Synthesis of a simplified analogue of eleutherobin *via* a Claisen rearrangement and ring closing metathesis strategy†

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The enantioselective synthesis of a simplified eleutherobin analogue 7 by ring closing metathesis (RCM) of the 2,9-divinyl-substituted tetrahydro-oxonin 5 is described; the analogue 7 and an advanced intermediate 15 revealed microtubule stabilising properties in the micromolar range.

Eleutherobin **1** (Fig. 1) has emerged as an exciting cytotoxic compound and was shown to possess microtubule stabilising properties similar to that of paclitaxel.[1,2] Its novel molecular architecture and its scarce availability from natural sources have already prompted the total synthesis of eleutherobin by both the Nicolaou[3] and Danishefsky groups.[4]

We demonstrate that a nine-membered medium ring lactone **3** available from our Claisen ring expansion methodology (Scheme 1‡)[5,6] can be elaborated to a simplified analogue of eleutherobin **7** that shows microtubule stabilising activity.

The design of our target was based upon comprehensive SAR studies on the sarcodictyins,[7] a class of compounds structurally related to eleutherobin. Thus, we elected to keep the crucial urocanic acid side chain attached to the core bicycle (see structure **7**). A key intermediate in the synthesis would be the aldehyde **4**,[6] which can be readily elaborated to a 2,9-divinyl derivative for ring closing metathesis[8,9] to the core bicycle **6**.

The synthesis of eleutherobin analogue **7** is depicted in Scheme 2.‡ From aldehyde **4**, Wittig methylenation afforded the alkene **8** (90%), which was then treated with buffered pyridinium

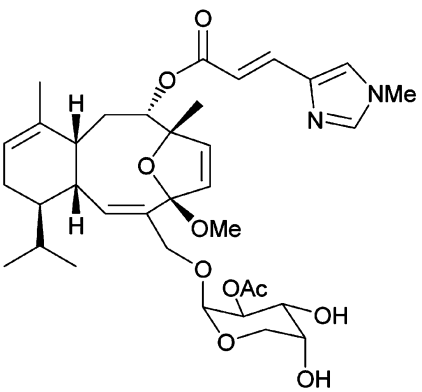
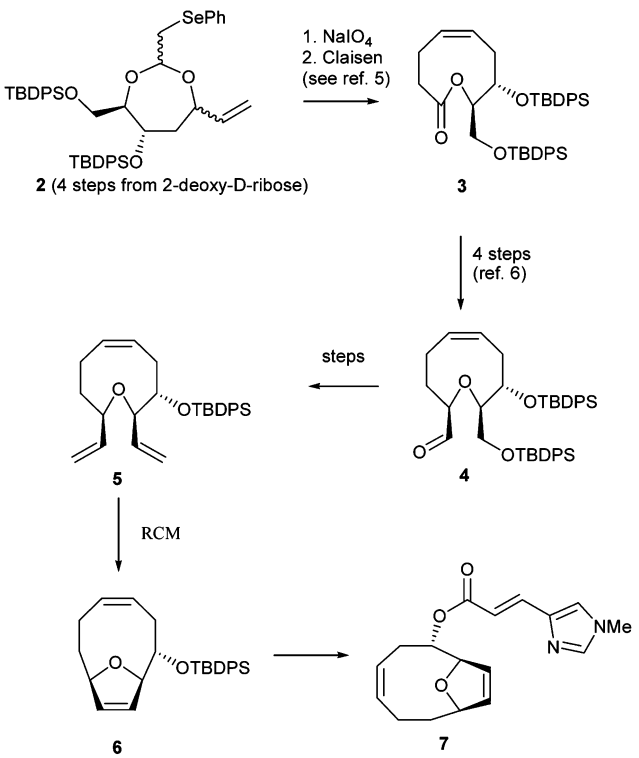


Fig. 1 Eleutherobin **1**.



