Total synthesis of a library of designed hybrids of the microtubule-stabilising anticancer agents taxol, discodermolide and dictyostatin†

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Received (in Cambridge, UK) 13th October 2009, Accepted 10th November 2009
First published as an Advance Article on the web 24th November 2009
DOI: 10.1039/b921237j

A hybrid library of the marine natural products dictyostatin and discodermolide, incorporating the taxol or taxotere side chains, were synthesised; preliminary biological evaluation in the PANC-1 cancer cell line revealed significant antiproliferative activity, demonstrating that a macrolide scaffold is an effective surrogate for the baccatin core of taxol.

In recent years, natural products have proved to be a rich source of therapeutic agents.[1,2] Indeed, the majority of new anticancer agents have been derived from natural products or their analogues. The diterpenoid Taxol® (paclitaxel, **1**, Fig. 1), along with its semi-synthetic analogue Taxotere® (docetaxel, **2**), are clinically important antimitotic drugs, widely used in the treatment of breast, ovarian and lung cancers, that target tubulin.[3] Discodermolide[4] (**3**) and dictyostatin[5] (**4**) meanwhile are structurally similar, marine sponge-derived polyketides, which share the same microtubule-stabilising mechanism as taxol, but lack the interaction with P-glycoprotein and maintain antiproliferative efficacy against taxol-resistant cancer cell lines. All three compounds bind to the same site on β-tubulin, with dictyostatin displaying the strongest assembly-inducing abilities.[6] Numerous conflicting models have been postulated for the pharmacophores and bioactive conformations of these molecules and for their binding interactions with tubulin. Recently, this question was re-examined with the aid of NMR studies, molecular modelling and docking calculations.[7] The subsequent results indicated a high degree of conformational overlap for tubulin-bound dictyostatin and discodermolide (Fig. 1, Image 1), and inspired our recently reported total synthesis[8] of the potent dictyostatin–discodermolide hybrid **5**.[9,10]

Inspection of the overlaid tubulin-bound conformations of the parent natural products indicated that the C13 *N*-benzoyl-(2′*R*,3′*S*)-3-phenylisoserine side chain of taxol occupied a region of the binding pocket that was not exploited by dictyostatin or discodermolide (Fig. 1, Image 2).[7] However, the C7 and C9 hydroxyls on dictyostatin were orientated to point into this vacant region, and presumably this would also be the case for the dictyostatin-like hybrid **5**. It was

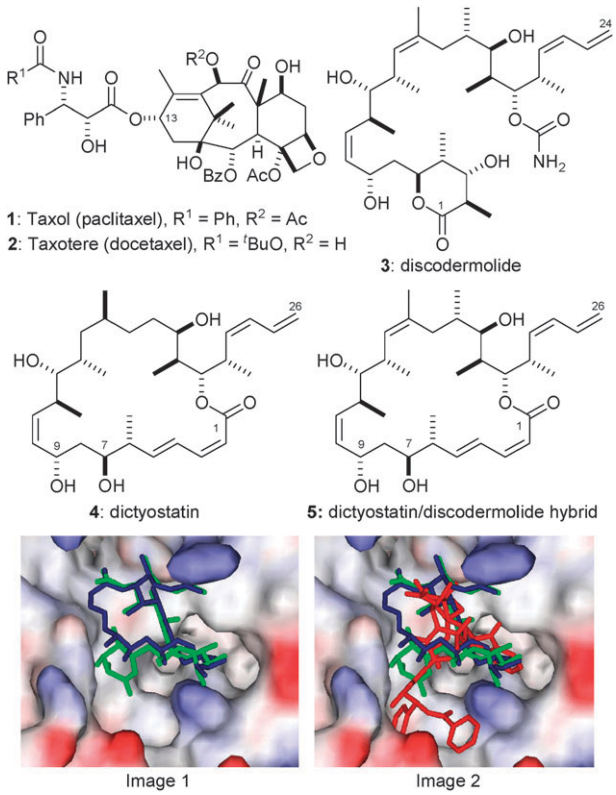

Fig. 1 Microtubule-stabilising anticancer agents taxol (**1**), taxotere (**2**), discodermolide (**3**), dictyostatin (**4**), and dictyostatin–discodermolide hybrid (**5**). The NMR-derived bioactive conformations of discodermolide (green) and dictyostatin (blue) superimposed at the taxoid binding site on β-tubulin (Image 1) and with that of taxol (red, Image 2).

