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Natural products with taxol-like anti-tumor activity: Synthetic approaches to eleutherobin and dictyostatin*

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Abstract: Eleutherobin and dictyostatin are antimitotic compounds which exert their cytotoxic activity by a taxol-like mode of action, i.e., hypernucleating tubulin assembly and interfering with the dynamic instability of the cytoskeleton during mitosis. A formal total synthesis of eleutherobin was accomplished by accessing a key intermediate reported by Danishefsky and coworkers in their 1998 synthesis of the natural product. The key step of our strategy, used for obtaining the [8.4.0] fused bicyclic ring system, is a ring-closing metathesis (RCM) reaction of a densely functionalized diene under forcing conditions, using Grubbs' second-generation catalyst. Synthetic approaches to dictyostatin are also described, and in particular the preparation of the C15–C23 fragment of the macrolide, containing 5 of its 11 stereocenters.

Keywords: antitumor agents; microtubule-stabilizing agents; natural products; ring-closing metathesis; stereocontrol; total synthesis.

INTRODUCTION

Cancer is a group of diseases characterized by the disregulate proliferation of abnormal cells that invade and disrupt surrounding tissues. Being the second leading cause of death in the United States and in most parts of Europe, its social and economical impact is overwhelming. While surgery is still the first and most effective treatment of cancer, chemotherapy is often used as a complement to surgical intervention, in combination with other types of therapy, such as radiation or immunotherapy, or as the only possible treatment in the case of disseminated and metastasized tumors. A number of programs for the screening of natural products against cytotoxic and anti-tumor activity have led in the past years to the isolation of interesting candidates and leads. The complex diterpene Taxol® (paclitaxel) is perhaps the most well known chemotherapeutic agent of natural origin that was introduced on the market following a natural product screening program [1] (Fig. 1). Approved by the FDA in 1992, Taxol, which inhibits cancer cell growth through the stabilization of cellular microtubules and interference with microtubule dynamics, is today recognized as the most effective clinical agent for the treatment of metastatic breast cancer, metastatic cancer of the ovary, Kaposi's sarcoma, and, more recently, non-small cell lung cancer (in combination with cisplatin). A close analog of Taxol, Taxotere® (docetaxel), is used in the treatment of lung and metastatic breast cancer. Unfortunately, treatment with taxanes is

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ineffective in colon and prostate cancer and is associated with severe side effects. Besides cancer cells, other rapidly dividing cells, such as white blood cells and hair cells, are attacked by the antimitotic action of paclitaxel, and chemotherapy with Taxol is therefore accompanied by suppression of the immune system (neutropenia), deadening of sensory nerves (peripheral neuropathy), hair loss (alopecia), haematological toxicities, and adverse cardiac effects [2]. The most severe limitation to the clinical application of Taxol is the emergence of tumor phenotypes resistant to taxanes as well as to other chemotherapeutic agents. This phenomenon, known as multiple drug resistance (MDR), results from two mechanisms: (a) over-expression of the P-glycoprotein (PgP), an ATP-dependent efflux pump that lowers the intracellular concentration of cytotoxic products, on the surface of neoplastic cells; (b) over-expression of tubulin isotypes that are less susceptible to induced polymerization and stabilization.

