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Streamlined Syntheses of (−)-Dictyostatin, 16-Desmethyl-25,26-dihydrodictyostatin, and 6-*epi*-16-Desmethyl-25,26-dihydrodictyostatin

Wei Zhu,[†] María Jiménez,[†] Won-Hyuk Jung,[†] Daniel P. Camarco,[‡] Raghavan Balachandran,[§] Andreas Vogt,[‡] Billy W. Day,*[,†,§] and Dennis P. Curran*[,†]

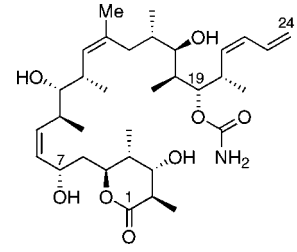
Departments of Chemistry, Pharmacology & Chemical Biology, and Pharmaceutical Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania 15261

Received April 26, 2010; E-mail: bday@pitt.edu; curran@pitt.edu

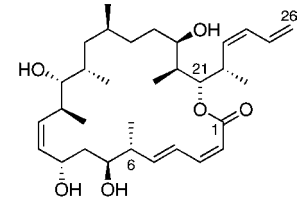
Abstract: The dictyostatins are a promising class of potential anti-cancer drugs because they are powerful microtubule-stabilizing agents, but the complexity of their chemical structures is a severe impediment to their further development. On the basis of both synthetic and medicinal chemistry analyses, 16-desmethyl-25,26-dihydrodictyostatin and its C6 epimer were chosen as potentially potent yet accessible dictyostatin analogues, and three new syntheses were developed. A relatively classical synthesis involving vinyllithium addition and macrocyclization gave way to a newer and more practical approach based on esterification and ring-closing metathesis reaction. Finally, aspects of these two approaches were combined to provide a third new synthesis based on esterification and Nozaki−Hiyama−Kishi reaction. This was used to prepare the target dihydro analogues and the natural product. All of the syntheses are streamlined because of their high convergency. The work provided several new analogues of dictyostatin, including a truncated macrolactone and a C10 *E*-alkene, which were 400- and 50-fold less active than (−)-dictyostatin, respectively. In contrast, the targeted 16-desmethyl-25,26-dihydrodictyostatin analogues retained almost complete activity in preliminary biological assays.

Introduction

After resting in the shadow of the important natural product (+)-discodermolide for about a decade,[1] (−)-dictyostatin (**1**) has recently emerged as a potentially useful anti-cancer agent in its own right. Pettit isolated dictyostatin in tiny quantities from a marine sponge in 1994, showed its potent anti-cancer activity, and provided its constitution.[2] Several years later, Wright isolated enough sample to garner information about the mechanism of action[3] and to collect a full battery of NMR spectra in collaboration with Paterson. These spectra were the basis for a complete assignment of configuration in 2004[4] that was confirmed almost immediately by simultaneous reports of total syntheses by Paterson[5] and our group.[6]



(+)-discodermolide



(−)-dictyostatin, **1**

These total syntheses provided precious samples and thereby paved the way for a detailed biological characterization of dictyostatin.[7] It is one of the most potent microtubule-stabilizing agents known, inducing the assembly of purified tubulin even more rapidly than paclitaxel. It inhibits the binding of paclitaxel, discodermolide, and epothilone B to microtubules. It is a powerful anti-proliferative agent in several standard and paclitaxel-resistant cancer cell lines.

There has also been a lively interplay between synthesis and structure−activity relationship (SAR) studies in the dictyostatin

[†] Department of Chemistry.
[‡] Department of Pharmacology & Chemical Biology.
[§] Department of Pharmaceutical Sciences.

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class. The original syntheses have been refined and improved,[8] new total syntheses by Phillips[9] and Ramachandran[10] have appeared, and partial syntheses of key fragments have provided additional new options.[11] Building on these foundations, our group[12] and Paterson's[13] have made an assortment of analogues and stereoisomers of dictyostatin either by modifications of existing synthetic routes or by introducing new routes. A clear outline of the dictyostatin SAR has emerged,[14] and several potent analogues with potential advantages over dictyostatin itself have been identified. Most importantly, in the first in vivo studies of any dictyostatin, synthetic analogue 6-*epi*-dictyostatin was more effective than paclitaxel in treating mice bearing human breast cancer xenografts.[15]

An important next step in the field would be to advance dictyostatin, 6-*epi*-dictyostatin, or another analogue farther down the road of preclinical development toward possible clinical trials. The current syntheses of dictyostatin are, however, at the outer limit of today's technology for scale-up, and the active analogues of dictyostatin are not that much simpler to make than is the parent. So, there is a need for streamlined syntheses of the class and for discovery of analogues with biological profiles comparable to that of dictyostatin but that are easier to make. Here we report advances toward both of these objectives.

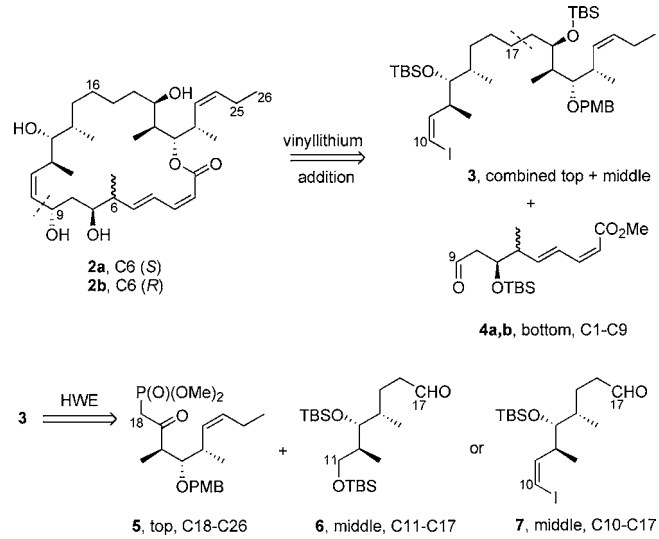

Figure 1. High-level plan of the vinyllithium approach.

Based on SAR and metabolic lability analyses,[12f,16] we identified 16-desmethyl-25,26-dihydrodictyostatin and its 6-epimer as potential analogues of dictyostatin that might be active yet considerably easier to make. We have developed three new, streamlined routes to this class of compounds that are based on vinyllithium addition, ring-closing metathesis (RCM), and Nozaki−Hiyama−Kishi (NHK) coupling. Along the way, we implemented a straightforward esterification method to make the problematic O22−C1 bond. In contrast to related macrolactonization reactions, no isomerization of the adjacent C2−C3 alkene was observed. To culminate the synthesis studies, the NHK approach was used to make (−)-dictyostatin itself. This is the first synthesis in which all of the carbon atoms of the molecule are built into the three main fragments so that none are introduced after fragment couplings begin. The increased convergency makes this the shortest current synthesis of dictyostatin. Finally, both of the new analogues exhibit interesting results in preliminary biological assays.

Results and Discussion

Analogue Design and Vinyllithium Addition Route. The new analogues targeted in this work are 16-desmethyl-25,26-dihydrodictyostatin (**2b**) and its 6-epimer, **2a** (see Figure 1). These were selected for several reasons. First, removal of the C16 methyl group deletes an isolated stereocenter, thereby simplifying the synthesis. 16-Desmethyldictyostatin is a known compound with an interesting biological profile.[12b,13c] Second, metabolism[16] and SAR work in the discodermolide area[17] suggested that modification of the terminal C25−C26 alkene of dictyostatin, while retaining good activity, might be possible. Reduction of this alkene provides a dihdyro analogue that is not much simpler than the parent. We felt, however, that

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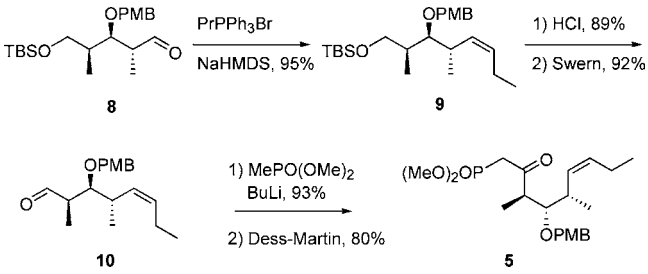
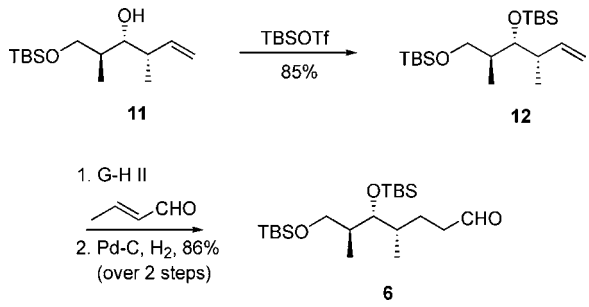
Scheme 1. Synthesis of Top Fragment **5**, C18−C26



Scheme 2. Synthesis of Middle Fragment **6**, C11−C17



synthetic opportunities to make such molecules could be greatly expanded because dictyostatin has five alkenes and a sixth is present during the synthesis. Due to its exposure and therefore high reactivity, the C25−C26 monosubstituted alkene of the terminal diene complicates reactions such as metathesis or hydrogenation that are used to make or manipulate other alkenes. Such complications have been avoided by late introduction of one (or both) of the two dienes, but this sacrifices convergency. Finally, the terminal double bond of discodermolide is a metabolic soft spot,[16] and other dihydro analogues of dictyostatin at C10,C11 and C2,C3 are biologically active.[13d,g]

We adopted the strategy shown in Figure 1 to make the new analogues **2a**,**b**. Three main fragments—top (C18−C26, **5**), middle (C11−C17, **6** or C10−C17, **7**), and bottom (C1−C9, **4**)—were targeted. The commonly used Horner−Wadsworth−Emmons (HWE) reaction[18] first joins the top and middle fragments to give **3**, while the vinyllithium addition later appends the bottom fragment. We have studied related vinyllithium approaches,[12d] and Ramachandran has used a vinylzinc addition as a key step in his synthesis.[10] We initially used known middle fragment **6** because it was available from prior work; however, it lacks a carbon atom of the target (C10) and therefore does not meet our objective of maximum convergency. We therefore later directly incorporated vinyl iodide **7** as the middle fragment to increase the convergency.

