Total synthesis of a potent hybrid of the anticancer natural products dictyostatin and discodermolide†‡

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A potent dictyostatin–discodermolide hybrid was designed and synthesised; it showed enhanced cell growth inhibitory activity relative to discodermolide in four human cancer cell lines including the Taxol-resistant NCI/ADR-Res cell line.

Discodermolide[1] (**1**, Fig. 1) and dictyostatin[2,3] (**2**) are marine sponge-derived antimitotic polyketides which exhibit potent growth inhibition against a wide range of human cancer cell lines, including multidrug-resistant cancer cells.[4] Functioning by the same microtubule-stabilising mechanism as Taxol, they cause an accumulation of cells in the G2/M phase and subsequent cell death *via* apoptosis. In a comparison of the tubulin polymerising ability of natural products that bind at the taxoid site on β-tubulin, discodermolide and dictyostatin were found to be the most potent, with dictyostatin displaying the strongest assembly inducing abilities.[3a] Notably, Novartis undertook the large-scale total synthesis of discodermolide[4a] and advanced it into clinical trials as a novel anticancer agent.

Recently, the bioactive conformations of dictyostatin and discodermolide were elucidated using a combination of NMR analysis, molecular modelling and docking studies.[5] The overlay of these tubulin-bound structures (Fig. 1) revealed some striking conformational similarities. The overlap is most pronounced from the common terminal diene moiety through to C9 on dictyostatin and C7 on discodermolide. Whereas, there appears to be minimal spatial correlation between the δ-lactone of discodermolide and the dienoate of dictyostatin. In addition, the AutoDock-derived model for tubulin binding indicates they both occupy the taxoid site and share similar interactions with the protein residues of the receptor.

With this information in hand and building on our previous synthetic work,[6,7] we sought to rationally design an active hybrid of these two anticancer natural products. Herein, we report an efficient total synthesis of the novel dictyostatin–discodermolide hybrid **3** (Fig. 1) and disclose that it has enhanced (low nanomolar) antiproliferative activity *in vitro* relative to discodermolide, which is retained against the Taxol-resistant NCI/ADR-Res cell line. As part of efforts to develop a practical dictyostatin synthesis, we also describe an improved route to a common C4–C10 intermediate exploiting our boron aldol methodology.

The structural similarities between dictyostatin and discodermolide coincide with the regions of best overlap on the tubulin-bound conformers. However, the superior binding ability of dictyostatin could potentially be aided by the dienoate, occupying a region where the polyketide-derived structures differ. Hence, in our designed hybrid **3**, the stereochemistry and substitution from C8 to C26 are identical to those of discodermolide (lacking the carbamate), while the C1 to C7 region, incorporating the dienoate moiety, and 22-membered macrolactone are dictyostatin[8] derived. Our retrosynthetic analysis (Scheme 1) for **3** envisaged a cross-coupling–macrolactonisation endgame, preceded by installation of the (10*Z*)-alkene by a complex Still–Gennari olefination between aldehyde **4**[7] and β-ketophosphonate **5**.[6]

Synthesis of phosphonate **5** was initiated by enolisation of the lactate-derived ketone **7**[9] with *c*-Hex_2BCl–Me_2NEt (Scheme 2) and addition of aldehyde **8**, affording the *anti*

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† Dedicated to Professor Andrew B. Holmes on the occasion of his 65th birthday.

