A "Methyl Extension" Strategy for Polyketide Natural Product Linker Site Validation and Its Application to Dictyostatin

Stephen Ho,[†] Dan L. Sackett,[‡] and James L. Leighton*,[†]

[†]Department of Chemistry, Columbia University, New York, New York 10027, United States
[‡]Program in Physical Biology, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892, United States

Ⓢ *Supporting Information*

ABSTRACT: An approach to the validation of linker strategies for polyketide natural products with few or no obvious handles for linker attachment, and its application to dictyostatin, are described. Analogues in which the C(6)- and C(12)-methyl groups were replaced by 4-azidobutyl groups were prepared and shown to retain the low nanomolar potency of dictyostatin. Further, conjugation of the C(6) analogue with a cyclooctyne resulted in only minor attenuations in potency. Together, these results shed light on the binding of dictyostatin to β-tubulin, establish a validated linker strategy for dictyostatin, and set the stage for the synthesis and study of dictyostatin conjugates.

Figure 1. (a) Dictyostatin, Paterson's C(9)-OMe analogue **1**, and the penultimate intermediate in our synthesis, **2**. (b) In crowded polypropionate arrays, alcohol acylation might result in significant structural perturbations, whereas methyl extension should not.

Strategies for the selective delivery of small-molecule cancer chemotherapeutic agents to tumor cells (e.g., antibody–drug conjugates[1]) hold promise as a way to, in effect, increase their therapeutic index. A requirement for the drug in many of these approaches is a validated linker strategy,[2] the most critical component of which is the identification of a site on the drug that may be modified without any deleterious impact on its activity. More broadly, the identification of such modifiable sites on bioactive natural products can facilitate chemical biology and mechanism of action studies and enable exploration of more novel linked constructs. Dictyostatin,[3−5] for which we developed a synthesis that proceeds in 14 steps in the longest linear sequence,[6] is a worthy candidate for linker strategy validation in that it is among the most potent of the microtubule-stabilizing agents (MSAs), retains significant potency against taxane-resistant cell lines, and has been shown to be a rare example of a brain-penetrant MSA.[7]

The principal challenge in the identification of modifiable sites on polyketide/polypropionate structures such as dictyostatin is that the hydroxyl groups may be the only readily modifiable groups (Figure 1a). Such an alcohol modification strategy would require the identification of an "innocent" hydroxyl group that is not critical for activity as well as a synthetic strategy to allow for selective modification of only that hydroxyl group. In this regard, we were aware of Paterson and Wright's demonstration that the C(9)-OMe analogue **1** mostly retains the low nanomolar potency of the natural product,[8] and mindful that the penultimate intermediate in our synthesis (**2**) is one in which the C(9)-OH group is, uniquely, unprotected (Figure 1a). Despite this, we rejected a C(9)-OH
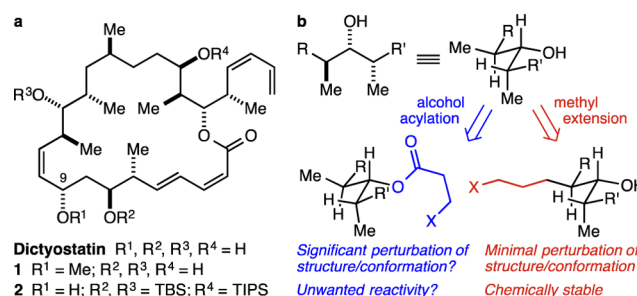
modification approach because we were concerned (1) that complex ether formation with our late stage intermediate might be difficult and (2) that acylation, the synthetically straightforward alternative, might be expected to subtly but significantly perturb the local electronic and steric structure and global conformation of the natural product[9] as well as raise concerns about acyl group migration or cleavage *in vivo* (Figure 1b). Indeed, such concerns are not strictly hypothetical, as Paterson has demonstrated that analogues of a dictyostatin/discodermolide hybrid in which the C(7)- and C(9)-OH groups were acylated with taxoid side chains were susceptible to both acyl migration and methanolysis, and were significantly less potent than the parent compound.[10] Having rejected an alcohol acylation strategy, we became intrigued by the notion that the ideal approach would entail modifying one of the ubiquitous methyl groups to a linker-bearing linear alkyl group (Figure 1b). In most contexts, linear alkyl groups are electronically and sterically equivalent to methyl groups, and this approach would be expected to result in as minimal a perturbation of the structure and conformation of the natural product as possible, while also obviating any concerns about O-acyl migration or cleavage.

