The Synthesis and Biological Evaluation of Novel Eunicellin Analogues

James E. P. Davidson,[a] Ryan Gilmour,[a] Sylvie Ducki,[b] John E. Davies,[a] Richard Green,[c] Jonathan W. Burton,*[a] Andrew B. Holmes*[a]

[a] Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge, CB2 1EW, UK
Fax +44(1223)334866; E-mail: abh1@cam.ac.uk

[b] Centre for Molecular Drug Design, Cockcroft Building, University of Salford, Salford, M5 4WT, UK

[c] GlaxoSmithKline, New Frontiers Science Park, Third Avenue, Harlow, Essex, CM19 5AW, UK

Received 15 March 2004

Dedicated to Professor Clayton H. Heathcock for his inspiration and example in organic chemistry

Abstract: The intramolecular Diels–Alder reaction of a 2,9-disubstituted hexahydro-$\Delta^{5,6}$-oxonin derivative **14** afforded two diastereomeric tricyclic eunicellin analogues **15** and **16**, which were desilylated to produce the alcohols **17** and **3**; these were found to be microtubule stabilising agents with activity in the micromolar range.

Key words: eunicellin, cycloaddition, lactone, microtubules

Eunicellin (**1**) and related diterpenoid marine natural products such as the antineoplastic sclerophytin A (**2**) have attracted considerable attention owing to their general cytotoxic properties.[1,2] A number of different synthetic strategies have been employed, ranging from the pioneering Prins-pinacol condensation-rearrangement combined with Nozaki–Hiyama–Kishi ring closure approach of Overman[3] to the furanone annelation combined with ring closing metathesis approach of Paquette[4] and the Lewis acid-mediated [4+3]-annulation of Molander.[5] Other important contributions have been made by Rainier,[6] Jung[7] and McIntosh.[8] The importance of synthesis in the revision of certain structures in this family has been demonstrated on several occasions.

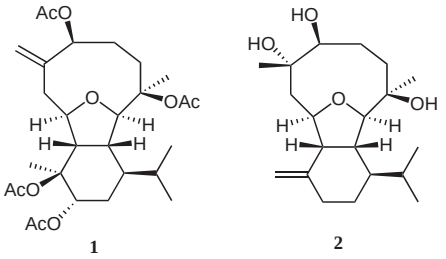
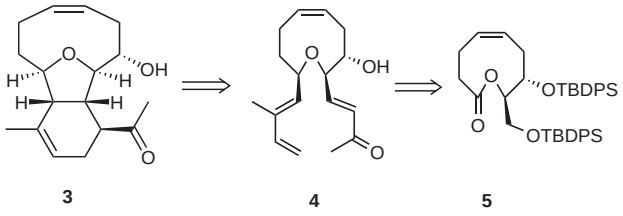


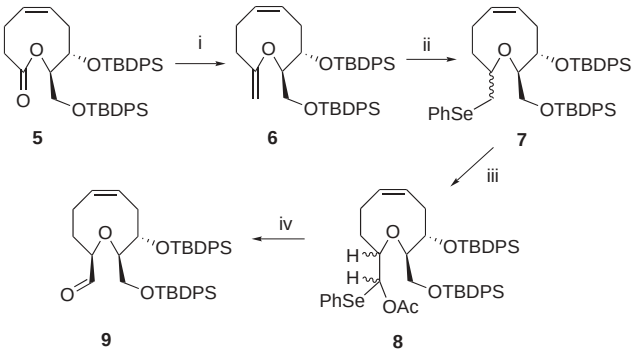
Figure 1 Eunicellin (**1**) and sclerophytin A (**2**)

Our strategy for the synthesis of the skeleton **3** (Scheme 1) represented by **1** and **2** (Figure 1) is based on the use of an intramolecular Diels–Alder reaction of a 2,9-disubstituted nine-membered ether derivative **4** which should be readily accessible from the corresponding $\Delta^{5,6}$-unsaturated 9-membered lactone precursor **5** which we have previously described.[9,10]



