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Total synthesis and biological evaluation of potent analogues of dictyostatin: Modification of the C2–C6 dienoate region

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ABSTRACT

By exploiting a Still–Gennari olefination of a common C11–C26 aldehyde with a C4–C10 or C1–C10 β-ketophosphonate, three modified C2–C6 region analogues of the 22-membered macrolide dictyostatin were synthesised and evaluated in vitro for growth inhibition against a range of human cancer cell lines, including the Taxol-resistant NCI/ADR-Res cell line. 6-Desmethyldictyostatin and 2,3-dihydrodictyostatin displayed potent (low nanomolar) antiproliferative activity, intermediate between dictyostatin and discodermolide, while 2,3,4,5-tetrahydrodictyostatin showed activity comparable to discodermolide. As with dictyostatin, these simplified analogues act through a mechanism of microtubule stabilisation, G2/M arrest and apoptosis.

The tubulin/microtubule system is an important target in current cancer chemotherapy.[1] Taxol® (**1**, Fig. 1) and its semi-synthetic derivative Taxotere® act by stabilising cellular microtubules and suppressing microtubule dynamics in cell division, and are widely used in the treatment of breast, ovarian and lung cancers. Despite the clinical utility of the taxane class of microtubule-stabilising agents, they have limited effectiveness towards multidrug-resistant cancers, prompting the search for new and improved antitumour agents.[2]

In a similar manner to Taxol, the antimitotic marine macrolide dictyostatin (**2**), first reported by Pettit in 1994,[3a] functions by microtubule stabilisation and G2/M arrest.[3b] Upon re-isolation from a Caribbean deep-sea sponge by Wright and co-workers,[3b] detailed NMR analysis enabled us to assign the full stereochemistry.[4] Concurrent total syntheses by the Paterson[5a] and Curran groups[5b] independently confirmed this structure, enabling more extensive biological evaluation of this promising anticancer agent,[6] as well as the initiation of SAR studies.[7,8] From elegant NMR studies by Jiménez-Barbero and co-workers,[9] the bioactive conformation of dictyostatin bound to the taxoid binding site on β-tubulin was recently proposed, highlighting its similarity to the tubulin-bound 3D structure of the structurally related polyketide discodermolide (**3**).[10]

To date, a variety of dictyostatin analogues,[7,8] together with hybrid structures with discodermolide,[11,12] have been synthesised and evaluated for their anticancer properties, leading to an evolving common pharmacophore model with discodermolide. Herein, we report the total synthesis and biological evaluation of 6-desmethyldictyostatin (**4**, Fig. 2), 2,3-dihydrodictyostatin (**5**) and 2,3,4,5-tetrahydrodictyostatin (**6**), incorporating modifications to the C2–C6 dienoate region of dictyostatin. We disclose that the novel dictyostatin analogues **4**, **5** and **6** display potent growth inhibitory activity in vitro against a range of human cancer cell lines, including the Taxol-resistant NCI/ADR-Res cell line. As with dictyostatin, these simplified analogues act through a mechanism of microtubule stabilisation, G2/M arrest and apoptosis.

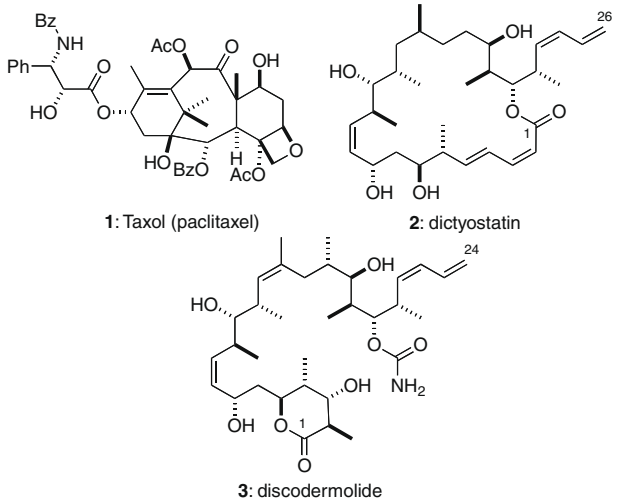

Figure 1. Structures of Taxol, dictyostatin and discodermolide.