For guidance as to the selection of appropriately disposed methyl groups we turned to the extensive structure–activity relationship (SAR) data for dictyostatin reported by Curran[11−14] and Paterson,[9,15,16] particularly as they related to the starkly contrasting models for the binding of dictyostatin in

the taxane binding site advanced by Curran and Snyder[17] and by Díaz and Jiménez-Barbero.[18] More specifically, we took note of Curran's demonstration that C(6)-*epi*-dictyostatin is as potent as the natural product and Paterson's demonstration that C(6)-normethyl-dictyostatin is only slightly less potent (≤1 order of magnitude) than the natural product. According to Curran and Snyder, only their model (Figure 2a) is fully
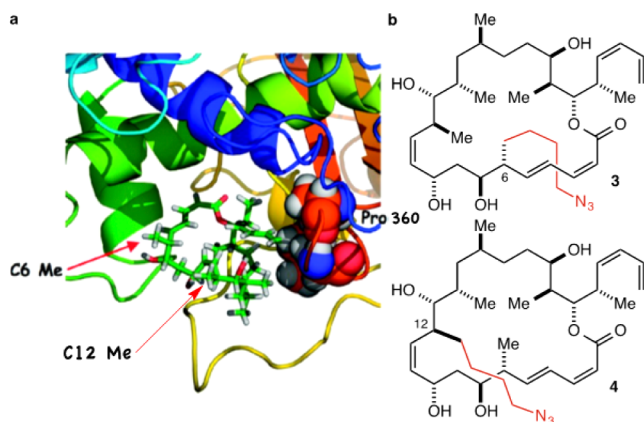

Figure 2. (a) Curran/Snyder model for the binding of dictyostatin in the taxane binding site. Reprinted with permission from ref 17. Copyright 2011 American Chemical Society. (b) The C(6)- and C(12)-methyl extended analogues 3 and 4.

consistent with this SAR data, as it places the C(6)-methyl group in a solvent exposed position without contacts to the receptor. Conversely, the Díaz/Jiménez-Barbero model places the C(6)-methyl group deeper into the binding pocket and in van der Waals contact with Pro360, which Curran and Snyder contend is inconsistent with the SAR data because deletion or epimerization of the C(6)-methyl group would remove this contact with Pro360 and be expected to lead to a significant decrease in potency. The models lead to similar conclusions regarding the C(12)-methyl group, though here more caution is warranted in that the Curran/Snyder model locates it in proximity to the M-loop (the yellow loop at the bottom of Figure 2a), which undergoes significant conformational changes upon the binding of an MSA in the taxane binding pocket.[19] Based on this analysis, we decided to target the C(6)- and C(12)-(4-azidobutyl) analogues 3 and 4 (Figure 2b), which we hoped would lead to a validated linker strategy for dictyostatin, and which would in the process provide support for the Curran and Snyder binding model.

In our synthesis of dictyostatin,[6] the C(6)-methyl group is installed in a Sc(OTf)$_3$-catalyzed crotylation[20] of aldehyde 5 to give ketone 6 (Scheme 1). To incorporate a 4-azidobutyl group instead, we employed a cross-metathesis reaction between allylsilane 7 and 6-chlorohex-1-ene using the second generation Hoveyda–Grubbs catalyst[21] (HG-II) to produce 8 (~3:1 *E:Z*), which was employed *in situ* in a Sc(OTf)$_3$-catalyzed allylation of aldehyde 5.[22,23] After ketal hydrolysis, 9 was isolated as a 3:1 mixture of diastereomers in 58% overall yield (90% ee for the major diastereomer). Subjection of 9 to a one-pot protection/bromination reaction gave 10, which was subjected to the Arbuzov/trans-esterification method we developed[6] to give Still–Gennari-type β-ketophosphonate 11. Displacement of the chloride with NaN$_3$ delivered 12, and Heck reaction with iodide 13 (TMSE = 2-trimethylsilylethyl) produced 14 in 58% overall yield. The diastereomers were separated at this stage, and we