Scheme 1 Retrosynthetic analysis of the eunicellin skeleton **3**

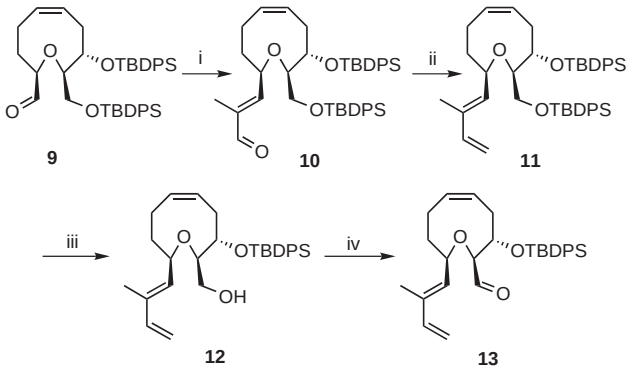
Petasis methylenation[11] of the lactone **5**[9,10] (Scheme 2) afforded the enol ether **6**. The enol ether was efficiently converted into the aldehyde **9** by a method adapted from that used in the synthesis of octahydrodeacetyldebromolaurencin.[12] Addition of phenylselenyl chloride to the enol ether **6** and subsequent reduction with lithium aluminium hydride gave a mixture of the phenylselenomethyl derivatives **7**. Selenoxide formation followed by Pummerer rearrangement and methoxide cleavage of **8** afforded the *cis*-2,9-disubstituted unsaturated oxonane **9**. The *cis*-configuration is predicted by molecular modelling to be the thermodynamically preferred, and was confirmed by NOE measurements, which are consistent with previous experience regarding the base-catalysed equilibration of related 2,9-disubstituted oxonane derivatives.[13]



Scheme 2 Synthesis of the 2,9-*cis*-disubstituted $\Delta^{5,6}$-oxonane. *Reagents and conditions*: i, Cp_2TiMe_2, toluene, 110 °C, 0.5 h, 83%; ii, PhSeCl, THF, then $LiAlH_4$, –78 °C, 52%; iii, *m*-CPBA, THF, NaOAc, Ac_2O, –78 °C → reflux; iv, MeOH, CH_2Cl_2, K_2CO_3, 16 h, 88% over two steps.

SYNLETT 2004, No. 8, pp 1434–1436
Advanced online publication: 08.06.2004
DOI: 10.1055/s-2004-825627; Art ID: Y02204ST

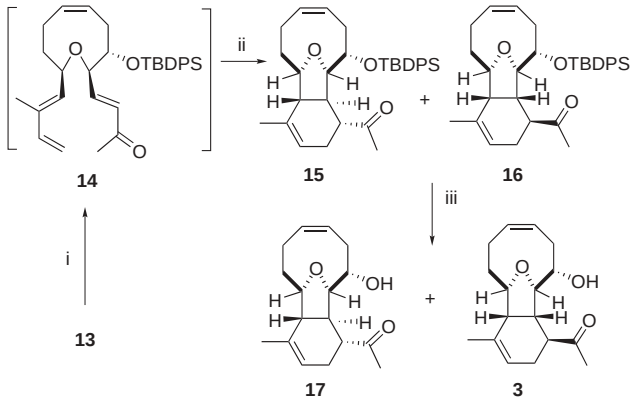
Synthesis of the diene component of the Diels–Alder precursor is illustrated in Scheme 3. The aldehyde **9** was converted by Wittig reaction into the *E*-enal **10**; Petasis methylenation[11] of the enal proved unsatisfactory, giving a mixture of products that were inseparable by HPLC. However, Wittig methylenation yielded the diene **11** in quantitative yield. This was deprotected to the alcohol and oxidised with perruthenate[14] to the aldehyde **13**.



Scheme 3 Synthesis of the aldehyde **13**. *Reagents and conditions*: i, 2-(Triphenylphosphoranylidene)propanal, toluene, 110 °C, 62%; ii, Ph_3PCH_3Br, *n*-BuLi, –78 °C, 2 h, 100%; iii, HF–pyridine, THF, 3 h, 89%; iv, TPAP, NMO, 4 Å molecular sieves, CH_2Cl_2, 0.5 h, 100%.

The aldehyde **13** was converted into the enone **14** by a Wittig reaction. Under the conditions employed the product **14** underwent an in situ intramolecular Diels–Alder reaction to afford two diastereomeric tricyclic adducts **15** and **16** in a 3:1 ratio and a combined overall yield of 84% (Scheme 4). Finally, cleavage of the silyl ether with HF–pyridine furnished the diastereomeric eunicellin analogues **17** and **3** in 77% and 64% yield, respectively.

The alcohol **17** derived from the major adduct **15** afforded crystals suitable for X-ray crystal structure analysis which revealed (Figure 2) that it corresponded to a non-natural eunicellin configuration, arising from one of the two possible *endo* transition states.



