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Synthetic Studies of Microcolin B

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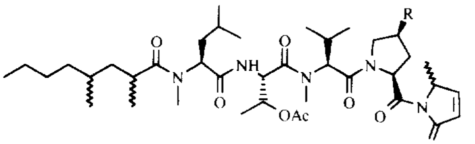
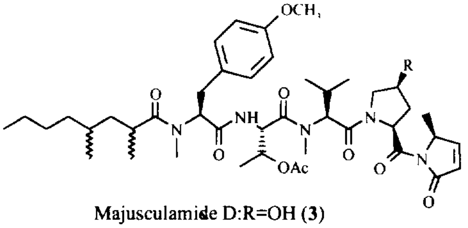
Abstract: The octanoyl and desacetyl analogue of the lipopeptide microcolin B isolated from *Lyngbya majuscula* was synthesized using a segment condensation strategy. To achieve this the unique 1-prolyl-methyl-2-pyrroline unit was prepared from Boc-Pro-Ala-OH in a five step synthesis. This segment was coupled to the dipeptide Boc-Thr-MeVal-OH using PyBoP®. Deprotection followed by PyBroP®-mediated coupling with octanoyl-MeLeu-OH finally led to the octanoyl analogue of desacetylmicrocolin B.

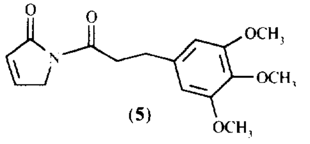
Numerous biologically active compounds have been isolated from marine organisms during the last several decades many of which are peptides containing unique amino acids or amino acid derived substructures.[2] Among these are microcolins A (**1**) and B (**2**), two lipopeptides isolated from the blue-green alga *Lyngbya majuscula*[3] which express potent cytotoxicity and immunesuppressive properties.



Microcolin A:R=OH (**1**)
Microcolin B:R=H (**2**)



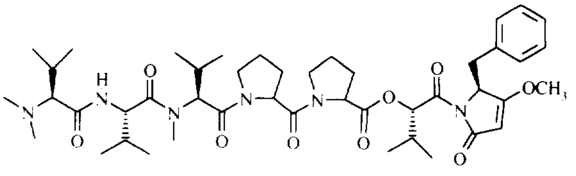
Majusculamide D:R=OH (**3**)
DesoxymajusculamideD:R=H (**4**)



(**5**)

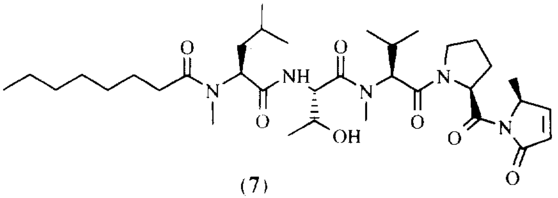
Both compounds contain an unsaturated lactam moiety which according to recently published studies[4] is mandatory for their biological activity.

The closely related lipopeptides, majusculamide D (**3**) and desoxymajusculamide D[5] (**4**), contain the same lactam substructure. The glycine derived analogue of this lactam, the pyrrolin-2-one, also occurs in an alkaloid isolated from the terrestrial plant *Piper demeraranum*[6] (**5**). However, more commonly found in natural products is the 4-methoxypyrrolin-2-one unit.[7] The most noteworthy compound containing this substructure derived from phenylalanine is dolastatin 15 (**6**) which is a remarkable cytotoxin. The isolation and synthesis of the dolastatins was subject of several papers by Pettit and coworkers.[8]



Dolastatin 15 (**6**)