Scheme 1. Synthesis of C(6) Analogue 3
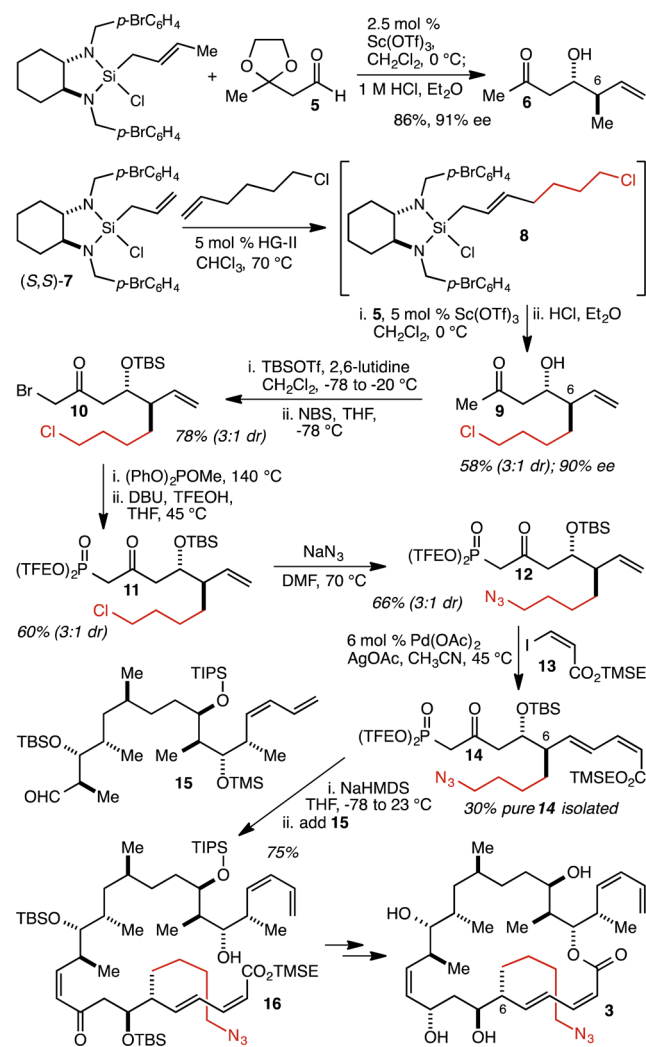
isolated 14 in 30% yield. Incorporation of 14 into the completed dictyostatin framework proved straightforward using our previously described synthesis.[6] Thus, Still-Gennari-type coupling of 14 with previously described aldehyde 15 resulted in the isolation of pure *Z*-isomer 16 in 75% yield. Four additional steps (ester deprotection, macrolactonization,[24] CBS reduction,[25] and global deprotection) delivered the targeted C(6)-(4-azidobutyl) analogue 3 (see the Supporting Information for details). Because we had previously prepared and stored a significant supply of aldehyde 15, this synthesis of 3 required just 10 total steps from 7, 5, and 6-chlorohex-1-ene.

To incorporate the 4-azidobutyl group at C(12), we adapted our recently reported two pot/three step protocol for the rapid and scalable synthesis of stereotriads.[26] Thus, silylformylation of 6-chlorohex-1-yne gave aldehyde 17 which was directly crotylated with (*S*,*S*)-*cis* EZ-CrotylMix[20] to give 18 in 90% yield and 95% ee (Scheme 2). Tamao oxidation/*anti*-diastereoselective tautomerization and protection of the aldehyde gave 19 in 66% yield over two steps. The remainder of the fragment synthesis followed our dictyostatin synthesis[6] with the added azide displacement step, and produced, by way of intermediates 20, 21, and 22, iodide 23. Iodide 23 was then used to produce analogue 4 (see the Supporting Information for details).

