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Synthesis and Biological Evaluation of (−)-16-Normethyldictyostatin: A Potent Analogue of (−)-Dictyostatin†

Youseung Shin,‡ Jean-Hugues Fournier,‡ Raghavan Balachandran,§ Charitha Madiraju,§ Brianne S. Raccor,‡ Guangyu Zhu,‡ Michael C. Edler,|| Ernest Hamel,|| Billy W. Day,*,‡,§ and Dennis P. Curran*,‡

Department of Chemistry and Department of Pharmaceutical Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania 15261, and Screening Technologies Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute at Frederick, National Institutes of Health, Frederick, Maryland 21702

curran@pitt.edu

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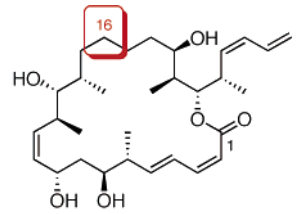
ABSTRACT



(−)-16-normethyldictyostatin, $IC_{50} < 1$ nm

(−)-16-Normethyldictyostatin has been made by total synthesis and is a potent antitumor agent in cells expressing wild-type tubulin and in one mutant cell line that is resistant to paclitaxel, but it is much less active than dictyostatin in another paclitaxel-resistant cell line where Val is substituted for Phe270. This provides strong evidence that the C16 methyl group of the dictyostatins is oriented toward Phe270 in the paclitaxel-binding site on β-tubulin.

Although it was first described by Pettit and co-workers in 1994,[1] the potent anticancer agent (−)-dictyostatin[2] has advanced slowly because it was only available in tiny quantities and because its complete stereostructure was not known. In 2004, Paterson and Wright proposed structure **1a** for dictyostatin.[3] Concurrent total syntheses of **1a** by our group and Paterson's confirmed the structure and have begun to address the supply issue.[4,5] Detailed in vitro and cell assays of dictyostatin **1a** suggest that it is as active as—possibly even more active than—its much more well studied cousin discodermolide **2a** (Figure 1).[6]

The stage is now set for study of structure/activity relationship in the dictyostatin series, and we have already described several active analogues that were made before the full structure of dictyostatin was known and that, in

† Dedicated to Prof. Iwao Ojima on his 60th birthday.
‡ Department of Chemistry, University of Pittsburgh.
§ Department of Pharmaceutical Sciences, University of Pittsburgh.
|| National Cancer Institute.

(1) (a) Pettit, G. R.; Cichacz, Z. A.; Gao, F.; Boyd, M. R.; Schmidt, J. M. *J. Chem. Soc., Chem. Commun.* **1994**, 1111−1112. (b) Pettit, G. R.; Cichacz, Z. A. US patent 5,430,053, 1995. (c) Isbrucker, R. A.; Cummins, J.; Pomponi, S. A.; Longley, R. E.; Wright, A. E. *Biochem. Pharm.* **2003**, *66*, 75−82.

(2) Originally called dictyostatin-1, the compound is now commonly called more simply dictyostatin.

(3) Paterson, I.; Britton, R.; Delgado, O.; Wright, A. E. *Chem. Commun.* **2004**, 632−633.

(4) (a) Paterson, I.; Britton, R.; Delgado, O.; Meyer, A.; Poullennec, K. G. *Angew. Chem., Int. Ed.* **2004**, *43*, 4629−4633. (b) Shin, Y.; Fournier, J.-H.; Fukui, Y.; Brückner, A. M.; Curran, D. P. *Angew. Chem., Int. Ed.* **2004**, *43*, 4634−4637.

(5) Model and fragment studies: (a) O'Neil, G. W.; Phillips, A. J. *Tetrahedron Lett.* **2004**, *45*, 4253−4256. (b) Kangani, C. O.; Brückner, A. M.; Curran, D. P. *Org. Lett.* **2005**, *7*, 379−382.

(6) Madiraju, C.; Edler, M. C.; Hamel, E.; Raccor, B. S.; Balachandran, R.; Zhu, G.; Giuliano, K. A.; Vogt, A.; Shin, Y.; Fournier, J.-H.; Fukui, Y.; Brückner, A. M.; Curran, D. P.; Day, B. W. Submitted for publication.

