Design, synthesis and biological evaluation of a macrocyclic discodermolide/dictyostatin hybrid†

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A 22-membered macrocyclic discodermolide/dictyostatin hybrid has been designed and synthesised; biological evaluation against a range of human cancer cell lines revealed significant levels of growth inhibition.

Discodermolide (**1**, Fig. 1), originally isolated from the deep-sea sponge *Discodermia dissoluta*, displays potent antiproliferative activity against a wide range of human cancer cell lines and inhibits the growth of drug-resistant solid tumours.[1] It shares a similar microtubule-stabilising mechanism to that of Taxol, while having a greater tubulin binding affinity,[2] and has progressed into clinical development as a novel anticancer agent.[3] Furthermore, the synergistic combination of Taxol and discodermolide induces tumour regressions and suppresses angiogenesis in animal models of ovarian carcinoma, supporting their potential use together in cancer therapeutics.[4]

Similarly, dictyostatin (**2**) displays further elevated levels of growth inhibition across the same wide range of human cancer cell lines, and has emerged as a new microtubule-stabilising agent with promising anticancer properties.[5,6] Strong structural similarities exist between discodermolide and dictyostatin, particularly with regard to stereochemical homology, as determined by our recent configurational assignment of the latter structure using detailed NMR analysis,[5c] suggesting that they interact in a similar fashion with the Taxol binding site on β-tubulin.[5d,6] Thus, building on initial encouraging findings reported by the Curran group,[7a] constraining the conformation of the more flexible open-chain structure of discodermolide into the macrocyclic ring motif of dictyostatin might provide active hybrids of these marine-sponge-derived polyketides. Herein, we report the synthesis of the designed 22-membered macrolide **3**, incorporating the full C2–C24 linear sequence of discodermolide and the (*Z*)-enoate of dictyostatin, and that it shows significant growth inhibitory activity against human cancer cell lines.

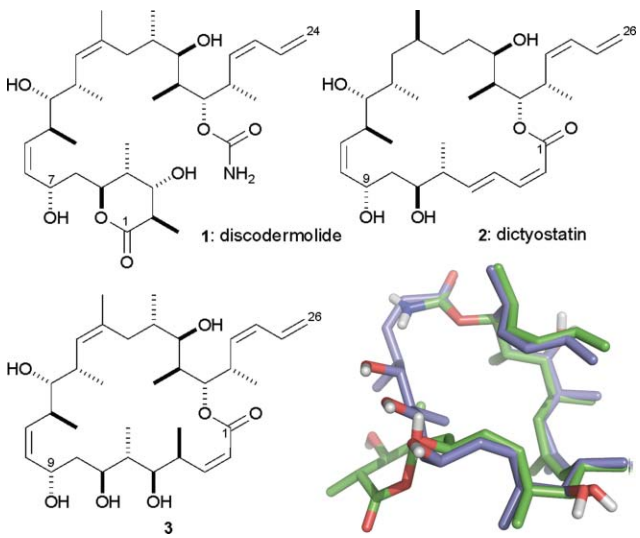

Fig. 1 Structures of discodermolide, dictyostatin and designed hybrid **3**. Overlay of the lowest energy conformation of hybrid **3** (purple) and the X-ray structure of discodermolide (green).

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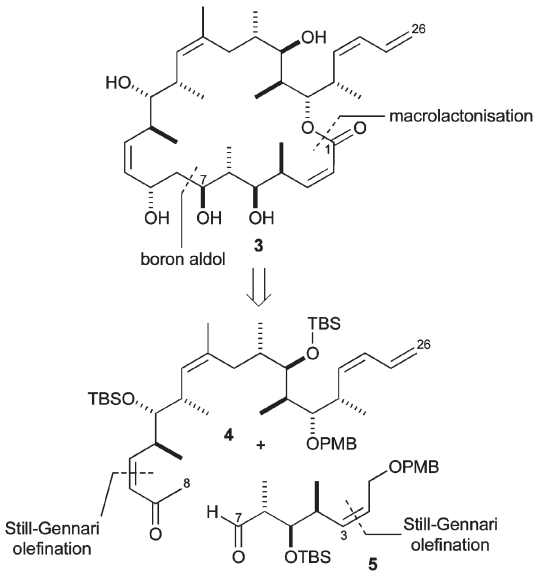
† Electronic supplementary information (ESI) available: Experimental and modelling details, and $^{1}H/^{13}C$ NMR data for compounds **3** and **10–15**. See DOI: 10.1039/b615122a

At the outset, we considered it essential to achieve a suitable overlay of the energetically preferred conformation of our designed hybrid with that of discodermolide. A 10 000 step Monte Carlo conformational search was performed using Macromodel (Version 8.0) with the MM2* force field and a Born/surface area (GB/SA) water solvent model. The calculated global minimum for analogue **3** (see the ESI†) correlates well with the X-ray crystal structure of discodermolide,[1a] as shown in the overlay in Fig. 1. The match for the C9–C26 region (corresponding to C7–C24 of discodermolide) region is particularly striking. Consequently, if the tubulin-bound conformation of discodermolide resembles its X-ray structure, the constrained macrocyclic analogue **3** may possess a similar binding affinity and cytotoxicity to that of the natural product.