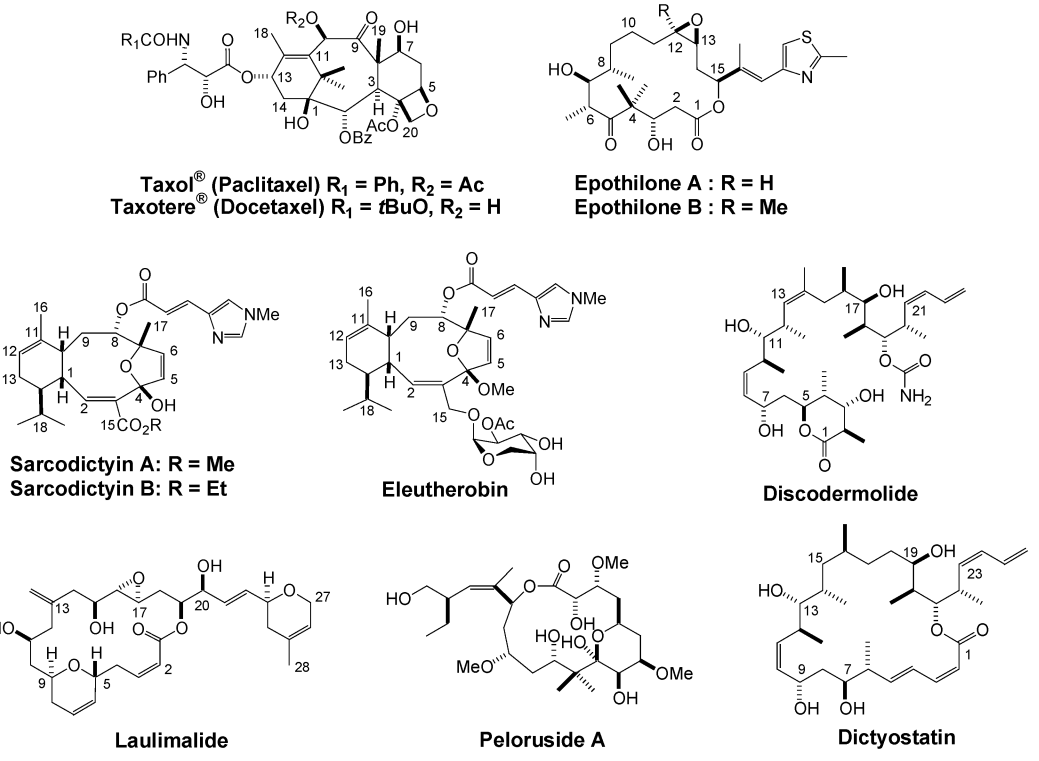


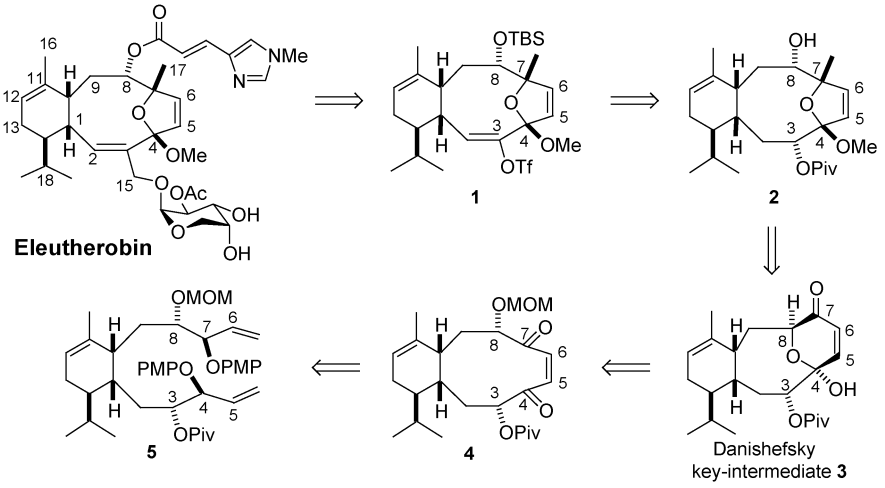
Fig. 1 Microtubule-stabilizing agents (MSAs).

The discovery of alternative natural products endowed with a paclitaxel-like mode of action is a relatively recent achievement [3]. In Fig. 1, the most representative elements of each structural family are represented. With the exception of paclitaxel (plant origin) and of the epothilones (bacterial origin), they are all derived from marine sources. As is often the case for natural products extracted from marine organisms, supply is insufficient for extensive in vitro studies, determination of structure–activity relationship (SAR), in vivo studies, and in general for advancement to clinical trials. The need for a partially or fully synthetic approach is therefore motivated by the scarcity rather than (as well as) by the fascination of their challenging molecular architecture. The overall pharmacological profile of the taxanes highlights the tremendous potential that microtubule-stabilizing agents (MSAs) offer, as well as the need to discover agents with fewer side effects and which retain potency against MDR cancers. Ideally, these would not only exhibit an improved pharmacological profile, but they should also be amenable to large-scale chemical synthesis.

