J|A|C|S ARTICLES

Published on Web 06/02/2010

Total Synthesis and Evaluation of a Key Series of C5-Substituted Vinblastine Derivatives

Porino Va, Erica L. Campbell, William M. Robertson, and Dale L. Boger*

Department of Chemistry and the Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037

Received April 1, 2010; E-mail: boger@scripps.edu

Abstract: A remarkably concise seven- to eight-step total synthesis of a systematic series of key vinblastine derivatives is detailed and used to characterize the importance and probe the role of the C5 ethyl substituent (R = H, Me, Pr, CH=CH_2, C≡CH, CH_2OH, and CHO vs Et). The analogues, which bear deep-seated structural changes accessible only by total synthesis, were prepared using a powerful intramolecular [4 + 2]/[3 + 2] cycloaddition cascade of 1,3,4-oxadiazoles ideally suited for use in the assemblage of the vindoline-derived lower subunit followed by their incorporation into the vinblastine analogues through the use of a single-step biomimetic coupling with catharanthine. The evaluation of the series revealed that the tubulin binding site surrounding this C5 substituent is exquisitely sensitive to the presence (Et > H, 10-fold), size (Me ≤ Et > Pr, 10-fold), shape (Et > CH=CH_2 and C≡CH, >4-fold), and polarity (Et > CHO > CH_2OH, >10−20-fold) of this substituent and that on selected occasions only a C5 methyl group may provide analogues that approach the activity observed with the naturally occurring C5 ethyl group.

Introduction

Vinblastine (**1**)[1] and vincristine (**2**) are the most widely recognized members of the class of vinca alkaloids as a result of their clinical use as antitumor drugs (Figure 1). They were originally isolated in trace quantities from the leaves of *Catharanthus roseus* (L.) G. Don,[2] and their biological properties were among the first to be shown to arise from inhibition of microtubule formation and mitosis that today is still regarded as one of the more successful targets for cancer therapeutic intervention.[3] Vinblastine and vincristine possess an identical velbanamine upper subunit and nearly identical vindoline-derived lower subunits differing only in the dihydroindole N-substituent. Despite this small structural distinction, vinblastine and vincristine differ in their antitumor properties and dose-limiting toxicities.[1,3] The major limitation on the use of the vinca alkaloids is the emergence of drug resistance derived principally from overexpression of phosphoglycoprotein (Pgp), an efflux pump that transports many of the major drugs out of the cell.[4] Thus, in addition to the identification of vinblastine and vincristine analogues that may address their dose-limiting toxicities, the development of a modified vinca alkaloid that is not a substrate for Pgp efflux and is efficacious against multidrug-resistant (MDR) tumors would constitute a major advance.

Although extensive derivatization of vinblastine has been conducted in the exploration of semisynthetic analogues of the natural products,[3,5] a more limited series of synthetic analogues that contain more deep-seated changes in the structure have been examined.[3,6] This reflects the structural complexity of the natural product and the intrinsic challenge in preparing such analogues. Recently, we reported the development of a concise

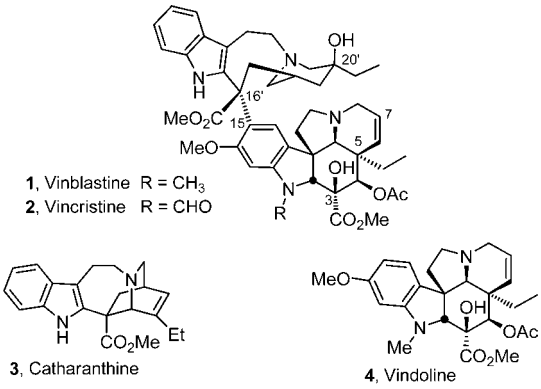


Figure 1. Natural products.

total synthesis of vindoline[7] enlisting a tandem [4 + 2]/[3 + 2] cycloaddition cascade of 1,3,4-oxadiazoles[8] that is applicable to the preparation of structural analogues[9] and the use of a single-pot, two-step biomimetic Fe(III)-promoted coupling with catharanthine and subsequent oxidation for their incorporation into vinblastine and its analogues.[10,11] Significantly, the approach

(1) Neuss, N.; Neuss, M. N. In *The Alkaloids*; Brossi, A., Suffness, M., Eds.; Academic Press: San Diego, CA, 1990; Vol. 37, p 229.