The top (C18−C26) fragment **5** was readily made in five steps from the well-known intermediate **8**[19] as summarized in Scheme 1. Briefly, *Z*-selective Wittig reaction to give **9**, then desilylation and Swern oxidation, provided an aldehyde (**10**) that was converted to the ketophosphonate **5** in the usual way. Full details of these and all reactions to make new intermediates and products are provided in the Supporting Information. Similarly, initial middle fragment **6** (Scheme 2) was made from homoallylic alcohol **11**[20] by cross-metathesis with crotonaldehyde[21] followed by reduction. Both epimers of the bottom fragment **4** were already in hand through an efficient six-step route based on cross-metathesis.[11i] All three fragments were readily made on a multigram scale.

The initial synthesis of combined top-middle fragment **3** (C10−C26) is summarized in Scheme 3. HWE coupling of fragments **5** and **6** gave enone **13** in 91% yield. The α,β-alkene of enone **13** was selectively reduced with Stryker's reagent in 95% yield.[22] Stereoselective reduction of the ketone with Li(*t*-BuO)$_3$AlH provided the alcohol as an 85:15 mixture of C19 epimers in 83% yield. After column chromatography to remove the minor epimer, alcohol **14** was then protected as the TBS ether in quantitative yield, and the primary TBS group was removed with a solution of HF·pyridine in pyridine/THF. The resulting alcohol was subsequently treated with Dess−Martin reagent to give aldehyde **15**, which was immediately subjected to Wittig reaction[23] to install the vinyl iodide functionality in top-middle fragment **3** in 66% yield over two steps.

The more convergent route to top-middle fragment **3**, featuring the complete middle fragment **7**, is summarized in Scheme 4. First, Marshall's palladium-catalyzed addition reaction of the allenylzinc reagent derived from (*R*)-2-mesyloxy-3-butyne to aldehyde **16**[24] gave alcohol **17** (82%, dr >95:5).[25] After protection of alcohol **17** as the TBS ether, the acetylene was deprotonated, iodinated, and reduced with *o*-nitrobenzenesulfonyl hydrazide[26] (NBSH) to provide *Z*-alkenyl iodide **18** (95%). Deprotection of primary TBS ether (97%) followed by Dess−Martin oxidation furnished the new middle fragment **7** (90%). This was then coupled to fragment **5** to provide top-middle fragment **3** (via enone **19** and alcohol **20**) in good overall yield by using the same sequence of steps as in Scheme 3. Note that only four steps are required to produce top-middle fragment **3** from the start of the fragment coupling (fragments **5** + **7**) in Scheme 4, whereas seven steps are needed to produce it from the same point (fragments **5** + **6**) in Scheme 3.

Coupling of the bottom fragments to top-middle fragment **3** and completion of the syntheses of 16-desmethyl-25,26-dihydrodictyostatin (**2b**) and its 6-epimer **2a** are summarized in Scheme 5. Yields and selectivities for both epimers were comparable (see data in the scheme), so we focus here only on the (6*S*)-epimer (the "**a**" series). Lithium−iodine exchange with top-middle fragment **3** followed by addition of aldehyde **4a** provided a mixture of three compounds that were separated by flash chromatography to provide C-9 epimers **21a-α** (29%) and **21a-β** (19%), together with the de-iodinated byproduct **22** (30%).

The established end-game was then followed with C9 α-epimer **21a-α** to complete the synthesis. Protection of the alcohol with TBSOTf (**23a**, 91%), followed by deprotection of the PMB group with DDQ, provided C21 alcohol **24a** in 85% yield. The C1 methyl ester was then hydrolyzed by using KOH

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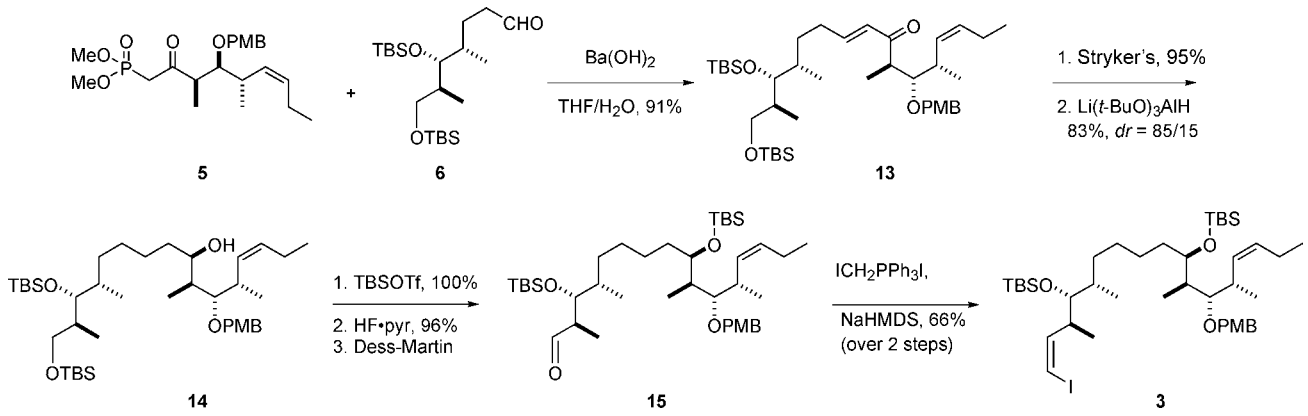
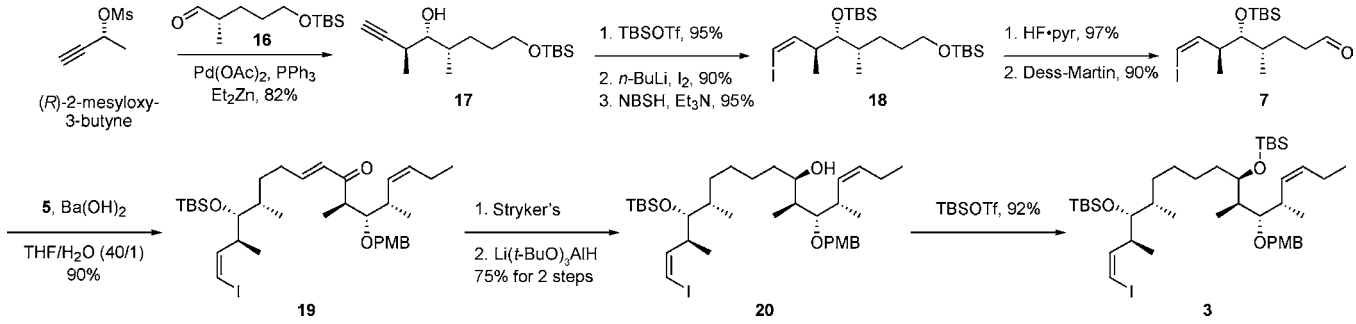
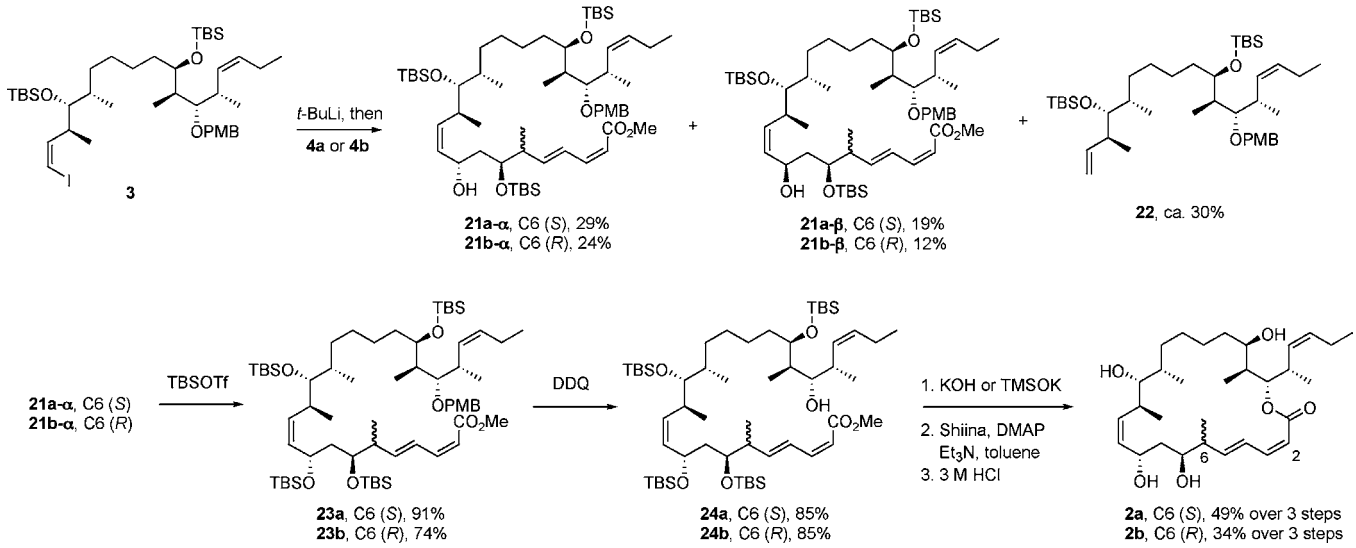
Scheme 3. Initial Synthesis of Top-Middle Fragment **3**, C10−C26



Scheme 4. Improved Synthesis of Top-Middle Fragment **3**, C10−C26



Scheme 5. End-game of the Vinyllithium Route



or TMSOK[27] (which gave a better yield) to produce a *seco*-acid. Macrolactonization of the *seco*-acid using the Shiina reagent (2,6-methylnitrobenzoyl anhydride)[28] in toluene gave the crude protected macrolactone in 93% yield as a 60:40 mixture of the target (2*Z*,4*E*)-dienyl lactone and its (2*E*,4*E*)-isomer (not shown). Global deprotection of the isomer mixture with HCl followed by isomer separation provided the target analogue **2a** (6 mg, 49% over three steps). Likewise, the (6*R*)-epimer **2b** was prepared on a 1.6 mg scale by a similar sequence of reactions starting from top-middle fragment **3** and bottom fragment **4b**. Again, (6*R*)-epimer **2b** had to be separated from its (2*E*,4*E*)-isomer.

