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Synthetic studies of Microcolin-B

M. Shiva Rama Krishna and B. Srinivasulu

Department of Chemistry, Rayalaseema University, Kurnool-518002(A P), India

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ABSTRACT

The octanoyl and acetyl analogue of the lipopeptide microlin B was synthesized from the suitably protected L-amino acids adopting segment condensation strategy. To achive this the unique Boc and Mem protected cyclic compound was prepared from N-Boc-Methyl-L-Leucil-L-threonin-benzyl ester in a five step synthesis. This segment was coupled to the readily available octanoic acid followed by deprotection employing standard mixed anhydride strategy. This resultant compound on debenzylation and coupling with pyrrolinone unit after acetylation led to the targeted moiety.

***Keyword*:** Immuno suppressive peptide, microcolin-B, analogues of microcolin B, synthetic studies, L-amino acids

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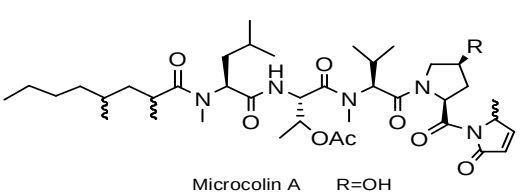
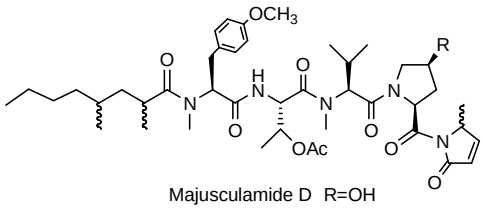
INTRODUCTION

Numerous biologically active compounds have been isolated from marine organisms during the last several decades many of which are peptides containing unique amino acids or amino acid derived substructures. Among these are microcolin A and B two lipopeptides isolated from the blue-green alga Lyngbya majuscule which express potent cytotoxicity and immunosuppressive properties.Immuno Suppressants which suppress the immune mechanism of the body thereby enabling it to accept the organ without any inhibition. Most of the immuno suppressats were introduced in early 1960's, which were cytotoxic agents, whose regiomen overlapped with anti cancer agents. Shortly there after carticosteroids were introduced, which were safer than cytotoxic agents but caused serious adrenal suppression which led to various physiological disorders. Immuno suppressive protocols have undergon a major change with the introduction of undecapeptide cyclosporine A (csA)3 in renal, cardiac, hepatic, pancreatic and bone marrow transplantation. Cyclosporin and FK-506 are the natural products have shown particular promise in the treatment of organ transplant rejection thru suppressing the immune system.Our present targeted analogue of immunosuppressive peptide microcolin B, has the similar structure to microcolin and majusculamide D.



Microcolin A R=OH

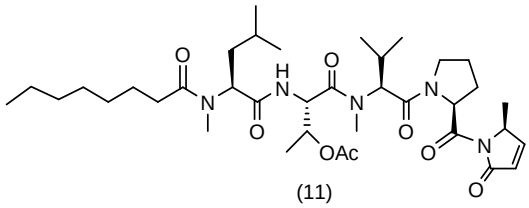
Microcolin B R=H



Majusculamide D R=OH

Desoxymajusculamide D R=H

Both compounds contain the pyrrolin-2-one unit which makes them biologically active in nature. We report herein the synthesis of the octanoyl derivatice (11) of the acylated microcolin B. The most challenging steps of the synthesis are the addition of and mem protected cyclic acid compound with pyrrolinone moiety followed by removal of mem by Ticl4, and acetylation of this moiety, because of low yields.Three of the stereo centres of microcolins are still to be determined. The stereo center at the C-5 in the pyrrolidinone group and stereochemistry in the 2,4-dimethyl octanoic acid were not assigned in the synthesis work. In the majusculamides pyrrolinone moiety was declared as "S" configuration based on the degradation studies.



(11)

Comparision of NMR and 13C-NMR chemical shifts with those of the corresponding majusculamide-D compounds seems to suggest that the C-4 stereochemistry may be assigned as "S" in microcolins. Consequently L-amino acids were used for the synthesis of the target moiety. Since the stereochemistry at C-2 and C-4 in the dimethyl octanoic acid portion was not yet determined. We used the readily available octanoic acid instead of synthesizing all four possible stereo isomers of dimethyl octanoic acid. The acetylated microcolin A was found to be a more potent immunosuppressive agent than microcolin A. Basing on this here in we report the syntheis of acetylated analogue of microcolin B.