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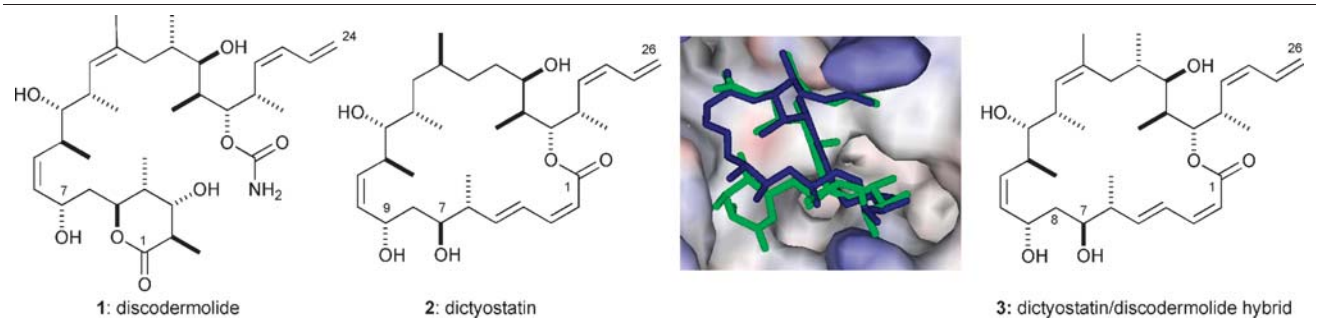
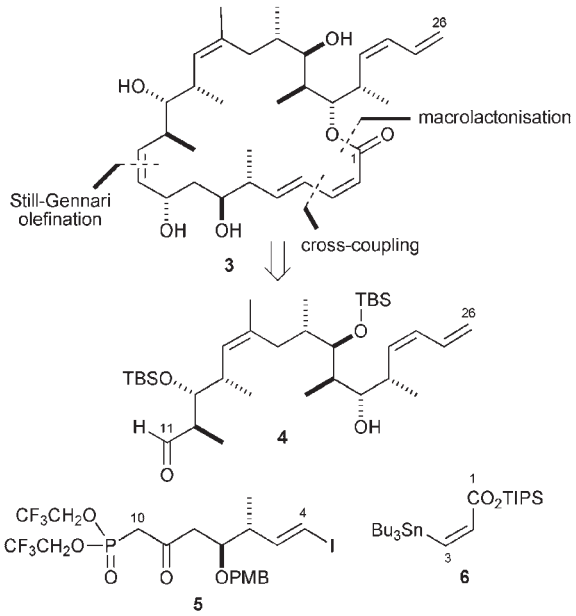


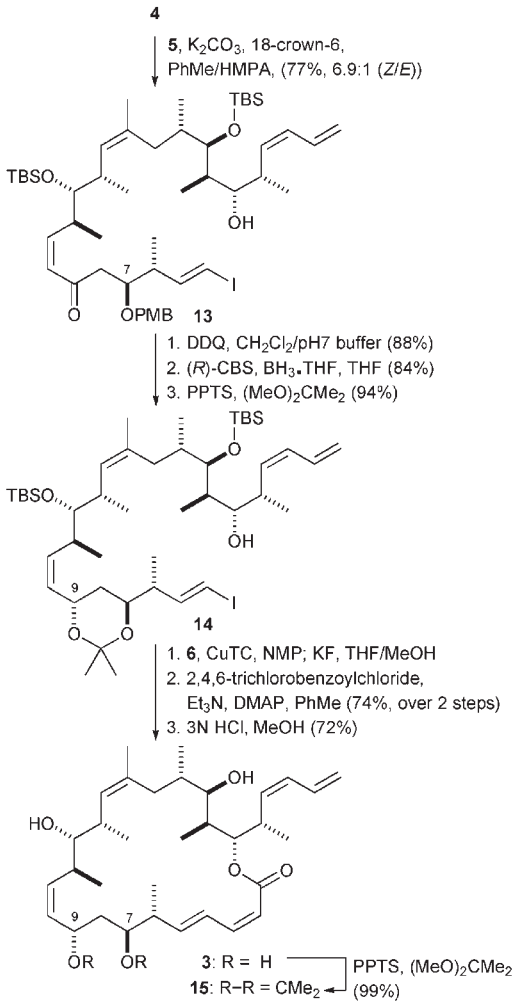
Fig. 1 Structures of discodermolide (**1**), dictyostatin (**2**) and designed hybrid analogue **3**. Overlay of the NMR-derived bioactive conformations of discodermolide (green) and dictyostatin (blue) at the taxoid binding site on β-tubulin.