(2) (a) Noble, R. L.; Beer, C. T.; Cutts, J. H. *Ann. N.Y. Acad. Sci.* **1958**, *76*, 882. (b) Noble, R. L. *Lloydia* **1964**, *27*, 280. (c) Svoboda, G. H.; Nuess, N.; Gorman, M. *J. Am. Pharm. Assoc. Sci. Ed.* **1959**, *48*, 659. For related naturally occurring bisindole alkaloids, see: (d) Leurosidine: Svoboda, G. H. *Lloydia* **1961**, *24*, 173. (e) Deoxyvinblastine: De Bruyn, A.; Sleechecker, J.; De Jonghe, J. P.; Hannart, J. *Bull. Soc. Chim. Belg.* **1983**, *92*, 485. Neuss, N.; Gorman, M.; Cone, N. J.; Huckstep, L. L. *Tetrahedron Lett.* **1968**, 783. (f) *N*-Desmethylvinblastine: Simonds, R.; De Bruyn, A.; De Taeye, L.; Verzele, M.; De Pauw, C. *Planta Med.* **1984**, *50*, 274. (g) Desacetoxyvinblastine: Neuss, N.; Barnes, A. J., Jr.; Huckstep, L. L. *Experientia* **1975**, *31*, 18. (h) Desacetylvinblastine: Svoboda, G. H.; Barnes, A. J., Jr. *J. Pharm. Sci.* **1964**, *53*, 1227. (i) Anhydrovinblastine: Goodbody, A. E.; Watson, C. D.; Chapple, C. C. S.; Vukovic, J.; Misawa, M. *Phytochemistry* **1988**, *27*, 1713.

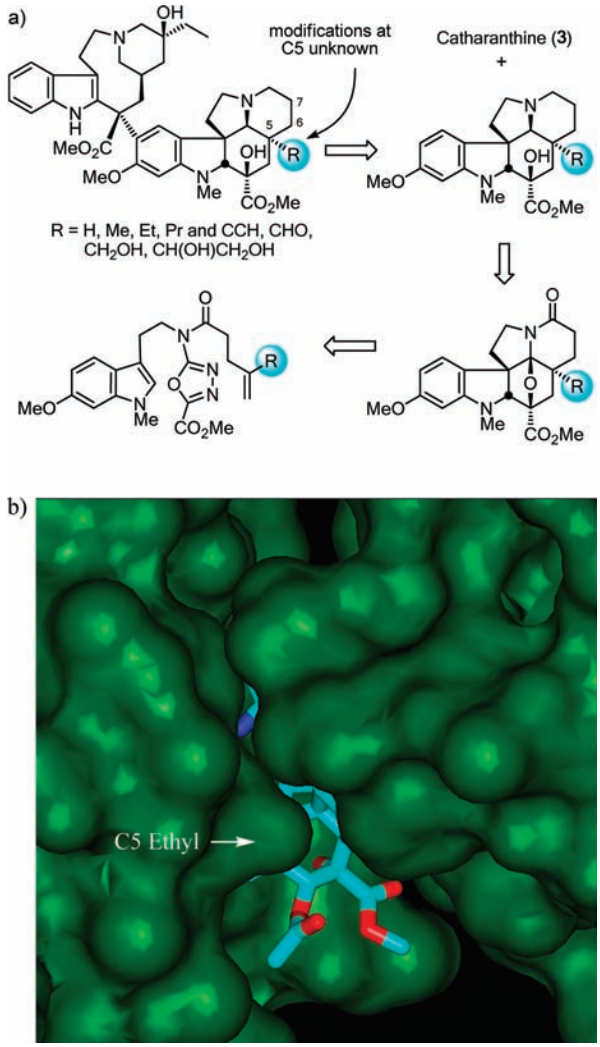


Figure 2. (a) Synthetic strategy for C5-substituted analogues. (b) X-ray crystal structure of vinblastine bound to tubulin[12] (an additional view is provided in the Supporting Information).