hypothesised that the addition of the taxol or taxotere side chain onto either of these hydroxyls would garner additional binding interactions (supported by a comparison of the affinities of baccatin III and taxol).[11] Herein, we report the synthesis of a library of novel natural product triple hybrids, utilising either the taxol or taxotere side chains attached to **5** through an ester linkage at C7 or C9.

As shown in Scheme 1, formation of each of the initially designed hybrids† began with diol **6** (obtained from a precursor to **5**[8]), and adapted the protocols developed for taxane side chain introduction onto the C13 hydroxyl of baccatin III.[12] Treatment of **6** with NaHMDS followed by addition of reactive β-lactam[13] **7** (taxol side chain, R = Ph) or **8** (taxotere side chain, R = O^{t}Bu) afforded an inseparable mixture of the

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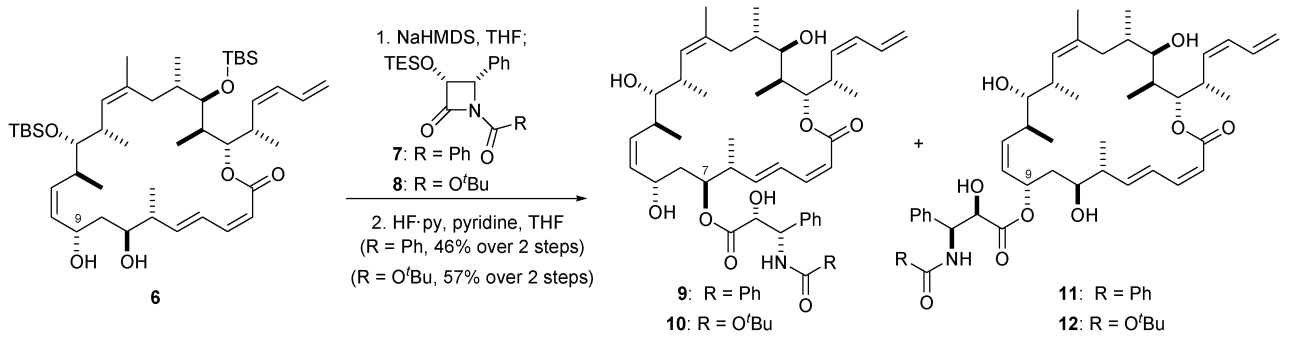
† Electronic supplementary information (ESI) available: Full synthetic details for all compounds, NMR data and spectra for **10–12**, **14–16** and **18–20**. See DOI: 10.1039/b921237j



Scheme 1 Synthesis of hybrids **9–12**.