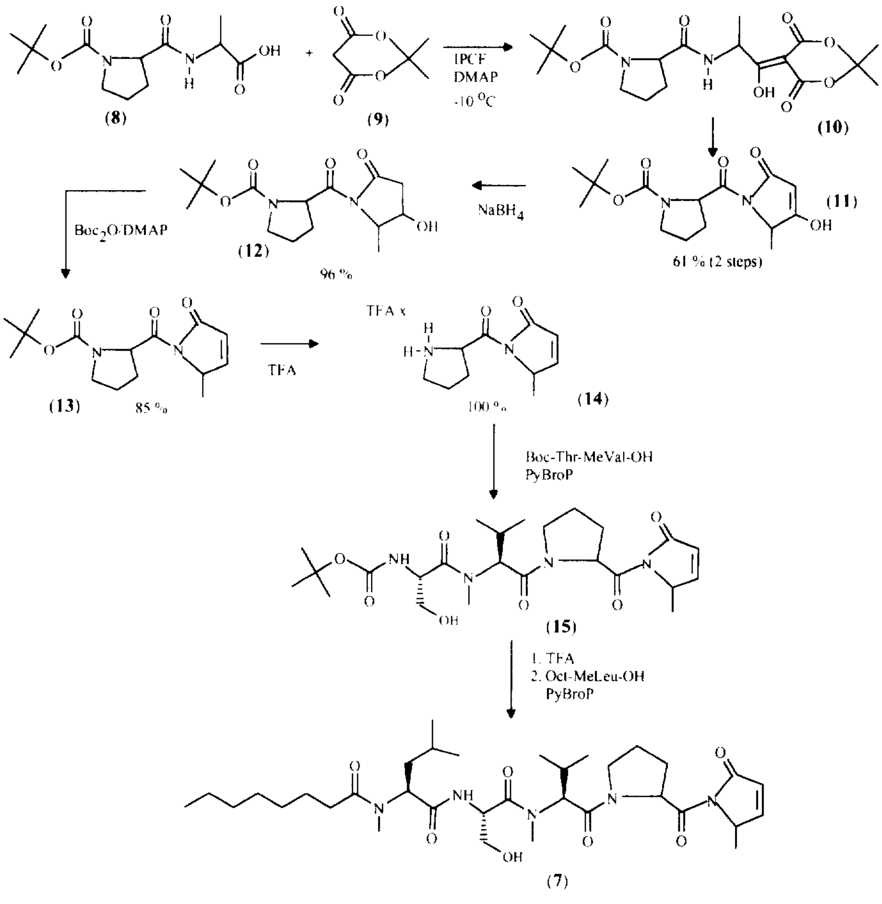
We report herein the synthesis of the octanoyl derivative (**7**) of desacetylmicrocolin B. The most challenging steps of this synthesis are the formation of the lactam moiety, the formation of the proline lactam bond and the coupling of the N-methylated amino acids. Three of the stereocenters of the microcolins are still to be determined: the stereocenter at the C-5 in the 2-pyrrolidinone group and the stereochemistry in the 2,4-dimethyloctanoic acid. In the majusculamides, the S configuration in the lactam was proved by degradation.[5]



(**7**)

Comparison of the NMR data of (**3**) and (**4**) with the data of microcolin A (**1**) and B (**2**) made it highly probable that the stereochemistry of the lactam residue in the microcolins was also S. Consequently, L-alanine was used for the synthesis of the target compound. The stereochemistry in all other amino acids of microcolin B was reported to be S. Since the stereochemistry at the C-2 and C-4 in the dimethyloctanoic acid portion was not yet determined, we used the readily available octanoic acid instead of synthesizing all four possible stereroisomers of dimethyloctanoic acid. Because the desacetylmicrocolin A was found to be a more potent immunosuppressive agent than microcolin A,[4] the desacetyl analogue of microcolin B was synthesized in this study.

Although there are elegant methods to acylate lactams such as replacement of the amide hydrogen with a trimethylsilyl group and reaction of the silylated lactam with acid chlorides[12] an alternate synthetic pathway to the proline-2-pyrrolinone portion was followed whereby the proline lactam bond had been formed already. Therefore, we began the synthesis of the lactam with the dipeptide Boc-Pro-Ala-OH (**8**) rather than using an N-protected alanine derivative and forming the proline lactam bond afterwards. To this end Meldrum's acid (**9**) was acylated with Boc-Pro-Ala-OH after activating this dipeptide with isopropenyl chloroformate in the presence of 4-dimethylaminopyridine following a procedure described by Castro *et al.* for N-protected amino acids.[9] The crude product (**10**) was refluxed in benzene to yield the 1-(Boc-Pro)-4-hydroxy-5-methyl-2-pyrrolinone (**11**). The use of methanol as solvent for the lactamization as suggested by Castro *et al.* led in our case to the formation of the β-ketoester. The same side reaction was also observed by Pettit *et al.*[8a] in a similiar reaction leading to the S-dolapyrrolidone unit. The NMR spectrum of the product (**11**) indicated that racemization had occurred at the 5-position in the lactam. These diastereomers could not be separated by chromatography at this point. However, the integration of the pair of the 5-methyl doublets indicated that the diastereomers were present in a ratio of approximately 5:1. It is reasonable to assume that the racemization occured during the activation of the dipeptide with isopropenyl chloroformate in the presence of the base 4-dimethylaminopyridine via α-proton abstraction.



The double bond in **11** was reduced with NaBH$_4$[9] to give pyrrolidinone (**12**) which was reacted with di-*tert.*butyl-dicarbonate in the presence of 4-dimethylaminopyridine to form the 1-(Boc-Pro)-5-methyl-2-pyrrolinone (**13**) in good yield. The elimination of water with di-tert.butyl-dicarbonate to yield **13** occured unexpectedly. We had originally planned to protect the hydroxy function with the Boc group and eliminate the so formed t-butyloxycarbonyloxy group with Cs_2CO_3 afterwards as described by Schmidt et al.[13] However, the elimination of the β-hydroxyl group with Boc_2O was reproducible and was also observed under the same conditions with N-Z-4-hydroxy-5-methyl-2-pyrrolidinone and N-Boc-4-hydroxy-5-methyl-2-pyrrolidinone (synthesized from Z-Ala and Boc-Ala) leading to N-benzyloxycarbonyl-5-methyl-2-pyrrolinone and its butyloxycarbonyl protected analogue. Further utilization of this reaction is currently under investigation.