This route is considerably shorter than other syntheses of dictyostatin, needing only 10 steps from the start of the fragment coupling to the end. The three fragments are also made in 10 steps or fewer, for a longest linear sequence of about 20 steps. On the down side, the key vinyllithium addition was low yielding and did not scale well,[29] and the formation of substantial

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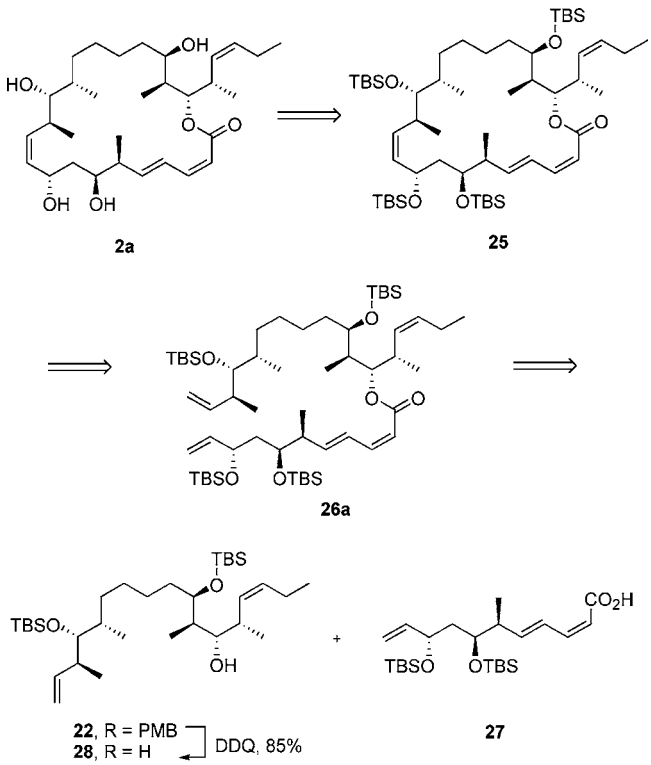


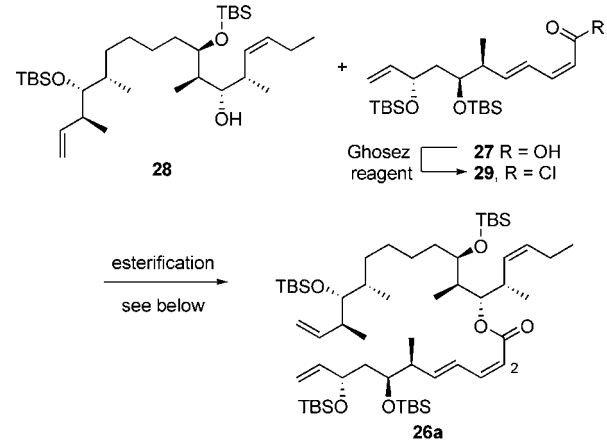
Figure 2. RCM route to the C10,C11 alkene.

amounts of (2*E*,4*E*)-isomer of the final macrolactonization was a persistent problem. (The *E*,*E*-isomers are not very active.[12d−f]) Because the final products **2a**,**b** showed promising preliminary biological activity (see below), we decided to pursue further improvements to the synthesis.

Esterification/Ring-Closing Metathesis Routes. Previous routes to dictyostatin and analogues almost all used macrolactonization as a penultimate step to close the ring. In the lone exception to date, O'Neil and Phillips closed the ring at the C2−C3 alkene by an intramolecular Still−Gennari reaction.[9] In recent years, the traditional late-stage lactonization strategy for making macrolactones has received competition from a strategy involving esterification followed by late-stage ring-closing metathesis.[30] This strategy is challenging for dictyostatin itself because chemoselectivity issues with the C10−C11 alkene and the two dienes arise. We[11b] and Phillips introduced RCM strategies to join bottom and middle fragments at the C10−C11 alkene, and Phillips carried his intermediate through to make dictyostatin.[9] In such approaches, however, both dienes have to be constructed after the initial fragment couplings.

On the basis of an understanding of cross-metathesis chemistry of dienyl esters,[11i,31] we hypothesized that the simple tactic of saturating the C25−C26 terminal alkene would enable late-stage RCM reactions to make molecules like targets **2a**,**b**. Accordingly, we drew up the strategy shown in Figure 2 involving fragments **27** and **28**. The top-middle fragment **28** (C11−C26) comes from deprotection of the PMB group of alkene **22** and is coupled with fragment **27** (C1−C10) to assemble the complete carbon skeleton of diene **26a**. Finally, RCM builds the C10−C11 bond and completes the macrocycle

Scheme 6. Bimolecular Esterification by Shiina Coupling and through an Acid Chloride



prec.	conditions	total yield	2*Z*/2*E*	yield **26a**
27	Shiina rgt DMAP, Et_3N	81%	58/42	47%
29	NaHDMS	75%	100/0	75%

25. The strategy is attractive because it could require as few as eight steps from the beginning of the fragment couplings to the target. Four of those steps (the top/middle coupling sequence, Scheme 4) were already well established and reliable. But, in addition to the chemoselectivity risk, there is also a risk regarding stereoselectivity. The previous RCM approaches to the C10−C11 *Z*-alkene used a temporary 10-membered lactone to ensure *Z*-selectivity in the RCM.[9,11b] Here, there is no such insurance. In part because we already had available both fragments **22** and **27**, we decided to take on the RCM challenge uninsured.

E/*Z*-Isomerization during macrolactonization has been a persistent problem in our and other syntheses of dictyostatins.[6,12] The use of the Shiina reagent[28] can sometimes suppress this isomerization, so we first tried esterification under the preferred conditions for macrolactonization. Alcohol **28** (1 equiv) and acid **27** (2 equiv) were reacted with the Shiina reagent (4 equiv) in the presence of excess Et_3N and DMAP (Scheme 6). An 81% yield of ester **26a** was obtained, but this was a 58:42 mixture of target (2*Z*,4*E*)- and isomerized (2*E*,4*E*)-dienyl esters. Apparently, the alkene isomerization is not associated with macrolactonization *per se*.

Roush has suggested that related isomerizations are induced by the acylation catalyst,[32] here DMAP, but Yamaguchi and Shiina reactions in this series do not work if the DMAP is omitted. Accordingly, we transformed acid **27** to the corresponding acid chloride **29** with the Ghosez reagent (1-choro-1-(dimethylamino)-2-methyl-2-propene).[33] Alcohol **28** (1 equiv) was deprotonated with NaHMDS, and then crude acid chloride **29** (1 equiv) was added. After standard workup and chromatography, the *Z*,*E*-isomer **26a** was obtained in 75% isolated yield along with 5% of recovered **28**. There was no evidence for formation of the *E*,*E*-isomer in the ^{1}H NMR spectrum of the crude product **26a**.

The acid chloride esterification method provided general access to the late-stage compounds needed for both this RCM

(29) Jung, W.-H. Ph.D. Thesis, University of Pittsburgh, 2007 (http://etd.library.pitt.edu/ETD/available/etd-03252008-125826/).
(30) Fürstner, A. *Angew. Chem., Int. Ed.* **2000**, *39*, 3012–3043.
(31) Funk, T. W.; Efskind, J.; Grubbs, R. H. *Org. Lett.* **2005**, *7*, 187–190.
(32) Roush, W. R.; Blizzard, T. A. *J. Org. Chem.* **1984**, *49*, 1772–1783.
(33) Devos, A.; Remion, J.; Frisque-Hesbain, A. M.; Colens, A.; Ghosez, L. *J. Chem. Soc., Chem. Commun.* **1979**, 1180–1181.