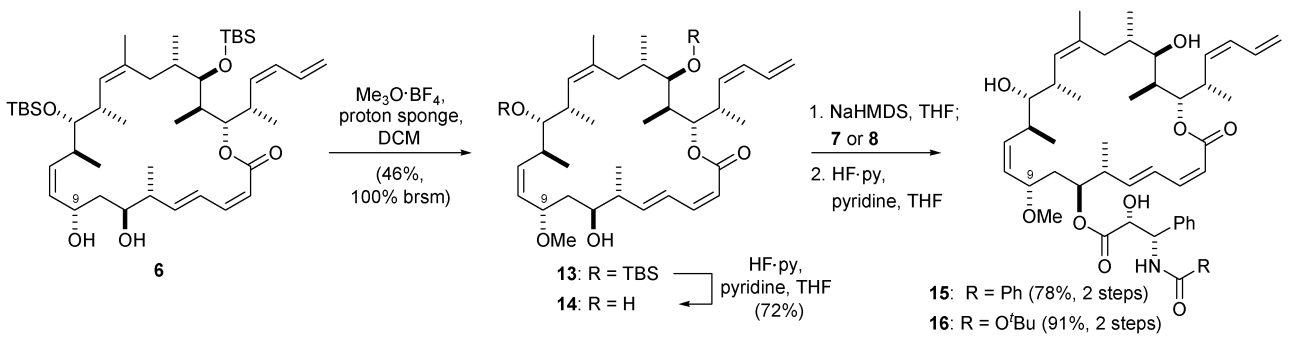
corresponding C7 and C9 esters. At −78 °C, coupling with the C7 hydroxyl was favoured (3 : 1 for **7**), but this selectivity was overturned at higher temperatures, generating predominantly the C9 product (2 : 1 at 0 °C). The mixtures of regioisomers were then subjected to deprotection using HF·py (buffered with pyridine), to generate the required triple hybrids **9–12** which were separated by HPLC and individually characterised.

Investigations into the stability of these new compounds yielded some unexpected results. Dissolving **9–12** in DMSO (the standard solvent used in biological assays) resulted in regiomerically pure hybrids undergoing transesterification to afford mixtures of C7 and C9 isomers. This transformation occurred irrespective of the starting hybrid used, and the ratio of isomers formed was found to be increasingly biased towards the C9 ester at elevated temperatures, presumably due to the less sterically hindered nature of this position. Intriguingly, in methanol the side chains were labile, undergoing transesterification to form the original dictyostatin–discodermolide hybrid **5** and the corresponding methyl ester derivative of the side chain.

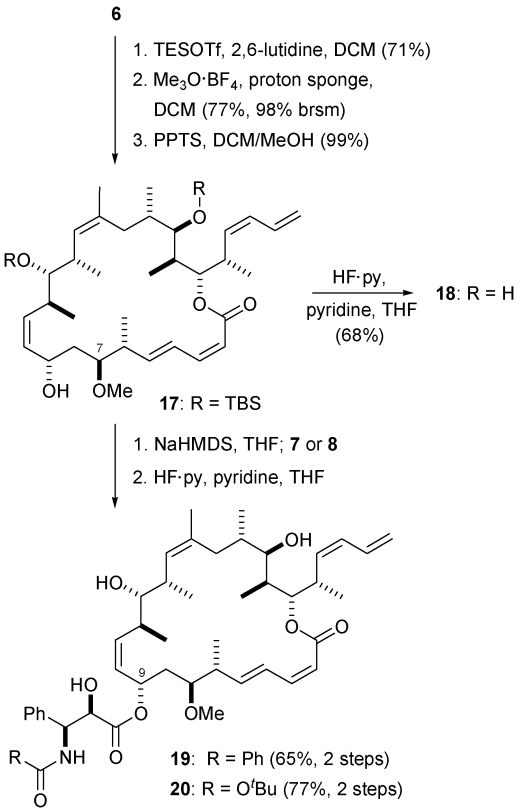
As a consequence of these findings, we decided to embark on the synthesis of a second series of methyl ether derivatives. Taking inspiration from the highly active 9-methoxydictyostatin analogue previously prepared,[14] it was presumed that capping the C7/C9 free hydroxyl as the methyl ether would prevent transesterification without significantly affecting cytotoxicity. Accordingly, treatment of diol **6** with Meerwein's salt and proton sponge yielded the C9 methyl ether **13**, with 30 : 1 selectivity over the C7 regioisomer (along with recovered starting material) (Scheme 2). Deprotection with HF·py (buffered with pyridine) gave the C9 methoxy analogue **14**. Alcohol **13** was treated with NaHMDS and β-lactam **7** or **8**, before subsequent deprotection resulted in the *O*-methylated triple hybrids **15** and **16** in 78% and 91% yield over two steps respectively.

