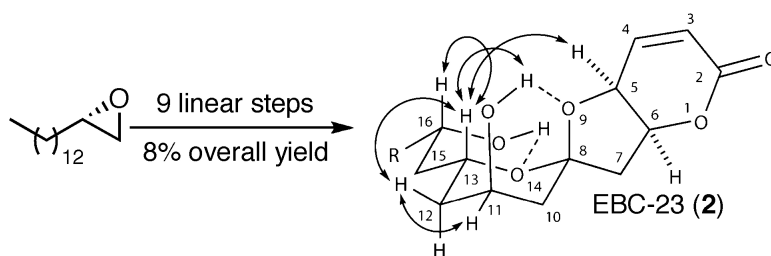


## Structure and Absolute Stereochemistry of the Anticancer Agent EBC-23 from the Australian Rainforest

Lin Dong, Victoria A. Gordon, Rebecca L. Grange, Jenny Johns, Peter G. Parsons, Achim Porzelle, Paul Reddell, Heiko Schill, and Craig M. Williams

*J. Am. Chem. Soc.*, **2008**, 130 (46), 15262-15263 • DOI: 10.1021/ja807133p • Publication Date (Web): 25 October 2008

Downloaded from <http://pubs.acs.org> on February 8, 2009



### More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

[View the Full Text HTML](#)



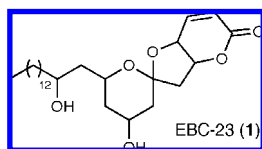
## Structure and Absolute Stereochemistry of the Anticancer Agent EBC-23 from the Australian Rainforest

Lin Dong,<sup>†</sup> Victoria A. Gordon,<sup>§</sup> Rebecca L. Grange,<sup>†</sup> Jenny Johns,<sup>‡</sup> Peter G. Parsons,<sup>‡</sup>  
Achim Porzelle,<sup>†</sup> Paul Reddell,<sup>§</sup> Heiko Schill,<sup>†</sup> and Craig M. Williams<sup>\*,†</sup>

School of Molecular and Microbial Sciences, University of Queensland, Brisbane, Queensland, Australia,  
Queensland Institute of Medical Research, PO Royal Brisbane Hospital, Brisbane, Queensland, Australia, and  
EcoBiotics Ltd, Yungaburra, Queensland, Australia

Received September 15, 2008; E-mail: c.williams3@uq.edu.au

Australian tropical rainforests are megadiverse and offer an opportunity for discovery of new bioactive compounds. During a screening program to identify biologically active candidates as potential treatments for cancer, the spiroacetal EBC-23 (**1**) was found in the fruit of *Cinnamomum laubatii* (family Lauraceae).<sup>1</sup> EBC-23 (**1**) displayed noticeable in vitro anticancer activity against a range of cancer cell lines [i.e., melanoma (MM96L), breast carcinoma (MCF7), prostate cancer (DU145)]. More significantly, xenografts of a human prostate tumor cell line (DU145) in immunodeficient mice when treated with **1** inhibited tumor growth with no observable side effects, suggesting the potential for the treatment of refractory solid tumors in adults.<sup>1</sup> Critical to the development of this lead candidate would be an implicit understanding of its chemical structure; however, only software generated<sup>2</sup> bond connectivity (i.e., **1**) was reported.<sup>1</sup> It was therefore imperative to confirm the gross structure and deduce both relative and absolute stereochemistry of this unique fused  $\alpha,\beta$ -unsaturated lactone spiroacetal.



Comparison of **1** with the known small lactone natural product osumundalactone<sup>3</sup> and the synthetic *syn*-isomer<sup>4</sup> confirmed the presence of a *syn* disubstituted lactone. Utilizing NOESY and 1D NOE experiments H5 ( $\delta_H$  4.5) correlated with H13 ( $\delta_H$  4.4), which in turn correlated with H16 ( $\delta_H$  3.8). This established the spiroacetal carbon (C8) as *EE*, and the side chain hydroxyl was  $\alpha$  configured. Even though a *syn* correlation was present between H13 ( $\delta_H$  4.4) and H12 $\beta$  ( $\delta_H$  1.8) the corresponding correlation to H11 ( $\delta_H$  4.1) was ambiguous. When a more diluted sample was measured by NMR a sharp doublet ( $\delta_H$  3.05) and singlet ( $\delta_H$  2.95) appeared for the two hydroxyl groups. With this development NOE correlations between H13 ( $\delta_H$  4.4), the C11 hydroxyl hydrogen, and H16 ( $\delta_H$  3.8) defined both configurations at C11 and C16 (i.e., H16 $\beta$ ) suggesting structure **2** (Figure 1).