MATERIALS AND METHODS

General: - Melting points were determined in a sulfuric acid-bath and are uncorrected. IR spectra were recorded in KBr on a Shimadzu 435 spectrometer, ^{1}H NMR spectra on a Varian Gemini 200 MHz spectrometer with TMS as an internal standard and mass spectra on a Perkin Elmer Hitachi RDO-62 and MS-30 instrument.

i.General Procedure for the synthesis N-BOC-N- Methyl-L-Leucine-L-threonine (OMEM) benzyl ester (2):
To compound **1** (3g, 6.8 mmol) in dry DCM (25 ml) at 0 ^{0}C was added di-isopropyl ethyl amine (1.8 ml, 10.3 mmol) and MEM-Cl (10.06 ml, 7.6 mmol), reaction mixture was stirred at room temperature for 18h, diluted with DCM, and washed with water, organic layer was dried over Na_2SO_4, concentrated and the residue was purified by column chromatography using 25% Eto-Ac:Hexane to give compound **2** (2.92g, 81%) as a colourles syrup.

Specific rotation $[\alpha]_D^{25}$: -49.14^0 (C=0.35 in $CHCl_3$).
^{1}*H NMR (CDC1$_3$, 200 MHz):* δ 7.35 (S, 5H Ar-H), 6.80 (hump, 1H,-NH), 5.15 (S, 2H,-CH_2,Ph),4.80-4.60(m,3H,-OCH_2O,andα–CH),4.53(d,J=6.80Hz, H,α –CH), 4.42-4.30 (m, 1H,-CHOMEM),3.56-3.40(m,4H–$OCH_2)_2$,3.35 (S, 3H, -OCH_3), 2.78 (H, -NCH_3), 1.76-1.55 (m, 3H, -OCH_3), 2.78 (s, 3H,-NCH_3), 1.76-1.55 (m, 3H, Leu β- CH_2 and γ-CH), 1.48(S, 9H, tBu), 1.15 (d, J=6.0 Hz, 3H, Thr–CH_3),0.93 (t, J=5.60 Hz, 6H Leu- $(CH_3)_2$ CH-).

ii. N-BOC-N- Methyl-L-Leucine-L-threonine (OMEM) -OH (3):
Compound **2** (2.7g, 5.2 mmol) was debenzylated using 10% pd-C (270 mg) in ethyl acetate (25 ml) under hydrogen at room temperature for 4h to give acid **3** (2.10g 94%) as thick syrup.

Specific rotation $[\alpha]_D^{25}$: -61.13^0 (C=1.42 in $CHCl_3$).
^{1}*H NMR (CDC1$_3$, 200 MHz)* : δ 6.85 (bs, 1H,-NH), 4.80-4.60 (m, 4H, -OCH_2O and 2x α –CH), 4.50-4.35 (m, 1H,CH-OMEM), 3.84-3.70 and 3.65-3.50 (2xm, 4H, -$O(CH_2)_2$O), 3.40 (s, 3H, - OCH_3), 2.80 -NCH_3), 1.80-1.65 (m, 3H, Leu β-CH_2 and γ-CH), 1.50 (s, 9H, tBu), 1.15 (d, J=6.0 Hz, 3H, Thr -CH_3), 0.95 (t, J=6.0 Hz, 6H, Leu $(CH_3)_2$).

iii. N-BOC-N-Methyl-L-Leucine-L-Threonin (OMEM)-N-methyl-L-Val-Prp-benzyl ester (6):
To an ice cold solution of acid **3** (1.4g, 3.2 mmol) and Et_3N (10.52 ml, 3.7 mmol) in dry THF (20 ml), trimethyl acetyl chloride (0.42 ml, 3.3 mmol) was added and stirred for 1h at the same temperature. Amine **5** (0.97g, 3.0 mmol) in THF (10ml) was added slowly to the reaction mixture and stirred at room temperature for 8h. Volatiles were removed in vaccuo, residue was dissolved in DCM, washed with water, dried over Na_2SO_4, then concentrated and purified by column chromatography using 4:6 ethyl acetate: Hexane mixture as eluent to give compound **6** (1.43g, 64%) as thick syrup.