Both because of the greater ease of its synthesis and because it was found to be more potent than the C(12) analogue 4 (see

Scheme 2. Synthesis of C(12) Analogue 4
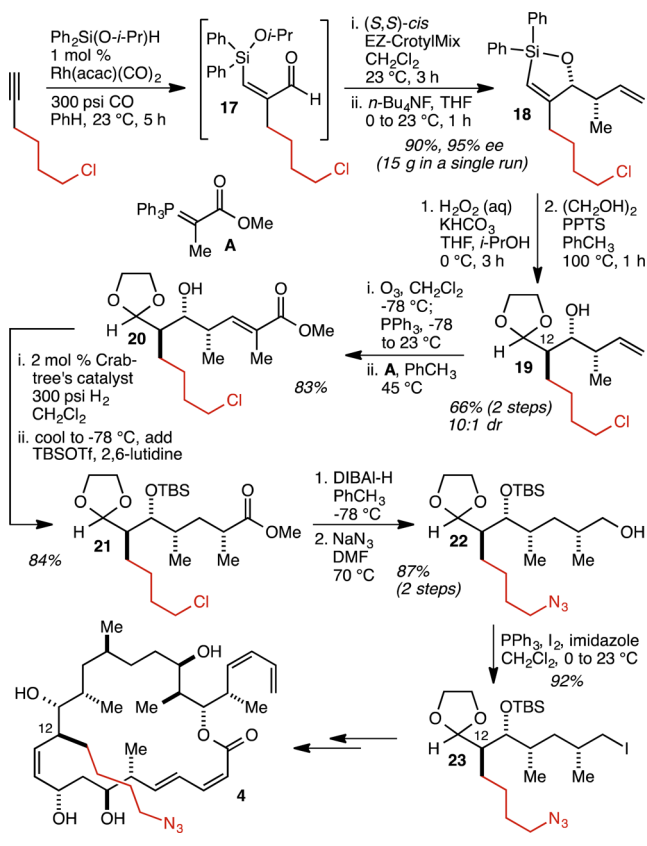
below), the C(6) analogue **3** was employed in model conjugation reactions. Treatment of **3** with **24**, van Delft's variant[27] of Bertozzi's cyclooctyne-based approach to metal-free click reactions,[28,29] afforded triazole **25** in 83% yield as the expected 1:1 mixture of diastereomers (Scheme 3). A traceless Staudinger ligation[30,31] was also carried out to give acetamide **26** in 35% yield.

Scheme 3. Model Conjugations with 3
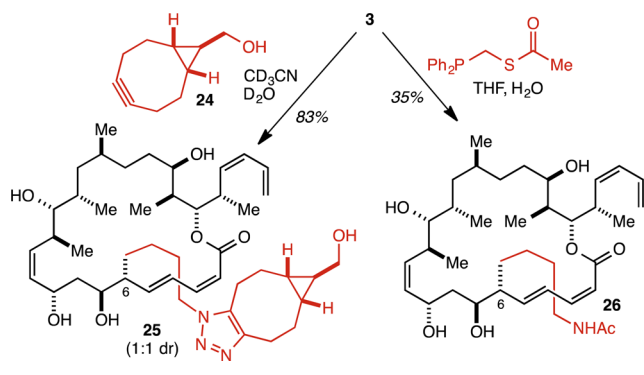
The C(6)- and C(12)-(4-azidobutyl) analogues **3** and **4** as well as triazole **25** and amide **26** were assayed for cell growth inhibition against four cell lines (PC3 (prostate), 1A9 (ovarian), DLD1 (colon), and A549 (lung)) alongside reference samples of synthetic dictyostatin and paclitaxel (Table 1). Consistent with our predictions, the GI$_{50}$ values for the C(6) analogue **3** were found to be only slightly attenuated relative to dictyostatin in all four cell lines, while the C(12) analogue **4** retains low nanomolar potency as well, albeit

Table 1. Cell Growth Inhibition GI$_{50}$ Values for 3, 4, 25, and 26[a]

compound	GI$_{50}$ (nM)			
	PC3	1A9	DLD1	A549
paclitaxel	1.9	0.4	15	1.2
dictyostatin	1.3	0.8	0.4	0.3
C(6) analogue **3**	1.9	2.2	1.1	1.1
C(12) analogue **4**	13	16	10	7.3
triazole **25**	4.8	8.4	13	25
amide **26**	50	30	32	22

