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Synthetic Studies on the Azinothricin Family of Antibiotics. 3. Enantioselective Synthesis of a Hexapeptide Precursor for Antitumour Antibiotic A83586C

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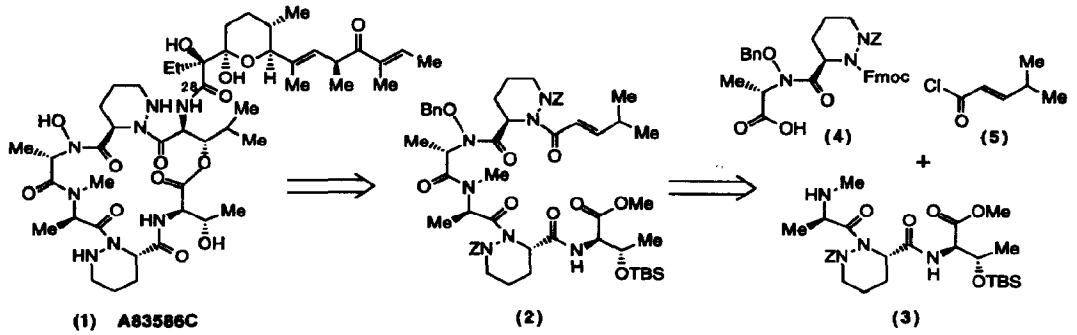
Abstract: A "3+2+1" fragment condensation strategy to a precursor of the hexapeptide found in antibiotic A83586C is described.

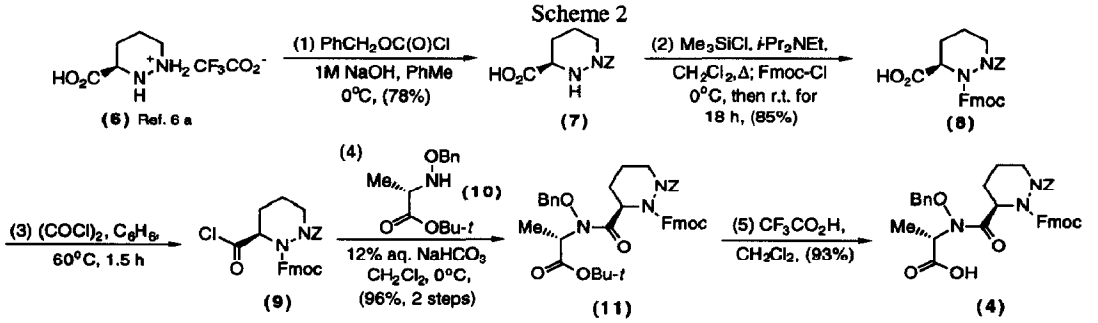
Whilst searching the culture filtrates of *Streptomyces karnatakensis* for biologically active metabolites, Smitka and coworkers[1] discovered a powerful new antibiotic that retarded the progression of a human T-cell leukaemia cell line, and which was active against Gram-positive bacteria. They termed this molecule A83586C (**1**), and determined its structure by single crystal X-ray analysis. A83586C is a rather unusual molecule on account of its novel 19-membered cyclodepsipeptide ring system, constructed from the rare building blocks of (*2S,3S*)-3-hydroxyleucine, D-threonine, *N*-methyl D-alanine, *N*-hydroxy-L-alanine, and the enantiomers of piperazic acid. Similar peptide sequences are found in the related antibiotics azinothricin,[2] variapeptin,[3] citropeptin,[3] L-156,602,[4] and verucopeptin.[5]

The mechanism by which A83586C exerts its antitumour effects is currently unknown, but studies[5] with the related cyclodepsipeptide, verucopeptin, suggest that members of this class might be operating through specific inhibition of DNA and RNA synthesis within cancer cells. In order to probe these biological effects further, particularly as a function of changing molecular structure, we decided to embark on a total synthesis of **1**.[6] In the present communication, we outline a synthesis of an advanced intermediate **2**, that we hope will serve as a precursor of the hexapeptide sequence found in A83586C, after application of an asymmetric dihydroxylation tactic. Our strategy for assembly of **2** is shown in Scheme 1, and is based upon a sequential [3+2+1] fragment condensation between the partially-protected intermediates **3** and **4**, and acid-chloride **5**.