Specific rotation $[\alpha]_D^{25}$ **:** 101.0^0 (C=0.6 in $CHCl_3$)
^1H *NMR(CDC1*$_3$*,200MHz)* :δ7.30(s,5H,Ar-H), 5.24-5.00 (m,3H,-CH_2,Ph) and (α-CH), 4.90-4.80(m,1Hα-CH),4.75-4.52(m,4Hα-CH,Thrβ-CH, and -OCH_2O of MEM), 4.48-4.38 (m, 1H, α–CH), 4.10-3.80 (2xm, 2H, Pro □ –CH_2),3.75-3.45(2xm,4H,-O$(CH_2)_2$O of MEM), 3.38 (s, 3H, -CH_3), 3.15(S, 3H, Val, -NCH_3), 2.78 (S, 3H , Leu N-CH_3), 2.40-1.80 (m, 5H, Pro β -CH_2, γ-CH_2, and Val β -CH), 1.70-1.55 (m, 3H, Leu β-CH_2 and γ-CH), 1.46 (S, 9H, tBu), 1.12 (d, J=6.32 Hz, 3H Thr-CH_3)1.00-0.75(m,12H,Leu-(CH_3)2,CH-and Val – $(CH_3)_2$, CH-)
EIMS: m/z: 735 $[MH]^+$

iv. N-Octanoyl-N-Methyl-L-Leucine-L-Threonin (OMEM)-N-methyl-L-Val-Pro-benzyl ester (7):
To an ice cold solution of compound **6** (1.20g, 5.35 mmol) in dry DCM (6ml) was added trifluoro acetic acid in dropwise manner. The resulting mixture was stirred at the same temperature for 3h. Solvent was evaporated under vaccuo and the residue was dissolved in water, basified with Sodium bicarbonate solution, Extracted with Ethylacetate. Organic layer was separated, dried over Na_2SO_4, Concentrated to afford compound (0.90g, 87%) as thick syrup, Which was immediately used in the next reaction without further purification and characterization. This thick syrupy compound was added to an ice cold solution of octanoic acid (0.215g, 1.48ml) and triethyl amine (0.240 ml, 1.72 mmol) in dry THF (10 ml) after 1h addition of acetyl chloride (0.108 ml, 5.0 mmol) at the same temperature by diluting in THF (10 ml). This reaction mixture was stirred at room temperature for 8h. Volatiles were removed in vaccuo, resude was dissolved in DCM, washed the organic layer with water, dried over Na_2SO_4, concentrated to get the residue. The residue was purified by Column Chromatography using Ethyl Acetate : Hexane (4:6) solvent mixture as eluent to give compound **8 (**0.626g, 70%) in pure state as thick syrup.

Specific rotation $[\alpha]_D^{25}$: 128.09 ^{0}C (C=0.92 in $CHCl_3$)
^1H *NMR (CDC1*$_3$*, 200 MHz)* :δ 7.30 (s, 5H, Ar-H), 7.15 (d, J=6.0 Hz, 1H, -NH), 5.18-4.93 (m, 4H, -CH_2 Ph and 2x α -CH), 4.80-4.70 (m, 1H, α –CH), 4.68-4.50 (m, 3H, Thr- β-CH and OCH_2O of MEM), 4.45-4.35 (m, 1H, α-CH), 4.08-3.78 (2xm, 2H, Pro γ-CH_2), 3.70-3.40 (2xm, 4H, O$(CH_2)_2$)O of MEM), 3.34 (2xs,3H,-OCH_3), 3.12(2xs, 3H, Val-NCH_3), 2.95 and 2.88 (2xs, 3H, Leu-N-CH_3), 2.30-1.75 (m, 7H,C_2, 2H, Pro β-CH_2, γ-CH_2 and Val β-CH), 1.68-1.48 (m, 3H, Leu β-CH_2, and γ- CH), 1.26 (S, 10H, C_3 - $(CH_2)_5$), 1.04(d, J=6.06 Hz, 3H, Thr-CH_3), 0.95-0.72 (m, 15H, Leu (CH_3)2 CH, Val (CH3)2 CH and C8-3H).

