Pergamon

Tetrahedron Letters, Vol. 38, No. 13, pp. 2197-2200, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)00336-5

Synthesis of Microcolin Analogs Using Trimethylsilylated Lactams

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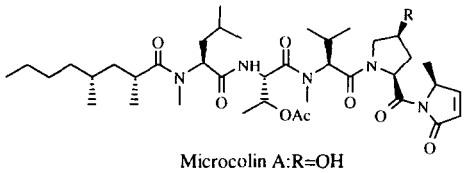
Abstract: The synthesis of microcolin analogs is described using an approach that could be of considerable practical interest for structure-activity studies on microcolin and related peptides. This synthetic pathway is more efficient than the methods reported to date, and allows the variation of the Xaa-pyrrolin-2-one unit of these molecules, which has been shown to be crucial for the biological activity. © 1997 Elsevier Science Ltd.

Microcolin A and B, two lipopeptides isolated from the blue-green alga *Lyngbya majuscula* expressed potent cytotoxicity and immunesuppressive properties.[1] Recently, we have reported the synthesis of an analog of microcolin B.[2]

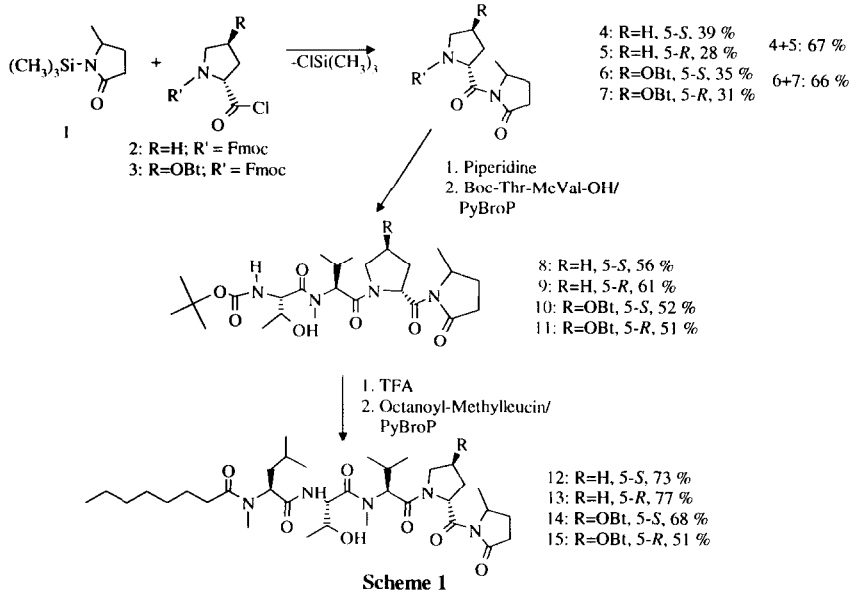


Microcolin A:R=OH
Microcolin B:R=H

The synthesis of the Pro-pyrrolin-2-one unit was achieved by acylation of Meldrum's acid with Boc-Pro-Ala-OH. The dipeptide was activated with isopropenyl chloroformate in the presence of 4-dimethylaminopyridine following a procedure originally described by Castro *et al.*[3] for N-protected amino acids and subsequently modified by Pettit and coworkers.[4] The reduction of the C=C bond and elimination of the OH group yielded the C-terminal Pro-lactam unit. In the meantime, the total synthesis of microcolin A has been described[5] applying an almost identical synthetic route for the Hyp-pyrrolinone part of this molecule starting from protected Hyp-Ala-OH. The synthetic routes using dipeptides to form the C-terminal unit are of considerable interest for the syntheses of compounds with an identical Xaa-pyrrolin-2-one unit. However, they are inconvenient for the syntheses of series of compounds with variations in this C-terminal part of the molecule. The degradation products of microcolin A have shown that this Hyp-pyrrolinone unit plays a crucial role for the biological activity.[1] Other biologically active natural products such as dolastatin 15[4] and majusculamide D[6] show similiar amino acid-lactam units. A more efficient way of modifying the C-terminal unit of these biologically active molecules is necessary in order to perform extensive structure-activity studies involving variations of the biologically important C-terminal unit. It would be desirable to form the Xaa-

lactam bond directly thus allowing the syntheses of a series of analogs simply by varying the amino acid preceeding the lactam. A 200-fold higher immunosuppressive activity of microcolin A compared to microcolin B[1b] demonstrates that these variations are of considerable interest for structure-activity studies.