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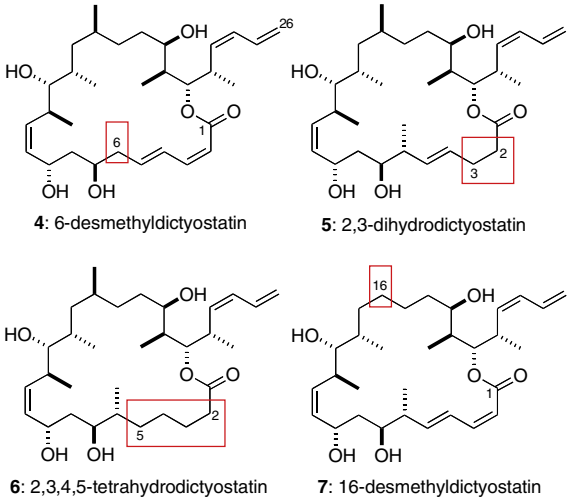
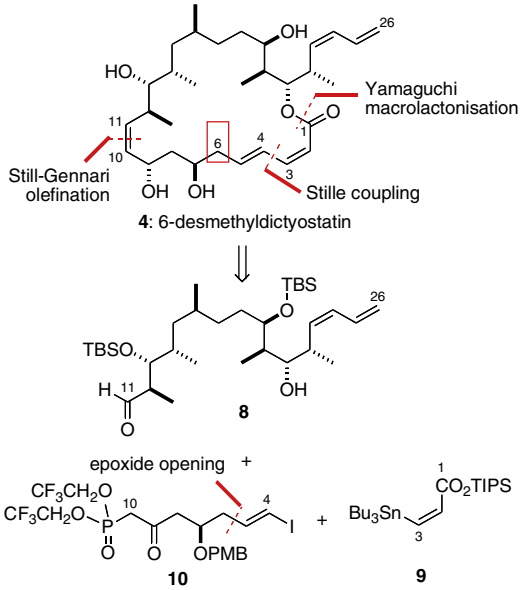


Figure 2. Structures of dictyostatin analogues.

In previous SAR work on dictyostatin, the importance of the C16 methyl substituent was probed through the synthesis of 16-desmethyldictyostatin (**7**, Fig. 2).[7a,8a] Our biological results[7a] for **7** led us to conclude that the C16 methyl group was both important in contributing to the low nanomolar levels of cytotoxicity of dictyostatin and permitting its circumvention of P-glycoprotein-mediated drug resistance. Looking towards achieving further structural simplifications, it was next proposed to explore the contribution of the C2–C6 dienoate region of dictyostatin to the pharmacophore by synthesising 6-desmethyldictyostatin (**4**) to evaluate its cytotoxicity in a panel of Taxol-sensitive and resistant cell lines.

Access to the targeted 6-desmethyl analogue **4** was planned by adapting our existing synthetic strategy for dictyostatin[5a] as used for preparing an initial set of analogues[7a] (Scheme 1). This relies on a pivotal Still–Gennari HWE fragment coupling with the fully elaborated C11–C26 aldehyde **8**, followed in turn by introduction of the (2*Z*,4*E*)-dienoate by a Stille coupling with stannane **9** and Yamaguchi macrolactonisation. By pursuing a similar strategy, the primary modification required to assemble 6-desmethyldictyostatin was envisaged to be the preparation of the novel C4–C10 phosphonate **10**, lacking a C6 methyl substituent and having a PMB ether[7a] at C7 (rather than the TBS ether used initially[5a]).