After cleaving the Boc group with trifluoroacetic acid the prolyl-2-pyrrolinone trifluoroacetate (**14**) was coupled with the dipeptide Boc-Thr-MeVal-OH using PyBroP®[11b] to yield **15**. The small amount of diastereomer obtained as a result of the racemization at C-5 of the lactam during the lactamization could now easily be separated by HPLC. After deprotection with trifluoroacetic acid the tetrapeptide trifluoroacetate was coupled with octanoyl-N-methylleucine using PyBroP® to yield (**7**). Other coupling reagents such as BOP chloride[10] and PyBOP®[11a] were used also but they were not as effective as PyBroP®. The synthesized octanoyl derivative of desacetylmicrocolin B (**7**) showed moderate activity in the mixed lymphocyte reaction assay (ED_{50}=3.8μg/mL) as well as in antitumor assays: A 549 (ED_{50}=3.3 μg/mL) and P 388 (ED_{50}=1.9 μg/mL). Structure-activity-relationships are the subject of our current studies and will be reported soon.

Experimental Section

Boc-Thr, MeVal-OBzl p-tosylate, MeLeu-OBzl p-tosylate, Boc-Pro, Ala-OMe were purchased from BACHEM Bioscience, Inc. and were used without further purification. PyBOP® and PyBroP® were bought from Novabiochem and all other reagents were obtained from Aldrich Chemical Company, Inc. Boc-Pro-Ala-OMe was synthesized from Boc-Pro and Ala-OMe with DCC and the methyl ester was cleaved with 1N NaOH in methanol. All solvents were HPLC grade purchased from either E.Merck or Baxter and were used for chromatography without further purification. For reactions, solvents were dried and redistilled. IR spectra were recorded on a Midac-M 200-FTIR. NMR spectra were measured on either a Bruker AMX-500 or a Bruker AM 360 and referenced to TMS=0. ^{13}C-NMR multiplicities as determined by DEPT are given as follows: s=C, d=CH, t=CH_2, q=CH_3. For the multiplicities in the ^1H-NMR the following abbreviations are used: s=singlet, d=doublet, t=triplet, dd=double doublet, q=quartet. Optical rotations were determined with a Jasco DIP-360 digital polarimeter. Thin layer chromatography was performed on E. Merck silica gel 60 F_{254} plates. Flash chromatography was performed on Merck silica gel 60 (230-400 mesh). HPLC was performed using refractive index detection. A Merck HiBar 250-10 mm Lichrosorb Si-60 7μm prepacked column was used for silica gel HPLC. Reversed phase chromatography was accomplished on a Vydac protein and peptide C-18 column.

Octanoyl-N-methylleucine benzylester:

MeLeu-OBzl p-tosylate (407 mg, 1 mMol) and octanoic acid (160 mg, 0.17 mL, 1.1 mMol) were dissolved in 2 mL of dry dichloromethane and the solution was cooled in an ice bath. BOP chloride (290 mg, 1.2 mMol) was added followed by 0.5 mL of absolute triethylamine. The reaction was stirred overnight, diluted with 20 mL of dichloromethane and extracted twice with 20 mL of saturated KHCO$_3$-solution, twice with saturated KHSO$_4$. The product was then washed with brine, dried over MgSO$_4$ and filtered. The solvent was evaporated and the crude product was purified by silica gel flash chromatography using ethyl acetate as solvent to yield 283 mg (78.2 %) of a colorless oil which was pure by TLC (ethyl acetate:heptane 1:1 and ethylacetate). ^{1}H-NMR (CDCl$_3$): 0.81(t, *J*=7.4 Hz, 3H, C-8, octanoic acid), 0.83 (d, *J*=6.7 Hz, 3 H, δ-Leu), 0.87 (d, *J*=6.7 Hz, 3H, δ-Leu), 1.21 (m, 10H, octanoic acid), 1.45 (m, 1H, γ-Leu), 1.61 (ddd, *J*=14.4, 10.1, 4.5 Hz, 1H, β-Leu), 1.71 (ddd, *J*=14.3, 10.1, 5.2 Hz, 1H, β-Leu), 2.33 (t, *J*=6.4 Hz, 2H, C-2, octanoic acid), 2.87 (s, 3H, N-Me), 5.08 (d, *J*=12.3 Hz, 1H, benzyl-CH$_2$), 5.18 (d, *J*=12.4 Hz, 1H, benzyl-CH$_2$), 5.41 (dd, *J*=10.2, 5.6 Hz, 1H, α-Leu), 7.33 (m, 5H, aromatic); ^{13}C-NMR (CDCl$_3$): 14.1 (q), 21.5 (q), 22.6 (t), 23.3 (q), 25.1 (d), 29.1 (t), 29.4 (t), 31.5 (q), 31.7 (t), 33.3, 33.7 (t), 37.4 (t), 54.3 (d), 66.7 (t), 128.1 (d), 128.2 (d), 128.6 (d), 128.7 (d), 135.8 (s), 172.1 (s), 174.1 (s); IR (film, KBr, cm^{-1}): 2953, 2929, 2868, 1743, 1646, 1118; LRFABMS [M+H]$^+$: 362; HRFABMS Calc. for C$_{22}$H$_{35}$NO$_3$: 362.2696, found: 362.2719 (Δmmu=2.3); $[\alpha]_D^{26}$ = -26.3° (c=1, CHCl$_3$).