Scheme 1

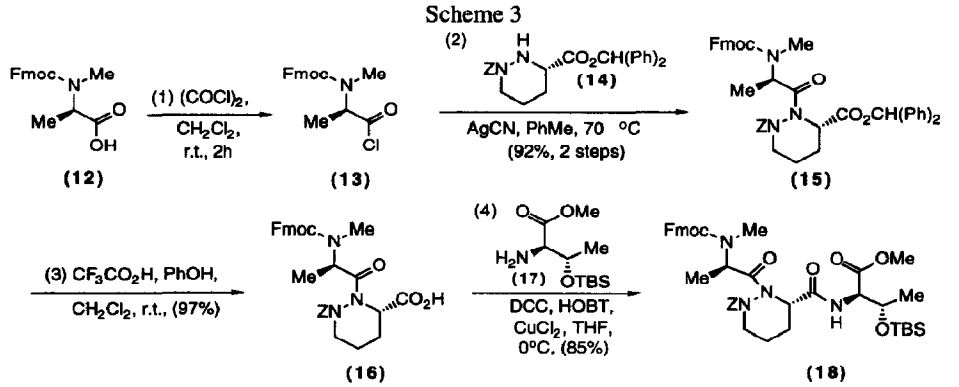


Scheme 2



The most satisfactory procedure for obtaining northern dipeptide **4** started with **6**[6a] and employed tactics used by Durette and Caldwell during their synthesis of L-156,602 (Scheme 2).[7b] This involved preparing the Fmoc-protected acid **8** from **7** by treatment with Me_3SiCl (2.0 equiv) and *i*-Pr_2NEt (1.7 equiv) in CH_2Cl_2 (*ca.* 0.43 M) at reflux for 2 h; Fmoc-Cl (1.3 equiv) was then added to the mixture in portions at 0°C, and the reactants allowed to stir at room temperature for 18 h. After aqueous work-up, **8** was converted to its acid chloride **9** (10.0 equiv of $(COCl)_2$, C_6H_6, 60°C, 1.5 h), and the latter condensed with α-hydroxamic acid derivative **10**[8] in a mixture of dichloromethane and 12% aqueous sodium bicarbonate.[9] The *t*-butyl ester was then detached from **11** by treatment with trifluoroacetic acid (15.0 equiv) in CH_2Cl_2 (*ca.* 0.6M) to give known acid **4**[7b] in 93% yield.

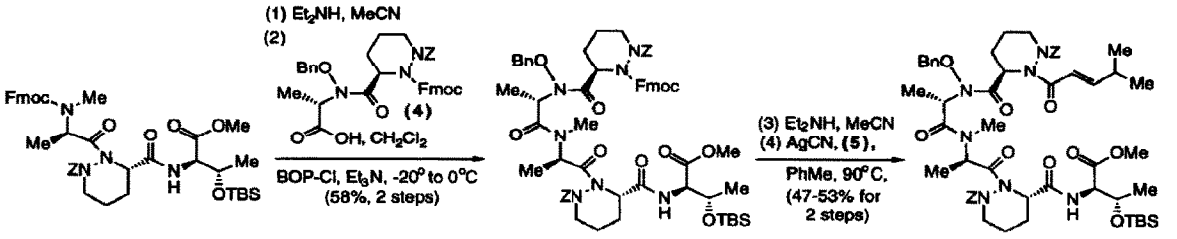
Scheme 3



Tripeptide **18** was synthesised according to the sequence shown in Scheme 3. The first step was preparation of acid chloride **13** from acid **12**[10] by reaction with oxalyl chloride (10.0 equiv) in dichloromethane at 25°C. After removal of excess reagents *in vacuo*, the crude acid chloride **13** (1.29 equiv) was immediately reacted with (*3S*)-piperazic acid derivative **14**[11] (1.0 equiv) in the presence of AgCN[7b] (1.61 equiv) in toluene at 70°C for 1h, to afford dipeptide **15** in 92% yield. Curiously, the Carpino[9] two-phase aq. $NaHCO_3$/acid chloride coupling conditions failed to deliver the desired dipeptide **15** from **13** and **14**. Likewise, other carboxyl activating agents such as DCC,[12] $Ph_2P(O)Cl$,[13] or $(PyS)_2/Ph_3P$[14] also failed to produce **15** when direct couplings were attempted between **12** and **14**. The latter failures can be attributed to the very poor nucleophilicity of the *N(2)*-atom in *N(1)*-acylated α-hydrazino acid derivatives. This is due to the strong electron-withdrawing effect of the *N(1)*-acyl unit, and the sterically hindered environment it creates around the *N(2)*-atom. The next step was cleavage of the diphenylmethyl ester[15] group from **15** with trifluoroacetic acid (12.5 equiv) and phenol (2.34 equiv) in dichloromethane; this furnished acid **16** in 97% yield. A wide range of coupling reagents and