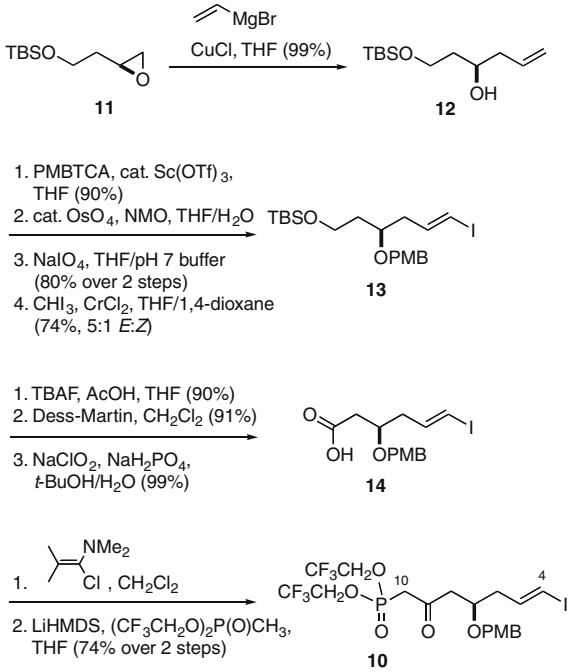


Scheme 1. Retrosynthetic analysis of 6-desmethyldictyostatin (**4**).

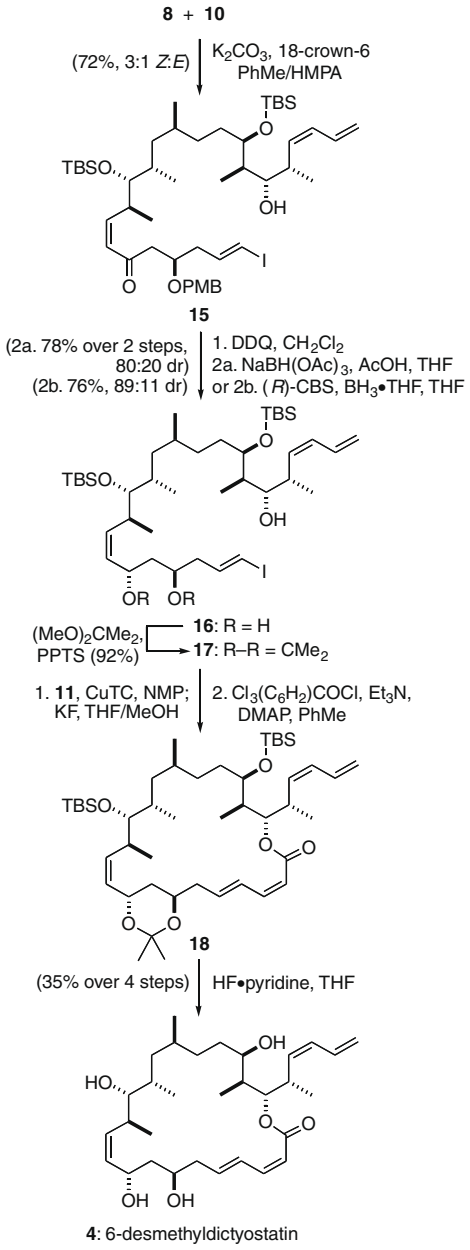
Preparation of the β-ketophosphonate **10** required for the Still–Gennari fragment coupling started out from the (*S*)-epoxide **11** (Scheme 2), which was obtained by a Jacobsen hydrolytic kinetic resolution reaction.[13] A CuCl-catalysed epoxide-opening of **11** with vinyl magnesium bromide afforded the secondary alcohol **12** in 99% ee. Following PMB ether formation, a sequence of oxidative olefin cleavage (OsO_4, NMO; $NaIO_4$) and Takai olefination[14] of the resulting aldehyde gave the (*E*)-vinyl iodide **13** (53% from **12**). After TBS ether cleavage (TBAF, AcOH), the resulting alcohol was converted via the carboxylic acid **14** into the acid chloride using the Ghosez chloroenamine reagent,[15] followed by the addition of $(CF_3CH_2O)_2P(O)CH_2Li$ to provide the β-ketophosphonate **10** (60% from **13**).