[a]Values are the average over two or three experiments; see the Supporting Information for details.

with a somewhat more substantial drop in potency. Importantly, the activity of triazole **25** against the PC3 and 1A9 cell lines is only slightly diminished from that of azide **3**, while more substantial (though not catastrophic) reductions in potency are observed in the DLD1 and A549 cell lines. Surprisingly, the potency of amide **26** is reduced by 1−2 orders of magnitude relative to azide **3** against all four cell lines. Thus, although the specific structure of the linking group (e.g., triazole, amide) can have an impact on potency in a somewhat cell line-dependent manner, the results for triazole **25** against the PC3 and 1A9 cell lines in particular constitute a convincing proof-of-concept that dictyostatin conjugates may be prepared from C(6)-(4-azidobutyl) analogue **3** with only minor reductions in potency.

Together, these results (1) establish a validated linker strategy for dictyostatin and (2) constitute compelling evidence in support of the Curran/Snyder binding model for dictyostatin. More broadly, our work demonstrates that even in cases where a synthetically convenient alcohol acylation strategy presents itself, the "methyl extension" strategy outlined here merits consideration, as it may be less likely to result in significant attenuations in potency and obviates any and all concerns about unwanted reactivity. Of course, it remains the case that this approach is more synthetic chemistry intensive, but in that regard we note that we have here demonstrated (1) that major improvements in step-economy and scalability such as in our dictyostatin synthesis can render this approach feasible in a far less time- and resource-intensive way, and (2) two ways in which our efficient and scalable polyketide synthesis methodologies may be adapted for the installation of 4-azidobutyl groups in place of methyl groups. We believe this strategy will prove applicable to other important polyketide/polypropionate natural products with few or no obvious handles for linker attachment, and we will report our findings in this regard in due course.

■ **ASSOCIATED CONTENT**

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.5b09869.

Experimental procedures, compound characterization data, and experimental details for the cell growth inhibition assays (PDF)

■ **AUTHOR INFORMATION**

Corresponding Author

*leighton@chem.columbia.edu

REFERENCES

(1) (a) Gerber, H.-P.; Koehn, F. E.; Abraham, R. T. *Nat. Prod. Rep.* **2013**, *30*, 625−639. (b) Perez, H. L.; Cardarelli, P. M.; Deshpande, S.; Gangwar, S.; Schroeder, G. M.; Vite, G. D.; Borzilleri, R. M. *Drug Discovery Today* **2014**, *19*, 869−881. (c) Chari, R. V. J.; Miller, M. L.; Widdison, W. C. *Angew. Chem., Int. Ed.* **2014**, *53*, 3796−3827.

(2) Ducry, L.; Stump, B. *Bioconjugate Chem.* **2010**, *21*, 5−13.

(3) Pettit, G. R.; Cichacz, Z. A.; Gao, F.; Boyd, M. R.; Schmidt, J. M. *J. Chem. Soc., Chem. Commun.* **1994**, 1111−1112.

(4) Isbrucker, R. A.; Cummins, J.; Pomponi, S. A.; Longley, R. E.; Wright, A. E. *Biochem. Pharmacol.* **2003**, *66*, 75−82.

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

(6) Ho, S.; Bucher, C.; Leighton, J. L. *Angew. Chem., Int. Ed.* **2013**, *52*, 6757−6761.

(7) Brunden, K. R.; Gardner, N. M.; James, M. J.; Yao, Y.; Trojanowski, J. Q.; Lee, V. M.-Y.; Paterson, I.; Ballatore, C.; Smith, A. B., III *ACS Med. Chem. Lett.* **2013**, *4*, 886−889.

(8) Paterson, I.; Gardner, N. M.; Poullennec, K. G.; Wright, A. E. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2443−2447.

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

(10) Paterson, I.; Naylor, G. J.; Gardner, N. M.; Guzmán, E.; Wright, A. E. *Chem. - Asian J.* **2011**, *6*, 459−473.