proved to be sufficiently concise that systematic explorations of each vinblastine structural feature not accessible by semisynthetic derivatization can be envisioned. One such site that remains unexplored is the C5 ethyl group of the lower vindoline subunit (Figure 2a). The recent X-ray crystal structure of vinblastine bound to tubulin[12] indicates that the tubulin binding pocket surrounding this ethyl substituent is composed of hydrophobic residues (i.e., Leu, Ala, Val) and yet potentially partially exposes the C5 ethyl group to solvent (Figure 2b). As a result, we sought not only to explore the removal (R = H), contraction (R = Me), or extension (R = *n*-Pr) of the C5 ethyl group and the incorporation of other unnatural functional groups at C5 (e.g., alkyne, hydroxymethyl, diol) but also to examine the incorporation of both polar and hydrophobic functional groups at C5 to further define the subtle features of this binding region. In addition, such changes (e.g., large hydrophobic groups) could impact Pgp export in resistant cell lines, addressing the one significant clinical limitation to the class. Access to the targeted series was anticipated to be provided through use of the [4 + 2]/[3 + 2] cascade recently introduced for the synthesis of vindoline (Figure 2).

Results and Discussion

The initial modifications were conducted on the 4-desacetoxy-6,7-dihydrovindoline scaffold, which is accessible in four steps from oxadiazole **5** and the corresponding 4-substituted 4-pentenoic acids **6** and in turn may be coupled with catharanthine in a single step to provide the synthetic vinblastine analogue in

(3) (a) Owellen, R. I.; Hartke, C. A.; Dickerson, R. M.; Haines, F. O. *Cancer Res.* **1976**, *36*, 1499. (b) Pearce, H. L. In *The Alkaloids*; Brossi, A., Suffness, M., Eds.; Academic Press: San Diego, CA, 1990; Vol. 37, p 145. (c) Borman, L. S.; Kuehne, M. E. In *The Alkaloids*; Brossi, A., Suffness, M., Eds.; Academic Press: San Diego, CA, 1990; Vol. 37, p 133. (d) Fahy, J. *Curr. Pharm. Des.* **2001**, *7*, 1181.

(4) Szakacs, G.; Paterson, J. K.; Ludwig, J. A.; Booth-Genthe, C.; Gottesman, M. M. *Nat. Rev. Drug Discovery* **2006**, *5*, 219.

(5) For recent examples, see: (a) Voss, M. E.; et al. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1245. (b) Shao, Y.; Zhang, H.; Ding, H.; Quan, H.; Lou, L.; Hu, L. *J. Nat. Prod.* **2009**, *72*, 1170. (c) Sheng, L. X.; Da, Y. X.; Long, Y.; Hong, L. Z.; Cho, T. P. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4602. (d) Passarella, D.; Giardini, A.; Peretto, B.; Fontana, G.; Sacchetti, A.; Silvani, A.; Ronchi, C.; Cappelletti, G.; Cartelli, D.; Borlak, J.; Danieli, B. *Bioorg. Med. Chem.* **2008**, *16*, 6269.

(6) For reviews, see: (a) Kuehne, M. E.; Marko, I. In *The Alkaloids*; Brossi, A., Suffness, M., Eds.; Academic Press: San Diego, CA, 1990; Vol. 37, p 77. (b) Potier, P. *J. Nat. Prod.* **1980**, *43*, 72. (c) Kutney, J. P. *Nat. Prod. Rep.* **1990**, *7*, 85. (d) Kutney, J. P. *Synlett* **1991**, 11. (e) Kutney, J. P. *Acc. Chem. Res.* **1993**, *26*, 559. For recent studies, see: (f) Kuehne, M. E.; Bornmann, W. G.; Marko, I.; Qin, Y.; Le Boulluec, K. L.; Frasier, D. A.; Xu, F.; Malamba, T.; Ensinger, C. L.; Borman, L. S.; Huot, A. E.; Exon, C.; Bizzarro, F. T.; Cheung, J. B.; Bane, S. L. *Org. Biomol. Chem.* **2003**, *1*, 2120. (g) Miyazaki, T.; Yokoshima, S.; Simizu, S.; Osada, H.; Tokuyama, H.; Fukuyama, T. *Org. Lett.* **2007**, *9*, 4737.