As deployed in our dictyostatin total synthesis, we were now ready to perform the Still–Gennari HWE coupling[16] with the C11–C26 aldehyde **8** (Scheme 3).[5a] Following treatment of a mixture of phosphonate **10** and aldehyde **8** with K_2CO_3 (18-crown-6, PhMe, HMPA), the required (*Z*)-enone **15** was obtained in an isolated yield of 54% (72%, 3:1 *Z*:*E*). Oxidative cleavage (DDQ, pH 7 buffer) of the PMB ether in **15** then released the β-hydroxyketone in readiness for a hydroxyl-directed reduction. Reduction with $NaBH(OAc)_3$[17] afforded the expected 1,3-*anti* diol **16** with moderate selectivity (78%, 80:20 dr). A switch to the Corey–Bakshi–Shibata (*R*)-oxazoborolidine-mediated reduction[18] gave the 1,3-*anti* diol **16** (76%, 89:11 dr) with improved levels of diastereoselectivity. After formation of the acetonide **17** ($Me_2C(OMe)_2$, PPTS), a CuTC-mediated Liebeskind–Stille coupling[19] with the vinyl stannane **9**[5a] installed the (2*Z*,4*E*)-dienoate and a Yamaguchi macrolactonisation[20] then gave the 22-membered macrolide **18**. Following global deprotection of **18** with HF•pyridine and HPLC purification, 6-desmethyldictyostatin (**4**) was isolated in 53% yield from **16** in readiness for biological testing.

The remarkable structural and stereochemical homology between the C8–C26 region of dictyostatin and the corresponding



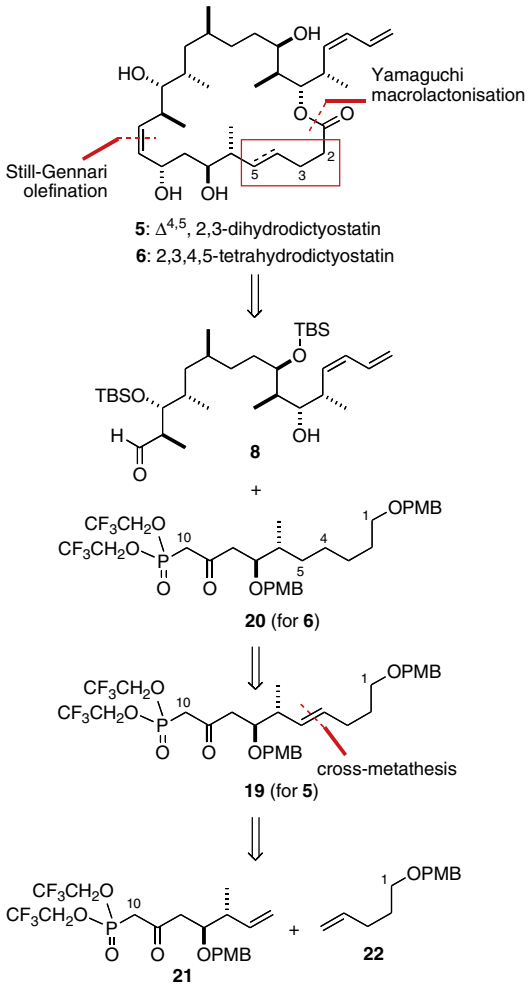
Scheme 2. Synthesis of C4–C10 β-ketophosphonate **13**.



Scheme 3. Completion of the synthesis of 6-desmethyldictyostatin (**4**).

C6–C24 region of discodermolide has previously been exploited in the design of novel dictyostatin analogues[7,8] and hybrid molecules.[11] In contrast, the discodermolide structure features a δ-lactone in the C1–C5 region, whereas the dictyostatin macrolide differs in having a (2*Z*,4*E*)-dienoate moiety. Hence, it was proposed to further probe the SAR associated with the unique C1–C5 region of dictyostatin through the total synthesis of two novel reduced analogues, 2,3-dihydrodictostatin (**5**) and 2,3,4,5-tetrahydrodictyostatin (**6**).