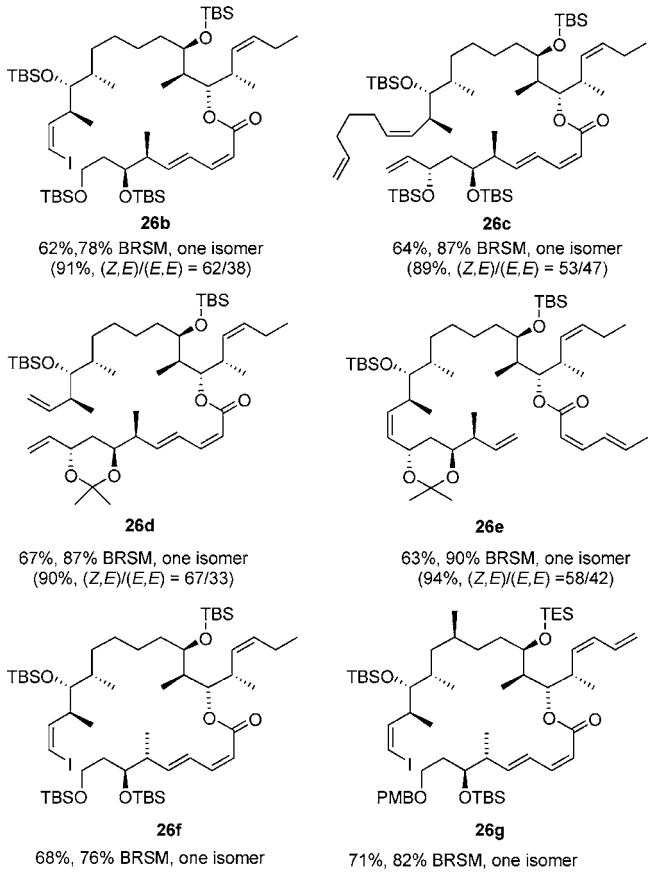
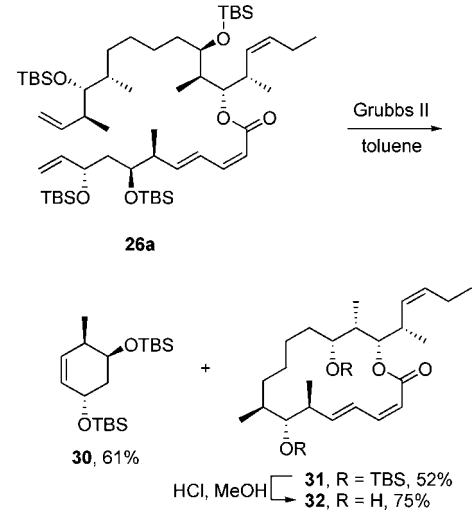


Figure 3. Esters made by the acid chloride route (with comparison data for the Shiina route in parentheses).

approach and the subsequent Nozaki−Kishi approach. Six additional esters were made by this method, as summarized in Figure 3. In four more cases (compounds **26b**−**e**), the acid chloride method was compared with the Shiina method for yields and stereoselectivity. The Shiina method uniformly provided excellent yields (89−94%) but poor stereoselectivities (53:47 to 67:33). Furthermore, the alkene isomers were not separable at this point. In contrast, the acid chloride provided lower total yields of ester (62−71%), but there was no alkene isomerization in any case. The moderate yields were offset by recovery of some amounts of the more complex starting alcohol. (We did not attempt to recover the acid **27**.) The acid chloride method was clearly superior because it provided a higher absolute yield of the *Z*,*E*-isomer and because it was much easier to separate the *Z*,*E*-isomer of **26** from the starting alcohol than from its *E*,*E*-isomer produced in the Shiina coupling. Furthermore, several of the acid chloride couplings in Figure 3 were only conducted once or twice on small scale, so prospects for optimization to increase conversion and yield remain.

We next examined the RCM reaction of diene **26a** under several conditions by varying the solvent, catalyst, and temperature. The target 22-membered ring product was not, however, detected in any case. In a typical experiment (Scheme 7), treatment of diene **26a** with 10% Grubbs II catalyst in refluxing toluene followed by cooling, concentration, and chromatography provided cyclohexene **30** and 16-membered ring product **31** in comparable yields (61% and 52%, respectively). The silyl groups of macrolactone **31** were removed with HCl/MeOH to provide the ring-contracted dictyostatin analogue **32** that was used for structure elucidation (see data in Supporting Information) and biological testing (see below).

Scheme 7. RCM Reaction of Diene **26a** Provides Truncated Products by RRCM



We ascribe the outcome of this RCM reaction to the initial formation of ruthenium carbene on C10 rather than C11, as shown in Figure 4. Instead of reacting with the terminal alkene at C11 to afford the 22-membered ring product, the carbene intermediate **33** reacted with the internal alkene at C5 to give a new ruthenium carbene (**34**) with extrusion of cyclohexene **30**. Carbene **34** then reacted with the terminal alkene to furnish the 16-membered ring product **31**. This failure nonetheless encouraged us because the C23−C24 alkene was a passive spectator in the process and because the structure of the side products suggested that both terminal alkenes could participate in RCM reactions.

In a sense, the ring-contracted product **31** was formed by a so-called "relay ring-closing metathesis" (RRCM) reaction.[34] We therefore first attempted to derail this RRCM reaction with a better RRCM option by designing substrate **26c**, which was readily prepared as shown in Scheme 8. Wittig reaction of aldehyde **15** and the ylide derived from **35** provided alkene **36** in 91% yield. Deprotection with DDQ gave alcohol **37** (92%), which was esterified as above to give diene **26c**. Assuming that the initial carbene is generated at the new terminal alkene of diene **26c**, this should be relayed in turn to a carbene at C11 (not C10), so the extrusion of cyclohexene **30** should be suppressed. It was not, however.

Again, several conditions were explored, but the RCM reactions of diene **26c** produced no major new products, giving instead two products that we already had in hand (Scheme 9). Starting from diene **26c**, we observed mostly product **26a**, in which the newly introduced side chain was simply clipped off. We also desilylated **26c** to tetraol **38**, but this gave similar results. Truncated product **39** was formed at short reaction times, followed by growth of its derived (doubly truncated) product **32**. In a typical experiment with diene **38** (Grubbs II, dichloromethane, reflux, 14 h or Grubbs II, toluene, reflux), we isolated **39**[35] and the 16-membered macrolactone **32** in 30% and 36% yields, respectively. On the basis of these results, we suppose

(34) Hoye, T. R.; Jeffrey, C. S.; Tennakoon, M. A.; Wang, J.; Zhao, H. *J. Am. Chem. Soc.* **2004**, *126*, 10210–10211.

(35) The sample of **39** isolated from this reaction still contained some impurities, but the structure assignment of **39** as the main component was clear from ^{1}H NMR spectroscopy and MS analysis (ESI *m*/*z* 548, $[M + Na]^+$).

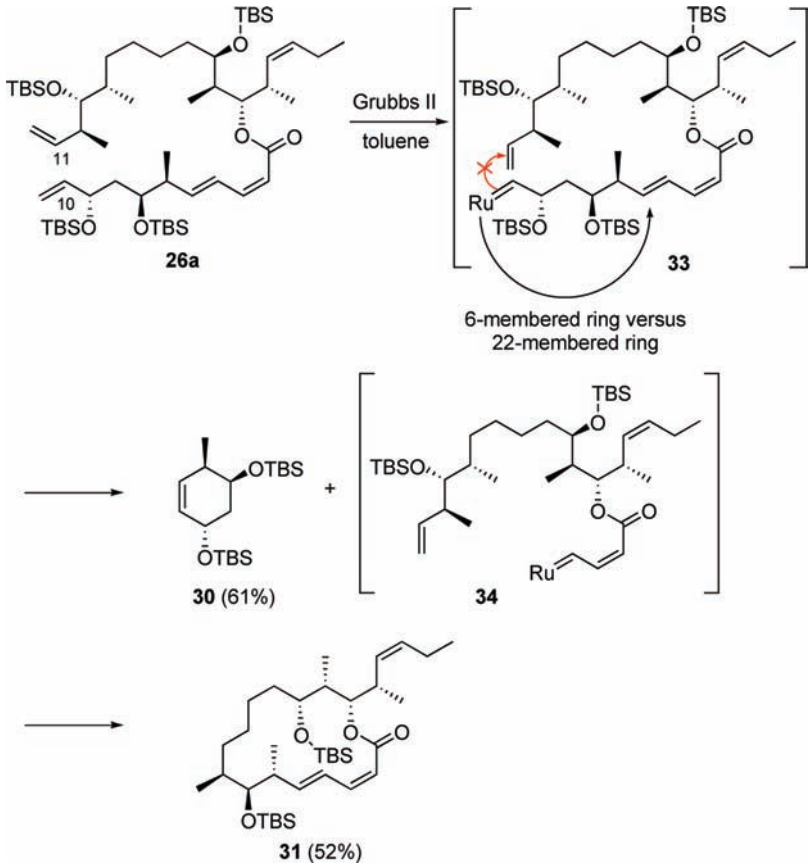
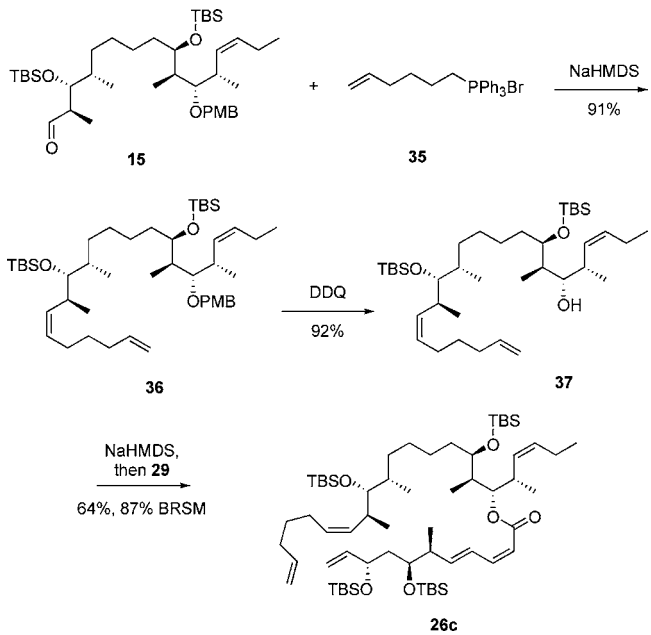


Figure 4. Suggested pathway for formation of truncated RCM products.