Scheme 4 Tandem Wittig/Diels–Alder sequence to prepare adducts **15** and **16**. *Reagents and conditions*: i, 1-Triphenylphosphoranylidene-2-propanone, toluene, 110 °C, 2 h, 84%; ii, heat; iii, HF (40% aq), MeCN, 48 h, **17** (77%), **3** (64%).

The parent natural products (**1** and **2**) possess a *cis-anti-cis*-relative stereochemistry of the bridgehead hydrogens in relation to the hydrogens at the angular positions that would arise from an *exo* transition state. Detailed ^{1}H NMR analysis[15] of the minor product **16** and comparison with the data for **15** suggests that the structure of **16** corresponds to the required natural configuration of the eunicellin skeleton, and efforts are in hand to adjust the diastereoselectivity of the intramolecular cycloaddition reaction.

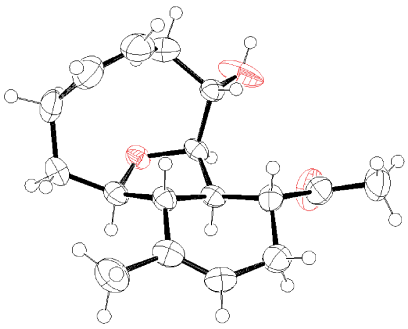


Figure 2 X-ray crystal structure of the alcohol **17**[16,17]

The effect of the new analogues **17** and **3** on the assembly of tubulin to form microtubules was assessed using paclitaxel™ as a reference (Table 1).[18] Both compounds were shown to induce microtubule assembly and stabilise them in the presence of $CaCl_2$.[19]

Table 1 Comparison of Tubulin Assembly Properties of **17** and **3** with that of Paclitaxel™.

Compound	ED_{90} (μM)[a]
Paclitaxel™	<0.5
3	3 ± 0.6
17	7 ± 1.4

[a] ED_{90}: Effective dose required to induce 90% tubulin assembly.[18]

The lower ED_{90} of **3** compared with **17** (Table 1), correlates with a more potent microtubule stabilising activity. This may be consistent with the fact that the minor adduct corresponds to the natural eunicellin configuration.

Acknowledgment

We thank EPSRC for financial support, provision of the Swansea National Mass Spectrometry Service, assistance with the purchase of the Rigaku AFC7R diffractometer, and a doctoral training award (RG). We thank GSK for the award of a studentship (JEPD), The Royal Society (University Research Fellowship to Dr J. W. Burton) and Dr S. J. Conway for helpful discussions. R. A. Robinson made early suggestions in this project.

References

(1) Kennard, O.; Watson, D. G.; Riva di Sanseverino, L.; Tursch, B.; Bosmans, R.; Djerassi, C. *Tetrahedron Lett.* **1968**, 2879.

(2) (a) Bernardelli, P.; Paquette, L. A. *Heterocycles* **1998**, *49*, 531. (b) Sung, P.-J.; Chen, M.-C. *Heterocycles* **2002**, *57*, 1705.

(3) (a) MacMillan, D. W. C.; Overman, L. E. *J. Am. Chem. Soc.* **1995**, *117*, 10391. (b) Overman, L. E.; Pennington, L. D. *Org. Lett.* **2000**, *2*, 2683. (c) MacMillan, D. W. C.; Overman, L. E.; Pennington, L. D. *J. Am. Chem. Soc.* **2001**, *123*, 9033. (d) Gallou, F.; MacMillan, D. W. C.; Overman, L. E.; Paquette, L. A.; Pennington, L. D.; Yang, J. *Org. Lett.* **2001**, *3*, 135. (e) Corminboeuf, O.; Overman, L. E.; Pennington, L. D. *Org. Lett.* **2003**, *5*, 1543. (f) Corminboeuf, O.; Overman, L. E.; Pennington, L. D. *J. Am. Chem. Soc.* **2003**, *125*, 6650.

(4) (a) Paquette, L. A.; Moradei, O. M.; Bernadelli, P.; Lange, T. *Org. Lett.* **2000**, *2*, 1875. (b) Friedrich, D.; Doskotch, R. W.; Paquette, L. A. *Org. Lett.* **2000**, *2*, 1879. (c) Bernardelli, P.; Moradei, O. M.; Friedrich, D.; Yang, J.; Gallou, F.; Dyck, B. P.; Doskotch, R. W.; Lange, T.; Paquette, L. A. *J. Am. Chem. Soc.* **2001**, *123*, 9021. (d) Friedrich, D.; Paquette, L. A. *J. Nat. Prod.* **2002**, *65*, 126.