Total synthesis was now key to authenticating structure **2** and determining absolute stereochemistry; however, six chiral centers partly imbedded in a unique fused  $\alpha,\beta$ -unsaturated lactone consisting of an acid labile spiroacetal unit required careful synthetic planning. Based on a plausible biosynthetic pathway, the retrosynthetic analysis initiated by ring opening of the spiroacetal moiety

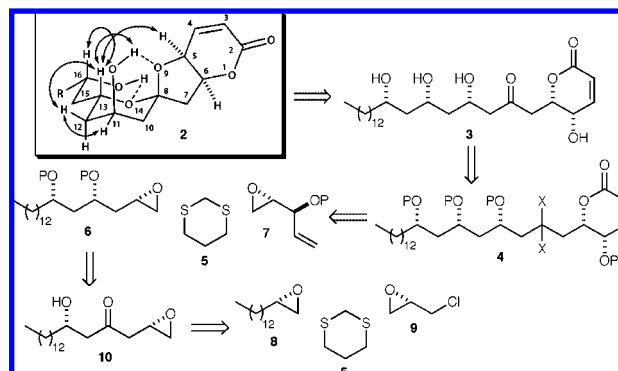


Figure 1. Deduced structure **2** and retrosynthetic analysis.

arrived at the polyketide **3**. The keto functionality in **3** serves as an acyl equivalent junction (i.e., **4**) potentially suited to a Tietze<sup>5</sup>–Smith<sup>6</sup> linchpin (i.e., dithiane **5**) convergence strategy (i.e., **6** and **7**). Ring closing metathesis (RCM) was employed as a feasible choice for lactone installation via epoxide **7**. Left hand fragment **6** requires significant stereochemical flexibility built into the synthetic approach in the event the relative stereochemical assignment of **2** is incorrect or structure–activity relationship studies are deployed. To achieve this goal linchpin methodology is again considered from enantiopure epoxides **8** and **9**, which would allow construction of ketone **10** in either enantiopode or desired diastereoisomer (i.e., **6**) selectively (Figure 1).

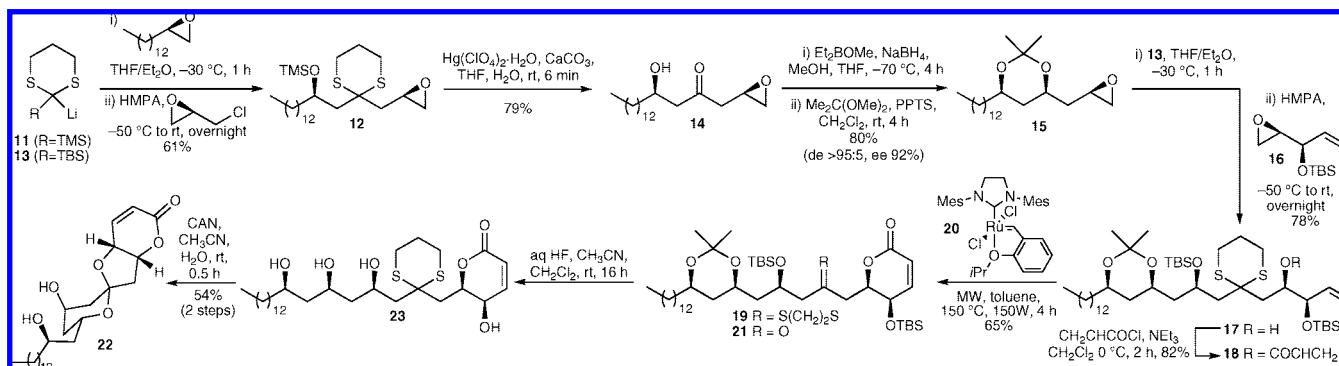
The all *R* isomer was targeted in the first instance. Linchpin coupling of the anion of TMS-dithiane (**11**) with (*R*)-pentadecene oxide,<sup>7</sup> and then (*R*)-epi-chlorohydrin afforded the disubstituted dithiane **12** in 61% yield. Although the TBS derivative (**13**) is reported to outperform **11** in linchpin reactions (i.e., affording higher yields),<sup>8</sup> *O*-TMS protection (and not TBS protection) was fortuitously found to undergo simultaneous deprotection when the keto functionality was unmasked [Hg(ClO<sub>4</sub>)<sub>2</sub>], giving hydroxyketone **14** in high yield (Scheme 1). *Syn* selective reduction,<sup>9</sup> followed by ketal protection,<sup>10</sup> proceeded smoothly in 80% yield (over 2 steps) affording the desired left-hand fragment **15** (i.e., *RRR* enantiomer of **6**). The second linchpin coupling, that is, reaction of **13** and **15** with **16**,<sup>11</sup> gave the coupled product **17** in 78% yield. Reaction of **17** with acryloyl chloride proceeded smoothly affording acrylate **18** in 82% yield. Surprisingly, RCM required considerable effort to facilitate the formation of the desired lactone (i.e., **19**). Six RCM catalysts (**A**–**E**,<sup>14a–e</sup> **20**<sup>14f</sup>) were extensively investigated, but only the Hoveyda–Grubbs second generation catalyst (i.e., **20**) under microwave irradiation afforded lactone **19** in substantial yield (65%). Even though RCM reactions have on occasion been performed in the presence of a dithiane ring system,<sup>15</sup> the

<sup>†</sup> University of Queensland.