In designing a synthetic route to analogues **5** and **6**, it was apparent that removal of the dienoate negated the requirement for the Stille coupling step and hence the presence of a vinyl iodide in the β-ketophosphonate fragment. By retaining the Yamaguchi macrolactonisation and Still–Gennari olefination with the pivotal aldehyde **8**, a unified route was planned to the phosphonates **19** and **20**, which differed only in the presence or absence of a $\Delta^{4,5}$-olefin, as required for the construction of analogues **5** and **6**, respectively (Scheme 4). It was envisaged that the key C1–C10

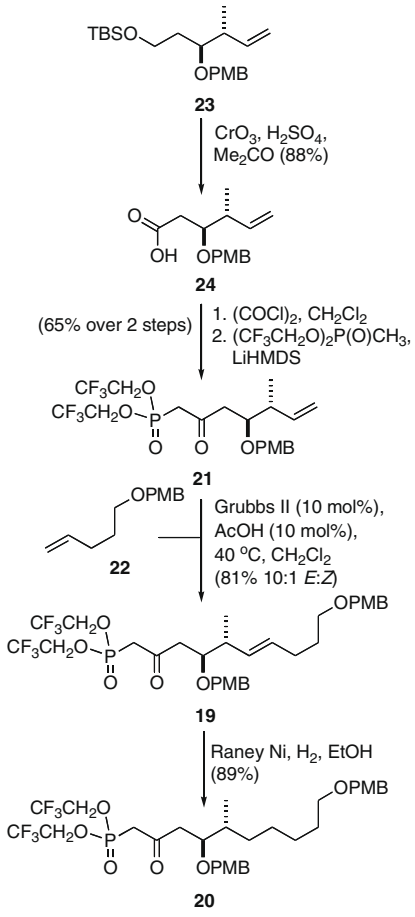


Scheme 4. Retrosynthetic analysis of 2,3-dihydrodictyostatin (**5**) and 2,3,4,5-tetrahydrodictyostatin (**6**).

building block **19** might arise from a cross-metathesis reaction between β-ketophosphonate **21** and the olefin **22**. This sequence was viewed as particularly attractive due to its enhanced convergence and versatility.

Starting from the previously reported PMB ether **23**[7a] obtained using a Brown crotylation sequence, treatment with Jones reagent (CrO_3, H_2SO_4) led to cleavage of the TBS ether and subsequent oxidation of the resulting alcohol to provide the acid **24** in 88% yield (Scheme 5). Conversion to the acid chloride was now possible using oxalyl chloride, and addition of $(CF_3CH_2O)_2P(O)CH_2Li$ provided the β-ketophosphonate **21** (65% from **24**) in readiness for the planned cross-metathesis reaction[21] with the terminal olefin **22**. Only when the Grubbs second-generation Ru catalyst (10 mol %) was used in the presence of AcOH[22] (10 mol %) did this afford the desired unsaturated phosphonate **19** in high yield (81%, 10:1 *E*:*Z*).[23] Raney nickel catalysed hydrogenation of alkene **19** then gave the corresponding saturated phosphonate **20** (89%), as required for the construction of 2,3,4,5-tetrahydrodictyostatin.

With the two β-ketophosphonates **19** and **20** in hand, the targeted analogues **5** and **6** were then completed in a parallel synthesis sequence (Scheme 6). First the phosphonates were each coupled with the fully elaborated C11–C26 aldehyde **8** under modified Still–Gennari olefination conditions,[16] providing the corresponding (*Z*)-enones, **19** → **26** (65%) and **20** → **27** (54%). DDQ-mediated oxidative cleavage of the C1 and C7 PMB ethers then gave the corresponding triols, **26** → **28** and **27** → **29**. In this situation, the