(5) Molander, G. A.; St Jean, D. J. Jr.; Haas, J. *J. Am. Chem. Soc.* **2004**, *126*, 1642.

(6) Xu, Q.; Weeresakare, M.; Rainier, J. D. *Tetrahedron* **2001**, *57*, 8029.

(7) (a) Jung, M. E.; Pontillo, J. *J. Org. Chem.* **2002**, *67*, 6848. (b) Jung, M. E.; Pontillo, J. *Tetrahedron* **2003**, *59*, 2729.

(8) Chai, Y.; Vicic, D. A.; McIntosh, M. C. *Org. Lett.* **2003**, *5*, 1039.

(9) Congreve, M. S.; Holmes, A. B.; Hughes, A. B.; Looney, M. G. *J. Am. Chem. Soc.* **1993**, *115*, 5815.

(10) Anderson, E. A.; Davidson, J. E. P.; Harrison, J. R.; O'Sullivan, P. T.; Burton, J. W.; Collins, I.; Holmes, A. B. *Tetrahedron* **2002**, *58*, 1943.

(11) Petasis, N. A.; Bzowej, E. I. *J. Am. Chem. Soc.* **1990**, *112*, 6392.

(12) Burton, J. W.; Clark, J. S.; Derrer, S.; Stork, T. C.; Bendall, J. G.; Holmes, A. B. *J. Am. Chem. Soc.* **1997**, *119*, 7483.

(13) Carling, R. W.; Clark, J. S.; Holmes, A. B. *J. Chem. Soc., Perkin Trans. 1* **1992**, 83.

(14) Griffith, W. P.; Ley, S. V.; Whitcombe, G. P.; White, A. D. *Chem. Commun.* **1987**, 1625.