v.N-octanoyl-N-Methyl-L-Leucyl-L-Threonin (OMEM)-N-methyl-L-Valyl-Prolyl-5S-methyl-2 pyrrolinone (9):
To an ice cold solution of compound **8** (0.24g, 0.35 mmol) Et_3N (0.05 ml, 0.4 mmol) in dry THF (2ml) was added trimethyl acetyl chloride (0.04ml, 0.35 mmol) and stirred at the same temperature for 1.3h. (S)-5-methyl pyrrolidin-2-one in dry THF (1 ml) was added to pre cooled (-78 ^{o}C), 2M n-Buli (0.2 ml) was added and stirred for 15min. This was slowly added to a pre cooled (-78 ^{o}C) solution of mixed anhydride via cannula. Reaction mixture was stirred at the same temperature for 2h, then quenched with saturated ammonium chloride solution. Organic layers was separated, dried over Na_2SO_4, concentrated and purified by column chromatography using 5% MeOH: CHCl3 mixture as eluent to give compound **9** (0.1g, 37%) as semi solid.

Specific rotation $[\alpha]_D^{25}$ **:**-160.0^0 (C 0.53 in $CHCl_3$)
^1H *NMR (CDC1*$_3$*, 200 MHz)* : δ 7.30 -7.20 (m, 1H, C3-H Pryrrolinone), 6.10 (d , J=6.45 Hz, 1H, C_2- H of Pyrrolinone), 5.55 (dd, J=4.3 and 8.6 Hz, 1H, C_8-H), 5.30-5.05 (m, 3H, 3x α -H), 4.96-4.60 (m, 4H, C_4-H of Pyrrolinone, Thr β–CH and OCH_2O of MEM), 4.20- 3.90 (m, 2H ,pro □-CH_2), 3.80-3.55 (m, 4H,-O$(CH_2)_2$O of MEM), 3.46 (s, 3H, OCH_3), 3.20 (S,3H,Val-NCH_3), 2.97 (s, 3H, Leu-NCH_3), 2.50-1.80 (2xm, 7H, C_{36}-2H , Pro β-CH_2, γ-CH_2 and Val β-CH), 1.75-1.60 (m, 3H, Leu β-CH_2 and γ-CH), 1.50-1.20 (m, 13H, C_6-3H and C_{37}-C_{41}-$(CH_2)_5$, 1.20-0.80 (2xm, 18H, Thr-CH_3, Leu $(CH_3)_2$, CH, Val $(CH_3)_2$ and C_{42}-3H)

vi.N-Octanoyl-N-Methyl-L-Leucyl-L-Threonyl-N-methyl-L-Valyl-Prolyl-5S-methyl-2 pyrrolinone (10):
To an ice cooled solution of **9** (0.05g, 0.072 mmol) in DCM-Pentane (1:0.5 ml) mixture was added TiCl4 (0.03 ml, 0.07 mmol). Reaction mixture was stirred at the same temperature for 5h. Then quenched with ammonium hydroxide solution. Volatiles were removed under vaccum residue was dissolved in chloroform, washed with water, dried over Na_2SO_4, and concentrated. Crude compound was purified by column chromatography using 5% MeOH: $CHCl_3$ mixture as eluent to give compound **10** (23mg, 52%) as syrup.

Specific rotation $[\alpha]_D^{25}$: -151.2^0 (C 0.50 in $CHCl_3$), Lit:148.50 (C 0.2 in $CHCl_3$)
^{1}*H NMR (CDC1$_3$, 200 MHz)* : δ 7.25-7.15 (m, 1H, C_3-H), 6.85 (d,J=6.08 Hz, 1H, -NH), 10(d, J=6.45 Hz, 1H, C_2-H), 5.55-5.45 (m, 1H, C_8-H), 5.30-5.00 (m, 3H ,3x α -H), 4.95-4.60 (m, 2H, C_4-11 and Thr- β-CH), 4.20-4.00 (m, 2H, Pro □-CH_2), 3.10 (2xS, 3H, Val-NCH_3), 2.90 (2xS, 3H, Leu –NCH_3), 2.40-1.80(2xm, 7H, C_{36}-2H, Pro β -CH_2, γ -CH_2, and Val- β -CH), 1.75-1.50 (m, 3H, Leu β-CH_2 and γ-CH), 1.50-1.20 (m, 13H, C_6-3H, and C_{37}-C_{41}-$(CH_2)_5$), 1.15-0.75 (2xm, 18H, Thr-CH_3, Leu $(CH_3)_3$, CH-Val (CH_3)-CH and C_{42}-3H)