conditions were then examined for establishing the amide linkage between **16** and D-threonine derivative **17**. The best results came when DCC (1.1 equiv), *N*-hydroxybenzotriazole (2.1 equiv), and cupric chloride[16] (0.11 equiv) were used in THF at 0°C. This regimen delivered tripeptide **18** in 85% yield after flash chromatography.

Scheme 4



At this juncture, the Fmoc-group was removed from **18**, and the unification of amine **3** with dipeptide **4** attempted (Scheme 4). To our dismay, none of the desired pentapeptide was formed when either DCC/HOBT[12,16] or $Ph_2P(O)Cl/Et_3N$[13] were used for carboxyl activation. However, when *freshly recrystallised* bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl)[17] (1.4 equiv) was employed along with triethylamine (2.9 equiv), the desired product **19** was isolated in 58% overall yield from **18**, with negligible racemisation. The Fmoc-group was then excised from pentapeptide **19**, and the partially liberated hydrazine reacted with acid chloride **5** (2.0 equiv) in toluene at 90°C, in the presence of AgCN[7b] (2.0 equiv). This afforded pure hexapeptide precursor **2** in 47-53% yield (2 steps), after reverse-phase preparative HPLC. The structure of **2** was confirmed by its high resolution mass spectrum, which contained an $(M+Na)^+$ peak at m/e 1120.5400 (Calcd. for $C_{57}H_{79}N_7O_{13}SiNa$, $(M+Na)^+$ 1120.5403), and by its 400 MHz ^1H NMR spectrum (DMSO-d_6) at 125°C. The latter exhibited the expected two olefinic double-doublets at δ 6.80 (J = 6.6, 15.5 Hz) and 6.13 (J =15.5, 1.0 Hz) which corroborated the presence of the (*E*)-α,β-unsaturated amide linkage. In addition, compound **2** gave a satisfactory C, H, and N combustion microanalysis for $C_{57}H_{79}N_7O_{13}Si$ (Calcd.: C, 62.33; H, 7.25; N, 8.93%. Found: C, 62.07; H, 7.29; N, 8.86%).