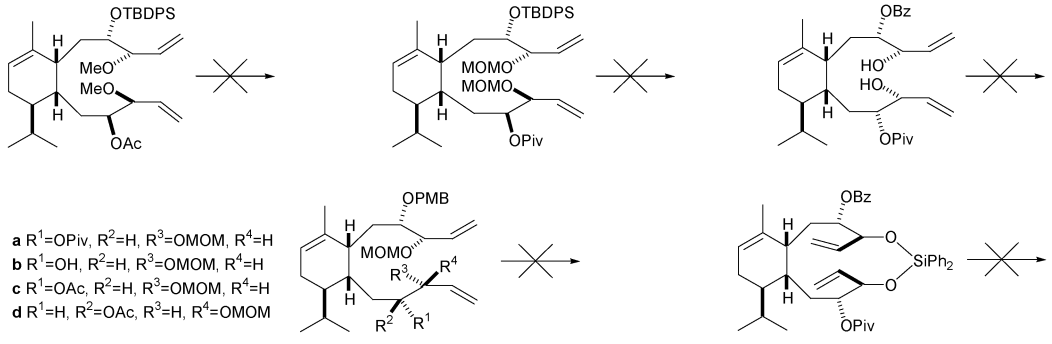
FORMAL TOTAL SYNTHESIS OF ELEUTHEROBIN

Sarcodictyins and eleutherobin (the "eleutheside" family of microtubule-stabilizing drugs, Fig. 1) are active against paclitaxel-resistant tumor cell lines and therefore hold potential as second-generation microtubule-stabilizing anticancer agents. Their scarce availability from natural sources makes their total synthesis vital for further biological investigations. To date, sarcodictyins A and B have been synthesized successfully by Nicolaou et al. [4], who have also exploited a similar route for accessing eleutherobin [5]. A subsequent report by Danishefsky and coworkers details an elegant alternative access to eleutherobin [6].

The preparation of **3**, a key intermediate in the synthesis reported by Danishefsky and coworkers [6], and thus a formal total synthesis of eleutherobin (Scheme 1) has been recently accomplished [7]. The key step of our strategy, used for obtaining the [8.4.0] fused bicyclic ring system **4**, is a ring-closing metathesis (RCM) reaction of the densely functionalized diene **5**. The RCM reaction of a number of densely functionalized diene cyclization precursors of type **5** (bearing protected and/or free alcohol functionalities at both the allylic and the homoallylic positions) had previously been investigated with a variety of catalysts, but no desired cyclized frameworks were ever obtained (Scheme 2) [8a].

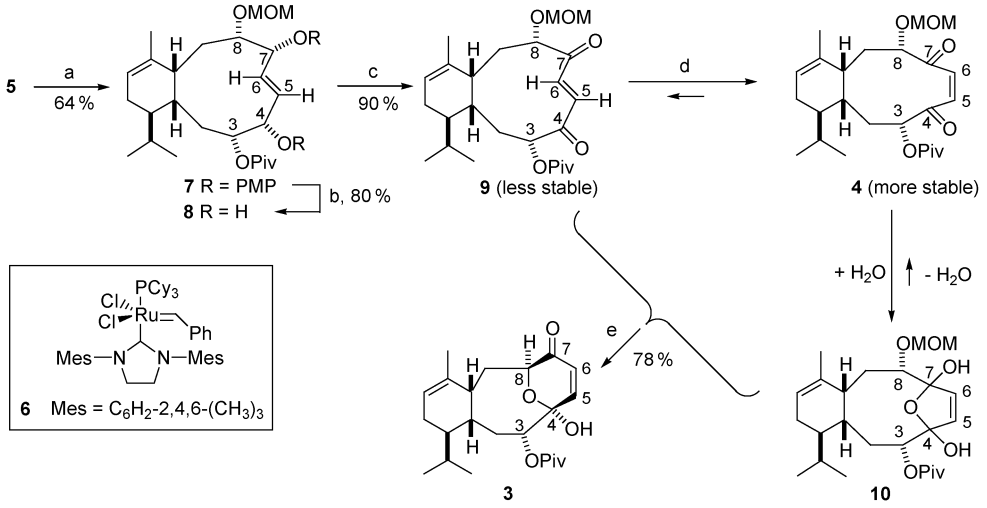


Scheme 1 Retrosynthetic analysis of eleutherobin.



Scheme 2 Failed RCM reactions.

However, none of the previously examined diene precursors had the allylic alcohols protected as *p*-methoxyphenyl (PMP) ethers, which were discovered to facilitate the RCM reaction with respect to other protective groups and the corresponding free alcohols [8a]. Based on these premises, diene **5** was treated with the "second-generation Grubbs" RCM catalyst **6** (Scheme 3). Under forcing conditions [slow addition by syringe pump (over 2.5 h) of a toluene solution of RCM catalyst **6** (30 mol %) to a boiling toluene solution of diene **5**, and additional stirring for 4 h at 110 °C], the *E*-stereoisomer **7** was formed and isolated in 64 % yield.