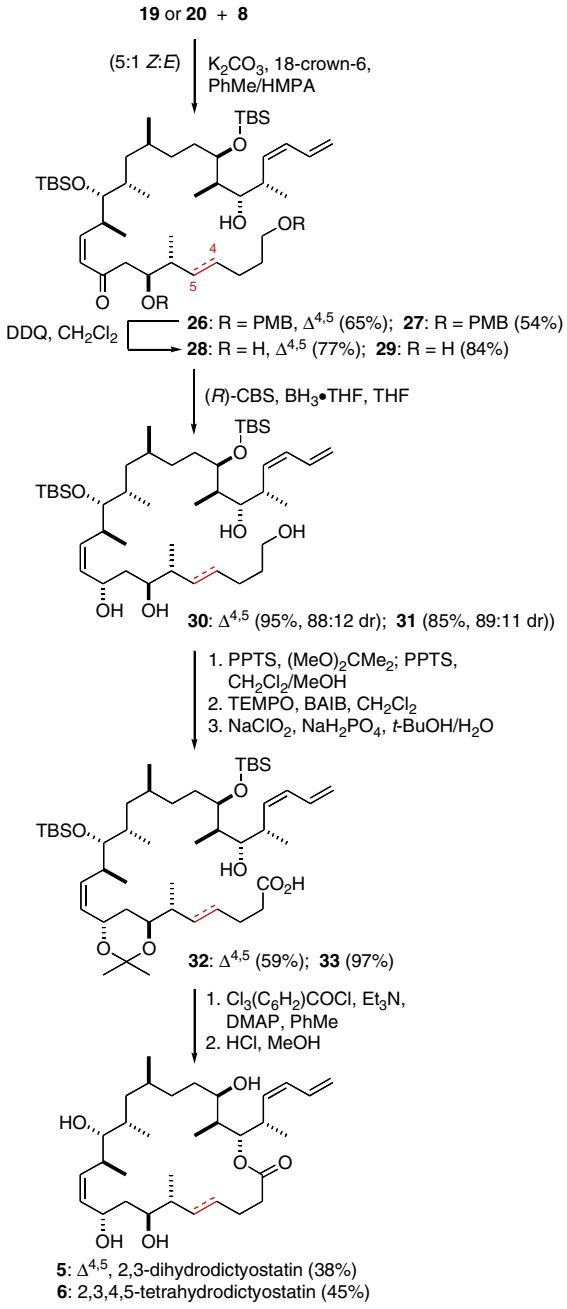


Scheme 5. Synthesis of β-ketophosphonates **19** and **20**.

Evans–Saksena hydroxyl-directed reduction proceeded with little or no stereocontrol. As previously observed, a switch to the CBS (*R*)-oxazoboroline-mediated reduction afforded the desired 1,3-*anti* diols, **28** → **30** (95%, 88:12 dr) and **29** → **31** (85%, 89:11 dr), with high diastereoselectivity. The 1,3-diols were then transformed into their acetonides, followed by chemoselective oxidation of the primary alcohol (TEMPO/BAIB; $NaClO_2$) to afford the *seco*-acids, **30** → **32** and **31** → **33**. Following macrolactonisation under Yamaguchi conditions,[20] global deprotection and HPLC purification afforded 2,3-dihydrodictyostatin (**4**, 39%) and 2,3,4,5-tetrahydrodictyostatin (**6**, 45%) in readiness for biological evaluation.

Biological evaluation. The cell growth inhibitory activities of the dictyostatin analogues **4**, **5** and **6** were evaluated in vitro relative to Taxol (**1**), dictyostatin (**2**), and discodermolide (**3**) against four cancer cell lines: AsPC-1 (pancreatic), DLD-1 (colon), PANC-1 (pancreatic) and NCI/ADR-Res (Taxol-resistant ovarian) (Table 1). Drug resistance is mediated in the NCI/ADR-Res cell line by the overexpression of a P-glycoprotein in the cell membrane, facilitating the removal of the drug agent from the cell.