Scheme 1 Retrosynthesis of dictyostatin–discodermolide hybrid **3**.

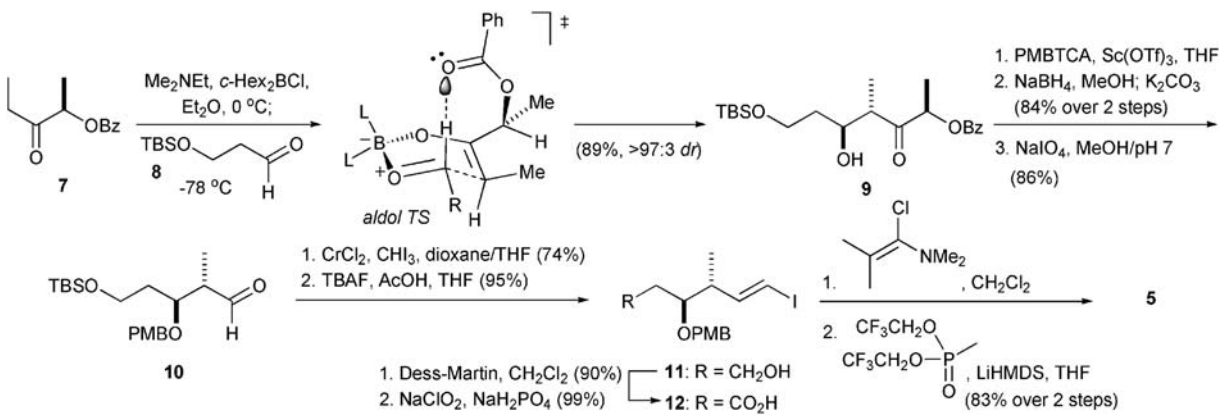
adduct **9** cleanly (89%, >97 : 3 dr) *via* the bicyclic aldol transition state shown. Following formation of the PMB ether, a one-pot reduction and hydrolysis sequence gave the corresponding 1,2-diol (84%). Periodate cleavage revealed the aldehyde **10**, which was then converted into the β-ketophosphonate **5** (52%) *via* **11** and **12** by a similar procedure to that developed previously.[6,8a] In our 2004 dictyostatin total synthesis,[6,10] the requisite *anti* C6/C7 stereocentres were configured through a Brown crotylation reaction. In comparison, this new aldol-based route to **5** was found to be more readily scaleable and achieved enhanced stereoselectivity.

With both **4** (a key intermediate in our discodermolide synthesis)[4a,7] and **5** in hand (Scheme 3), the pivotal Still–Gennari olefination[11] was performed on a gram scale to afford the desired (*Z*)-enone **13** in 67% isolated yield with good selectivity (6.9 : 1 *Z*/*E*). Oxidative cleavage of the PMB ether at C7 afforded the corresponding β-hydroxy ketone in readiness for reduction to install the C9 stereocentre. Following related studies,[12a] use of (*R*)-CBS[13] and BH_3·THF generated the required 1,3-*anti* diol cleanly (>95 : 5 dr), which was transformed into acetonide **14** (70% from **13**).



Scheme 3 Completion of the synthesis of **3** and **15**.

The endgame commenced with a copper-mediated Stille cross-coupling[14] between vinyl iodide **14** and stannane **6** to install the (2*Z*,4*E*)-dienoate. Macrolactonisation under Yamaguchi conditions then smoothly afforded the protected macrolactone (74% from **14**). While HF·pyridine gave inconsistent results, global deprotection with HCl–MeOH (1 : 3) proved more reliable, generating the hybrid **3** (72%) with negligible translactonisation onto the C19 hydroxyl. In order



Scheme 2 Synthesis of β-ketophosphonate **5**.