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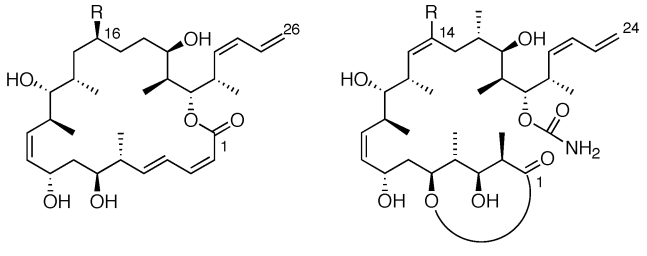


(–)-dictyostatin, **1a**, R = Me
16-normethyldictyostatin, **1b**, R = H

(+)-discodemolide, **2a**, R = Me
14-normethyldiscodermolide, **2b**, R = H

Figure 1. Structures of dictyostatin, discodermolide, and their normethyl analogues.

hindsight, are closely related.[7] Among the many possible analogues to target, we focused on 16-normethyldictyostatin **1b** because Smith has already shown that the analogous discodermolide analogue **2b** is highly active[8,9] and especially because the isolated stereocenter at C16 is the most difficult one to introduce. If this stereocenter were not needed for activity, then synthesis of analogues for SAR and drug development would be facilitated. We report herein the syntheses and biological characterization of (−)-16-normethyldictyostatin **1b**. This compound proves to be highly potent, with very interesting features.

The strategy to make 16-normethyldictyostatin **1b** is similar to that used for the parent **1a**[4b] and is summarized in Figure 2. The molecule is cut into large fragments at the

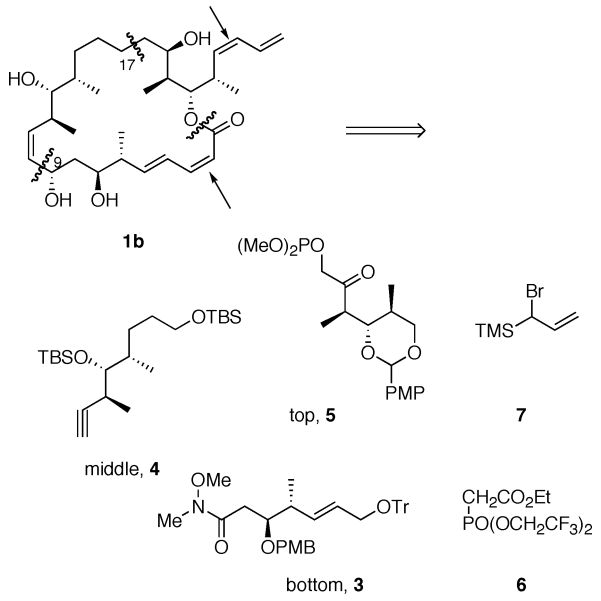
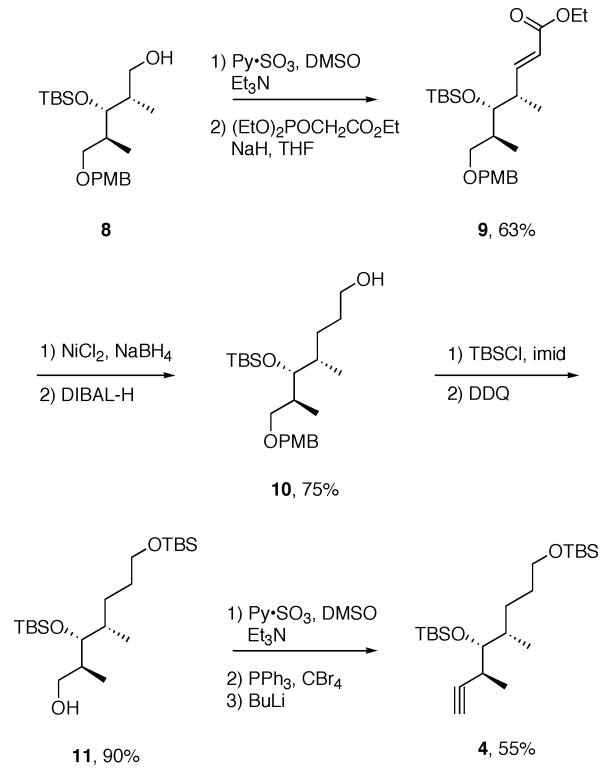


Figure 2. Synthetic strategy for 16-normethyldictyostatin.