Octanoyl-N-methylleucine:

Octanoyl-N-methylleucine benzylester (140 mg, 0.4 mMol) was dissolved in 10 mL of methanol. Palladium on activated carbon (30 mg) was added and then hydrogen was bubbled through the solution over a period of 3 h. After this time no starting material could be detected by TLC. Nitrogen was then bubbled through the solution to remove the excess of hydrogen. The catalyst was filtered off and the solution was evaporated under reduced pressure to give the free acid as white needles in 95 % yield. Mp: 83-85 °C.^{1}H-NMR (CDCl$_3$): 0.85 (t, *J*=7.2 Hz, 3H, C-8, octanoic acid), 0.91 (d, *J*=6.4 Hz, 3H, δ-Leu), 0.94 (d, *J*=6.4 Hz, 3 H, δ-Leu), 1.25 (m, 3H), 1.28 (m, 10H, octanoic acid), 1.46 (m, 1H, γ-Leu), 1.61 (ddd, *J*=14.3, 10.4, 5.1 Hz, 1H, β-Leu), 1.74 (ddd, *J*=14.1, 10.3, 5.6 Hz, 1H, β-Leu), 2.34 (t, *J*=6.5 Hz, 2H, C-2, octanoic acid), 2.91 (s, 3H, N-Me) 5.24 (q, *J*=10.3, 5.4 Hz, 1H, α-Leu), 7.75 (br s); ^{13}C-NMR (CDCl$_3$): 13.9 (q), 21.3 (q) , 22.5 (t), 23.1 (q), 24.9 (d), 28.9 (t), 29.1 (t), 33.6 (t), 37.1 (t), 54.5 (d), 174.9 (s), 175.2 (s); IR (film, KBr, cm^{-1}): 2956, 2930, 1731, 1602, 1464, 1411, 1196; LRFABMS: [M+H]$^+$: 272; HRFABMS Calc. for C$_{15}$H$_{30}$NO$_3$: 272.2225, found: 272.2213 (Δmmu= 1.2); $[\alpha]_D^{20}$= -34.9° (c=1, CHCl$_3$).

tert. Butyloxycarbonylthreonyl-N-methylvaline benzylester:

Boc-Thr (219.2 mg, 1mMol) and N-MeVal-OBzl p-tosylate (380 mg, 1mMol) were dissolved in 2 mL of dichloromethane and cooled to 0 °C in an ice bath. PyBOP® (520.4 mg, 1mMol) followed by triethylamine (0.84 mL) was added. The reaction mixture was stirred for 4 h and the solvent was removed under reduced pressure. The residue was redissolved in ethyl acetate and extracted twice with saturated $KHSO_4$ solution, twice with saturated $KHCO_3$ solution and then washed with brine, dried over $MgSO_4$ and filtered. After the solvent was evaporated the crude material was filtered over basic aluminum oxide with ethyl acetate as solvent and finally purified by flash chromatography on silica gel with a 1:1 mixture of ethyl acetate and heptane to give 329.7 mg (80.6 %) of a colorless oil which was pure by HPLC (ethyl acetate:heptane 1:1 on silica gel). ^{1}H-NMR ($CDCl_3$): 0.84 (d, J=6.5 Hz, 3H, γ-Val), 1.02 (d, J=6.4 Hz, 3H, γ-Val), 1.12 (d, J=6.6 Hz, 3H, γ-Thr), 1.43 (s, 9H, Boc), 2.24 (m, 1H, β-Val), 3.02 (s, 3H, N-Me), 3.96 (dq, J=3.7, 6.5 Hz, 1H, β-Thr), 4.42 (q, J=9.7, 3.4 Hz, 1H, α-Thr), 4.90 (d, 1H, J=10.5 Hz, α-Val), 5.06 (d, J=12.3 Hz, 1H, benzyl-CH_2), 5.18 (d, J=12.4 Hz, 1H, benzyl-CH_2), 7.33 (m, 5H, aromatic); ^{13}C-NMR ($CDCl_3$): 18.6 (q), 19.8 (q), 27.3 (q), 28.2 (d), 31.6 (q), 53.6 (d), 61.8 (t), 66.7 (t), 67.3 (d), 80.1 (s), 128.4 (d), 128.5 (2d), 128.6 (d), 135.5 (s), 156.2 (s), 170.3 (s), 173.6 (s); IR (film, KBr, cm^{-1}): 3422, 2922, 2930, 1743, 1719, 1634, 1500, 1166 ; LRFABMS: 424 $[M+H]^+$; HRFABMS Calc. for $C_{22}H_{35}N_2O_6$: 423.2496, found: 423.2571 (Δmmu=7.5); $[\alpha]_D^{26}$= -71.4° (c=1, $CHCl_3$).