Gratifyingly, all three synthetic dictyostatin analogues, **4**, **5** and **6**, showed low nanomolar antiproliferative activity in both the Taxol-sensitive and resistant cell lines. As anticipated, 6-desmethyldictyostatin (**4**) proved to be the most potent of the analogues studied, and was intermediate between dictyostatin and discodermolide. This result is consistent with the findings of the Curran group,[8b] where 6-*epi*-dictyostatin was found to be essentially equipotent to dictyostatin in its antiproliferative activity against the Taxol-resistant ovarian 1A9/Ptx22 cell line. Moreover, both sets of results suggest that the C6 substituent is located in a relatively open region of the binding site on β-tubulin, where it forms no strong interactions.



Scheme 6. Completion of the synthesis of 2,3-dihydrodictyostatin (**5**) and 2,3,4,5-tetrahydrodictyostatin (**6**).

Table 1
Cytotoxicity of Taxol (**1**), dictyostatin (**2**), discodermolide (**3**)[7a] and dictyostatin analogues **4**, **5** and **6** in cultured human cancer cells as determined by MTT metabolism following 72 h exposure to the test agent

Compound	IC_{50} (nM)[a]			
	AsPC-1 pancreatic	DLD-1 colon	PANC-1 pancreatic	NCI/ADR-Res
1	89 (±23)	22 (±3.5)	9.9 (±3.6)	1300 (±345)
2	6.2 (±1.7)	2.2 (±0.9)	4.2 (±1.7)	6.6 (±1.0)
3	98 (±34)	29 (±8)	59 (±34)	160 (±34)
4	56 (±25)	8.1 (±3.8)	17 (±10)	43 (±13)
5	94 (±28)	22 (±13)	42 (±7)	66 (±11)
6	120 (±25)	55 (±13)	64 (±33)	130 (±66)

[a] Values are ±standard deviation (in parenthesis) from a minimum of four separate experiments.

Partial saturation of the (2*Z*,4*E*)-dienoate in 2,3-dihydrodictyostatin (**5**) was found to be well tolerated and led to low nanomolar antiproliferative activity in both the Taxol-sensitive and resistant cell lines, again intermediate between dictyostatin and discodermolide. In comparison, 2,3,4,5-tetrahydrodictyostatin (**6**) showed cell growth inhibitory activity resembling that of discodermolide. We attribute this trend to a decreased entropic component of the binding energy as a result of the greater rotational freedom of the C1–C5 region upon saturation. As a nanomolar level of antiproliferative activity was still observed for both these analogues, the presence of the (2*Z*)-olefin or (4*E*)-olefin does not appear to be critical. In a separate series of incubatory experiments performed on the PANC-1 cell line, the three analogues **4**, **5** and **6** were shown to act in an analogous fashion to dictyostatin, through a mechanism of microtubule stabilisation, causing both an accumulation of cells at the G2/M phase and formation of characteristic dense intracellular microtubule bundles.

In conclusion, we have synthesised three potent new dictyostatin analogues by modification of the C2–C6 dienoate region. Removal of the C6 methyl substituent, as in 6-desmethyldictyostatin (**4**), and partial saturation of the (2*Z*,4*E*)-dienoate, as in 2,3-dihydrodictyostatin (**5**), were found to be well tolerated and led to low nanomolar antiproliferative activity in both the Taxol-sensitive and resistant cell lines, intermediate between dictyostatin and discodermolide. Full saturation of the (2*Z*,4*E*)-dienoate region, as in 2,3,4,5-tetrahydrodictyostatin (**6**), led to cell growth inhibitory activity resembling that of discodermolide. These new SAR results and that reported for other analogues,[7,8] combined with consideration of the bioactive conformation of dictyostatin,[9] should facilitate the design and synthesis of further simplified dictyostatin analogues that retain a low nanomolar cytotoxicity profile comparable to the natural product.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.09.109.

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23. Without the AcOH additive, the cross-metathesis reaction proceeded only in low yield (ca. 10%). It is proposed that the in situ generation of ruthenium hydride facilitates olefin isomerisation, preventing the metathesis proceeding. See: Hong, S. H.; Wenzel, A. G.; Salguero, T. T.; Day, M. W.; Grubbs, R. H. *J. Am. Chem. Soc.* **2007**, *129*, 7961.