<sup>§</sup> EcoBiotics Ltd.

<sup>‡</sup> PO Royal Brisbane Hospital.

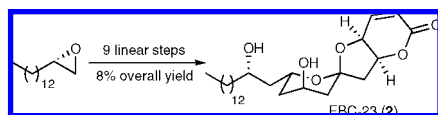
Scheme 1. Total Synthesis of ent-EBC-23 (22)



unprecedented difficulties described herein are most likely attributed to the close proximity of S atoms to the reaction center allowing S coordination to Ru preventing catalyst turnover, an aspect of RCM requiring further development.<sup>16</sup> All attempts to remove the dithiane ring at earlier stages (i.e., **17** and **18**) failed, as did suitable alternative lactone formation protocols. With the carbon backbone in place (i.e., lactone **19**) deprotection cyclization strategies could now be investigated. The first approach commenced with dithiane (i.e., **19**) deprotection (95%). However, cyclization of the subsequent ketone (i.e., **21**) with a variety of acids (i.e., aq HF, CSA) gave unacceptably low yields of the target (i.e., **22**) along with a plethora of unidentified products. Direct methods<sup>17</sup> for conversion of **19** to **22** (i.e., HF·py, aq HF or HCl) provided little improvement. Fortunately, following the work of Nakata,<sup>18</sup> TBS and acetonide deprotection performed with aq HF afforded the polyol **23**, which on treatment with CAN<sup>19</sup> revealed the target in 54% yield over two steps (PIFA<sup>20</sup> failed) (Scheme 1). Considering the effort required to drive spiroacetal formation with a myriad of acid promoters, and the fact that CAN performed this task extremely well, presumably mediated by metal (i.e., Ce) templated preorganization,<sup>21,22</sup> suggests CAN should be utilized more often for such transformations.

Comparison of the optical rotation of both the synthetic material **22** (−16.6) and the natural material (+16.6) confirmed the non-natural enantiomer had been synthesized. The synthesis was then repeated starting from (*S*)-pentadecene oxide (Scheme 2), which afforded synthetic material (+16.3) matching (<sup>1</sup>H and <sup>13</sup>C NMR are identical) both the relative and absolute stereochemistry of natural EBC-23 (**2**) (Figure 1).

Scheme 2. Total Synthesis of EBC-23 (2)



In conclusion, the deduced structure of the prostate anticancer agent **2** has been completely matched in terms of bond connectivity and relative and absolute stereochemistry. Key to this exercise were extensive NOE experiments and chemical synthesis of both enantiopodes. Total synthesis further demonstrated the value of linchpin technology, provided valuable insights into RCM reactions, and allows for significant stereochemical flexibility to initiate structure–activity relationship studies.

**Acknowledgment.** We thank EcoBiotics Ltd and the University of Queensland for financial support; Prof. W. Kitching for constructive discussions; Dr. L. Lambert for assistance with NMR experiments; Dr. A. Savchenko for attempts to crystallize EBC-23.

**Supporting Information Available:** Full experimental and characterization data, <sup>1</sup>H and <sup>13</sup>C NMR spectra, complete ref 4, and structures of RCM catalysts A–E. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## References