Scheme 8. Synthesis of RRCM Precursor **26c**



that the initial metathesis was initiated at the terminal alkene and then relayed to C11 as expected. This carbene (not shown) then suffered cross-metathesis to give **39** rather than RCM to give the target macrolactone. In turn, **32** is a secondary reaction product derived from **39**.

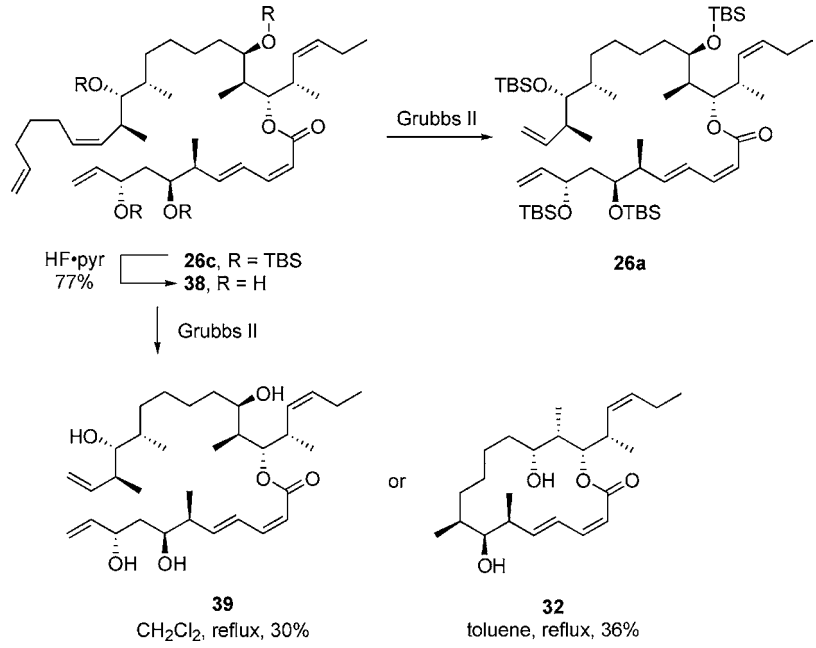
Noting the demonstration that RCM reactions could be initiated at C10, we prepared precursor **26d** (Scheme 10) with a dioxane acetal protecting group on O7 and O9 in place of the two TBS groups. The RRCM contraction reaction that took place with **26a** should now be prevented (because the extruded product would be a *trans*-bicyclo[3.3.0] ring). The acetal **40** was easily made from the bis-silyl ether by desilylation and acetonide formation (see Supporting Information). The esterification reaction to make **26d** was already shown in Figure 3.

Initial reactions of **26d** with Grubbs II catalyst provided little or no conversion. Speculating that this might be caused by the increased Lewis basicity of the acetal oxygens compared to the prior silyl ethers,[36] we added Lewis acids to interfere with potential chelate formation of ruthenium carbene intermediates. Indeed, treatment of **26d** with 10% Grubbs II catalyst and 25% $Ti(O^iPr)_4$ cleanly produced a new 22-membered ring macrocycle **42** in 72% isolated yield. Analysis of a set of 2D NMR spectra of **42** provided assignments for all of the protons, and the coupling constant $J_{10,11}$ was extracted. Its 15 Hz magnitude showed that the RCM reaction produced exclusively the 10*E*-isomer. Indeed, desilylation of **42** did not produce **2a** but gave instead its stereoisomer **43**. Paterson has recently reported 10,11-dihydrodictyostatin,[13d] and **43** is the first 10,11-*E* isomer of the dictyostatin family. The dihdyro analogue is active, but the *E*-isomer is not (see below).

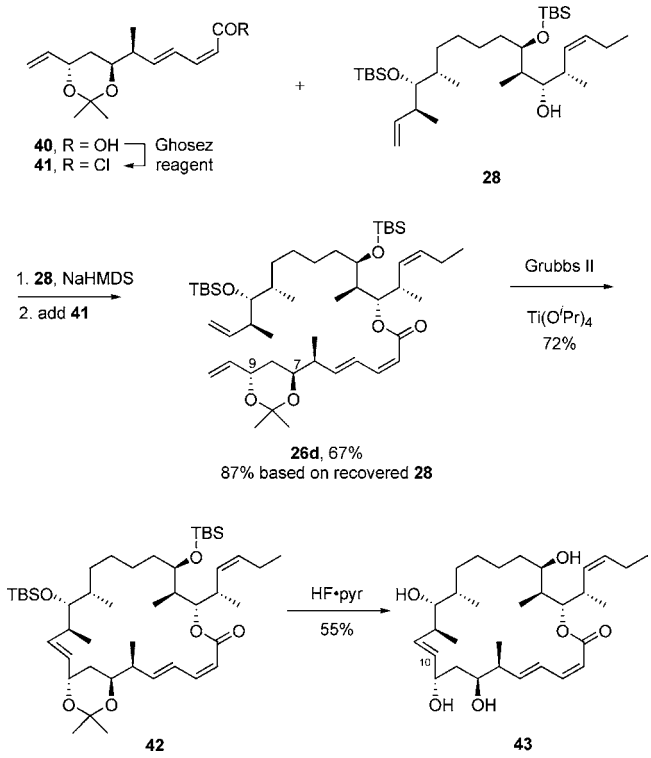
On the basis of what we learned from this series of RCM reactions, we redesigned the RCM approach to form the C4−C5 *E*-alkene via **26e**, as shown in Figure 5. In the syntheses of the bottom fragments above, we routinely used a related cross-metathesis reaction. This early-stage precedent suggests that a late-stage application could work if other pathways for carbene

(36) Fürstner, A.; Langemann, K. *J. Am. Chem. Soc.* **1997**, *119*, 9130–9136.

Scheme 9. Typical RCM Reaction of **26c**



Scheme 10. Synthesis and RCM Reaction of **26d**



reaction are not available. On the surface, substrate **26e** has major design problems since initiation of the RCM reaction at three different places (C2, C10, and C24) could be followed by rapid extrusion of a cyclohexene and a truncated carbene. We expected the reaction of **26e** to initiate at C5, however, so the lone extrusion reaction to derail is the RRCM reaction starting there (which would form the same product **30** as in Scheme 7, though in the reverse direction). We again initiated this path by using the *trans*-acetal protecting group.

The synthesis and RCM reaction of **26e** are shown in Scheme 11. Generation of the vinyllithium reagent from iodide **3** (C10−C26) and addition of aldehyde **46** (C5−C9) provided a mixture of epimeric alcohols at C9 that was not easily separated. This product was subjected to Dess−Martin oxidation to afford the enone **47** in 52% yield for two steps. After DDQ deprotection of the bis-PMB ether, the enone was diastereoselectively reduced with Evans−Saksena conditions[37] to give the 1,3-*anti* triol **48** (90% yield, dr 75:25). Triol **48** was then protected with 2,2-dimethoxypropane to give the acetonide **49** in 94% yield. The esterification reaction between acetonide **49** and (2*Z*,4*E*)-hexa-2,4-dienoyl chloride[38] gave RCM precursor **26e** in 63% yield (90% BRSM). The RCM reaction was run with $Ti(O^iPr)_4$ as additive, and product **50** was isolated in 65% yield. Global deprotection of macrolactone **50** with HF•pyridine gave 6-*epi*-16-desmethyl-25,26-dihydrodictyostatin **2a** (70%), whose analytical and spectroscopic data (R_f, ^1H NMR spectra, and co-injection on chiral HPLC) matched those of the previous synthetic sample, thus confirming the structure and stereochemical outcome of this RCM reaction.

Because top-middle fragment **3** is an intermediate in both the RCM and vinyllithium synthesis of **2**, it is instructive to compare and contrast the approaches. Recall that **3** is already four steps in from the start of the fragment couplings. The vinyllithium route requires six more steps to provide **2**, whereas the RCM route requires eight. The yields are about the same (8 and 9%, respectively). The RCM route is apparently less convergent because what was the "bottom fragment" in prior approaches (C1−C9) is now introduced in two pieces rather than one. The two "extra" steps in RCM route are, however, misleading because we separated the epimeric C9 alcohols directly in Scheme 1 but added the steps of oxidation and reduction to get the target epimer in Scheme 11. Therefore, the step count of the two routes is effectively the same.

(37) (a) Evans, D. A.; Chapman, K. T.; Carreira, E. M. *J. Am. Chem. Soc.* **1988**, *110*, 3560–3578. (b) Saksena, A. K.; Magiaracina, P. *Tetrahedron Lett.* **1983**, *24*, 273–276.

(38) For the synthesis of the corresponding acid, see: Smith, A. B., III; Safonov, I. G.; Corbett, R. M. *J. Am. Chem. Soc.* **2002**, *124*, 11102–11113.