Scheme 1‡

hydrofluoride to effect silyl group removal (89%). Our initial attempts used TBAF as the reagent for silyl deprotection, but this was found to be non-chemoselective and gave a diol. IBX oxidation then yielded an aldehyde (94%), which was subsequently methylenated to afford the triene **5** (93%). RCM of **5** using the first generation Grubbs catalyst[8] **10** yielded two compounds, the required fused bicyclic medium-ring ether **6** (69%) and the isomeric bis-cyclopentene **9** (22%).

To investigate whether the bis-cyclopentene was formed directly from the triene **5** or from the bicyclic compound **6**, the latter was resubjected to the RCM conditions. After 3 days, ^{1}H NMR analysis of the reaction mixture showed a 20% conversion to **9**. This result suggests that equilibration of **6** to the thermodynamically more stable **9** was possible, but the slow conversion suggested that **9** was formed directly from the triene **5** and not through **6** as an intermediate.

An alternative route to the synthesis of **6** was explored in order to avoid the formation of the bis-cyclopentene **9**. The strategy was to protect the interfering endocyclic ring double bond with an

† Electronic supplementary information (ESI) available: experimental procedure for the conversion of **5** into **6** and **9** and spectroscopic data for **6** and **7**. See http://www.rsc.org/suppdata/cc/b4/b413426e/

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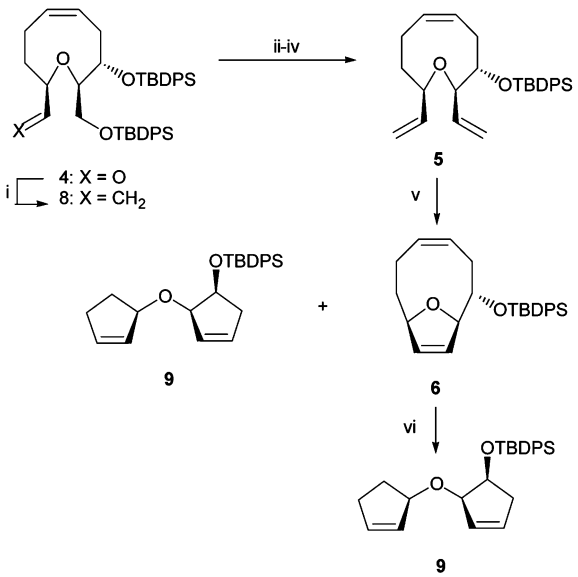
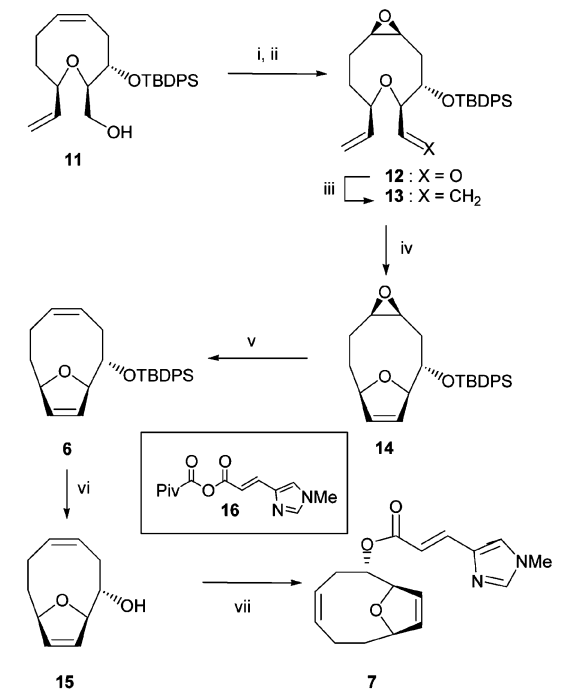