tert. Butyloxycarbonylthreonyl-N-methylvaline:

Boc-Thr-MeVal-OBzl (230 mg, 0.55 mMol) was dissolved in 50 mL of methanol. Pd on activated carbon (85 mg) was added and hydrogen was bubbled through the solution over a period of 3 h. Nitrogen was finally bubbled through the solution to remove the excess of hydrogen. The Pd was filtered off and the methanol was removed under reduced pressure to give 178.5 mg (98.6 %) of a white solid which was pure by TLC (ethyl acetate:acetone:water 5:5:1). Mp: 101-103 °C. ^{1}H-NMR ($CDCl_3$): 0.87 (d, J=6.5 Hz, 3H, γ-Val), 1.05 (d, J=6.5 Hz, 3H, γ-Val), 1.28 (d, J=6.8 Hz, 3H, γ-Thr), 1.43 (s, 9H, Boc), 2.21 (m, 1H, β-Val), 3.12 (s, 3H, N-Me), 4.13 (dq, J=3.7, 6.5 Hz, 1H, β-Thr), 4.58 (dd, J=8.6, 3.1 Hz, 1H, α-Thr), 4.78 (d, J=10.8 Hz, 1H, α-Val), 5.95 (d, J=7.2 Hz, 1H, N-H), 6.38 (bs, 1H); ^{13}C-NMR ($CDCl_3$): 18.6 (q), 19.8 (q), 27.3 (q), 28.2 (d), 31.6 (q), 53.6 (d), 61.8 (t), 66.7 (t), 67.3 (d), 80.1 (s), 156.2 (s), 173.6 (s); IR (film, KBr, cm^{-1}): 3422, 2988, 1701, 1628, 1160, LRFABMS: 333 $[M+H]^+$; HRFABMS Calc. for $C_{15}H_{29}N_2O_6$: 333.2025, found: 333.2005 (Δmmu=2.0); $[\alpha]_D^{20}$= -65.3° (c=1, $CHCl_3$).

1-(*tert.* Butyloxycarbonylprolyl)-4-hydroxy-5-methyl-2-pyrrolinone (11):

Boc-Pro-Ala-OH (1.4 g, 4.9 mMol) was dissolved in 15 mL of absolute dichloromethane. DMAP (1.5 g, 12.3 mMol) and Meldrum's acid (0.8 g, 5.5 mMol) were added. The solution was cooled to -15 °C. Isopropenyl chloroformate (0.65 mL, 0.6 mMol) dissolved in 5 mL of absolute dichloromethane was dropped into this solution over a period of 45 min. During this period the temperature of the reaction mixture was held strictly below -10 °C. Afterwards the mixture was stirred for 2 h keeping the temperature between -5 and -10 °C. The reaction was quenched by adding 30 mL of saturated $KHSO_4$-solution and then extracted with 1N HCl and washed with brine. Evaporation of the solvent under reduced pressure yielded 1.992 g of crude material **10** which was then dissolved in 50 mL of absolute benzene and refluxed for 2 h. The benzene was removed in vacuo and the residue was dried overnight to yield 1.57 g of an orange colored material. The purification included gradient flash chromatography on silica gel with ethyl acetate/methanol mixtures as solvent systems and reversed phase chromatography using acetonitrile/water 3:1. HPLC analysis on reversed phase with various acetonitrile/water mixtures and on silica gel with ethyl acetate/heptane in various ratios showed a shoulder which could not be separated in any of the used solvent systems. Study of the NMR spectra led to the conclusion that this shoulder was the diastereomer formed by racemization on the carbon 5 in the lactam residue. 925 mg (61 % over two steps) of diastereomeric mixture was obtained. The ratio estimated from the ^{1}H-NMR is approximately 5:1. Otherwise the compound was pure by TLC (ethylacetate and ethylacetate: acetone:water 5:5:1). ^{1}H-NMR ($CDCl_3$): 1.39 (d, *J*=6.8 Hz, 3H, 5-CH_3), 1.47 (s, 9H, Boc), 1.91 (m, 2H, γ-Pro), 2.40 (m, 2H, β-Pro), 3.48 (ddd, *J*=16.1, 10.2, 6.6 Hz, 1H, δ-Pro), 3.56 (ddd, *J*=16.4, 10.6, 6.3 Hz, 1H, δ-Pro), 4.51 (qdd, J=6.8, 1.8, 1.8 Hz, 1H, C-5), 5.01 (d, *J*=11.2 Hz, 1H, C-3), 5.36 (dd, *J*=8.5, 5.4 Hz, 1H, α-Pro); ^{13}C-NMR (DMSO-d_6): 16.8 (q), 22.4 (t), 27.8 (q), 30.0 (t), 46.5 (d), 55.2 (d), 59.2 (d), 78.2 (s), 92.4 (d), 152.9 (s), 170.1 (s), 171.6 (s), 180.5 (s); IR (film, KBr, cm^{-1}): 3446, 2984, 1689, 1615, 1409, 1263, 1159; HRFABMS Calc. for $C_{15}H_{23}N_2O_5$: 311.1606, found: 311.1570 (Δmmu=3.6); $[\alpha]_D^{20}$= -83° (c=1, $CHCl_3$).