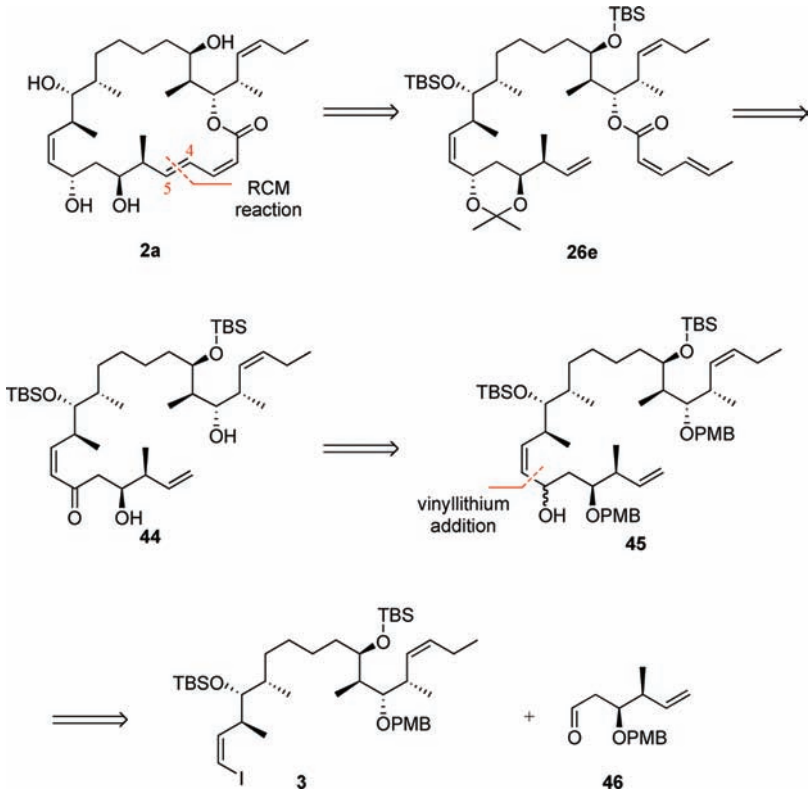
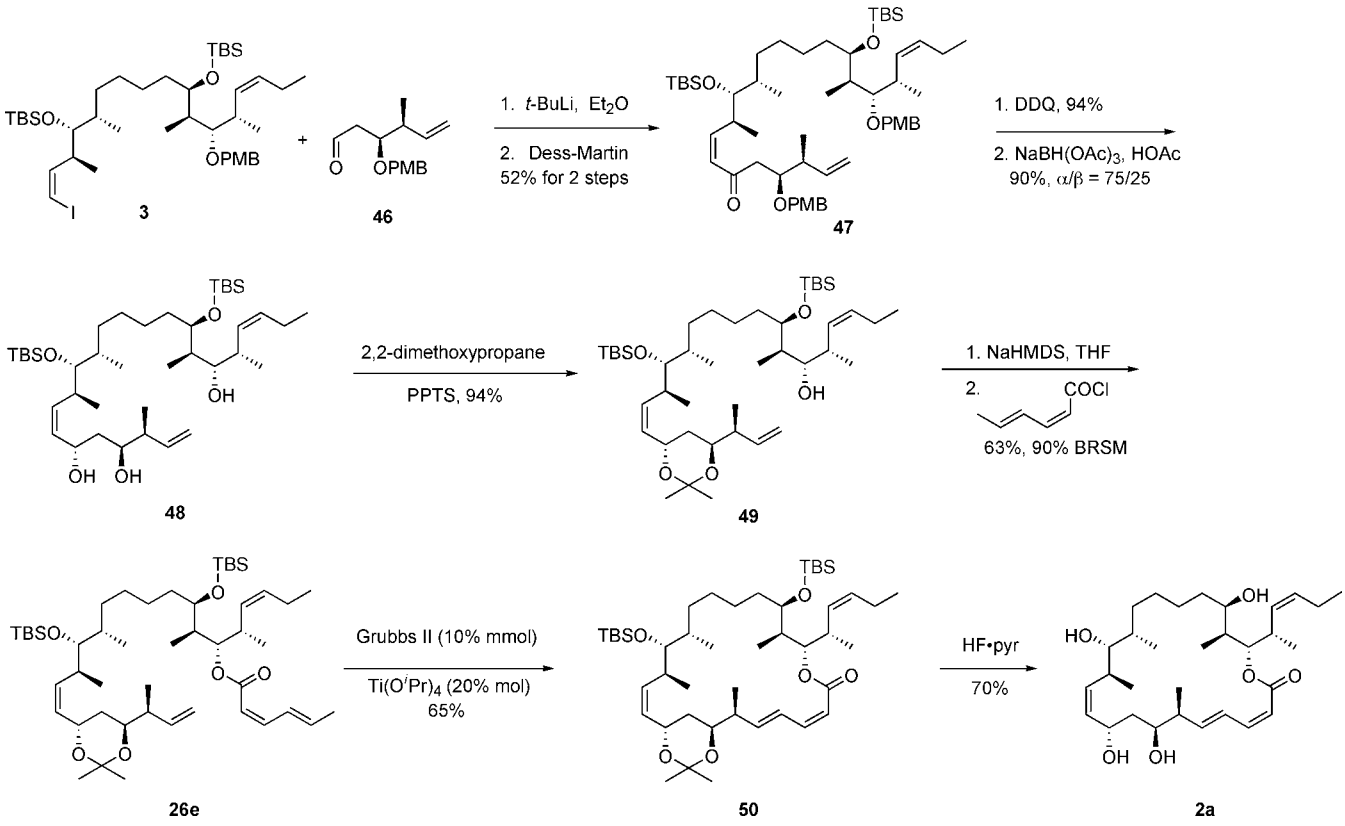


Figure 5. RCM route to the C4−C5 alkene.

Scheme 11. Successful RCM Route to **2a**



Furthermore, while the vinyllithium route is already maximally convergent (assuming no changes to the basic fragment assembly strategy), the successful RCM route is not. The convergency and step count of this synthesis could be considerably improved by appending the fragment **46** (C5−C9) to the middle fragment (see **7**) and the dienyl ester fragment (C1−C4) to the top fragment (see

5) prior to the fragment couplings. This could provide a very efficient synthesis based on two (rather than three) main fragments with as few as six steps for the end game.

Nozaki−Hiyama−Kishi Route. Although the RCM route has many attractive features coupled with potential for further improvement, it still probably cannot directly accommodate the C25−C26 terminal alkene present in both the natural product dictyostatin and the preclinical candidate 6-*epi*-dictyostatin. Accordingly, we pursued a third approach based on macrocyclization by Nozaki−Hiyama−Kishi reaction[39,40] that is a hybrid of the first two approaches. Like the RCM approach, the esterification is done before making the macrocycle. Like the vinyllithium approach, the macrocycle is made from an alkenyl iodide and an aldehyde. Maier proposed a related approach and prepared key fragments but did not yet describe the NHK reaction.[11d,e]

The plan for the NHK approach to dihydro analogues **2a**,**b** is summarized in Figure 6. Cyclization of alkenyl iodide **52** should produce alcohol **51**, which is very similar to the usual penultimate intermediate **25** (one fewer TBS group). In turn, **52** is made by esterification of **54** (C10−C26) and **55** (C1−C9), followed by selective desilylation and oxidation. An attractive feature of the plan is the small number of steps needed from the beginning of the fragment coupling to the end of the synthesis (only five). The key concern is the viability and stereoselectivity of the proposed NHK reaction. Myles and coworkers used a bimolecular NHK reaction in their synthesis of discodermolide.[41] We had previously explored a bimolecular NHK approach to dictyostatin with limited success.[42] The model addition reactions succeeded, but the stereoselectivity at C9 was very low.

The fragment coupling and execution of the NHK reaction are shown in Scheme 12. Common intermediate **3** was first deprotected with DDQ to give alcohol **54** in 94% yield, and then **54** was coupled with the acid chloride **55a** (prepared from the corresponding acid **53a** and the Ghosez reagent) to afford the ester **26b** (62%, 78% BRSM). The primary TBS ether was removed with HF·pyridine, and the resulting alcohol was oxidized to **52a** (92%) with the Dess−Martin reagent. Epimer **52b** was prepared by a similar sequence of reactions in comparable yields.

The key intramolecular NHK reaction[40] of **52a** was then examined under various conditions, and details of this survey

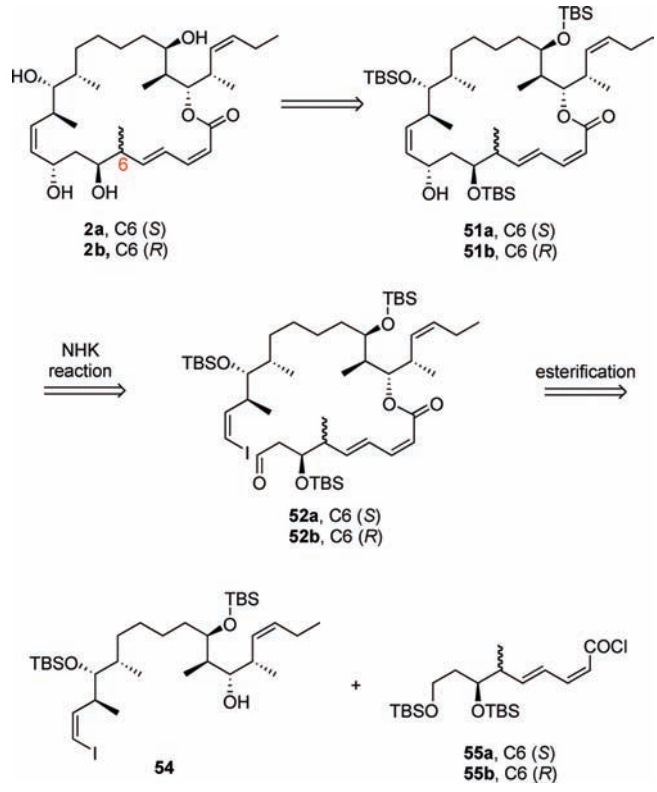


Figure 6. Plan for the NHK route to **2**.

are contained in the Supporting Information. Under the best conditions identified, **52a** was reacted with excess chromium chloride and 4,4′-di-*tert*-butyl-2,2′-dipyridyl (15 equiv each) and $NiCl_2$(dppf) (0.2 equiv) in THF at room temperature. After workup and flash chromatography, macrocycle **51a** was isolated in 53% yield as a single stereoisomer. The configuration of NHK product **51a** was confirmed by global deprotection as usual to provide **2a**, which was identical to the previous two samples.