C−O bond of the macrolactone, C9−C10 and C17−C18 (see wavy lines), and then the dienes are truncated (see arrows). This leaves large top **5** (C18−C23), middle **4** (C10−C17), and bottom (C3−C9) fragments **3** along with small olefinating reagents **6** and **7**.

The synthesis of the middle fragment **4** is simpler than that of its counterpart in the dictyostatin synthesis[4b] and is summarized in Scheme 1. Starting from the popular disco-

Scheme 1. Synthesis of the Middle Fragment **4**



dermolide and dictyostatin intermediate **8**,[10] oxidation and Horner−Wadsworth−Emmons (HWE) reaction provided unsaturated ester **9**. This was reduced with nickel boride to saturate the alkene, and then the ester was reduced to give **10**. Protection of the free alcohol as a TBS ether followed by removal of the PMB group gave **11**. Oxidation, Corey−Fuchs olefination, and subsequent elimination then provided key alkyne **4** in 26% overall yield (unoptimized) from **8**.

The fragment coupling and completion of the synthesis of **1b** follow the parent synthesis[4b] with comparable or better yields (Scheme 2). Briefly, fragment coupling of **3** and **4** followed by Noyori and Lindlar reductions provided **12** ready

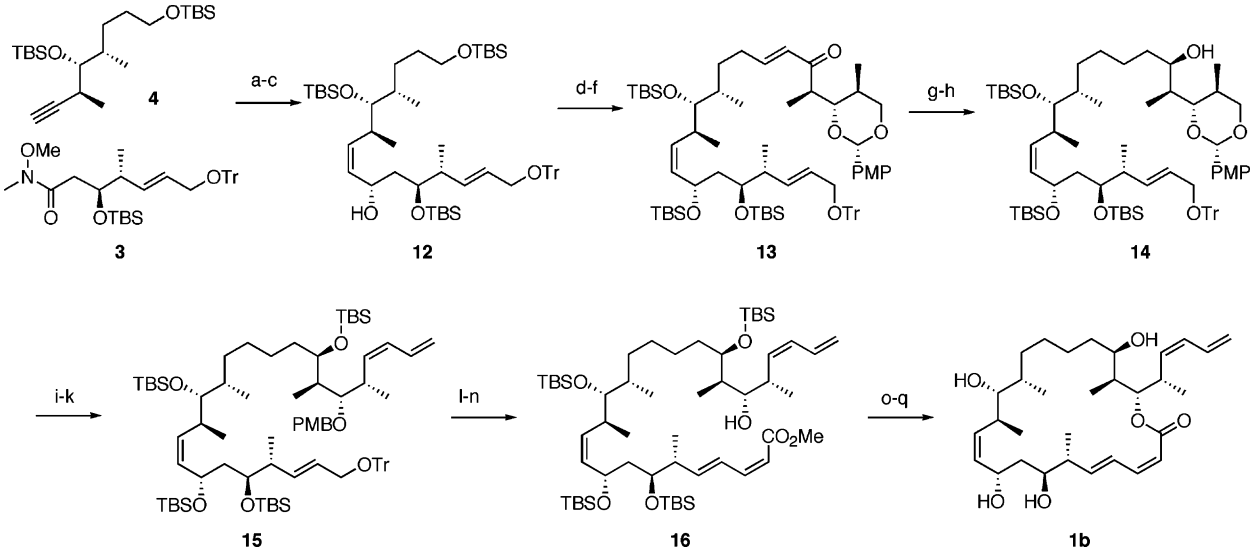
(7) Shin, Y.; Choy, N.; Turner, T. R.; Balachandran, R.; Madiraju, C.; Day, B. W.; Curran, D. P. *Org. Lett.* **2002**, *4*, 4443−4446.

