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Design, Synthesis, and Evaluation of Carbamate-Substituted Analogues of (+)-Discodermolide

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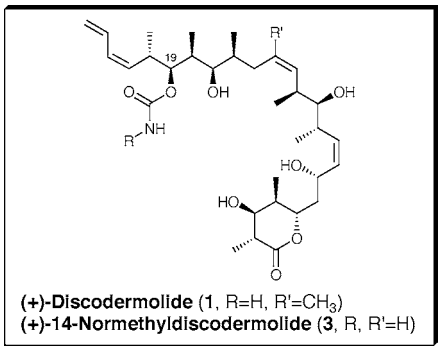
ABSTRACT



(+)-Discodermolide (1, R=H, R'=CH_3)
(+)-14-Normethyldiscodermolide (**3**, R, R'=H)

The design, syntheses, and biological evaluation of 22 totally synthetic analogues of the potent microtubule-stabilizing agent (+)-discodermolide (1) have been achieved. Structure–activity relationships of the C(19) carbamate were defined, exploiting two synthetically simplified scaffolds, as well as the parent (+)-discodermolide framework.

In 1990, Gunasekera and co-workers reported the isolation and structural elucidation of (+)-discodermolide (**1**, Scheme 1) from the deep sea marine sponge *Discodermia dissoluta.*[1] Several years later (1996), Ter Haar and co-workers and Schreiber et al. independently revealed that (+)-discodermolide possesses potent antimitotic activity,[2] with a mechanism of action that, akin to the clinically proven anticancer agent Taxol (**2**), entails binding and stabilization of microtubules.[2] Importantly, (+)-discodermolide displays both significant tumor cell growth inhibitory activity against Taxol-resistant cells[3] and cytotoxic synergy with Taxol in a variety of cell lines.[4] Interest in (+)-discodermolide both as a synthetic target[5] and a lead chemotherapeutic agent[6] remains strong, as evidenced by five total syntheses[5i−n] and ten papers devoted to analogues[6g−p] published since 2001.

In parallel with our efforts to develop an ever more practical (i.e., scalable) total synthesis of (+)-discodermolide

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(1) Gunasekera, S. P.; Gunasekera, M.; Longley, R. E.; Schulte, G. K. *J. Org. Chem.* **1990**, *55*, 4912−4915. Correction: *J. Org. Chem.* **1991**, *56*, 1346.

(2) (a) ter Haar, E.; Kowalski, R. J.; Hamel, E.; Lin, C. M.; Longley, R. E.; Gunasekera, S. P.; Rosenkranz, H. S.; Day, B. W. *Biochemistry* **1996**, *35*, 243−250. (b) Hung, D. T.; Chen, J.; Schreiber, S. L. *Chem. Biol.* **1996**, *3*, 287−293.

(3) Kowalski, R. J.; Giannakakou, P.; Gunasekera, S. P.; Longley, R. E.; Day, B. W.; Hamel, E. *Mol. Pharm.* **1997**, *52*, 613−622.

(4) Martello, L. A.; McDiad, H. M.; Regl, D. L.; Yang, C. H.; Meng, D.; Pettus, T. R.; Kaufman, M. D.; Arimoto, H.; Danishefsky, S. J.; Smith, A. B., III.; Horwitz, S. B. *Clin. Cancer Res.* **2000**, *6*, 1978−1987.

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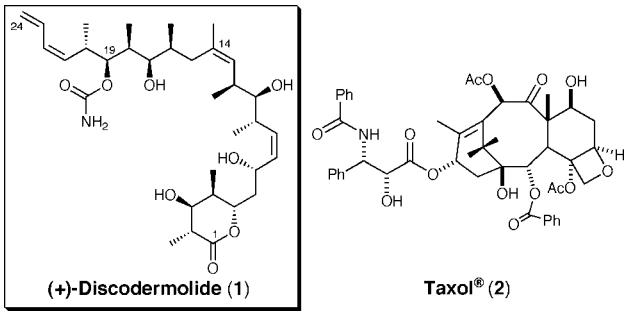
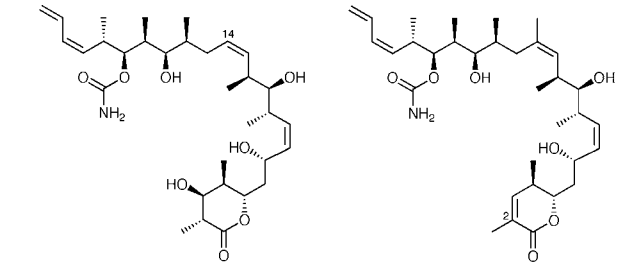
Scheme 1