(11) Fukui, Y.; Brückner, A. M.; Shin, Y.; Balachandran, R.; Day, B. W.; Curran, D. P. *Org. Lett.* **2006**, *8*, 301−304.

(12) Shin, Y.; Fournier, J.-H.; Brückner, A. M.; Madiraju, C.; Balachandran, R.; Raccor, B. S.; Edler, M. C.; Hamel, E.; Sikorski, R. P.; Vogt, A.; Day, B. W.; Curran, D. P. *Tetrahedron* **2007**, *63*, 8537−8562.

(13) Jung, W.-H.; Harrison, C.; Shin, Y.; Fournier, J.-H.; Balachandran, R.; Raccor, B. S.; Sikorski, R. P.; Vogt, A.; Day, D. P.; Day, B. W. *J. Med. Chem.* **2007**, *50*, 2951−2966.

(14) Zhu, W.; Jiménez, M.; Jung, W.-H.; Camarco, D. P.; Balachandran, R.; Vogt, A.; Day, B. W.; Curran, D. P. *J. Am. Chem. Soc.* **2010**, *132*, 9175−9187.

(15) Paterson, I.; Gardner, N. M.; Naylor, G. J. *Pure Appl. Chem.* **2009**, *81*, 169−180.

(16) Paterson, I.; Gardner, N. M.; Guzmán, E.; Wright, A. E. *Bioorg. Med. Chem.* **2009**, *17*, 2282−2289.

(17) Jogalekar, A.; Damodaran, K.; Kriel, F. H.; Jung, W.-H.; Alcaraz, A. A.; Zhong, S.; Curran, D. P.; Snyder, J. P. *J. Am. Chem. Soc.* **2011**, *133*, 2427−2436.

(18) Canales, A.; Matesanz, R.; Gardner, N. M.; Andreu, J. M.; Paterson, I.; Díaz, J. F.; Jiménez-Barbero, J. *Chem. - Eur. J.* **2008**, *14*, 7557−7569.

(19) Prota, A. E.; Bargsten, K.; Zurwerra, D.; Field, J. J.; Fernando Díaz, J.; Altmann, K.-H.; Steinmetz, M. O. *Science* **2013**, *339*, 587−590.

(20) Kim, H.; Ho, S.; Leighton, J. L. *J. Am. Chem. Soc.* **2011**, *133*, 6517−6520.

(21) Garber, S. B.; Kingsbury, J. S.; Gray, B. L.; Hoveyda, A. H. *J. Am. Chem. Soc.* **2000**, *122*, 8168−8179.

(22) Goldberg, S. D.; Grubbs, R. H. *Angew. Chem., Int. Ed.* **2002**, *41*, 807−810.

(23) Huber, J. D.; Perl, N. R.; Leighton, J. L. *Angew. Chem., Int. Ed.* **2008**, *47*, 3037−3039.

(24) Shiina, I.; Kubota, M.; Oshiumi, H.; Hashizume, M. *J. Org. Chem.* **2004**, *69*, 1822−1830.

(25) Corey, E. J.; Helal, C. J. *Angew. Chem., Int. Ed.* **1998**, *37*, 1986−2012.

(26) Foley, C. N.; Leighton, J. L. *Org. Lett.* **2014**, *16*, 1180−1183.

(27) Dommerholt, J.; Schmidt, S.; Temming, R.; Hendriks, L. J. A.; Rutjes, F. P. J. T.; van Hest, J. C. M.; Lefeber, D. J.; Friedl, P.; van Delft, F. L. *Angew. Chem., Int. Ed.* **2010**, *49*, 9422−9425.

(28) Agard, N. J.; Prescher, J. A.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2004**, *126*, 15046−15047.

(29) Lutz, J.-F. *Angew. Chem., Int. Ed.* **2008**, *47*, 2182−2184.

(30) Nilsson, B. L.; Kiessling, L. L.; Raines, R. T. *Org. Lett.* **2000**, *2*, 1939−1941.

(31) Saxon, E.; Armstrong, J. I.; Bertozzi, C. R. *Org. Lett.* **2000**, *2*, 2141−2143.