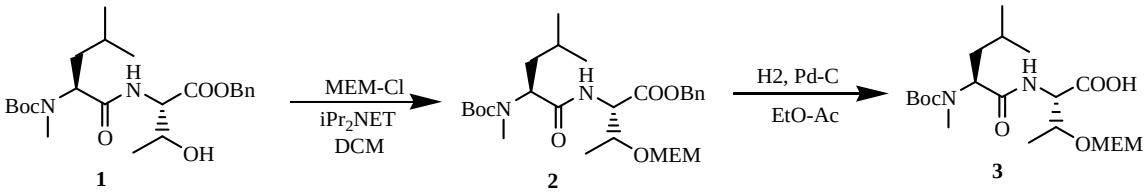
vii.N-Octanoyl-N-Methyl-L-Leucyl-L-Threonyl-(O-Acetyl)-N-methyl-L-Valyl-Prolyl-5S-methyl-2-pyrrolinone (11):
To a mixture of compound **10** (15mg, 0.2mmol) and pyridine (0.004ml, 0.04 mmol) in dry DCM (1ml) at 0^{0}C acetic-anhydride (0.003 ml,0.03 mmol) was added and the reaction mixture was stirred at room temperature for 5h. Reaction mixture was then diluted with DCM (5ml) washed with water, dried over Na_2SO_4, and concentrated in vaccuo. The residue was purified by column chromatography using 5% MeOH: CHCl3, mixture as eluent to give compound **11** (6mg, 40%) as thick syrup.

Specific rotation $[\alpha]_D^{25}$: -146.06^0 (C=0.33 in $CHCl_3$)
^{1}*H NMR (CDC1$_3$, 400 MHz)* : δ 7.16 (d, J=6.60 Hz, 1H, C_3-H,Pyrrolinone), 6.82 (d, J=6.85 Hz, 1H,-NH), 6.00 (d, J=6.45 Hz, 1H, C_2-H of pyrrolinone), 5.58-5.50 (m, 1H, C_8-H), 5.22-5.10 (m, 2H, CH-OAc and α -CH), 4.98 (d, J=10.05 Hz, 1H, α -CH), 4.92-4.85 (m, 1H, α -CH), 4.82-4.72 (m, 1H,C_4-H), 3.68-3.58 (m, 2H, Pro □ -CH_2), 3.05 (S, 3H, Val-NCH_3), 2.85 (S, 3H,Leu-NCH_3), 2.40-2.15 (m, 5H Pro β- CH_2, Val β-CH and C_{36}–2H), Val- NCH_3), 2.85 (S, 3H, Leu-NCH_3), 2.05- 1.70 (m, 5H, Pro γ -CH_2 and –CH_3 of acetate), 1.60-1.40 (m, 3H, Leu β-CH_2 and γ-CH), 1.35 (d, J=6.10 Hz, 3H,C_6-3H), 1.30-1.10 (m, 10H, C_{37}-C_{41} $(CH_2)_5$), 1.10 (d, J=6.15 Hz, 3H, Thr-CH_3), 0.98 (d, J=6.12 Hz, -3H, Val-CH_3), 0.90-0.70 (m, 12H, Val-CH_3, Leu - $(CH_3)_2$, -CH and C_{42}-3H)
EIMS: m/z : 704 $[MH]^+$