1-(*tert.*Butyloxycarbonylprolyl)-4-hydroxy-5-methyl-2-pyrrolidinone (12):

1-(*tert.*butyloxycarbonyl)-4-hydroxy-5-methyl-2-pyrrolinone (**11**, 700 mg, 2.25 mMol) were dissolved in a mixture of 70 mL of dichloromethane and 7 mL of acetic acid and this solution was cooled in an ice bath to 0 °C. $NaBH_4$ (1.4 g, 37 mMol) was added in small portions over a period of 1 h at this temperature. The reaction mixture was stirred at 0 °C for another 4 h and overnight at room temperature. The solution was poured into 30 mL of cold water, the organic layer was separated and washed with water. Evaporation of the solvent yielded 661 mg of a yellowish oil (93.8 %) which was pure by TLC (ethylacetate and ethylacetate:acetone: water 5:5:1). ^{1}H-NMR ($CDCl_3$): 1.32 (d, *J*=6.4 Hz, 3H, 5-CH_3), 1.45 (s, 9H, Boc), 1.85 (m, 4H), 2.35 (m, 1H), 2.72 (m, 1H), 3.42 (ddd, *J*=16.2, 10.1, 6.6 Hz, 1H, δ-Pro), 3.56 (ddd, *J*=16.4, 10.3, 5.8 Hz, 1H, δ-Pro), 4.48 (q, *J*=9.7 Hz, 1H, C-5) 5.27 (dd, *J*=8.5, 5.2 Hz, 1H, α-Pro); ^{13}C-NMR ($CDCl_3$): 12.4 (q), 22.8 (t), 23.7

(t), 28.4 (q), 29.6 (q), 30.4 (t), 39.6 (d), 47.0 (d), 56.7 (d), 57.6 (d), 60.4 (d), 65.0 (d), 79.8 (s), 153.9 (s), 154.6 (s), 172.3 (s), 174.4 (s), IR (film, KBr, cm^{-1}): 3440, 2986, 1737, 1676, 1415; HRFABMS Calc. for $C_{15}H_{25}N_2O_5$: 313.1763, found: 313.1808 (Δmmu=4.4); $[\alpha]_D^{20}$= -72° (c=1, CHCl$_3$).

1-(*tert.*Butyloxycarbonylprolyl)-5-methyl-2-pyrrolinone (13):

1-(*tert.*butyloxycarbonylprolyl)-4-hydroxy-5-methyl-2-pyrrolidinone (**12**, 650 mg, 2.1 mMol) was dissolved in 35 mL of dry THF. Di-*tert.*butyl dicarbonate (1.4 g , 6.4 mMol) and DMAP (700 mg, 5.7 mMol) were added and the reaction mixture was stirred for 30 h at room temperature. The solvent was evaporated, the residue redissolved in ethyl acetate and extracted twice with 1 N HCl and saturated $KHCO_3$ solution, dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. The crude product was redissolved in ethyl acetate and filtered over silica gel. The final purification was achieved by HPLC on silica gel using ethyl acetate as solvent. 428 mg (69.9 %) of a colorless oil was obtained. The compound contained the diastereomer formed by racemization at C-5, but appeared to be otherwise pure by ^{1}H-NMR and TLC (ethyl acetate:acetone: water 5:5:1). ^{1}H-NMR (CDCl$_3$): 1.32 (d, *J*=6.5 Hz, 3H, 5-CH$_3$), 1.45 (s, 9H, Boc), 1.85 (m, 3H, β,γ-Pro), 2.38 (m, 1H, β-Pro), 3.55 (m, 2H, δ-Pro), 4.76 (dq, *J*=1.8, 6.8 Hz, 1H, C-5), 5.27 (dd, *J*=8.6, 5.3 Hz, 1H, α-Pro), 6.08 (dd, *J*=6.1, 1.5 Hz, 1H, C-3), 7.24 (dd, *J*=6.0, 2.1 Hz, 1H, C-4); ^{13}C-NMR (CDCl$_3$): 17.8 (q), 23.3 (t), 28.3 (q), 29.9 (t), 46.8 (d), 57.8 (d), 60.1 (d), 79.4 (s), 128.4 (d), 154.3 (s), 169.4 (s), 173.2 (s); IR (film, KBr, cm^{-1}): 3442, 2981, 1762, 1634, 1348, 1311, 1159; LR FABMS: 295 $[M+H]^+$, 239, 195 $[M\text{-}Boc+H]^+$; HRFABMS Calc. for $C_{15}H_{23}N_2O_4$: 295.1658, found: 295.1660 (Δmmu=0.2): $[\alpha]_D^{20}$=-92°(c=1, CHCl$_3$);