In this paper, we describe a synthetic route to form the bond between amino acid and lactam directly thus allowing more efficient variations of both the lactam and the preceeding amino acid. It has been shown by Rothe and coworkers that the nucleophilicity of a lactam nitrogen can be increased by trimethyl silylation.[7] We have applied this methodology to enhance the lactam reactivity and successfully synthesized four saturated analogs of microcolin A and B (**12-15**, scheme 1) using commercially available racemic 5-methyl-pyrrolidin-2-one.

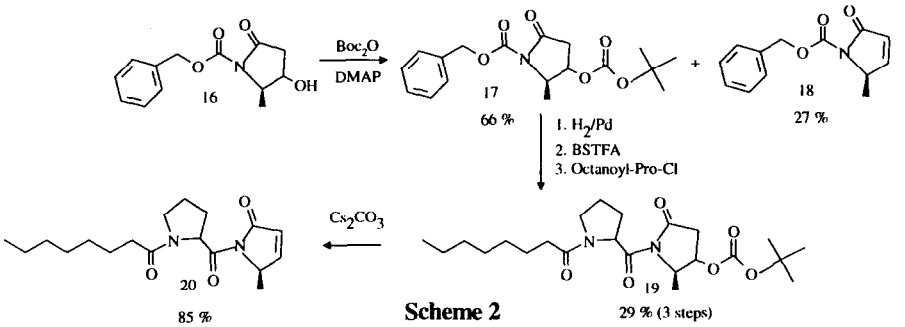


Scheme 1

The 5-methyl-pyrrolidin-2-one was silylated using chlorotrimethylsilane / triethylamine. The silylated lactam was purified by distillation under argon and then reacted with Fmoc-Pro-Cl (**2**)and Fmoc-Hyp(OBt)-Cl (**3**), which were synthesized from the Fmoc-Xaa-OH and PCl_5 in diethyl ether at -20°.[8] The chlorides were used since Pro and Hyp have very little tendency to racemize. The diastereomers 5-*R*- and 5-*S*-Fmoc-Xaa-5-methylpyrrolidin-2-one (**4-7**) were separated by HPLC. After the removal of the Fmoc protecting group with piperidine, the products were reacted with Boc-Thr-MeVal-OH in the presence of PyBroP. Removal of the N-terminal and OH protecting group with TFA and coupling with octanoyl-N-methylleucine lead to compounds **12-15**.

The OH-protected 4-hydroxy-pyrrolidin-2-ones, which are easily accessible from amino acids using Castro's methodology,[3] can be used as starting materials for this reaction. To demonstrate the use of this synthetic pathway for the synthesis of unsaturated analogs of microcolin B we have synthesized N-

(octanoylprolyl)-5-methyl-pyrrolin-2-one, a compound that we had previously synthesized using the dipeptide approach.[9] 4-(*tert.*butyloxycarbonyloxy)-5methylpyrrolin-2-one was synthesized from Z-Ala as described by Castro[3] followed by protection of the OH group with Boc_2O / DMAP and hydrogenation to remove the Z-group. Because we had experienced β-elimination of the *tert.*butyloxycarbonyloxy in the presence of a base[9] we used bis(trimethylsilyl)trifluoroacetamide (BSFTA) as the silylating agent instead of trimethyl chlorosilane / triethylamine to silylate the O-protected lactam. The silylated lactam was reacted with octanoylprolyl chloride. Finally the elimination of the *tert.* butyloxycarbonyloxy group was carried out with Cs_2CO_3 as described by Schmidt *et al.*[10] to yield the N-(octanoylprolyl)-5-methyl-pyrrolin-2-one **20**.