A similar sequence of reactions in the C6-(*R*) series starting from **53b** provided NHK precursor **52b** in comparable yields. This time, however, the NHK reaction was not as stereoselective, and a 75:25 mixture of **51b** and its C9 epimer (not shown) was produced. The mixture was desilylated, and the target C9 epimer **2b** was isolated in 47% yield after careful purification. Apparently the stereoselectivity of the NHK reaction is modulated by the configuration at C6; however, even in the less selective (*R*) series, the stereoselectivity is still better than in related bimolecular model reactions.[42]

The NHK route is both convergent and efficient compared to the previous two routes. Starting from the same intermediate **3**, the NHK approach provides **2a** in six steps in 15.7% overall yield (six steps, 8.3% yield for vinyllithium addition approach; eight steps, 9% yield for RCM approach, respectively). In addition, we expected that dictyostatin analogues with the C25−C26 diene could be made by this route. To demonstrate this, we synthesized dictyostatin itself to culminate the work.

The new synthesis of dictyostatin is summarized in Schemes 13 and 14. The combined top-middle fragment **63** (C10−C26) was prepared from alcohol **56**, a readily available intermediate from our first synthesis of dictyostatin (Scheme 13).[6] The TBS group in **56** was removed with HCl to give a diol that was protected as bis-TES ether **57** (86% yield, two steps). Removal of the PMB group with DDQ gave the corresponding alcohol (90% yield), which was oxidized with SO_3·pyridine, DMSO,

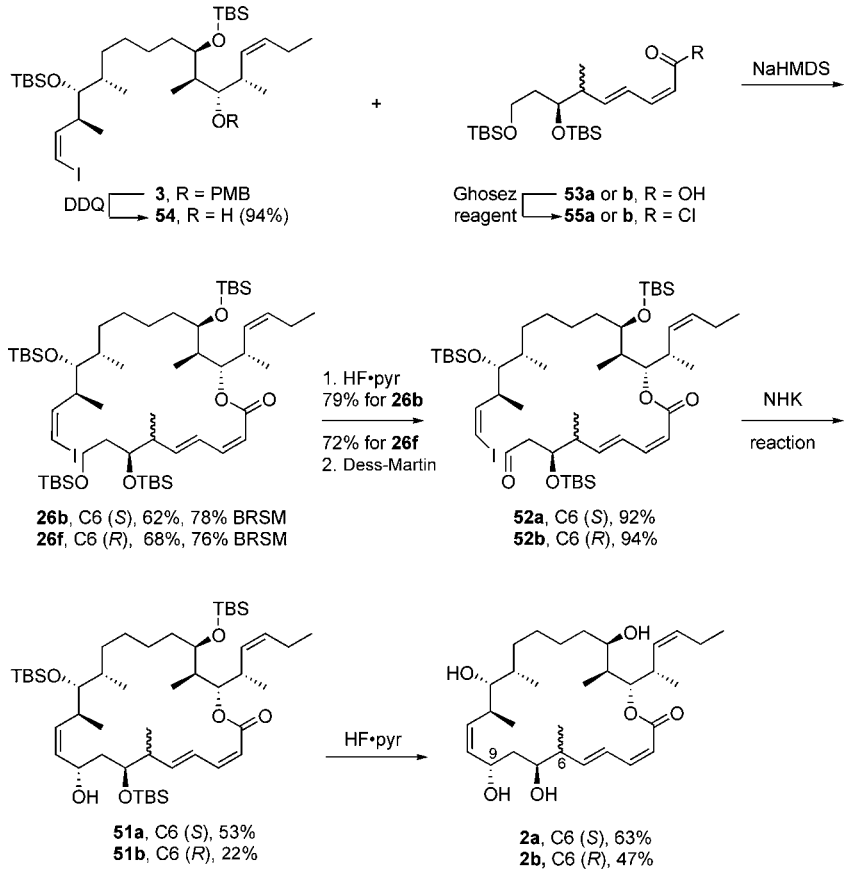
(39) (a) Okude, Y.; Hirano, S.; Hiyama, T.; Nozaki, H. *J. Am. Chem. Soc.* **1977**, *99*, 3179–3181. (b) Takai, K.; Tagashira, M.; Kuroda, T.; Oshima, K.; Utimoto, K.; Nozaki, H. *J. Am. Chem. Soc.* **1986**, *108*, 6048–6050. (c) Fürstner, A. *Chem. Rev.* **1999**, *99*, 991–1046.

(40) For recent examples of intramolecular Nozaki−Hiyama−Kishi reactions, see: (a) Takao, K.-I.; Hayakawa, N.; Yamada, R.; Yamaguchi, T.; Morita, U.; Kawasaki, S.; Tadano, K.-I. *Angew. Chem., Int. Ed.* **2008**, *47*, 3426–3429. (b) Marshall, J. A.; Eidam, P. M. *Org. Lett.* **2008**, *10*, 93–96. (c) Pelphrey, P. M.; Bolstad, D. B.; Wright, D. L. *Synlett* **2007**, *17*, 2647–2650. (d) Matsuda, M.; Yamazaki, T.; Fuhshuku, K.-I.; Sugai, T. *Tetrahedron* **2007**, *63*, 8752–8760. (e) Venkatraman, L.; Salomon, C. E.; Sherman, D. H.; Fecik, R. A. *J. Org. Chem.* **2006**, *71*, 9853–9856. (f) Suzuki, K.; Takayama, H. *Org. Lett.* **2006**, *8*, 4605–4608. (g) Bian, J.; Van Wingerden, M.; Ready, J. M. *J. Am. Chem. Soc.* **2006**, *128*, 7428–7429. (h) Namba, K.; Kishi, Y. *J. Am. Chem. Soc.* **2005**, *127*, 15382–15383. (i) Araki, K.; Saito, K.; Arimoto, H.; Uemura, D. *Angew. Chem., Int. Ed.* **2004**, *43*, 81–84. (j) Njardarson, J. T.; Biswas, K.; Danishefsky, S. J. *Chem. Commun.* **2002**, *23*, 2759–2761. (k) Pilli, R. A.; Victor, M. M. *Tetrahedron Lett.* **1998**, *39*, 4421–4424.

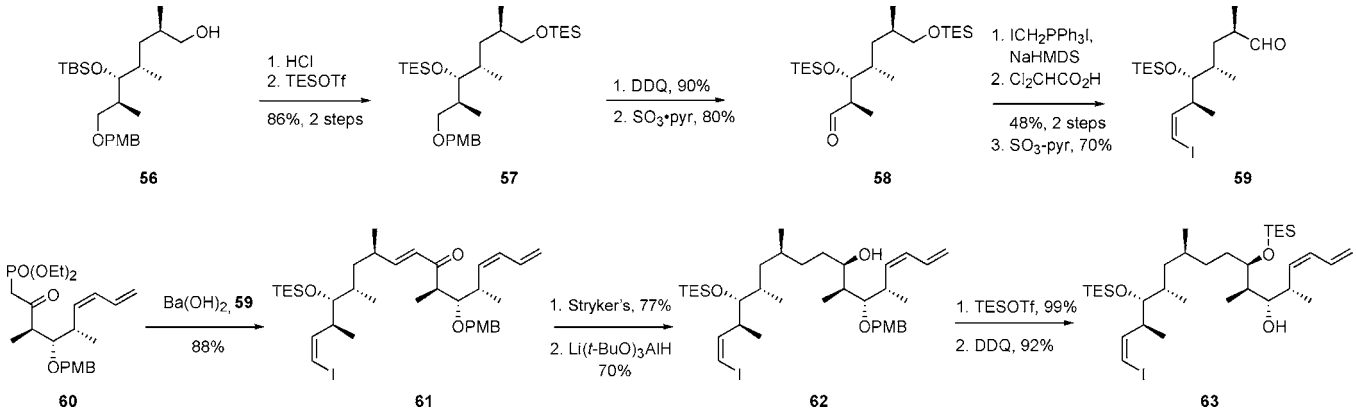
(41) (a) Harried, S. S.; Yang, G.; Strawn, M. A.; Myles, D. C. *J. Org. Chem.* **1997**, *62*, 6098–6099. (b) Harried, S. S.; Lee, C. P.; Yang, G.; Lee, T. I. H.; Myles, D. C. *J. Org. Chem.* **2003**, *68*, 6646–6660.

(42) Moura-Letts, G. Ph.D. Thesis, University of Pittsburgh, 2007 (http://etd.library.pitt.edu/ETD/available/etd-11162007-001904/).

Scheme 12. Synthesis of **2a**,**b** by the Nozaki–Hiyama–Kishi Route



Scheme 13. NHK Route to Dictyostatin: Synthesis of the Top-Middle Fragment **63**, C10–C26



and TEA (Parikh–Doering conditions) to provide the aldehyde **58** (80% yield). Next, Wittig olefination (ICH_3PPh_3, I_2, BuLi, and NaHMDS)[43] gave a (*Z*)-vinyl iodide, which was directly treated with dichloroacetic acid to give a primary alcohol (48% yield, two steps). Oxidation under Parikh–Doering conditions provided the C10–C17 aldehyde **59** (70% yield).