(7) (a) Ishikawa, H.; Elliott, G. I.; Velcicky, J.; Choi, Y.; Boger, D. L. *J. Am. Chem. Soc.* **2006**, *128*, 10596. (b) Choi, Y.; Ishikawa, H.; Velcicky, J.; Elliott, G. I.; Miller, M. M.; Boger, D. L. *Org. Lett.* **2005**, *7*, 4539. (c) Kato, D.; Sasaki, Y.; Boger, D. L. *J. Am. Chem. Soc.* **2010**, *132*, 3685.

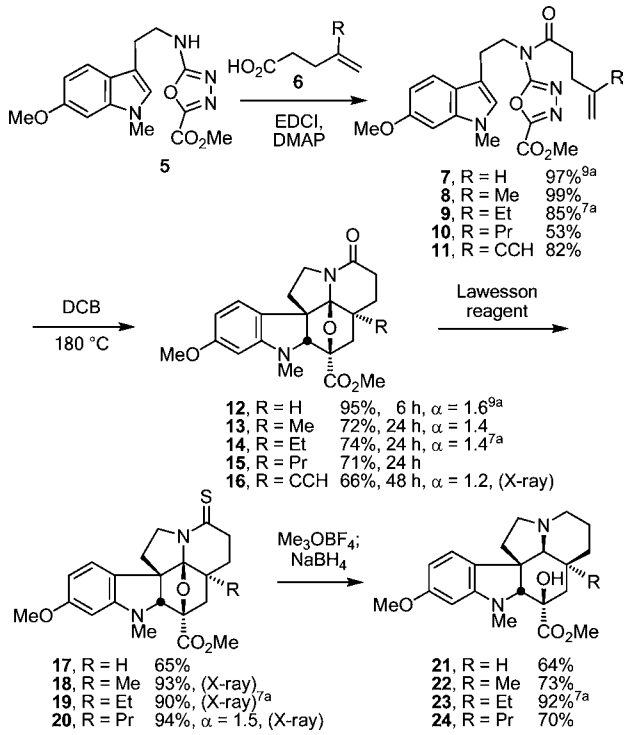
(8) (a) Wilkie, G. D.; Elliott, G. I.; Blagg, B. S. J.; Wolkenberg, S. E.; Soenen, D. B.; Miller, M. M.; Pollack, S.; Boger, D. L. *J. Am. Chem. Soc.* **2002**, *124*, 11292. (b) Elliott, G. I.; Fuchs, J. R.; Blagg, B. S. J.; Ishikawa, H.; Tao, H.; Yuan, Z.; Boger, D. L. *J. Am. Chem. Soc.* **2006**, *128*, 10589. For reviews of heterocyclic azadiene cycloaddition reactions, see: (c) Boger, D. L. *Tetrahedron* **1983**, *39*, 2869. (d) Boger, D. L. *Chem. Rev.* **1986**, *86*, 781. (e) Boger, D. L.; Weinreb, S. M. *Hetero Diels−Alder Methodology in Organic Synthesis*; Academic Press: San Diego, CA, 1987.

(9) (a) Ishikawa, H.; Boger, D. L. *Heterocycles* **2007**, *72*, 95. (b) Elliott, G. I.; Velcicky, J.; Ishikawa, H.; Li, Y.; Boger, D. L. *Angew. Chem., Int. Ed.* **2006**, *45*, 620. (c) Yuan, Z.; Ishikawa, H.; Boger, D. L. *Org. Lett.* **2005**, *7*, 741. (d) Campbell, E. L.; Zuhl, A. M.; Liu, C. M.; Boger, D. L. *J. Am. Chem. Soc.* **2010**, *132*, 3009.