Scheme 2

Compound **20** showed moderate activity in antitumor assays: A 549 (4.95μg/mL) and P388 (2.04 μg/mL). No activity in the mixed lymphocyte reaction assay (MLR) has been detected.

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. PyBroP® was bought from Novabiochem and all other reagents were obtained from Aldrich Chemical Company, Inc. Octanoyl-N-methylleucine and *tert.* Butyloxycarbonylthreonyl-N-methylvaline were synthesized as described in Ref.2. (*S*)-N-benzyloxycarbonyl-4-*tert.* butyloxycarbonyloxy-5-methylpyrrolidin-2-one was synthesized as described in Ref 9.

Synthesis of **1:**

5-Methyl-2-pyrrolidinone (9.9 g, 0.1 Mol) was dissolved in 100 mL dichloromethane and chloro trimethylsilane (25.4 mL, 0.2 Mol) and triethylamine (27 mL, 0.2 Mol) was added. The solution was stirred for 4 hours and the precipitated triethylammonium hydrochloride was filtered under argon. The dichloromethane was removed by distillation and the product was distillled in vacuo to give the pure N-trimethylsilyl-5-methyl-2-pyrrolidinone in 78 % yield. ^{1}H-NMR ($CDCl_3$): 0.05 (s, 9H, $Si(CH_3)_3$) 1.34 (d, *J*=6.8, 3H, 5-Me), 1.80 (m, 1H, H4), 2.15 (m, 1H, H4), 2.58 (ddd, *J*=16.2, 9.1, 4.3, 1H, H3), 2.69 (ddd, *J*=16.2, 10.4, 4.3, 1H, H3), 4.32 (m, 1H, H5); bp.: 94°C (17 Torr).

General procedure for the synthesis of compounds **4-7**:

Fmoc-Xaa-OH (2 mMol) was dissolved in 15 mL diethyl ether and the solution was cooled to -20°. PCl_5 (2 mMol) was added under argon and the solution was stirred for 30 min. Afterwards, the solution was filtered under argon and the diethyl ether and the phosphorous oxychloride was removed by distillation. The remaining product was redissolved in diethyl ether, the solvent was evaporated and the residue was dried in vacuo. This procedure was repeated three times to remove traces of phosphorous oxychloride. The product was not characterized due to its moisture sensitivity but was dissolved in 25 mL anhydrous dichloromethane and directly reacted with trimethylsilyl-5-methyl-2-pryrrolidinone (2 mMol). The mixture was stirred at room temperature for 12 h. The solvent was evaporated, the reaction mixture was dissolved in ethyl acetate and filtered over basic aluminum oxide to remove unreacted material. The crude product was purified by HPLC on silica gel using ethyl acetate / heptane 2:1 to give the two diastereomers. Comparison of the ^{1}H chemical shift of the 5-methyl signal with those reported for dihydromicrocolin A and B[1b] was used to determine the stereo chemistry of the diastereomers.

4: ^{1}H-NMR($CDCl_3$): 7.83-7.24 (8H, Fmoc aromatic H), 5.48 (dd, *J* = 5.6, 9.2, 1H, α-Pro), 4.53-4.23 (m, 4H, Fmoc H3+H4, lactam H5), 3.84 (ddd, *J* = 15.8, 10.6, 7.1, 1H, δ-Pro), 3.48 (ddd, *J* = 15.8, 10.6, 6.5, 1H, δ-Pro), 2.70 (ddd, *J* = 16.8, 11.0, 6.3, 1H, H3), 2.45 (ddd, *J* = 16.8, 10.5, 7.3, 1H, H3), 2.37 (m, 1H, β-Pro), 2.20 (m, 1H, H4), 1.90 (bm, 3H, β- and γ-Pro), 1.75 (m, 1H, H4), 1.38 (d, *J* = 6.3, 3H, 5-Me); FABMS: 371 $[M+H]^+$; $[\alpha]_D^{20}$= -61.8° (c = 0.2, $CHCl_3$).