To access the regioisomeric C7 methyl ether, a selective monoprotection–methylation–deprotection strategy was employed. Reaction of diol **6** with TESOTf and 2,6-lutidine at −78 °C generated the allylic silyl ether in 5 : 1 regioselectivity; while cooling to −98 °C increased this to >10 : 1 (Scheme 3). Methylation of the C7 hydroxyl, followed by selective cleavage of the TES group (PPTS in MeOH–DCM), afforded the desired C7 methyl ether **17** in 99% yield. At this stage, global deprotection led to the novel dictyostatin–discodermolide hybrid **18**, whilst ring opening of β-lactam **7** or **8** and subsequent deprotection gave the esterified triple hybrids **19** and **20** in 65% and 77% yield respectively.[15]

Following HPLC purification, the antiproliferative activities were evaluated *in vitro* against the PANC-1 human pancreatic ductal carcinoma cell line (Fig. 2). All of the compounds showed IC_{50} values <1 μM and retained the ability to induce cell cycle arrest at the G2/M phase of mitosis. Confocal imaging to observe the microtubule network in the PANC-1 cell line (Fig. 2, Images 1 and 2) also confirmed the presence of microtubule bundling, as expected for tubulin stabilising compounds. Some general trends could be discerned in this preliminary study: triple hybrids with the taxol side chain were somewhat more active than those bearing the taxotere side chain, and the methyl ether triple hybrids were less active than the non-methylated. The 7-taxotere compound **10** was the most potent of the triple hybrids (IC_{50} = 86 nM); while intriguingly, the *O*-methylated dictyostatin–discodermolide double hybrids **14** and **18** were the most active new compounds, with **14** displaying cytotoxicity (IC_{50} = 14 nM) that was directly comparable to that of the parent compound **5** and nearing that of dictyostatin (**4**).



Scheme 2 Generation of C9 methoxy analogues **14–16**.



Scheme 3 Completion of C7 methoxy analogues **18–20**.

Cytotoxicity IC_{50}/nM	
	PANC-1
1	20
3	59
4	3
5	15
9	100
10	86
11	100
12	181
14	14
15	150
16	250
18	70
19	330
20	520

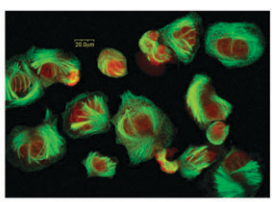


Image 1
100 nm dictyostatin (4)

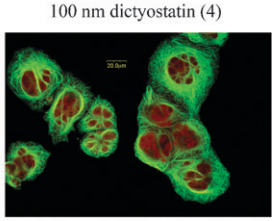


Image 2
100 nm 7-taxotere triple hybrid (**10**)

Fig. 2 The table shows human cancer cell growth inhibitory properties of hybrids **9–12**, **14–16**, **18–20**, relative to taxol (**1**), discodermolide (**3**),[8] dictyostatin (**4**) and dictyostatin–discodermolide hybrid (**5**). Images: 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 (Image 1) and analogue **10** (Image 2). Dense microtubule bundling (green) can be seen around the nuclei (red) in both images.

In conclusion, we have designed and synthesised the first triple hybrids of the anticancer natural products taxol, dictyostatin and discodermolide. Significantly, we have demonstrated that the polycyclic baccatin core of taxol can be replaced with a macrolide template whilst maintaining pronounced cytotoxicity, indicating the possibility to develop further scaffolds for attachment to the taxol side chain. Currently, more extensive biological evaluation is being pursued and efforts are ongoing to identify and synthesise further hybrids of these important anticancer agents.

Financial support was provided by the EPSRC and NIH Grant no. R56 CA093455. We thank Dr J. Fernando Díaz (CSIC, Madrid) for providing AutoDock 3D structures, Dr Stuart Mickel (Novartis) for chemicals, Professor Eiichi Nakamura (University of Tokyo) for arranging a student exchange (TF), and Tara Pitts and Pat Linley (HBOI) for biological assays.

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