Our synthetic strategy leading to analogue **3** was adapted from previous work on the total synthesis of discodermolide.[8] A complex aldol coupling of enone **4** and aldehyde **5**, derived from previously prepared advanced intermediates, would thus be used to assemble a suitable linear precursor for macrolactonisation (Scheme 1).

Synthesis of the enone **4** started from bis-PMB ether **6**,[8a] which incorporates the stereochemistry required for the C11–C26 region (Scheme 2). Selective removal of the primary PMB ether was achieved with BCl_3·DMS,[9] followed by oxidation of the resulting alcohol with TEMPO/PhI$(OAc)_2$ and Still–Gennari olefination[10] to give enone **4** (73%). Synthesis of the aldehyde partner **5** began with the selective primary oxidation of diol **7**,[8b,c] again making use of TEMPO/PhI$(OAc)_2$, followed by a Still–Gennari olefination to provide (*Z*)-enoate **8** (60%). Removal of the PMB ether,[11] bis-silylation of the corresponding diol with TBSOTf/2,6-lutidine and DIBALH reduction of the methyl ester generated allylic alcohol **9** (48%). Finally, PMB ether formation with PMBTCA/Sc$(OTf)_3$, selective primary TBS cleavage and Dess–Martin oxidation

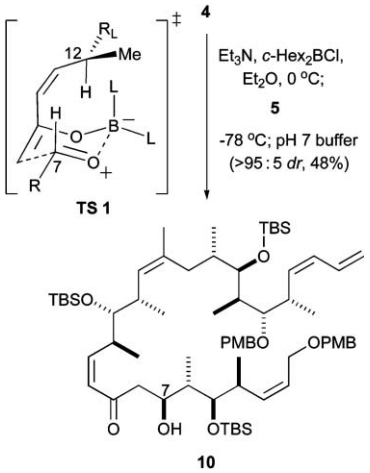


Scheme 1 Retrosynthetic analysis of hybrid analogue **3**.

provided aldehyde **5** (68%) in readiness for the pivotal aldol coupling step.

Enolisation of enone **4** with *c*-Hex_2BCl/Et_3N in Et_2O, and subsequent addition of aldehyde **5**, gave adduct **10** with >95 : 5 dr in favour of the desired (7*S*)-adduct (48%, Scheme 3). This anti-Felkin–Anh outcome is consistent with our earlier work on 1,6-induction in similar boron aldol reactions, and can be rationalised by invoking the favoured transition state model **TS 1**.[8b,c]

Reduction of β-hydroxyketone **10** proved problematic, affording mixtures of epimeric alcohols at C9 when subjected to standard Evans–Saksena conditions (Scheme 4).[12] An improvement was found by following the same protocol as previously employed for the reduction of similarly troublesome β-hydroxyketones[8b] using stoichiometric quantities of (*R*)-CBS and $BH_3{\cdot}THF$,[13] which provided the desired isomer **11** in a 75 : 25 dr.[14] The 1,3-*anti* diol **11** was protected as its acetonide; subsequent oxidative PMB ether cleavage (with DDQ) and sequential oxidation of the resultant

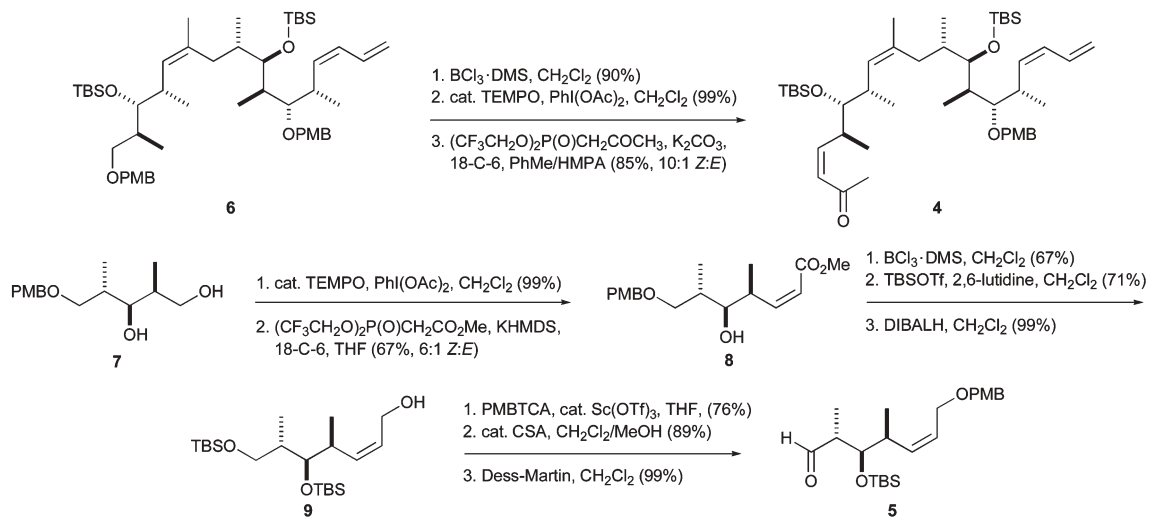


Scheme 3 Key boron aldol coupling.