(15) **Wittig Elaboration of 13 and Intramolecular Cycloaddition of 14 to Give 15 and 16.** To a stirred solution of the aldehyde **13** (93 mg, 0.2 mmol) in toluene (15 mL) was added 1-triphenylphosphoranylidene-2-propanone (187 mg, 0.59 mmol). The resultant mixture was heated to reflux for 2 h 15 min, and then allowed to cool to ambient temperature. The solvent was removed in vacuo and purification by flash chromatography (CH_2Cl_2) yielded the title compounds **15** (64 mg, 63%) and **16** (21.4 mg, 21%) as colourless oils.
Data for **15**: R_f = 0.41 (CH_2Cl_2); $[\alpha]_D^{25}$ –24.6 (*c* 0.65 in $CHCl_3$). IR ($CHCl_3$): ν_{max} = 3022 (w), 3019 (m), 2932 (s), 2859 (m), 1713 (s) (CO), 1473 (m), 1428 (m), 1358 (m), 1111 (s), 1089 (m), 1043 (s), 1021 (m), 943 (w), 823 (m), 610 (w) cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 7.64–7.71 (4 H, m, Ar), 7.33–7.44 (6 H, m, Ar), 5.55–5.64 (1 H, m, H-6), 5.30–5.50 (1 H, br s, H-5), 5.20–5.24 (1 H, m, H-12), 4.65 (1 H, dd, *J* = 8.4 and 1.1 Hz, H-2), 3.95–4.03 (1 H, m, H-9), 3.48 (1 H, d, *J* = 8.4 Hz, H-3), 2.72–2.81 (1 H, m, H-10), 2.54 (1 H, dt, *J* = 11.0 and 6.8 Hz, H-14), 2.10–2.47 (4 H, m, H-1, H-4, H-13 and H-7), 2.02–2.11 (2 H, m, H-4 and H-8), 1.87–1.96 (1 H, m, H-13), 1.79 (3 H, s, CH_3CO), 1.67–1.75 (1 H, m, H-8), 1.66 (3 H, s, CH_3C=C) and 1.04 [9 H, s, $C(CH_3)_3$]. ^{13}C NMR (62.5 MHz, $CDCl_3$): δ = 209.2 (C=O), 136.0, 136.0, 134.8, 134.6, 130.9, 129.5, 129.5, 127.6 and 127.47 (Ar), 133.7 (C-11), 130.9 (C-6), 127.3 (C-5), 120.5 (C-12), 84.6 (C-2), 80.4 (C-9), 71.8 (C-3), 48.9 (C-14), 45.9 (C-1), 43.6 (C-10), 31.9 (C-4), 30.8 (C-13), 27.8 (CH_3CO), 27.5 (C-8), 27.0 [$C(CH_3)_3$], 21.4 (C-7), 21.2 (CH_3C=C) and 19.2 [$C(CH_3)_3$]. MS (EI): *m/z* (%) = 514 (20) [M^+], 491 (15), 472 (10), 457 (100) [M – *t*-Bu]$^+$, 215 (10), 199 (85), 135 (40) and 77 (20). $C_{33}H_{42}O_3Si$ requires [M]: 514.2903; found [M^+]: 514.2884.
Data for **16**: R_f = 0.55 (CH_2Cl_2), $[\alpha]_D^{25}$ +0.8 (*c* 0.76 in $CHCl_3$). IR ($CHCl_3$): ν_{max} = 2931 (s), 2859 (s), 1709 (s) (CO), 1472 (w), 1428 (m), 1170 (w), 1112 (s), 1071 (s), 999 (w), 824 (m), 612 (m) cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 7.68–7.73 (4 H, m, Ar), 7.34–7.45 (6 H, m, Ar), 5.51–5.59 (1 H, m, H-6), 5.40–5.50 (1 H, m, H-5), 5.31 (1 H, br s, H-12), 4.03 (1 H, t, *J* = 6.2 Hz, H-9), 3.84 (1 H, d, *J* = 5.8 Hz, H-2), 3.78 (1 H, dd, *J* = 9.0 and 2.9 Hz, H-3), 2.38–2.73 (4 H, m, H-4, H-10, H-7 and H-1), 2.20–2.28 (1 H, m, H-4), 2.07–2.20 (2 H, m, H-7 and H-8), 1.96–2.05 (1 H, m, H-13), 1.94 (3 H, s, CH_3CO), 1.84–1.92 (2 H, m, H-14 and H-13), 1.58–2.66 (1 H, m, H-8), 1.59 (3 H, s, CH_3C=C) and 1.07 [9 H, s, $C(CH_3)_3$]. ^{13}C NMR (62.5 MHz, $CDCl_3$): δ = 210.2 (C=O), 136.1, 136.0, 135.5, 130.8 and 127.5 (Ar), 134.1 (C-11), 129.6 (C-6), 126.4 (C-5), 118.7 (C-12), 90.3 (C-2), 84.5 (C-9), 74.1 (C-3), 48.5 (C-14), 45.5 (C-10), 41.5 (C-1), 31.2 (C-8), 30.6 (C-4), 28.7 (CH_3CO), 27.1 [$C(CH_3)_3$], 25.2 (C-13), 22.7 (CH_3C=C), 21.7 (C-7) and 19.3 [$C(CH_3)_3$]. MS (CI, NH_3): *m/z* (%) = 532 (10) [M + NH_4]$^+$, 515 (2) [M + H]$^+$, 437 (15), 259 (100). $C_{33}H_{46}NO_3Si$ requires [M]: 532. 3247. Found [M + NH_4]$^+$: 532.325.

(16) Crystal data for **17**: $C_{17}H_{24}O_3$, *M* = 276.36, trigonal, space group $P3_1$, (no. 144), *a* = *b* = 10.041 (3), *c* = 12.585 (5) Å, *α* = *β* = 90°, *γ* = 120°, *U* = 1098.8 (6) Å^3, *Z* = 3, μ(MoK_α) = 0.084 mm^{-1}, 1450 reflections measured at 150 (2) K using an Oxford Cryosystems Cryostream cooling apparatus, 1332 unique (R_{int} = 0.039); R_1 = 0.073, wR_2 = 0.189 [I>2σ(I)]; goodness-of-fit on F^2, *S* = 1.030. The structure was solved with *SHELXS-97* and refined with *SHELXL-97*[17] (CCDB 233639).

(17) Sheldrake, G. M. *SHELXS-97/SHELXL-97*; University of Göttingen: Germany, **1997**.

(18) Beumer, R.; Bayón, P.; Bugada, P.; Ducki, S.; Mongelli, N.; Sirtori, F. R.; Telser, J.; Gennari, C. *Tetrahedron* **2003**, *59*, 8803.

(19) Microtubules did not depolymerise at 10 °C.