Scheme 2

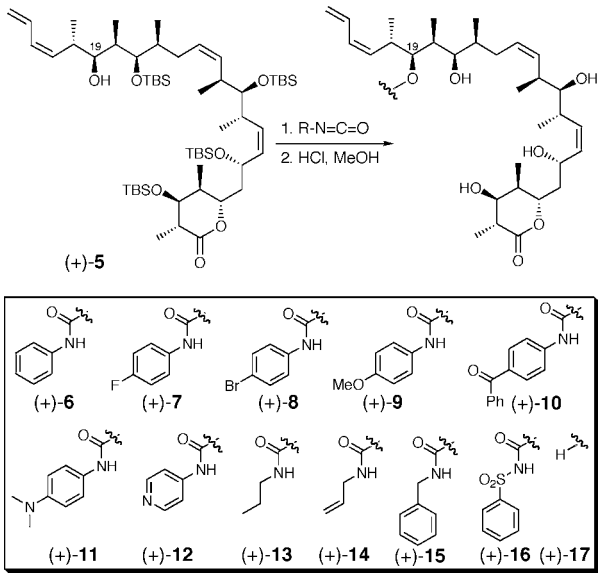


to provide material for clinical development, we broadened our program to include the production of analogues designed to probe the structure−activity relationship, as well as to define the minimum critical structural element necessary for tumor cell growth inhibition.

Toward this end, we reported in 2001 that the simplified congeners (+)-14-normethyldiscodermolide (**3**, Scheme 2) and (+)-2,3-anhydrodiscodermolide (**4**) display cell growth inhibitory activity rivaling that of the natural product.[6f] Herein we describe the design, syntheses, and biological evaluation of derivatives of (+)-**1**, (+)-**3**, and (+)-**4**, which explore the influence of substitution at the C(19) carbamate.

Employing the synthetically simplified 14-normethyldiscodermolide scaffold for our initial investigations, we treated the previously disclosed secondary alcohol (+)-**5**[6f] with a range of sterically and electronically varied isocyanates, followed by global deprotection to furnish the corresponding carbamates **6**−**16** in good to excellent yield (Scheme 3). Additionally, direct deprotection of (+)-**5** furnished descarbamoyl congener (+)-**17**.

Scheme 3



In three of the four cell lines tested (Table 1), attachment of the phenyl, *p*-fluorophenyl, *p*-bromophenyl, or pyridyl substituents (**6**−**8**, **12**) led to a slight reduction in cell growth inhibition (a factor of 2−3), whereas carbamates possessing either a benzophenone moiety (**10**), electron-rich aromatics (**9**, **11**), or nonpolar substituents such as *n*-propyl, allyl, and benzyl (**13**−**15**), yielded analogues with potency comparable to that of the natural product. Alternatively, the phenyl sulfonamide congener **16** displays significantly reduced cytotoxicity, as does the carbamate-deletion analogue **17**.[7]