Scheme 2 Synthesis of **6**. *Reagents and conditions*: (i) CH_3PPh_3Br, *n*BuLi, THF, −78 °C, 20 min, 90%; (ii) HF·py, THF, pyridine, 16 h, 89%; (iii) IBX, DMSO, rt, 16 h, 94%; (iv) CH_3PPh_3Br, *n*BuLi, THF, −78 °C, 1 h, 93%; (v) $[(PCy_3)_2Cl_2Ru(=CHPh)]$ **10**, CH_2Cl_2, 18 h, 69% **6**, 22% **9**; (vi) $[(PCy_3)_2Cl_2Ru(=CHPh)]$ **10**, CH_2Cl_2, 72 h, 20% conv. IBX = *o*-iodoxybenzoic acid.‡

epoxide group, then perform the RCM reaction and then deoxygenate the epoxide back to the alkene **6** (Scheme 3‡).



Scheme 3 Epoxidation strategy and completion of **7**. *Reagents and conditions*: (i) *m*CPBA, THF, 0 °C → rt, 80% (major 63%); (ii) IBX, DMSO, rt, 20 h, 73%; (iii) CH_3PPh_3Br, *n*BuLi, THF, −78 °C, 20 min, 98%; (iv) $[(PCy_3)_2Cl_2Ru(=CHPh)]$ **10**, CH_2Cl_2, 18 h, rt, 86%; (v) WCl_6, *n*BuLi (2 equiv.), −78 °C → rt, 53%; (vi) TBAF, THF, 36 h, 0 °C → rt, 61%; (x) **16**, DMAP, NEt_3, THF, 20 h, 50%. IBX = *o*-iodoxybenzoic acid.‡

Starting from the alcohol **11**, epoxidation furnished a 3 : 1 mixture of epoxides (80%), which were separated by flash chromatography. Only the major isomer was carried forward in the synthesis. IBX oxidation (73%) gave aldehyde **12**, which was followed by a Wittig methylenation (98%) to give the epoxide protected RCM precursor **13**. RCM of **13** with the first generation Grubbs catalyst[8] **10** efficiently gave the desired ring-closed product **14** (86%). Epoxide removal was accomplished by treatment with WCl_6–*n*BuLi[10] to give the previously isolated bicyclic compound **6**, albeit in only a moderate yield (53%). Although the epoxide protection route was less efficient than our original route, the sequence served to confirm independently our assignments of the major and minor products of the RCM of **5**.

Completion of the synthesis of the eleutherobin analogue **7** was accomplished by silyl deprotection (61%) to give the crystalline alcohol **15** {61%, mp 85–86 °C (from ether)}, followed by coupling to the mixed anhydride **16**[3] (50%). The X-ray crystal structure (Fig. 2) of the bicycle **15**[11,12] provides insight into the conformation.

The eleutherobin analogue **7** and the advanced intermediate **15** were investigated for tubulin stabilising properties.[9] The results show that the analogue **7** exhibits microtubule stabilising properties, but with less potency ($ED_{90} = 6 \pm 1.2$ μM) than paclitaxel ($ED_{90} < 0.5 \pm 1.2$ μM). Nevertheless, this is an important result since the cyclohexene ring of eleutherobin is believed to be an important determinant for its antimitotic activity[7] and that fragment is not present in the analogue **7**. In addition, even the alcohol **15** was more potent ($ED_{90} = 3 \pm 1.2$ μM) than **7**. This result could imply that it is possible to design further eleutherobin analogues without the urocanic ester linkage and still maintain microtubule stabilising properties.[13]

In summary, the synthesis of a simplified eleutherobin analogue **7** has been accomplished. Biological data indicated that **7** exhibits microtubule stabilising properties in the micromolar range. In addition, compound **15**, which lacks the crucial urocanic ester side chain shows greater potency than **7**. Efforts to synthesise more potent analogues are currently under way.

