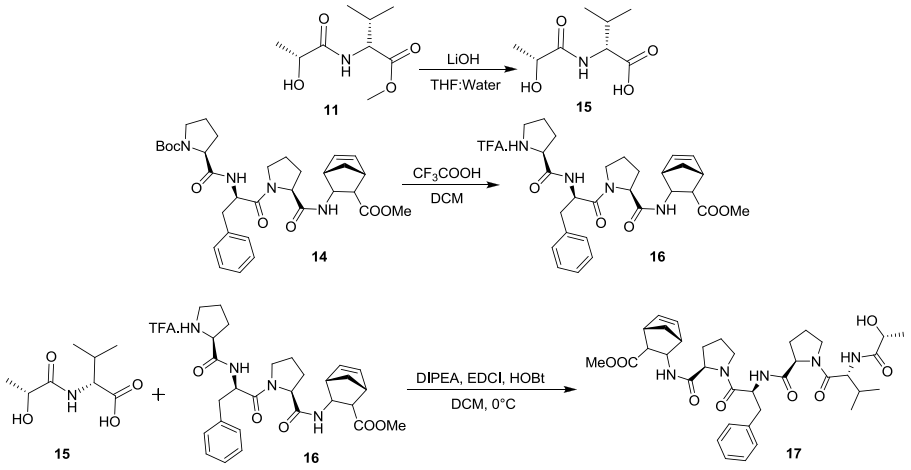
The Dimer **4** was hydrolysed by using LiOH in THF:Water (1:1) to obtain acid **12** and dimer **7** was treated with TFA in DCM solution to obtain Boc deprotected ester dimer **13 (Scheme 4)**.



Scheme 4

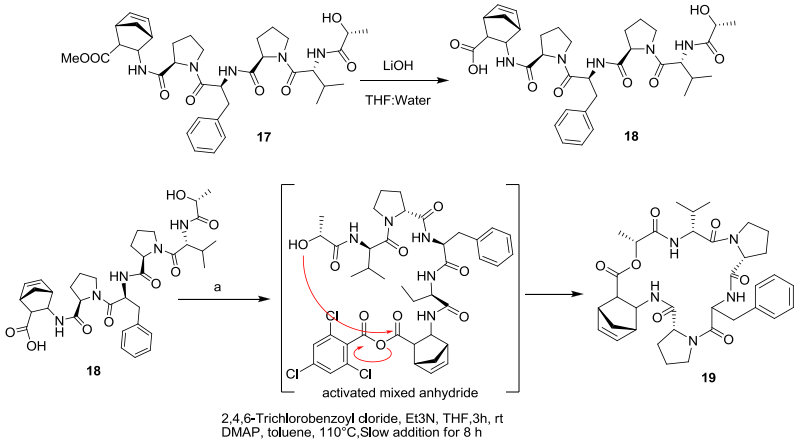
The tetramer **14** is synthesized by coupling dipeptide acid **12** and dipeptide amine salt **13** using EDCI, HOBt and DIPEA in dry DCM as solvent, to obtain **14** in 78 % yield (**Scheme 4**). The ^{1}H NMR spectrum of **14** showed the aromatic protons resonated at δ 7.28-7.24and 7.21-7.18. Double bond protons resonated at δ 6.25-6.17 as multiplet. ^{13}C NMR spectrum displayed five signals at δ 174.4, 174.1, 171.1, 170.9, and 156.8 corresponding to carbonyl carbon. ESIMS also showed a peak at 610 $[M+H]^+$.

Dimer **11** was ester hydrolysed to obtain **15** and tetramer **14** was Boc deprotected by using to obtain **16** (**Scheme 5**). The hexamer **17** was synthesized by EDCI, HOBt coupling of **15** and **16** (**Scheme 5**). The ^{1}H NMR spectrum of **17** showed the aromatic protons resonated at δ 7.32-7.28. Double bond protons resonated at δ 6.25-6.19 as multiplet. Boc and ester OMe protons resonated at δ 1.45 and δ 3.67 as singlets respectively. Boc and ester OMe protons resonated at δ 1.45 and δ 3.66 as singlets respectively. ^{13}C NMR spectrum displayed five signals at 172.7, 171.6, 171.0, 170.7, and 170.2 corresponding to six carbonyl carbon. ESIMS also showed a peak at 703 $[M+Na]^+$.