(10) (a) Ishikawa, H.; Colby, D. A.; Boger, D. L. *J. Am. Chem. Soc.* **2008**, *130*, 420. (b) Ishikawa, H.; Colby, D. A.; Seto, S.; Va, P.; Tam, A.; Kakei, H.; Rayl, T. J.; Hwang, I.; Boger, D. L. *J. Am. Chem. Soc.* **2009**, *131*, 4904.

(11) For studies of the coupling reaction, see: (a) Potier, P.; Langlois, N.; Langlois, Y.; Gueritte, F. *J. Chem. Soc., Chem. Commun.* **1975**, 670. (b) Langlois, N.; Gueritte, F.; Langlois, Y.; Potier, P. *J. Am. Chem. Soc.* **1976**, *98*, 7017. (c) Kutney, J. P.; Ratcliffe, A. H.; Treasurywala, A. M.; Wunderly, S. *Heterocycles* **1975**, *3*, 639. (d) Kutney, J. P.; Hibino, T.; Jahngen, E.; Okutani, T.; Ratcliffe, A. H.; Treasurywala, A. M.; Wunderly, S. *Helv. Chim. Acta* **1976**, *59*, 2858. (e) Kutney, J. P.; Choi, L. S. L.; Nakano, J.; Tsukamoto, H.; McHugh, M.; Boulet, C. A. *Heterocycles* **1988**, *27*, 1845. (f) Magnus, P.; Stamford, A.; Ladlow, M. *J. Am. Chem. Soc.* **1990**, *112*, 8210. (g) Magnus, P.; Mendoza, J. S.; Stamford, A.; Ladlow, M.; Willis, P. *J. Am. Chem. Soc.* **1992**, *114*, 10232. (h) Kuehne, M. E.; Matson, P. A.; Bornmann, W. G. *J. Org. Chem.* **1991**, *56*, 513. (i) Bornmann, W. G.; Kuehne, M. E. *J. Org. Chem.* **1992**, *57*, 1752. (j) Kuehne, M. E.; Zebovitz, T. C.; Bornmann, W. G.; Marko, I. *J. Org. Chem.* **1987**, *52*, 4340. (k) Schill, G.; Priester, C. U.; Windhovel, U. F.; Fritz, H. *Tetrahedron* **1987**, *43*, 3765. (l) Yokoshima, S.; Ueda, T.; Kobayashi, S.; Sato, A.; Kuboyama, T.; Tokuyama, H.; Fukuyama, T. *J. Am. Chem. Soc.* **2002**, *124*, 2137. (m) Kuboyama, T.; Yokoshima, S.; Tokuyama, H.; Fukuyama, T. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 11966. (n) Szantay, C., Jr.; Balazs, J.; Bolcskei, J.; Szantay, C. *Tetrahedron* **1991**, *47*, 1265. (o) Sundberg, R. J.; Hong, J.; Smith, S. Q.; Sabato, M.; Tabakovic, I. *Tetrahedron* **1998**, *54*, 6259. (p) Sundberg, R. J.; Gadamsetti, K.; Hunt, P. J. *Tetrahedron* **1992**, *48*, 277.

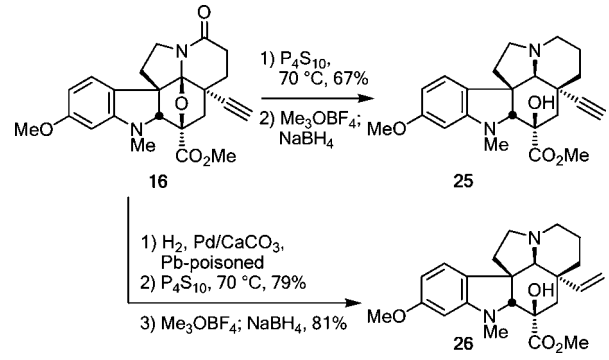
Scheme 1



a remarkably concise five-step synthesis. When the synthesis of the common oxadiazole **5** (in two or three steps from *N*-methyl-6-methoxytryptamine) and a direct chromatographic enantiomer resolution of the intermediate cycloadducts are included, this approach provides the vinblastine analogues in an overall seven- to eight-step synthetic sequence that is especially suitable for systematic examination of the importance of the vindoline C5 substitutent and highlighting the generality of the synthetic methodology.