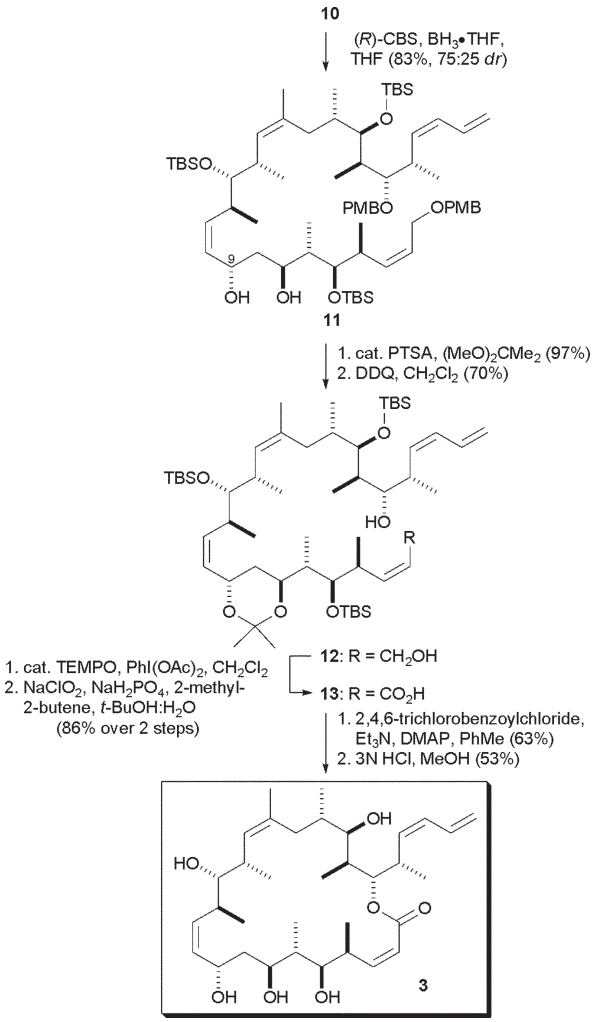
alcohol **12** with TEMPO/$PhI(OAc)_2$ then $NaClO_2/NaH_2PO_4$ provided *seco*-acid **13** (58%).

Finally, a Yamaguchi macrolactonisation of **13** (65%) and global deprotection using 3M HCl in MeOH (53%) afforded the 22-membered macrolide **3** after HPLC purification. Full proton and carbon assignments were carried out using COSY and HMQC NMR experiments. The close correlation between the calculated (by molecular modelling) and observed $^3J_{H-H}$ coupling constants of **3** (see the ESI†) suggests that the conformation adopted is similar to our modelled prediction.

The cell growth inhibitory activity of macrocyclic discodermolide/dictyostatin hybrid **3** was evaluated *in vitro* against three cancer cell lines: MDA-MB-231 (breast), A549 (non-small cell lung) and HT29 (colon) (Table 1). Notably, analogue **3** displayed significant antiproliferative activity against these human carcinoma cells, with a cytotoxicity around one-tenth that of discodermolide. This preliminary data is consistent with the conformation adopted by **3** closely resembling the X-ray structure of discodermolide, which itself has now been reported to correlate strongly with the NMR-derived conformation of discodermolide bound to tubulin.[15]



Scheme 2 Synthesis of enone **4** and aldehyde **5**.



Scheme 4 Completion of the synthesis of hybrid analogue **3**.

Table 1 Human cancer cell growth inhibitory properties of macrocyclic discodermolide analogue **3** relative to discodermolide (**1**)

	$GI_{50}/\mu M$[a]		
	MDA-MB-231 (breast)	A549 (non-small cell lung)	HT29 (colon)
1	0.029	0.020	0.015
3	0.208	0.399	0.170

[a] 50% growth inhibitory concentration after 72 h of continuous incubation.

In conclusion, we have designed and synthesised the most active macrocyclic discodermolide/dictyostatin hybrid reported to date.[7,16] The encouraging antiproliferative activity of analogue **3** can be attributed to its constrained (dictyostatin-like) macrocyclic structure, which bears a strong resemblance to the bioactive conformation of discodermolide.[15] In ongoing work, this rational design approach is being extended to the synthesis of further novel hybrid analogues of discodermolide and dictyostatin.

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