1-Prolyl-5-methyl-2-pyrrolinone trifluoro acetate (14):

1-(*tert.*butyloxycarbonylprolyl)-5-methyl-2-pyrrolinone (**13**, 50 mg, 0.17 mMol) was dissolved in 2 mL of trifluoroacetic acid and stirred for 2 h at room temperature. The trifluoroacetic acid was removed in vacuo and the residue was dried in a desiccator over KOH to give 52 mg of the trifluoroacetate **14** as a colorless oil (quantitative yield). ^{1}H-NMR (CDCl$_3$): 1.41 (d, *J*=6.5 Hz, 3H, 5-CH$_3$), 1.95 (m, 2H, β,γ-Pro), 2.13 (m, 1H, γ-Pro), 2.7 (m, 1H), 3.45 (m, 2H, δ-Pro), 4.80 (dd, *J*=6.8, 1.8 Hz, 1H, C-5), 5.28 (dd, *J*=8.4, 5.1 Hz, 1H, α-Pro), 6.05 (dd, *J*=6.2, 1.7 Hz, 1H, C-3), 7.35 (dd, *J*=6.2, 1.9 Hz, 1H, C-4); ^{13}C-NMR (CDCl$_3$): all peaks were doubled 17.0 (q), 17.1 (q), 23.7 (t), 23.8 (t), 29.2 (q), 29.5 (q), 46.5 (d), 46.7 (d), 58.0 (d), 58.5 (d), 61.0 (d), 61.3 (d), 124.6 (d), 124.7 (d), 155.4 (s), 167.9 (s), 168.0 (s), 169.6 (s), 169.7 (s); LRLSIMS: 195; HRFABMS Calc. for $C_{15}H_{15}N_2O_2$: 195.1155, found: 195.1144 (Δmmu=1.1).

1-(*tert.*Butyloxycarbonylthreonyl-N-methylvalylprolyl)-5-methyl-2-pyrrolinone (15):

1-prolyl-5-methyl-2-pyrrolinone trifluoroacetate (**14**, 52 mg, 0.17 mMol) and Boc-Thr-MeVal-OH (54 mg, 0.17 mMol) were dissolved in 2 mL of absolute dichloromethane, PyBrOP® (170 mg, 0.36 mMol) and diisopropylethylamine (0.1 mL) were added and the mixture was stirred at room temperature for 8 h. The solvent was evaporated and the residue was redissolved in ethyl acetate and filtered over a column with silica gel. Final purification was achieved by HPLC on silicagel using ethyl acetate as solvent. The two diastereomers obtained during the lactam formation could be separated at this step. 36.3 mg (42.5%) of the desired compound and 8.6 mg (10.1%) of the diastereomer were isolated. Both compounds were colorless oils which appeared to be pure by TLC (ethylacetate:acetone:water 5:5:1) and ^{1}H-NMR. ^{1}H-NMR ($CDCl_3$): 0.89 (d, *J*=6.4 Hz, 3H, γ-Val), 1.17 (d, *J*=6.4 Hz, 3H, γ-Val), 1.26 (d, *J*=6.8 Hz, 3H, γ-Thr), 1.37 (s, 9H, Boc), 1.45 (d, *J*=6.8 Hz, 3H, 5-CH_3) 1.98 (m, 3H), 2.28 (m, 2H), 3.15 (s, 3H, N-Me), 3.68 (m, 2H, δ-Pro), 4.04 (dt, J=7.2, 5.4 Hz, 1H), 4.45 (d, *J*=7.2 Hz, 1H, α-Thr), 4.80 (q, *J*=6.8, 1.8 Hz, 1H, C-5), 5.03 (d, *J*=11.2 Hz, 1H, α-Val), 5.37 (d, *J*=6.6 Hz, 1H), 5.58 (dd, *J*=6.5, 3.7 Hz, 1H, α-Pro) 5.86 (d, *J*=9.2 Hz, 1H, N-H), 6.04 (dd, *J*= 6.0, 1.7 Hz, 1H, C-3), 7.20 (dd, *J*= 6.0, 2.1 Hz, 1H, C-4); ^{13}C-NMR ($CDCl_3$): 17.1 (q), 17.3 (q), 18.7 (q), 19.1 (q), 24.6 (t), 27.6 (d), 28.3 (t), 29.1 (q), 31.9 (q), 43.6 (q), 46.7 (d), 47.7 (d), 53.5 (d), 55.6 (d), 57.9 (d), 59.4 (d), 60.1 (d) , 61.4 (d), 80.1 (s), 125.4 (d), 154.1 (s), 168.6 (d), 169.7 (s), 170.8 (s), 172.2 (s); IR (film, KBr, cm^{-1}): 2971, 1701, 1628, 1245, 837; LRFABMS: 509 $[M+H]^+$; HRFABMS Calc. for $C_{25}H_{41}N_4O_7$: 509.2985, found: 509.2980 (Δmmu=0.5); $[\alpha]_D^{26}$= -150.5 (c=0.2, $CHCl_3$).