Scheme 5

The hexamer ester **17** was ester hydrolysed to obtain acid **18.** Acid functionality in **18** is activated to its trichlorobenzoic anhydride, and then this mixed anhydride is slowly added with syringe pump to refluxing toluene and DMAP to generate the macrolide **19** with 50% yield (**Scheme 6**). The ^{1}H NMR spectrum of **19** showed the aromatic protons resonated at δ 8.15-7.40. Double bond protons resonated at δ 5.40-5.33 as multiplet. Briged head protons resonated at δ 1.23-1.19 as multiplet. ^{13}C NMR spectrum displayed six signals δ 176.0, 174.9, 174.2, 173.8, 170.3, and 169.3 corresponding to carbonyl carbon. ESIMS also showed a peak at 648 $[M+H]^{+}$.



Scheme 6

EXPERIMENTAL SECTION:

General experimental procedures

Ester hydrolysis of the amino esters **4, 11, 17** was performed as per the reported procedures.[24]

N-Boc deprotection of Boc protected peptide esters **7, 14** were performed according to the reported procedures.[24]

Preparation of dipeptides **4,7,11** from monomers were performed as per the reported procedures.[16]

Preparation of tetrapeptide **14** from dipeptides and hexamer **17** were performed as per the reported procedures.[16]

Dimer (4): mp 78-80°C; (KBr) ν_{max} 1685, 1216, 1123cm^{-1}; 1H NMR (500 MHz, $CDCl_3$) δ 7.35-7.23 (m, 3H), 7.16-7.09 (m, 2H), 6.57(br.s, 1H), 4.99-4.83(s, 1H),4.39- 4.19(m, 1H), 3.75(s, 3H), 3.39-3.29(m, 2H), 3.26-3.02(dd, 2H, *J*=5.6, 6.8Hz), 2.35-1.95(m, 2H)1.89-1.74(m, 2H),1.46(s, 9H); ^{13}C NMR(125MHz,$CDCl_3$) δ 171.6, 171.2, 154.0, 135.6, 128.7, 128.0, 126.6, 79.9, 60.6, 59.4, 52.9, 51.7, 46.5, 37.5, 27.8, 22.9; ESI-MS: *m/z* 377 $[M+H]^{+}$.

Dimer (7): mp 75-77°C; (KBr) ν_{max} 1719, 1682, 1242, 1175; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.58-7.36(m, 1H), 6.28-6.17(m, 2H), 4.32-4.00(m, 2H), 3.68(s, 3H), 3.55-3.28(m, 2H), 2.96(s, 1H), 2.68-2.57(m, 2H), 2.01-1.81(m, 4H), 1.60-1.54(m, 2H), 1.45(s, 9H); ^{13}C NMR(125MHz,$CDCl_3$) δ 174.6, 172.6, 155.3, 138.2, 137.1, 80.2, 60.2, 51.8, 50.9, 48.5, 47.0, 46.1, 44.3, 29.6, 28.8, 24.4; ESI-MS: *m/z* 365 $[M+H]^{+}$.

Dimer (11): mp 77-79°C; (KBr) ν_{max} 3392, 2958, 2928, 2856, 1746, 1681; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.13 (br.s, 1H), 4.53(dd, 1H, *J*=5.2, 3.7Hz), 4.36-4.22 (m,1H), 3.96 (br.s,1H), 3.74(s, 3H), 2.25-2.14 (m, 1H), 1.43 (d, 3H, *J*=6.8Hz), 1.00-0.92 (m, 6H); ^{13}C NMR(125MHz,$CDCl_3$) δ 175.1, 172.2, 68.1, 56.4, 51.9, 20.9, 18.7; ESI-MS: *m/z* 204 $[M+H]^+$.