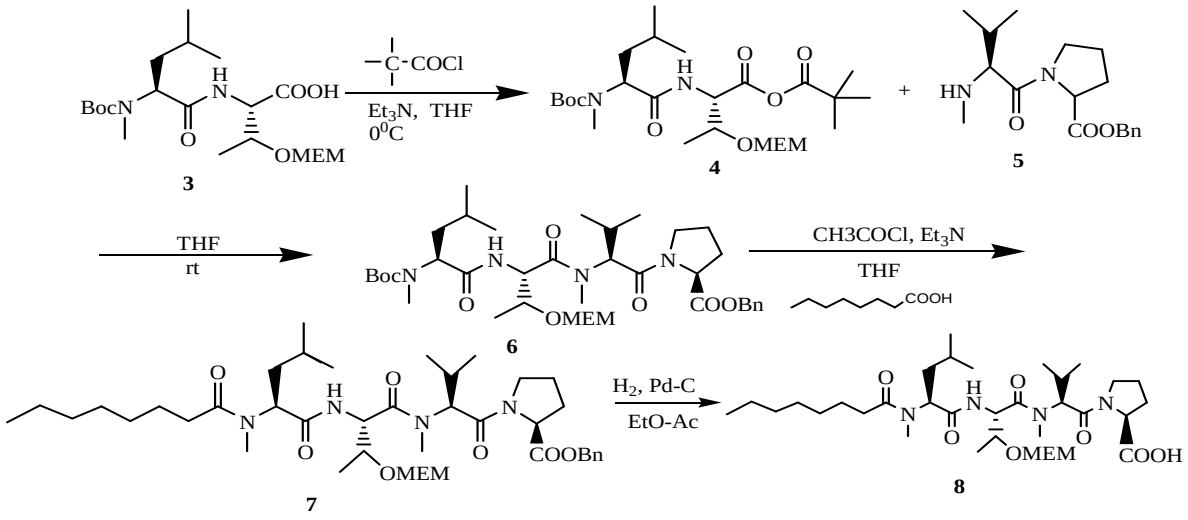
RESULTS AND DISCUSSION

Synthesis of N-Octanoyl-N-Methyl-L-Leucyl-L-Threonyl-(O-Acetyl)-N-methyl-L-Valyl-Prolyl-5S-methyl-2-pyrrolinone (11):
To a mixture of compound **10** (15mg, 0.2mmol) and pyridine (0.004ml, 0.04 mmol) in dry DCM (1mL) at 0^{0}C acetic-anhydride (0.003 ml,0.03 mmol) was added and the reaction mixture was stirred at room temperature for 5h. Reaction mixture was then diluted with DCM (5ml) washed with water, dried over Na_2SO_4, and concentrated in vaccuo. The residue was purified by column chromatography using 5% MeOH: $CHCl_3$, mixture as eluent to give compound **11** (6mg, 40%) as thick syrup.

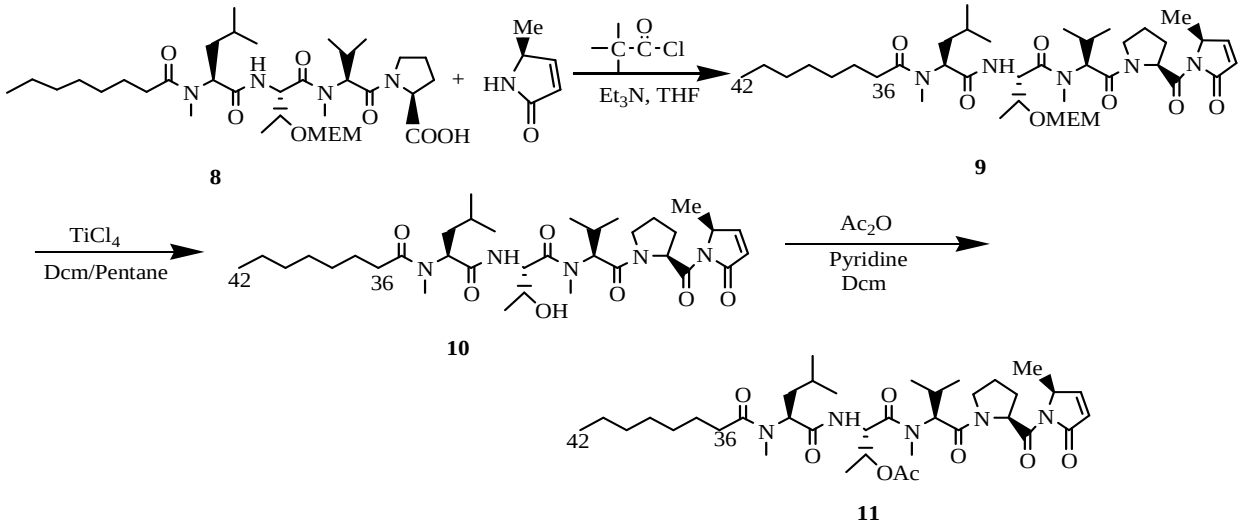
Scheme-1



Scheme-2



Scheme-3



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