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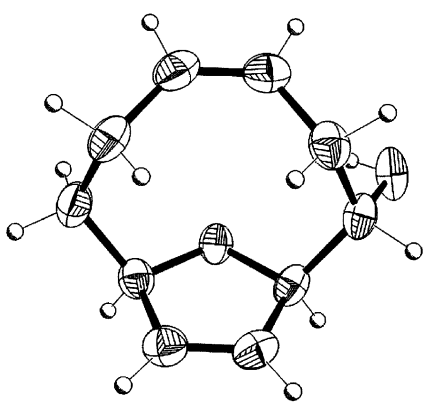

Fig. 2 X-Ray crystal structure of **15**.

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Notes and references

‡ All new compounds exhibited satisfactory spectroscopic and analytical and/or exact mass data.
§ CCDC 249051. See http://www.rsc.org/suppdata/cc/b4/b413426e/ for crystallographic data in .cif or other electronic format.

1 T. Lindel, P. R. Jensen, W. Fenical, B. H. Long, A. M. Casazza, J. Carboni and C. R. Fairchild, *J. Am. Chem. Soc.*, 1997, **119**, 8744.
2 B. H. Long, J. M. Carboni, A. J. Wasserman, L. A. Cornell, A. M. Casazza, P. R. Jensen, T. Lindel, W. Fenical and C. R. Fairchild, *Cancer Res.*, 1998, **58**, 1111.
3 K. C. Nicolaou, T. Ohshima, S. Hosokawa, F. L. van Delft, D. Vourloumis, J. Y. Xu, J. Pfefferkorn and S. Kim, *J. Am. Chem. Soc.*, 1998, **120**, 8674.
4 X. T. Chen, S. K. Bhattacharya, B. Zhou, C. E. Gutteridge, T. R. R. Pettus and S. J. Danishefsky, *J. Am. Chem. Soc.*, 1999, **121**, 6563.
5 M. S. Congreve, A. B. Holmes, A. B. Hughes and M. G. Looney, *J. Am. Chem. Soc.*, 1993, **115**, 5815.
6 J. E. P. Davidson, R. Gilmour, S. Ducki, J. E. Davies, R. Green, J. W. Burton and A. B. Holmes, *Synlett*, 2004, 1434.
7 K. C. Nicolaou, N. Winssinger, D. Vourloumis, T. Ohshima, S. Kim, J. Y. Xu and T. Li, *J. Am. Chem. Soc.*, 1998, **120**, 10814.
8 P. Schwab, M. B. France, J. W. Ziller and R. H. Grubbs, *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 2039.
9 For other ring closing metathesis approaches to eunicellin analogues and the evaluation of their tubulin stabilising properties see: R. Beumer, P. Bayon, P. Bugada, S. Ducki, N. Mongelli, F. R. Sirtori, J. Telser and C. Gennari, *Tetrahedron*, 2003, **59**, 8803 and references cited therein.
10 K. B. Sharpless, M. A. Umbreit, T. H. Nieh and T. C. Flood, *J. Am. Chem. Soc.*, 1972, **94**, 6538.
11 Crystal data for **15**: $C_{10}H_{14}O_2$, M = 166.21, tetragonal, space group $P4_32_12$, $a = b$ = 8.2007(4), c = 26.5896(9) Å, $\alpha = \beta = \gamma$ = 90 °, U = 1788.19(14) Å^3, Z = 8, μ(Mo–Kα) = 0.084 mm^{-1}, 4922 reflections measured at 180(2) K using an Oxford Cryosystems Cryostream cooling apparatus, 1997 unique (R_{int} = 0.066); R_1 = 0.0532, wR_2 = 0.118 [$I > 2\sigma(I)$]; goodness-of-fit on F^2, S = 1.187. The structure was solved with SHELXS-97 and refined with SHELXL-97[12].§
12 G. M. Sheldrick, *SHELXS-97*/*SHELXL-97*, University of Göttingen, Germany, 1997.
13 Microtubules did not depolymerise at 10 °C.