(5) (a) Nerenberg, J. B.; Hung, D. T.; Somers, P. K.; Schreiber, S. L. *J. Am. Chem. Soc.* **1993**, *115*, 12621−12622. (b) Smith, A. B., III; Qiu, Y.; Jones, D. R.; Kobayashi, K. *J. Am. Chem. Soc.* **1995**, *117*, 12011−12012. (c) Harried, S. S.; Yang, G.; Strawn, M. A.; Myles, D. C. *J. Org. Chem.* **1997**, *62*, 6098−6099. (d) Marshall, J. A.; Johns, B. A. *J. Org. Chem.* **1998**, *63*, 7885−7892. (e) Smith, A. B., III; Kaufman, M. D.; Beauchamp, T. J.; LaMarche, M. J.; Arimoto, H. *Org. Lett.* **1999**, *1*, 1823−1826. (f) Halstead, D. P. Ph.D. Thesis, Harvard University, Cambridge, MA, 1999. (g) Paterson, J.; Florence, G. J.; Gerlach, K.; Scott, J. *Angew. Chem., Int. Ed.* **2000**, *39*, 377−380. (h) Smith, A. B., III; Beauchamp, T. J.; LaMarche, M. J.; Kaufman, M. D.; Qiu, Y.; Arimoto, H.; Jones, D. R.; Kobayashi, K. *J. Am. Chem. Soc.* **2000**, *122*, 8654−8664. (i) Paterson, I.; Florence, G. J.; Gerlach, K.; Scott, J. P.; Sereinig, N. *J. Am. Chem. Soc.* **2001**, *123*, 9535−9544. (j) Harried, S. S.; Lee, C. P.; Yang, G.; Lee, T. I. H.; Myles, D. C. *J. Org. Chem.* **2003**, *68*, 6646−6660. (k) Paterson, I.; Delgado, O.; Florence, G. J.; Lyothier, I.; Scott, J. P.; Sereinig, N. *Org. Lett.* **2003**, *5*, 35−38. (l) Smith, A. B., III; Freeze, B. S.; Brouard, I.; Hirose, T. *Org. Lett.* **2003**, *5*, 4405−4408. (m) Mickel, S. J.; Sedelmeier, G. H.; Niederer, D.; Daeffler, R.; Osmani, A.; Schreiner, K.; Seeger-Weibel, M.; Berod, B.; Schaer, K.; Gamboni, R.; Chen, S.; Chen, W.; Jagoe, C. T.; Kinder, F. R., Jr.; Loo, M.; Prasad, K.; Repic, O.; Shieh, W.-C.; Wang, R.-M.; Waykole, L.; Xu, D. D.; Xue, S. *Org. Proc. Res. Dev.* **2004**, *8*, 92−130 and references therein. (n) Paterson, I.; Lyothier, I. *Org. Lett.* **2004**, *6*, 4933−4936.

(6) (a) Hung, D. T.; Nerenberg, J. B.; Schreiber, S. L. *Chem. Biol.* **1994**, *1*, 67−71. (b) Hung, D. T.; Nerenberg, J. B.; Schreiber, S. L. *J. Am. Chem. Soc.* **1996**, *118*, 11054−11080. (c) Paterson, I.; Florence, G. J. *Tetrahedron Lett.* **2000**, *41*, 6935−6939. (d) Gunasekera, S. P.; Longley, R. E.; Isbrucker, R. A. *J. Nat. Prod.* **2001**, *64*, 171−174. (e) Isbrucker, R. A.; Gunasekera, S. P.; Longley, R. E. *Cancer Chemother. Pharmacol.* **2001**, *48*, 29−36. (f) Martello, L. A.; LaMarche, M. J.; He, L.; Beauchamp, T. J.; Smith, A. B., III; Horwitz, S. B. *Chem. Biol.* **2001**, *8*, 843−855. As they were not included in the original paper, full experimental details for the compounds reported therein are provided in the Supporting Information of this work. (g) Curran, D. P.; Furukawa, T. *Org. Lett.* **2002**, *4*, 2233−2235. (h) Gunasekera, S. P.; Longley, R. E.; Isbrucker, R. A. *J. Nat. Prod.* **2002**, *65*, 1830−1837. (i) Gunasekera, S. P.; Paul, G. K.; Longley, R. E.; Isbrucker, R. A.; Pomponi, S. A. *J. Nat. Prod.* **2002**, *6*, 1643−1648. (j) Minguez, J. M.; Giuliano, K. A.; Balachandran, R.; Madiraju, C.; Curran, D. P.; Day, B. W. *Mol. Cancer Ther.* **2002**, *1*, 1305−1313. (k) Shin, Y.; Choy, N.; Balachandran, R.; Madiraju, C.; Day, B. W.; Curran, D. P. *Org. Lett.* **2002**, *4*, 4443−4446. (l) Choy, N.; Shin, Y.; Nguyen, P. Q.; Curran, D. P.; Balachandran, R.; Madiraju, C.; Day, B. W. *J. Med. Chem.* **2003**, *46*, 2846−2864. (m) Minguez, J. M.; Kim, S.-Y.; Giuliano, K. A.; Balachandran, R.; Madiraju, C.; Day, B. W.; Curran, D. P. *Bioorg. Med. Chem.* **2003**, *11*, 3335−3357. (n) Paterson, I.; Delgado, O. *Tetrahedron Lett.* **2003**, *44*, 8877−8882. (o) Burlingame, M. A.; Shaw, S. J.; Sundermann, K. F.; Zhang, D.; Petryka, J.; Mendoza, E.; Liu, F.; Myles, D. C.; LaMarche, M. J.; Hirose, T.; Freeze, B. S.; Smith, A. B., III. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2335−2338. (p) Gunasekera, S. P.; Mickel, S. J.; Daeffler, R.; Niederer, D.; Wright, A. E.; Linley, P.; Pitts, T. *J. Nat. Prod.* **2004**, *67*, 749−756.