5: ^{1}H-NMR($CDCl_3$): 7.85-7.23 (8H, Fmoc aromatic H), 5.46 (dd, *J* = 5.4, 9.6, 1H, α-Pro), 4.56-4.25 (m, 4H, Fmoc H3+H4, lactam H5), 3.81 (ddd, *J* = 15.4, 10.2, 7.3, 1H, δ-Pro), 3.48 (ddd, *J* = 15.4, 10.6, 6.5, 1H, δ-Pro), 2.70 (ddd, *J* = 15.6, 11.3, 6.3, 1H, H3), 2.41 (ddd, *J* = 15.8, 10.5, 7.2, 1H, H3), 2.34 (m, 1H, β-Pro), 2.23 (m, 1H, H4), 1.94 (bm, 3H, β- and γ-Pro), 1.78 (m, 1H, H4), 1.21 (d, *J* = 6.3, 3H, 5-Me); FABMS: 371 [M+H]+; $[\alpha]_D^{20}$= -41.4° (c = 0.2, $CHCl_3$).

6: ^{1}H-NMR($CDCl_3$): 7.80-7.26 (8H, Fmoc aromatic H), 5.41 (dd, *J* = 2.4, 9.7, 1H, α-Hyp), 4.53-4.23 (m, 5H, Fmoc H3+H4, γ-Hyp, lactam H5), 3.83 (dt, *J* = 11.2, 2.3, 1H, δ-Hyp), 3.69 (dt, *J* = 11.2, 6.5, 1H, δ-Hyp), 2.70 (ddd, *J* = 14.7, 10.3, 5.8, 1H, H3), 2.48 (ddd, *J* = 14.7, 10.3, 7.2, 1H, H3), 2.40 (m, 1H, β-Hyp), 2.21 (m, 1H, H4), 1.96 (bm, 1H, β-Hyp), 1.75 (m, 1H, H4), 1.37 (d, *J* = 6.3, 3H, 5-Me) 1.25 (s,9H, OBut) ; FABMS: 491 $[M+H]^+$; $[\alpha]_D^{20}$= -93.4° (c = 0.2, $CHCl_3$).

7: ^{1}H-NMR($CDCl_3$): 7.80-7.26 (8H, Fmoc aromatic H), 5.43 (dd, *J* = 2.7, 9.4, 1H, α-Hyp), 4.57-4.23 (m, Fmoc H3+H4, 5H, γ-Hyp, lactam H5), 3.81 (dt, *J* = 11.4, 2.8, 1H, δ-Hyp), 3.67 (dt, *J* = 11.4, 6.9, 1H, δ-Hyp), 2.75 (ddd, *J* = 14.3, 9.8, 5.5, 1H, H3), 2.48 (ddd, *J* = 14.3, 10.1, 7.2, 1H, H3), 2.33 (m, 1H, β-Hyp), 2.08 (m, 1H, H4), 1.96 (bm, 2H, β-Hyp), 1.71(m, 1H, H4), 1.29 (s, 9H, OBut), 1.28 (d, *J* = 6.5, 3H, 5-Me) ; FABMS: 491 $[M+H]^+$; $[\alpha]_D^{20}$= -73.8° (c = 0.2, $CHCl_3$).

Characterization of **12** and **14**:

12: ^{1}H-NMR ($CDCl_3$): 0.77 (d, *J* = 6.3, 3H, γ-Val), 0.81 (t, *J* = 6.5, 3H, C-8, octanoic acid), 0.93 (d, *J* = 6.7, 3H, δ-Leu), 0.95 (d, *J*= 6.8, 3H, δ-Leu), 1.03 (d, *J* = 6.4, 3H, γ-Val) 1.06 (d, *J* = 6.6, 3H, γ-Thr), 1.23 (t, 2H), 1.28 (m, 10H, octanoic acid), 1.37 (d, *J* = 6.8, 3H, 5-CH_3,), 1.59 (ddd, *J* = 14.1, 10.3, 4.8, 1H, β-Leu), 1.65 (ddd, *J* = 14.1, 10.4, 4.3, 1H, β-Leu), 1.79 (m, 1H, H4), 1.87 (m, 1H, β-Pro),1.95 (m, 2H, γ-Pro), 2.28 (m, 1H, H4), 2.35 (m, 1H, β-Pro), 2.48 (ddd, 1H, *J* = 16.3, 10.4, 7.1, H3) 2.70 (ddd, 1H, *J* = 16.3, 10.4, 6.3, H3), 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, 1H, β-Thr), 4.76 (dd, *J* = 6.8, 1.8, 1H, H5), 5.02 (d, *J* = 6.4, 1H, α-Thr), 5.17 (t, *J* = 10.4, 1H, α-Leu), 5.46 (dd, *J* = 8.6, 5.3, 1H, α-Pro), 6.90 (d, *J* = 9.1, 1H, N-H); $[\alpha]_D^{26}$ = -134.3° (c = 0.2, $CHCl_3$); FABMS: 664 $[M+H]^+$.