1-(Octanoyl -N-methylleucylthreonyl-N-methylvalylprolyl)-5-methyl-2-pyrrolinone (7):

1-(*tert.*butyloxycarbonyl-threonyl-N-methylvalyl-prolyl)-5-methyl-2-pyrrolinone (**15**, 20 mg, 0.04 mMol) was dissolved in 1 mL of trifluoroacetic acid and the solution was stirred at room temperature for 2 h. The trifluoroacetic acid was removed in vacuo and the residue was dried over KOH. The trifluoroacetate was dissolved in 1 mL of dichloromethane and octanoyl-N-methylleucine (11 mg, 0.04 mMol) was added. After addition of PyBroP® (37 mg, 0.08 mMol) and 20 μl of diisopropylethylamine the mixture was stirred for 24 h. The solution was diluted with 5 mL of dichloromethane and extracted twice with 1 N HCl , saturated $KHCO_3$ solution, washed with brine, dried over $MgSO_4$ and filtered. After the solvent was removed in vacuo, the product was purified by flash chromatography on silica gel using ethyl acetate followed by final purification on a reversed phase column with acetonitrile:water-7:3 as solvent. 20.8 mg (78.6 %) of a colorless oil were obtained. The compound appeared to be pure by HPLC, TLC (ethyl acetate:acetone:water 5:5:1) and ^{1}H-NMR. ^{1}H-NMR ($CDCl_3$): 0.77 (d, *J*=6.4 Hz, 3H, γ-Val), 0.81 (t, *J*=6.7 Hz, 3H, C-8, octanoic acid), 0.91 (d, *J*=6.8 Hz, 3H, δ-Leu), 0.95 (d, *J*=6.8 Hz, 3H, δ-Leu), 1.01 (d, *J*=6.4 Hz, 3H, γ-Val) 1.06 (d, *J*=6.7 Hz, 3H, γ-Thr), 1.23 (t, 2H), 1.28 (m, 10H, octanoic acid), 1.45 (d, *J*=6.8 Hz, 3H, 5-CH_3, pyrrolinone), 1.59 (ddd, *J*=14.2, 10.1, 4.5 Hz, 1H, β-Leu), 1.65 (ddd, *J*=14.2, 10.4, 4.8 Hz, 1H, β-Leu), 1.95 (m, 2H, γ-Pro), 1.87 (m, 1H) 2.35 (m, 1H), 2.83 (s, 3H, N-Me-Leu), 3.10 (s, 3H, N-Me-Val), 3.82 (m, 2H, δ-Pro), 4.10 (dq, *J*=2.2, 6.4 Hz, 1H, β-Thr), 4.76 (dd, *J*= 6.8, 1.8 Hz, 1H, C-5, pyrrolinone), 5.02 (d, *J*=6.4 Hz, 1H, α-Thr), 5.17 (t, *J*=10.4, 1H, α-Leu), 5.46 (dd, J=8.6, 5.3 Hz, 1H, α-Pro), 6.05 (dd, *J*=6.0, 1.8 Hz, 1H, C-3 pyrrolinone), 6.90 (d, *J*=9.1, 1H, N-H), 7.23 (dd, J=6.0, 2.1 Hz, 1H, C-4, pyrrolinone); ^{13}C-NMR (CDCl$_3$):14.0 (q), 17.1 (q), 18.3 (q), 18.8 (q), 19.1 (q), 22.1 (q), 22.6 (t), 22.9 (q), 24.6 (t), 25.0 (d), 27.5 (d), 28.8 (q), 29.0 (q), 29.3 (t), 29.7, 30.7, 30.9 (q), 31.7, 33.8 (t), 36.5 (d), 47.9 (d), 52.2 (d), 54.3 (d), 58.1 (d), 59.4 (d), 60.0 (d), 67.3 (d), 125.4 (d), 153.8 (d), 161.9 (s), 168.3 (s), 171.3 (s), 172.8 (s), 174.5 (s); IR (film, KBr, cm^{-1}): 2960, 2923, 1731, 1701, 1646, 1643, 1452, 1384; $[\alpha]_D^{26}$: -148.5° (c=0.2, CHCl$_3$); HRFABMS Calc. for $C_{35}H_{60}N_5O_7$: 662.4573, found: 662.4533 (Δmmu=4.0).

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References and Notes

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1. Abbreviations: Boc: *tert.*butoxycarbonyl-; BOP chloride: Bis(2-oxo-3-oxazolidinyl)phosphinic chloride; OBzl: benzyl ester, PyBOP®: Benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate; PyBroP®: Brom-tris-pyrrolidino-phosphonium hexafluorophosphate; Boc$_2$O: Di-*tert.*butyl-dicarbonate.

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