Tetramer (14): mp 61-63°C; (KBr) ν_{max} 1679, 1156, 1118, 725,610; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28-7.24(m, 3H), 7.21-7.18(m, 2H), 6.25-6.17(m, 2H), 5.06-4.91(br.s, 1H), 4.55-4.40(s, 1H), 4.19-4.14(t, 1H, *J*=9.1, 8.5Hz), 4.11-4.07(t, 3H, *J*=8.3, 8.0Hz), 3.73-3.69(m, 1H), 3.66(s, 3H), 3.37-3.24(brs, 2H), 3.18-3.12(m, 1H), 2.99(s, 1H),2.95-2.89(m, 2H) 2.79-2.68(m, 1H), 2.65-2.59(m, 1H), 2.23(s, 1H), 2.15-2.12(d, 1H, *J*=10.2Hz) 2.04-1.82(m, 7H), 1.62-1.52(d, 2H, *J*=9.3Hz), 1.45(s, 9H); ^{13}C NMR(125MHz,$CDCl_3$) δ 174.4, 174.1, 171.1, 170.9, 156.8, 138.3, 138.2, 137.1, 129.3, 128.3, 126.7, 60.4, 60.25, 51.8, 51.3, 48.3, 47.3, 46.9, 46.7, 46.2, 46.0, 44.6, 38.7, 28.3, 27.8, 25.0, 24.6; ESI-MS: *m/z* 610 $[M+H]^+$.

Hexamer (17): mp 57-59°C; (KBr) ν_{max} 3314, 3140, 2924, 1605, 1400, 1121, 771, 610; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32-7.28 (m, 5H), 6.25-6.19(m, 2H), 4.18-4.08 (m, 1H), 3.70-3.68(m, 2H), 3.67(s, 3H), 3.66-3.63(m, 2H), 3.22-3.18 (m, 1H), 3.17-3.14(m, 1H), 3.00-2.59(m, 6H),2.54-2.17(m, 2H), 2.15-2.12(m, 1H), 2.05-1.86(m, 7H), 1.43-1.28(m, 3H), 1.25(s, 6H), 1.17-1.13(m, 3H); ^{13}C NMR(125MHz,$CDCl_3$) δ 172.7, 171.6, 171.0, 170.7, 170.2, 136.9, 136.6, 135.9, 128.6, 127.7, 125.9, 68.5, 67.8, 66.8, 60.1, 54.7, 52.2, 51.0, 49.8, 49.5, 45.1, 43.6, 41.2, 37.7, 31.4, 29.5, 24.1,19.3, 18.7; ESI-MS: *m/z* 703 $[M+Na]^+$

Lactone (19): mp 53-55°C; (KBr) ν_{max} 3314, 3140, 2924, 1748, 1605, 1400, 1121, 771, 610; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.15-8.11(m, 3H),7.43-7.40(m, 2H),5.40-5.33(m, 2H),4.31-4.11(m, 3H), 2.79-2.75(m, 2H), 2.41-2.37(m, 2H), 2.36-2.28(m, 5H), 2.06-2.03(d,2H, *J*=7.0Hz), 1.68-1.63(m, 6H), 1.23-1.19(m, 5H), 0.92-0.85(m, 6H); ^{13}C NMR(125MHz,$CDCl_3$) δ 176.0, 174.9, 174.2, 173.8, 170.3, 169.3, 136.9, 136.6, 135.9, 128.6, 127.7, 125.9, 79.7, 68.5, 67.8, 60.3, 56.0, 51.2, 49.7, 49.5, 45.1, 43.6, 41.1, 37.7, 31.4, 29.5, 24.1, 19.2, 18.7; ESI-MS: *m/z* 648 $[M+H]^+$.

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