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18. All new compounds reported in this paper gave satisfactory 400 MHz ^1H and 100 MHz ^{13}C NMR and IR spectra, as well as HRMS and/or microanalyses within 0.4%. Selected physical data:
(11) is a foam: $[\alpha]_D$ -6.1° (*c* 1, CH_2Cl_2); IR (KBr): 3065 (w), 3044 (w), 2976 (m), 2946 (m), 1736 (s), 1710 (s), 1676 (s), 1452 (s), 1406 (s), 1367 (s), 1296 (s), 1254 (s), 1156 (s), 741 (s), 698 (m); 400 MHz ^1H NMR (Me_2SO-d_6) at 125°C: δ 7.88-7.15 (complex m, 18H), 5.19 (br s, 1H), 5.10 (br d, 1H), 4.99 (br d, 1H), 4.91 (br s, 2H), 4.51 (apparent br d, 3H), 4.19 (t, *J* = 5.9 Hz, 1H), 4.00 (br d, 1H), 2.85 (br t, 1H), 2.10 (br m, 1H), 1.80 (br, 1H), 1.65 (br, 1H), 1.41 (s superimposed on m, 10H), 1.36 (d, *J* = 6.4 Hz, 3H); FAB HRMS Calcd for $C_{42}H_{46}N_3O_8$ $(M+H)^+$ 720.3285; Found 720.3280; Anal. Calcd. for $C_{42}H_{45}N_3O_8$: C, 70.08; H, 6.30; N, 5.84%. Found: C, 69.96; H, 6.17; N, 5.78%. **(4)** is a foam; $[\alpha]_D$ -24.5° (*c* 1, CH_2Cl_2); IR (KBr): 3431 (br w), 3191 (br m), 3065 (m), 3034 (m), 2949 (m), 1711 (br s), 1680 (s), 1452 (s), 1409 (s), 1295 (s), 1255 (s), 1195 (s), 741 (s), 698 cm^{-1} (m); FAB HRMS Calcd for $C_{38}H_{38}N_3O_8$ $(M+H)^+$ 664.2659; Found: 664.2653. Anal. Calcd. for $C_{38}H_{37}N_3O_8$: C, 68.77; H, 5.62; N, 6.33%. Found: C, 68.89; H, 5.55; N, 6.17%. **(18)** is a foam: $[\alpha]_D$ +21.6° (*c* 0.25, CH_2Cl_2); IR (KBr) : 3400 (br w), 3325 (m), 2952, (m), 2924 (m), 2854 (w), 1753 (m), 1720 (s), 1688 (s), 1531 (m), 1451 (m), 1404 (m), 1249 (s), 1156 (s), 1091 (m), 838 (m), 740 cm^{-1}(m); 400 MHz ^1H NMR (Me_2SO-d_6) at 75°C: δ 8.23 (s), 7.85 (d, Ph), 7.79-7.2 (complex m, Ph), 5.00 (br), 4.75 (br), 4.55-4.15 (br), 3.96 (br), 3.64 (small s, OMe), 3.55 (s, OMe), 2.67 (br s, NMe), 1.96-1.3 (br), 1.3-0.94 (br), 1.05 (d, *J* = 6.2 Hz, Me), 0.82 (d, Me) partially superimposed on 0.81 (s, Bu-*t*), 0.00 (s, TBS-Me), -0.02 (s, TBS-Me); FAB HRMS Calcd. for $C_{43}H_{56}N_4O_9SiNa$ $(M+Na)^+$ 823.3714; Found: 823.3709; Anal. Calcd. for $C_{43}H_{56}N_4O_9Si$: C, 64.48; H, 7.05; N, 6.99%; Found: C, 64.22; H, 7.10; N, 6.90%. **(19)** is a foam: $[\alpha]_D$ -37.8° (*c* 0.4, CH_2Cl_2); 3395 (br w), 3318 (w), 3072 (w), 3030 (w), 2952 (m), 2924 (m), 2854 (w), 1722 (br s), 1673 (s), 1652 (s), 1525 (w), 1447 (m), 1405 (m), 1356 (m), 1244 (s), 1194 (m), 1082 (m), 836 (w), 751 (m), 737 (m), 688 (m); HRMS Calcd. for $C_{66}H_{81}N_7O_{14}SiNa$ $(M+Na)^+$ 1246.5508; Found: 1246.5504. Anal. Calcd. for $C_{66}H_{81}N_7O_{14}Si$: C, 64.74; H, 6.67; N, 8.01%. Found: C, 64.41; H, 6.74; N, 7.82%. **(2)** is a foam: $[\alpha]_D$ -55.0° (*c* 0.2, CH_2Cl_2); 400 MHz ^1H NMR (Me_2SO-d_6) at 125°C: δ 7.85 (br), 7.43-7.3 (complex m, Ph), 6.80 (2x dd, *J* =15.5, 6.6 Hz), 6.71 (smaller dd), 6.13 (dd, *J* = 15.5, 1.0 Hz, superimposed on small d), 5.76 (br m), 5.4-4.64 (complex br m), 4.47 (br m), 4.37 (br m), 4.34-3.74 (complex br m), 3.62 (s, OMe), 3.58 (s, OMe), 3.06 (br m), 2.93 (s), 2.40 (m), 2.08 (br), 1.82 (br m), 1.73 (br m), 1.46 (br m), 1.31 (br), 1.22 (d, *J* =7.0 Hz), 1.10 (d, *J* = 6.0 Hz), 1.04 (d, *J* = 6.2 Hz), 0.96 (apparent t), 0.86 (large s, Bu-*t*), 0.04 (large s, TBS-Me), 0.03 (small s, TBS-Me), 0.02 (large s, TBS-Me), -0.02 (small s, TBS-Me).

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