(8) (a) Martello, L. A.; LaMarche, M. J.; He, L.; Beauchamp, T. J.; Smith, A. B.; Horwitz, S. B. *Chem. Biol.* **2001**, *8*, 843−55. (b) Smith, A. B.; Freeze, B. S.; LaMarche, M. J.; Hirose, T.; Brouard, I.; Rucker, P. V.; Xian, M.; Sundermann, K. F.; Shaw, S. J.; Burlingame, M. A.; Horwitz, S. B.; Myles, D. C. *Org. Lett.* **2005**, *7*, 311−314. (c) Smith, A. B.; Freeze, S. B.; LaMarche, M. J.; Hirose, T.; Brouard, I.; Xian, M.; Sundermann, K. F.; Shaw, S. J.; Burlingame, M. A.; Horwitz, S. B.; Myles, D. C. *Org. Lett.* **2005**, *7*, 315−318.

(9) The backbone of dictyostatin has two more carbons than discodermolide, so C16 of **1b** corresponds to C14 of **2b**.

(10) Mickel, S. J.; Sedelmeier, G. H.; Niederer, D.; Daeffler, R.; Osmani, A.; Schreiner, K.; Seeger-Weibel, M.; Berod, B.; Schaer, K.; Gamboni, R. *Org. Process Res. Dev.* **2004**, *8*, 92−100.

Scheme 2. Fragment Coupling and Synthesis of (−)-16-Normethyldictyostatin[a]



[a] Key: (a) *n*-BuLi, THF, 86%; (b) (*S*,*S*)-Noyori catalyst (20 mol %), *i*-PrOH, 95%; (c) Lindlar catalyst, H_2 (balloon), toluene, 91%; (d) TBSOTf, 2,6-lutidine, CH_2Cl_2, 96%; (e) HF−pyridine, pyridine, THF, 0 °C, 1 day, 71%; (f) (i) Dess−Martin oxidation, (ii) $Ba(OH)_2$, **5**, THF−H_2O, 83% (two steps); (g) $NiCl_2$, $NaBH_4$, MeOH−THF, 65%; (h) $Li(OtBu)_3H$,THF, 95% (β), 5% (α); (i) TBSOTf, 2,6-lutidine, CH_2Cl_2, 92%; (j) DIBALH, CH_2Cl_2, 90%; (k) (i) Dess−Martin oxidation, (ii) CH_2═CHCH(TMS)Br, $CrCl_2$, THF, (iii) NaH, THF, 82% (three steps); (l) $ZnBr_2$, CH_2Cl_2−MeOH, 89%; (m) (i) Dess−Martin oxidation, (ii) $(CF_3CH_2O)_2P(O)CH_2CO_2Me$, KHMDS, 18-crown-6, THF, 88% (two steps); (n) DDQ, CH_2Cl_2−H_2O, 82%; (o) 1 N KOH, EtOH−THF; (p) 2,4,6-trichlorobenzoyl chloride, Et_3N, THF then 4-DMAP (10 equiv), toluene, 76% (two steps); (q) 3 N HCl−MeOH, THF, 24%.

for addition of the top fragment. This was accomplished by deprotection, oxidation, and HWE olefination with **5** to give **13**. Reduction of **13** with nickel boride was followed by ketone reduction with $Li(OtBu)_3H$[11] to give **14** in 95% yield; a small amount of the minor α-isomer was easily removed by flash chromatography. Elaboration of the top diene to give **15** was followed by completion of the bottom diene to give **16**. Deprotection of the PMB group and ester hydrolysis, followed by Yamaguchi lactonization,[7] provided the protected macrolactone. Global deprotection followed by careful purification resulted in some material loss but provided 25 mg of **1b** (24% yield) in excellent purity.

16-Normethyldictyostatin **1b** was thoroughly characterized spectroscopically by the usual means (see the Supporting Information). Essentially all the resonances in the ^1H NMR spectrum of **1b** could be assigned by 2D NMR experiments, allowing a direct comparison with **1a**. Interestingly, while the proton−proton coupling constants of the two molecules are very similar, the chemical shifts of a number of protons, including several remote from C16, are quite different.

Biological analyses of **1b** provided exciting results. The antiproliferative activity of the compound was examined in human ovarian carcinoma 1A9 cells and in clones 1A9PTX10 and 1A9PTX22 of this line resistant to paclitaxel due to mutations in β-tubulin at the paclitaxel binding site (Table 1).[11] The 1A9PTX10 line expresses β-tubulin with a Phe270→Val mutation, while the 1A9PTX22 line expresses Ala364→Thr β-tubulin. Neither of these mutations has ever been discovered in clinical cancer samples, but they are important cell biology tools to establish orientations of ligands within the taxoid binding site.