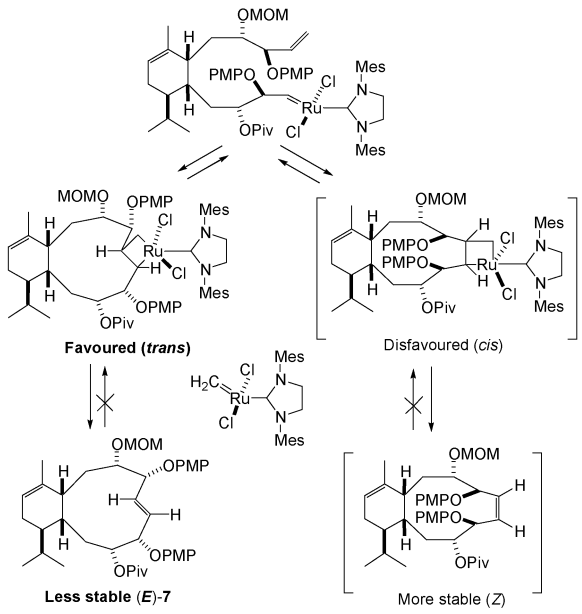


Scheme 3 *Reagents and conditions*: (a) cat. **6** (30 % mol), toluene, 110 °C, 6.5 h, 64 %; (b) CAN, CH_3CN/H_2O (4/1), 0 °C, 80 %; (c) DMP, CH_2Cl_2, 25 °C, 90 %; (d) $CDCl_3$, 25 °C; (e) $BF_3{\cdot}Et_2O$, Me_2S, CH_2Cl_2, –78 °C → –20 °C, 78 %. CAN = ceric ammonium nitrate; DMP = Dess–Martin periodinane.

This result contrasts sharply with many other *Z*-selective RCM reactions of diene cyclization precursors less densely functionalized than diene **5**, which possessed protected and/or free alcohol functionalities at both the homoallylic positions and at only one allylic position (eight examples, see ref. [8]). In the presence of a second-generation Grubbs catalyst, these dienes lead to the more stable *Z*-cyclized products under thermodynamic control.

Confident that the greater stability of the *Z* 10-membered carbocycle would eventually prevail, we continued our planned synthesis by removal of the PMP groups (CAN, 80 %) and oxidation of the allylic diol (DMP, 90 %). Enedione **9** showed remarkable properties: while recording its ^{1}H NMR spectrum in $CDCl_3$ it cleanly isomerized to the more thermodynamically stable *Z*-stereoisomer **4** ($t_{1/2}$ = 63 h). By addition of a catalytic amount of I_2 (10 mol %) a complete isomerization was observed in a shorter time (24 h). Bis-hemiacetal **10** was obtained as the only product after flash chromatography of the *Z*-enedione **4**, showing the propensity of **4** to add a molecule of water and equilibrate with its hydrated form. Finally, the MOM protective group of the **9/4/10** mixture was removed ($BF_3{\cdot}Et_2O$, Me_2S) to give compound **3** (78 %), which produced analytical data identical to those previously reported by Danishefsky and coworkers (^{1}H NMR, ^{13}C NMR, HRMS, IR, R_f, $[\alpha]_D$) [6c].

Our working hypothesis (supported by computational studies, Scheme 4) for the formation of the less stable *E*-stereoisomer **7** in the RCM reaction is the following: the *trans*-ruthenacyclobutane intermediate is more stable and formed preferentially than the *cis*-isomer, leading to the less stable *E*-stereoisomer under kinetic control (DFT calculations). Once formed, the *E* double bond of **7**, flanked by two bulky –OPMP groups, is too sterically hindered to react again with the ruthenium–methylidene complex by means of [2+2] cycloaddition and cycloreversion (according to the generally accepted



Scheme 4 RCM reaction under kinetic control.

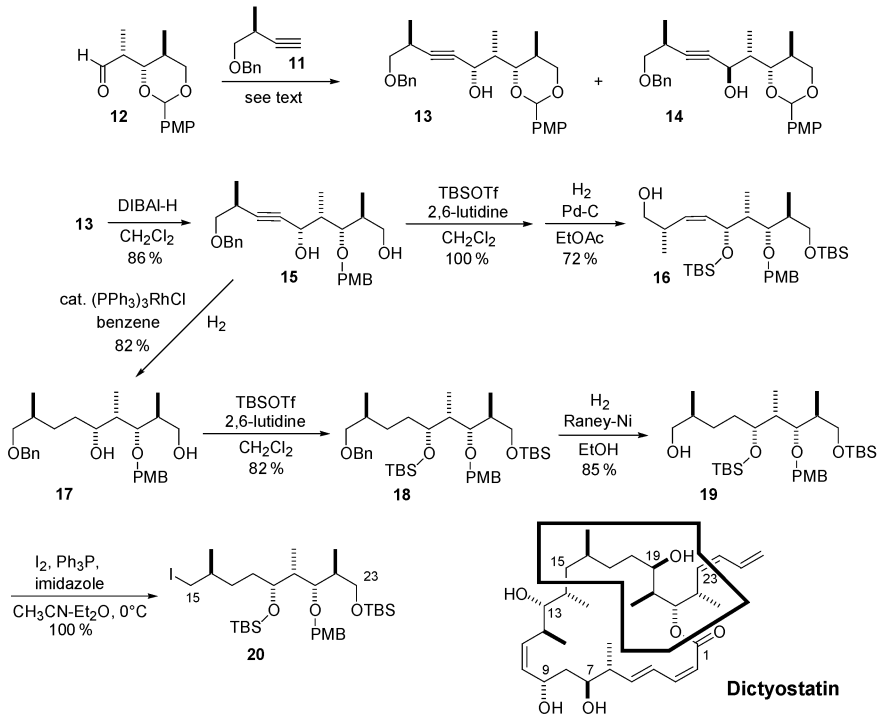
Hérisson–Chauvin mechanism). Thus, the equilibrium between the ring-closed and ring-opened products is arrested and thermodynamic control is inhibited [7b].