Table 1 Human cancer cell growth inhibitory properties of hybrid **3** and acetonide derivative **15** relative to discodermolide (**1**),[8a] dictyostatin (**2**) and Taxol, as determined by MTT metabolism after 72 h exposure to the test agent

	Cytotoxicity IC_{50}/nM			
	PANC-1	AsPC-1	DLD-1	NCI/ADR-Res
1	59 ± 34	98 ± 34	29 ± 8	160 ± 34
2	4.2 ± 0.5	6.2 ± 0.6	2.2 ± 0.5	6.6 ± 0.4
Taxol	9.9 ± 1.3	150 ± 32	22.4 ± 1.4	1260 ± 140
3	12.9 ± 2.0	33.9 ± 6.4	5.9 ± 1.1	66.4 ± 15.2
15	4860 ± 150	4850 ± 450	2350 ± 180	2930 ± 300

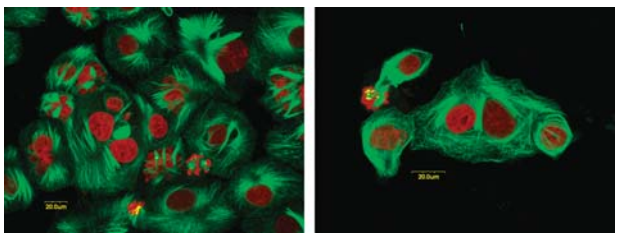


Fig. 2 Immunofluorescence images of PANC-1 cells stained with anti-α-tubulin (green) and propidium iodide (red) and observed by confocal microscopy. Cells were exposed to 100 nM concentrations of dictyostatin (left image) and analogue **3** (right image). Typical dense intracellular bundling of microtubules (green) can be seen around the nuclei (red) in both images.

to probe the contribution of the C7,C9-diol to the pharmacophore, **3** was treated with 2,2-dimethoxypropane–PPTS to reinstate the acetonide in **15**.

Following HPLC purification, the antiproliferative activities of **3** and **15** were evaluated *in vitro* against four human cancer cell lines (Table 1): PANC-1 (pancreatic), AsPC-1 (pancreatic), DLD-1 (colon), and NCI/ADR-Res (Taxol-resistant ovarian). Importantly, hybrid **3** demonstrated low nanomolar cell growth inhibitory activity that was intermediate between that measured for discodermolide (**1**)[8a] and dictyostatin (**2**) and similarly maintained this potent activity against the NCI/ADR-Res cell line (IC_{50} = 66.4 ± 15.2 nM), where the overexpression of a P-glycoprotein drug efflux pump in the cell membrane gives rise to Taxol resistance. As with dictyostatin and discodermolide, hybrid **3** led to an accumulation of cells at the G2/M phase. In contrast, acetonide **15** was found to have greatly reduced cytotoxicity (low micromolar), suggesting that one or both of the C7,C9 hydroxyls plays a key role in interacting with tubulin or in maintaining the bioactive conformation. Anti-α-tubulin staining of PANC-1 pancreatic carcinoma cells treated with 100 nM of hybrid **3** (Fig. 2) shows the characteristic patterns of microtubule bundling observed for other tubulin polymerising agents such as Taxol, discodermolide and dictyostatin.[2b] Similar to what is observed for dictyostatin, treatment with 10 nM of **3** shows a large number of cells undergoing apoptosis as evidenced by high levels of nuclear fragmentation observed in the confocal images and a large sub-G0 population in the cell cycle analysis.

In conclusion, we have completed an efficient total synthesis of the most potent cytotoxic hybrid of dictyostatin and discodermolide reported to date.[12] We attribute the enhanced cell growth inhibitory activity of **3** relative to discodermolide to the more constrained macrocyclic structure and the dictyostatin-like C1–C7 region playing a significant role in binding to tubulin. Efforts are ongoing to further probe the pharmacophore and anticancer profiles of these fascinating marine natural products and their hybrids.

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