Table 1. Cytotoxicity Observed for Analogues **6**−**17**

	cytotoxicity IC_{50}, nM			
	MCF-7	NCI/ADR	A549	SKOV-3
(+)-**1**	28	240	22	21
(+)-**3**	46	8200	50	35
(+)-**6**	48	>1000	135	47
(+)-**7**	42	>1000	125	47
(+)-**8**	84	>1000	247	90
(+)-**9**	23	>1000	89	23
(+)-**10**	23	>1000	67	36
(+)-**11**	6.2	2000	27	12
(+)-**12**	27	>1000	110	38
(+)-**13**	22	>1000	73	20
(+)-**14**	17	4000	55	10
(+)-**15**	27	4000	51	30
(+)-**16**	>1000	>1000	>1000	>1000
(+)-**17**	365	>1000	>1000	200

Also of significance, in marked contrast with (+)-discodermolide (**1**), which retains significant cell growth inhibitory activity in the Taxol-resistant *p*-glycoprotein-overexpressing cell line (NCI/ADR), (+)-14-normethyldiscodermolide and carbamoyl derivatives thereof generally demonstrate cytotoxicity only at concentrations greater than 1 μM in this cell line.

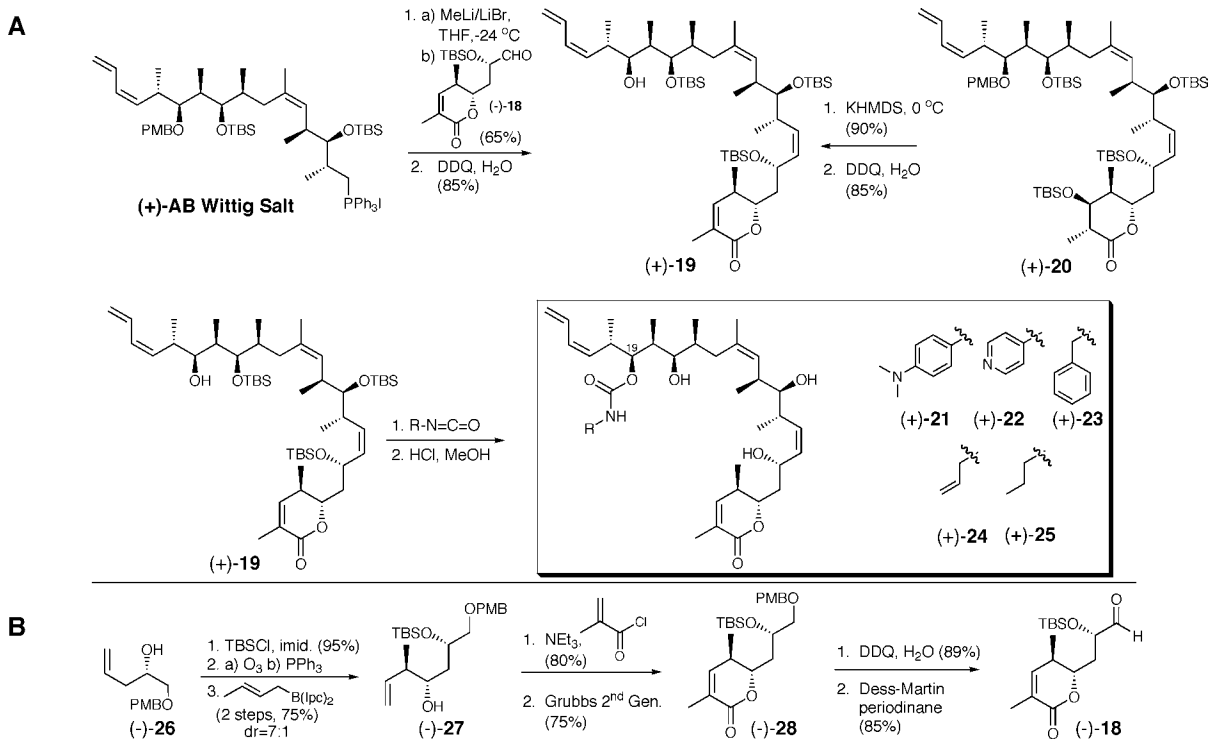
(+)-2,3-Anhydrodiscodermolide (**4**), isolated as a minor byproduct of global deprotection during our successful campaign to synthesize one gram of (+)-discodermolide, was found to be a more potent inhibitor of tumor cell growth than the natural product.[6f] Thus, analogue (+)-**4** and carbamoyl derivatives thereof were selected for synthesis and biological evaluation. Required for this venture was secondary alcohol (+)-**19** (Scheme 4A), accessible via Wittig reaction of the previously described phosphonium salt (+)-**AB**[5e] with the corresponding α,β-unsaturated lactone aldehyde (−)-**18**, followed by oxidative removal of the secondary PMB ether. Alternatively, (+)-**19** can be obtained via base-promoted elimination of TBSOH from lactone (+)-**20**, again an advanced intermediate in our gram-scale synthesis of (+)-discodermolide,[5e] followed by DDQ-mediated removal of the PMB group. Aldehyde (−)-**18**, the requisite C(1)−C(8) fragment for our modular synthesis, was available in seven steps from known alcohol (−)-**26**[8] (Scheme 4B).

Table 2. Cytotoxicity Observed for Analogues **21**−**25**

	cytotoxicity IC_{50}, nM			
	MCF-7	NCI/ADR	A549	SKOV-3
(+)-**1**	28	240	22	21
(+)-**4**	5.6	463	8.6	3.4
(+)-**21**	5.6	260	9.4	7.6
(+)-**22**	120	>1000	400	250
(+)-**23**	290	>1000	410	380
(+)-**24**	630	>1000	2000	790
(+)-**25**	660	>1000	2000	610

In this series, the presence of the *N*,*N*-dimethylaniline moiety as in (+)-**21** produced an analogue that was again superior in potency to the natural product (+)-**1** in three of the four tested cell lines (Table 2). However, carbamates with pyridyl, benzyl, allyl, or propyl substituents [**22**−**25**], changes

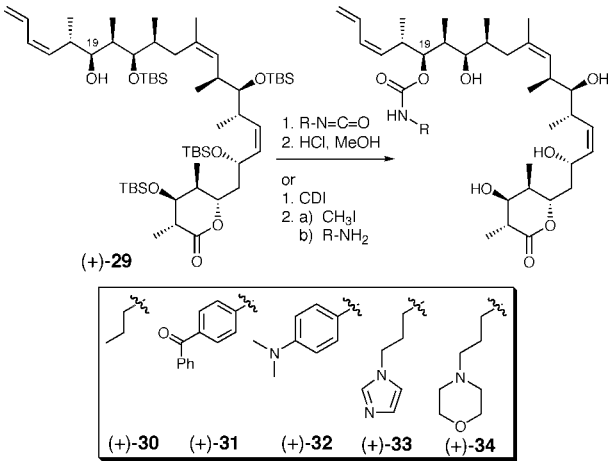
Scheme 4



that had minimal effect on the cytotoxicity in the 14-normethyl series (Table 1, **12**−**15**), severely degraded cell growth inhibitory activity when applied to the α,β-unsaturated lactone scaffold.