APPROACHES TO DICTYOSTATIN

The sponge-derived macrolide dictyostatin (Fig. 1) has been reported to exhibit paclitaxel-like effects on cellular microtubules and to inhibit human cancer cell proliferation at low nanomolar concentrations and with activity superior to discodermolide (ED_{50} 0.38 nM, P338 leukemia cells) [9]. The structure of dictyostatin with full stereochemical assignments was recently established [10], and two total syntheses were completed in 2004 by the Paterson [11] and the Curran groups [12a,12b,13]. More recently, in 2006, Phillips and O'Neil reported another total synthesis [14], Curran and coworkers published a fluorous mixture synthesis of (–)-dictyostatin and three of its diastereomers [15], while Maier and coworkers described an alternative approach to the C1–C9 and C10–C23 segments of this interesting natural product [16]. The development of a practical and flexible synthesis of dictyostatin is still an important goal, particularly as the natural supply is extremely scarce. With the recent withdrawal of discodermolide from clinical development [17], the importance of dictyostatin increases further.

Our synthetic efforts toward dictyostatin were initially focused on the preparation of the C15–C23 fragment of the macrolide, containing 5 of its 11 stereocenters (Scheme 5).

Alkyne **11** {prepared from methyl (*R*)-3-benzyloxy-2-methylpropionate in 67 % yield by consecutive addition of (i) DIBAl-H, (ii) MeOH, (iii) Bestmann–Ohira reagent, (iv) MeONa in THF, following a procedure described by Trost [18]} was treated with *n*-BuLi in THF at –78 °C and then with aldehyde **12** [19] to afford a mixture of the two diastereomeric alcohols **13** and **14** in a 6:4 ratio (50 % yield). Alternatively, a Carreira asymmetric alkynylation [$Zn(OTf)_2$, Et_3N, toluene, RT] [20] was carried out with either of the two enantiomers of *N*-methyl-ephedrine: the reaction with (+)-(1*S*,2*R*)-*N*-methyl-ephedrine gave the addition product in 33 % yield, with a diastereomeric ratio of 9:1 in favor of the undesired *R*-alcohol **14**. On the contrary, the Carriera coupling with (–)-(1*R*,2*S*)-*N*-methyl-ephedrine gave the desired *S*-alcohol **13** in 96 % yield, as the only diastereomer. Hydrogenation of alcohol **13** in the presence of a catalytic amount (10 %) of Wilkinson's catalyst in benzene, gave the desired saturated



Scheme 5 Synthesis of the C15–C23 fragment of dictyostatin.

compound in a low yield (30–40 %), which was then cleaved with DIBAl-H in dichloromethane [19] to yield diol **17** (85 %). Alternatively, acetal **13** was cleaved with DIBAl-H to generate diol **15** in 86 % yield. Subsequent hydrogenation with Wilkinson's catalyst gave the saturated compound **17** in a much improved 82 % yield. When diol **15** was first silylated (TBSOTf, 2,6-lutidine, CH_2Cl_2) and then reduced (H_2, Pd-C, EtOAc), *Z*-alkene **16** was obtained cleanly, with concomitant benzyl removal, but could not be further reduced to the corresponding alkane (**19**). Double protection of diol **17**, by reaction with TBSOTf in the presence of 2,6-lutidine, gave compound **18** in 82 % yield. Selective removal of the benzyl group over the PMB group (H_2, Raney-Ni, EtOH) [21], gave alcohol **19** in 85 % yield. Finally, alcohol **19** was treated with I_2, PPh_3, imidazole to give iodide **20** (C15–C23) in a quantitative yield, ready for further elongation of the carbon chain.

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