Paclitaxel (Ptx) showed subnanomolar potency against the parental 1A9 cells, but as expected the mutant cells showed about 90- and 70-fold resistance to the drug. Discodermolide **2a** was 2- to 4-fold less potent than **1a**. Normethyldictyostatin **1b** appeared to be essentially equipotent to its parent **1a** against the parental 1A9 cells and the 1A9PTX22 cells, but

Table 1. Biological Test Results of **1a**, **1b**, **2a**, and Paclitaxel (Ptx)

	antiproliferative $GI_{50} \pm SD$, nM[a] (fold resistance relative to 1A9)			% inhib of [^{3}H]Ptx binding ± SD[b]	tubulin assembly $EC_{50} \pm SD$[c] (μM)
compd	1A9	1A9PTX10	1A9PTX22		
1b	0.41 ± 0.52	470 ± 70 (1146)	5.6 ± 4.7 (14)	48 ± 3	14 ± 7
1a	0.69 ± 0.80	3.2 ± 2.4 (4.6)	1.3 ± 1.0 (1.9)	78 ± 2	3.1 ± 0.2
2a	1.7 ± 1.2	6.2 ± 3.6 (3.6)	7.0 ± 8.4 (4.1)	80 ± 2	3.6 ± 0.4
Ptx	0.71 ± 0.11	64 ± 8 (90)	51 ± 9 (72)	20 ± 1	25 ± 3

[a] Concentration of agent that caused 50% growth inhibition of cultures after 72 h ($N = 8$). [b] Percent inhibition by 4 μM test agent of binding of 2 μM [^{3}H]paclitaxel to a stoichiometric amount of microtubule polymer ($N = 9$). [c] Bovine brain tubulin (10 μM) in 0.2 M monosodium glutamate, 15 min at 20 °C, centrifugation, and Lowry determination of remaining soluble tubulin. The EC_{50} is the drug concentration required to polymerize 50% of the tubulin ($N = 3$).

(11) Hung, D. T.; Nerenberg, J. B.; Schreiber, S. L. *J. Am. Chem. Soc.* **1996**, *118*, 11054-11080.

experienced over 1000-fold resistance from the Phe270→Val β-tubulin mutant 1A9PTX10 cells. This remarkable result strongly suggests that the presence of a β-C16-methyl group in dictyostatin **1a** yields favorable interactions with either a phenylalanine or a valine at postion 270 in β-tubulin but that its absence in **1b** is tolerated only by the Phe270 wild type and not the Val270 mutant.

Examination of effects against isolated bovine brain tubulin showed **1b** to have about 60% of the ability of **1a** to inhibit the binding of [^{3}H]paclitaxel to microtubules,[6] and it was only 4.5-fold less active than **1a** in its ability to cause formation of tubulin polymer.[13] In each case, **1b** was more potent than paclitaxel in these activities found with isolated tubulin.

These results show that 16-normethyldictyostatin is an important member of the dictyostatin family. The middle fragment of this molecule is easier to make than that of dictyostatin, and this simplifies its synthesis. This analogue exhibits clear and powerful discodermolide/dictyostatin behavior. It is a potent antitumor agent in cells expressing wild-type tubulin and in one mutant cell line that is resistant to paclitaxel, but is much less active than dictyostatin in another paclitaxel resistant cell line. This provides strong evidence that the C16 methyl group of the dictyostatins, and by inference the C14 methyl group of the discodermolides, is oriented toward Phe270 in the paclitaxel binding site on β-tubulin.

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Supporting Information Available: Characterization data and copies of spectra for 16-normethyldictyostatin. This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) Giannakakou, P.; Sackett, D. L.; Kang, Y. K.; Zhan, Z.; Buters, J. T.; Fojo, T.; Poruchynsky, M. S. *J. Biol. Chem.* **1997**, *272*, 17118−17125.

(13) Lin, C. M.; Jiang, Y. Q.; Chaudhary, A. G.; Rimoldi. J. M.; Kingston, D. G. I.; Hamel, E. *Cancer Chemother. Pharmacol.* **1996**, *38*, 136−140.