- (1) Reddell, P. W.; Gordon, V. A. WO 2007070984 A1 20070628 PCT Int. Appl. 2007.
- (2) ACD/Structure Elucidator, Advanced Chemistry Development, Inc., Toronto ON, Canada.
- (3) Hashimoto, T.; Arakawa, T.; Tanaka, M.; Asakawa, Y. *Heterocycles* **2002**, *56*, 581–588.
- (4) Ley, S. V.; et al. *J. Chem. Soc., Perkin Trans. 1* **1991**, 667–692.
- (5) Tietze, L. F.; Geissler, H.; Gewert, J. A.; Jakobi, U. *Synlett* **1994**, 511–512.
- (6) Smith, A. B., III; Adams, C. M. *Acc. Chem. Res.* **2004**, *37*, 365–377.
- (7) (*R*)- and (*S*)-Pentadecene oxide were obtained from (*R*)- and (*S*)-epichlorohydrin using the method of Lepoittevin; see: Choukchou-Braham, N.; Asakawa, Y.; Lepoittevin, J.-P. *Tetrahedron Lett.* **1994**, *35*, 3949–3952.
- (8) Smith, A. B., III; Pitram, S. M.; Boldi, A. M.; Gaunt, M. J.; Sfougatakis, C.; Moser, W. H. *J. Am. Chem. Soc.* **2003**, *125*, 14435–14445.
- (9) Chen, K.-M.; Hardtmann, G. E.; Prasad, K.; Repic, O.; Shapiro, M. J. *Tetrahedron Lett.* **1987**, *28*, 155–158.
- (10) Lipshutz, B. H.; Kozlowski, J. A. *J. Org. Chem.* **1984**, *49*, 1149–1151.
- (11) Acquisition of **16** was achieved through direct transformation of a known epoxide,<sup>12</sup> via Mitsunobu inversion,<sup>13</sup> subsequent hydrolysis, and TBS protection.
- (12) For example: Schreiber, S. L.; Schreiber, T. S.; Smith, D. B. *J. Am. Chem. Soc.* **1987**, *109*, 1525–1529.
- (13) Albert, B. J.; Sivaramakrishnan, A.; Naka, T.; Czaicki, N. L.; Koide, K. *J. Am. Chem. Soc.* **2007**, *127*, 2648–2659.
- (14) (a) Trnka, T. M.; Grubbs, R. H. *Acc. Chem. Res.* **2001**, *34*, 18–29. (b) Scholl, M.; Ding, S.; Woo Lee, C.; Grubbs, R. H. *Org. Lett.* **1999**, *1*, 953–956. (c) Kingsbury, J. S.; Harrity, J. P. A.; Bonitatebus, P. J. J.; Hoveyda, A. H. *J. Am. Chem. Soc.* **1999**, *121*, 791–99. (d) Jafarpour, L.; Schanz, H.-J.; Stevens, E. D.; Nolan, S. P. *Organometallics* **1999**, *18*, 5416–5419. (e) van der Schaaf, P. A.; Hafner, A.; Mühlebach, A. US 640719 02002. (f) Garber, S. B.; Kingsbury, J. S.; Gray, B. L.; Hoveyda, A. H. *J. Am. Chem. Soc.* **2000**, *122*, 8168–8179.
- (15) For example, see: (a) Fürstner, A.; Fasching, B.; O’Neil, G. W.; Fenster, M. D. B.; Godbout, C.; Ceccon, J. *Chem. Commun.* **2007**, *304*, 5–3047. (b) Nicolaou, K. C.; Kofits, T. V.; Vyskocil, S.; Petrovic, G.; Ling, T.; Yamada, Y. M. A.; Tang, W.; Frederick, M. O. *Angew. Chem., Int. Ed.* **2004**, *43*, 4318–4324. (c) Garbaccio, R. M.; Stachel, S. J.; Baeschlin, D. K.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2001**, *123*, 10903–10908.
- (16) Smulik, J. A.; Giessert, A. J.; Diver, S. T. *Tetrahedron Lett.* **2002**, *43*, 209–211.
- (17) Aho, J. E.; Pihko, P. M.; Rissa, T. K. *Chem. Rev.* **2005**, *105*, 4406–4440.
- (18) Terauchi, T.; Terauchi, T.; Sato, I.; Tsukada, T.; Kanoh, N.; Nakata, M. *Tetrahedron Lett.* **2000**, *41*, 2649–2653.
- (19) Ho, T.-L.; Ho, H. C.; Wong, C. M. *J. Chem. Soc., Chem. Commun.* **1972**, 791.
- (20) (a) Peuchmaur, M.; Wong, Y.-S. *Synlett* **2007**, 2902–2906. (b) Jacobs, M. F.; Glenn, M. P.; McGrath, M. J.; Zhang, H.; Brereton, I.; Kitching, W. *ARKIVOC [online computer file]* **2001**, 2 (vii), 114–137.
- (21) Smith, A. B., III; Doughty, V. A.; Lin, Q.; Zhuang, L.; McBriar, M. D.; Boldi, A. M.; Moser, W. H.; Murase, N.; Nakayama, K.; Sobukawa, M. *Angew. Chem., Int. Ed.* **2001**, *40*, 191–195.
- (22) (a) Evans, D. A.; Trotter, B. W.; Coleman, P. J.; Coté, B.; Dias, L. C.; Rajapakse, H. A.; Tyler, A. N. *Tetrahedron* **1999**, *55*, 8671–8726. (b) Evans, D. A.; Coleman, P. J.; Diaz, L. C. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2738–2741.

JA807133P