14: ^{1}H-NMR ($CDCl_3$): 0.76 (d, *J* = 6.4, 3H, γ-Val), 0.84 (t, *J* = 6.2, 3H, C-8, octanoic acid), 0.93 (d, *J* = 6.4, 3H, δ-Leu), 0.96 (d, *J* = 6.4, 3H, δ-Leu), 1.04 (d, *J* = 6.4, 3H, γ-Val) 1.07 (d, *J* = 6.6, 3H, γ-Thr), 1.24 (t, 2H), 1.28 (m, 10H, octanoic acid), 1.31 (d, *J* = 6.8, 3H, 5-CH_3), 1.61 (ddd, *J* = 13.5, 10.3, 4.3, 1H, β-Leu), 1.65 (ddd, *J* = 13.5, 10.4, 4.6, 1H, β-Leu), 1.80 (m, 1H, H4), 1.96 (m, 1H, β-Hyp), 2.24 (m, 1H, H4), 2.39 (m, 1H, β-Hyp), 2.47 (ddd, 1H, *J* = 15.6, 10.3, 7.1, H3) 2.68 (ddd, 1H, *J* = 15.6, 9.5, 6.1, H3), 2.80 (s, 3H, N-Me-Leu), 3.10 (s, 3H, N-Me-Val), 3.79 (m, 2H, δ-Hyp), 4.12 (dq, *J* = 2.2, 6.4, 1H, β-Thr), 4.35 (m, 1H, γ-Hyp), 4.46 (dd, *J* = 6.3, 2.3, 1H, H5), 5.05 (d, *J* = 6.2, 1H, α-Thr), 5.13 (t, *J* = 9.6, 1H, α-Leu), 5.36 (dd, *J* = 9.6, 4.9, 1H, α-Hyp), 6.90 (d, *J* = 9.1, 1H, N-H); $[\alpha]_D^{26}$ = -112.1° (c = 0.2, $CHCl_3$); FABMS: 680 $[M+H]^+$.

Synthesis of **20**:

The benzyloxycarbonyl group in **16** was removed by hydrogenation and the deprotected lactam was dried in vacuum. The free lactam (200 mg, 2 mMol) was dissolved in anhydrous dichloromethane and BSTFA (1.06 mL, 4 mMol) was added. The mixture was stirred for 4 h. The solvent was removed by distillation and excess of BSTFA and MSTFA formed during the reaction were removed in high vacuo. The crude product was dissolved in 2 mL dichloromethane and reacted with 2mMol octanoylprolyl chloride (synthesized as described for the Fmoc-Xaa chlorides). The mixture was stirred at room temperature for 12 h and the solvent was evaporated. The reaction mixture was dissolved in ethyl acetate and filtered over basic aluminum oxide. The product was purified by silicagel HPLC using ethyl acetate. Elimination of the *tert.* butyloxycarbonyloxy group was carried out with Cs_2CO_3 in MeOH using the described method.[10]

Acknowledgement

We would like to thank Dr. David Powell of the University of Florida, Gainesville, for recording the mass spectral data and Dr. Ross Longley ans Ms. Pat Linley for performing the bioactivity tests. This publication is Harbor Branch Oceanographic Institution Contribution No. 1176.

References and Notes

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(*Received in USA* 9 *January* 1997; *revised* 5 *February* 1997; *accepted* 6 *February* 1997)