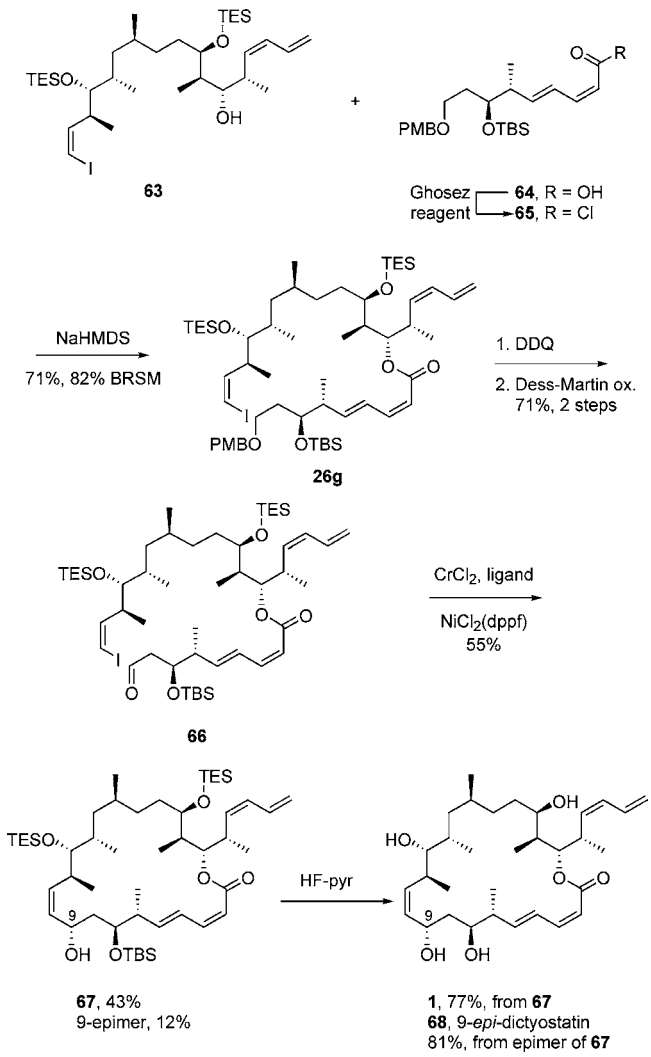
The usual C18–C26 phosphonate **60**[12c] was coupled with aldehyde **59**, mediated by $Ba(OH)_2$, to produce the enone **61** (88% yield). Again following established conditions, the C17–C18 alkene was reduced with the Stryker's reagent to give a ketone (77% yield), which was reduced with Li(*t*-BuO)$_3$AlH to afford two C19 epimers in a 78:22 ratio favoring the C19 β-epimer **62** (70%). Alcohol **62** was protected with TESOTf to give the TES ether (99%), followed by DDQ deprotection to afford fragment **63** (92%).

To start the end game, acid **64**[44] was converted to the acid chloride **65**, and this was coupled with alcohol **63** as usual to form the ester **26g** (71%, 82% BRSM). DDQ deprotection of PMB ether **26g** gave a primary alcohol, which was oxidized to aldehyde **66** (71% over two steps) by the Dess–Martin reagent. Treatment of **66** under optimal NHK reaction conditions gave macrocyclized product **67** in 55% combined yield as two separable C9 isomers in 78:22 in favor of the α isomer. Global deprotection of the major isomer provided natural dictyostatin **1** in 77% yield, identical with previous natural and synthetic

(43) Stork, G.; Zhao, K. *Tetrahedron Lett.* **1989**, *30*, 2173–2174.

(44) The synthesis of acid **64** is described in the Supporting Information of ref 12d.

Scheme 14. NHK Route to Dictyostatin: End-game Steps



samples. Global deprotection of the minor C9 isomer provided 9-*epi*-dictyostatin **68** (81%), whose spectra matched well those reported by Paterson.[13b] This synthetic route provides natural dictyostatin over a 22-step linear sequence with a 3.3% overall yield.

Preliminary Biological Testing Results. The target compounds 6-*epi*-16-desmethyl-25,26-dihydrodictyostatin (**2a**) and 16-desmethyl-25,26-dihydrodictyostatin (**2b**) were tested in comparison to 16-desmethyldictyostatin and paclitaxel for anti-proliferative activity against 1A9 human ovarian carcinoma cells and their paclitaxel-resistant subclones 1A9/PTX10 and 1A9/PTX22, as well as for microtubule-stabilizing activity in HeLa human cervical cancer cells. The side products 9-*epi*-dictyostatin (**68**), 6-*epi*-10,11-*E*-16-desmethyl-25,26-dihydrodictyostatin (**43**), and the 16-member macrolactone **32** were also examined. As shown in Table 1, both **2a** and**2b** caused microtubule bundling in HeLa cells and retained submicromolar growth inhibitory activity against 1A9 cells. In results that parallel those obtained with (−)-dicytostatin, the 1A9/PTX10 cells were cross-resistant to 25,26-dihydro derivatives, but the 1A9/PTX22 cells were not. This suggests that this saturation of the terminal alkene does not alter the mode of binding to tubulin. Interestingly, both analogues appeared to be much less susceptible to cross-resistance in both 1A9/PTX10 and 1A9/PTX22 than was 16-desmethyldictyostatin.

Conclusions

In summary, we have developed three new routes to the dictyostatin class of natural products. A relatively classical route based on vinyllithium addition and macrocyclization was first used to make 16-desmethyl-25,26-dihydrodictyostatin **2b** and its 6-epimer **2a**. That route is short and highly refined, but the two key reactions are problematic—the vinyllithium addition was low-yielding and not stereoselective, while the macrolactonization was high-yielding but marred by a nagging isomerization problem at the C2−C3 alkene.

Several new approaches to the 25,26-dihydro series of analogues based on esterification and ring-closing metathesis (RCM) were investigated. Synthesis of a bottom fragment acid chloride and esterification occurred in good yield and without isomerization at the C2−C3 alkene. This solution to the isomerization problem proved quite general and opened the door to both RCM and Nozaki−Hiyama−Kishi (NHK) approaches. RCM approaches to make the C10−C11 bond were problematic, with formation of truncated products or *E*-isomers prevailing. In contrast, an approach to make the C4−C5 *E*-alkene succeeded well, avoiding pitfalls like formation of truncated products and isomerization of the C2−C3 *Z*-alkene. This RCM approach already shows excellent potential for making diverse 25,26-dihydrodictyostatins, and room remains for improvement with increased convergency.

Finally, the discovery of practical esterification conditions allowed us to acylate intermediates from the initial vinyllithium approach and then close the macrocycle on the acylated products by an intramolecular NHK reaction. Though modest yields (up to about 50%) in the key NHK reaction have been obtained at this point, the route is attractive because it is highly convergent and because there is moderate to excellent stereoselectivity for

Table 1. Cell-Based Activities of New Analogues in Comparison to Paclitaxel and 16-Desmethyldictyostatin

test agent	MDEC[b] for tubulin polymer increase, nM ± SD (*N*)	GI_{50},[b] nM + SD (fold resistance)		
		1A9	1A9/PTX10	1A9/PTX22
2b	43.2 ± 10.3 (3)	285 ± 18	2817 ± 37 (10)	445 ± 27 (1.6)
2a	54.2 ± 3.1 (3)	241 ± 7	4090 ± 21 (17)	193 ± 4 (0)
9-*epi*-dictyostatin	74.4 ± 42.5 (4)	79 ± 1	2160 ± 160 (27)	160 ± 45 (2)
32	>5000 (2)	17000 ± 0.2	35000 ± 0.1 (2)	23000 ± 0.2 (1.4)
43	713.0 ± 145.3 (3)	820 ± 10	2610 ± 210 (3)	1370 ± 170 (1.7)
16-desmethyl-dictyostatin	25 ± 9 (3)	0.41 ± 0.52	470 ± 70 (1146)	5.6 ± 4.7 (14)
(−)-dictyostatin	12.8 ± 3.7 (4)	0.69 ± 0.80	3.2 ± 2.4 (4.6)	1.3 ± 1.0 (1.9)
6-*epi*-dictyostatin	9.1 ± 6.3 (9)	0.85 ± 0.03	4.5 ± 0.3 (5)	0.81 ± 0.17 (0)
paclitaxel	5.2 ± 0.4 (3)	0.71 ± 0.11	64 ± 8 (70)	51 ± 9 (72)

[a] Minimum detectable effective concentration of the test agent in HeLa cells after 21 h of continuous exposure. [b] Fifty percent growth inhibitory concentration after 72 h of continuous exposure to the test agent ($N = 4$).

formation of a C9 secondary alcohol stereocenter. The NHK route was used to produce both C6 epimers of 25,26-dihydrodictyostatin and dictyostatin itself.

The synthetic work marks significant advances toward the practical synthesis of dictyostatin and analogues for both drug discovery and development purposes. The "three fragment" approaches are all based on related fragments that can be made in 10 steps or fewer on large scale and that can be coupled, refunctionalized, and deprotected in less than 10 steps. Accordingly, the longest linear sequences of steps are 20 or less, and the total numbers of steps (from three commercial starting materials) are 36−40. All the approaches maximize convergency by building the complete carbon skeleton into the fragments; no carbons are added after the fragment couplings begin. The NHK approach to dictyostatin is the first synthesis of the natural product to meet this target, and it is currently the shortest synthesis of the natural product.

Both of the new 6-epimers of 25,26-dihydrodictyostatin exhibit considerable activity in preliminary biological assays. The saturation of the potentially troublesome 25,26-terminal alkene also opens new options for synthetic manipulations. Accordingly, the further investigation of the medicinal chemistry of this series of analogues is an attractive line of research.

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Supporting Information Available: Detailed descriptions of the synthesis and characterization of all new compounds, along with copies of key NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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