To extend the above carbamoyl substitutions to the natural (+)-discodermolide scaffold, the *n*-propyl, benzophenone, and *N*,*N*-dimethylaniline congeners **30**−**32** were prepared from the corresponding secondary alcohol (+)-**29**, a related late-stage intermediate in our synthesis of (+)-discodermolide[5e] (Scheme 5). In addition, to probe further the effect of heteroatom incorporation in the carbamate moiety, imidazole (+)-**33** and morpholine (+)-**34** were also constructed and evaluated.

Scheme 5



In this series, activity was again diminished relative to (+)-discodermolide (**1**) in the NCI/ADR drug-resistant cell line (Table 3). Conversely, in the three sensitive cell lines, the *n*-propyl-substituted analogue (+)-**30** proved to be only slightly less potent than (+)-discodermolide (**1**), while the benzophenone [(+)-**31**] and *N*,*N*-dimethylaniline [(+)-**32**] derivatives displayed a 3- to 14-fold increase in cell growth inhibitory activity. Introduction of alternate heteroatomic substructures as in (+)-**33** and (+)-**34** led to compounds that were generally equipotent with the natural product in the sensitive cell lines.

To summarize, 22 new, totally synthetic analogues of (+)-discodermolide (**1**) have been prepared and evaluated for tumor cell growth inhibitory activity in four human tumor cell lines. The NCI/ADR multidrug-resistant cell line appears to be very strict with respect to substitution at the carbamate, as only two analogues (**21** and **32**) retained submicromolar activity in this cell line. Potent cytotoxicity is, however, retained in the drug-sensitive cell lines when the carbamate is appended with a range of sterically and electronically varied substituents. In general, compounds derived from the parent (+)-discodermolide scaffold (**1**) and from the 14-normethyl variant (**3**) were highly potent, while analogues stemming from (+)-2,3-anhydrodiscodermolide (**4**), which is itself more cytotoxic than the natural product, proved to be over an order of magnitude less active than (+)-discodermolide. In conclusion, on the basis of the tolerance demonstrated by substitution at the C(19) carbamate, we believe that this region of the discodermolide skeleton holds considerable promise as a structural locale to alter favorably the pharmacokinetic profile of this potentially important class of chemotherapeutic agents.

Table 3. Cytotoxicity Observed for Analogues **30**−**34**

	cytotoxicity IC_{50}, nM			
	MCF-7	NCI/ADR	A549	SKOV-3
(+)-**1**	28	240	22	21
(+)-**30**	27	>1000	54	34
(+)-**31**	8.4	>1000	10	8
(+)-**32**	1.9	450	5.1	1.8
(+)-**33**	33	>1000	58	31
(+)-**34**	10	>1000	30	14

Acknowledgment. Financial support was provided by the National Institutes of Health (Institute of General Medical Sciences) through Grant GM-29028, the Department of the Army through Grant DAMD 17-00-1-0404, and by a Sponsored Research Agreement between the University of Pennsylvania and Kosan Biosciences, Inc., where Professor Smith is a member of the Scientific Advisory Board. We thank Fengua Liu for performing the cytotoxicity assays. We also thank Pharmacia for a Graduate Scholar Fellowship to M.J.L., the NIH for a Postdoctoral Fellowship to P.V.R., and Ministerio de Educación Cultura y Deportes (Spain) for a Postdoctoral Fellowship to I.B.

Supporting Information Available: Representative procedures, spectral data, and analytical data for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(7) For comparison, Gunasekera and co-workers recently reported the isolation of the natural product 19-descarbamoyldiscodermolide, which exhibits cytotoxicity 4−5-fold lower than that of (+)-discodermolide in the P388 and A549 cell lines. Gunasekera, S. P.; Paul, G. K.; Longley, R. E.; Isbrucker, R. A.; Pomponi, S. A. *J. Nat. Prod.* **2002**, *65*, 1643−1648.

(8) Keck, G. E.; Krishnamurthy, D. *Org. Synth.* **1998**, *75*, 12−18.