The requisite 4-substituted 4-pentenoic acids **6** were coupled to oxadiazole **5** to afford cycloaddition substrates **7**−**11**, representing the series with R = H,[9a] Me, Et,[7a] Pr, and ethynyl, respectively (Scheme 1). Warming solutions of the substrates **7**−**11** in 1,2-dichlorobenzene (DCB) afforded single diastereomers of the corresponding [4 + 2]/[3 + 2] cycloadducts **12**−**16**. Consistent with prior studies, the exclusive formation of a single diastereomer of the cascade cycloadduct is a result of indole endo [3 + 2] cycloaddition in which the intermediate 1,3-dipole is directed to the sterically less encumbered face of the stabilized carbonyl ylide opposite the newly formed lactam. The reaction of the unsubstituted substrate **7** (R = H) was considerably faster than those of **8**−**11** (6 vs 24 h), reflecting the relative ease of the rate-determining [4 + 2] cycloaddition initiated by the tethered dienophile. Although this was not examined in detail, all of the reactions were conducted in refluxing DCB under dilute conditions (1−4 mM) to preclude competitive intermolecular reactions and, in the cases examined, displayed a modest concentration dependence within the narrow range examined (see the Supporting Information). Finally, it is notable that in substrate **11**, which also bears a pendant alkyne on the dienophile tether, only the olefin served to undergo the [4 + 2] cycloaddition with the oxadiazole.[13] Treatment of cycloadducts **12**−**15** with Lawessons' reagent afforded thioamides **17**−**20**. The racemic intermediates were resolved by chiral-phase HPLC (2 × 25 cm ChiralCel OD, α = 1.2−1.6) after the cycloaddition for **12**−**14** (R = H, Me, Et) and **16** (R = alkynyl) or after treatment with Lawessons' reagent for thioamide **20** (R = Pr).[14] Treatment of thioamides **17**−**20** with Meerwin's salt followed by sodium borohydride reduction of the resulting *S*-methyliminium ion served to reductively remove the thioamide with concomitant diastereoselective opening of the oxido bridge, affording the C5-substituted vindoline analogues **21**−**24** (R = H, Me, Et, Pr). Interestingly, treatment of thioamide **16** (R = alkynyl) with Lawesson's reagent afforded the corresponding thioamide in only 25% yield. In this case, a higher yield (67%) was obtained utilizing P_4S_{10} (Scheme 2), and subsequent treatment with Meerwin's salt and then sodium borohydride gave the C5 alkyne **25**. The corresponding vindoline analogue **26** containing a C5 vinyl substituent was also obtained from **16** utilizing an analogous synthetic sequence after an initial Lindlar reduction of the C5 alkyne (Scheme 2).

Scheme 2



Without efforts to optimize the conversions, the vindoline analogues **21**−**24** were coupled with catharanthine (**3**) through Fe(III)-promoted coupling and subsequent oxidation[10] to afford the vinblastine analogues **27**, **30**, **33**, and **36** representing the series with H, Me, Et, and Pr substituents, respectively, at C5 (Scheme 3). In addition to the vinblastine analogues shown, the corresponding C20′ epimeric leurosidine analogues (**28**, **31**, **34**, **37**) were generated with the now characteristic ∼2:1 β/α diastereoselectivity for the introduction of the C20′ alcohol, and the intermediate anhydrovinblastines (**29**, **32**, **35**, and **38**) were also isolated in yields ranging from 6 to 22%.

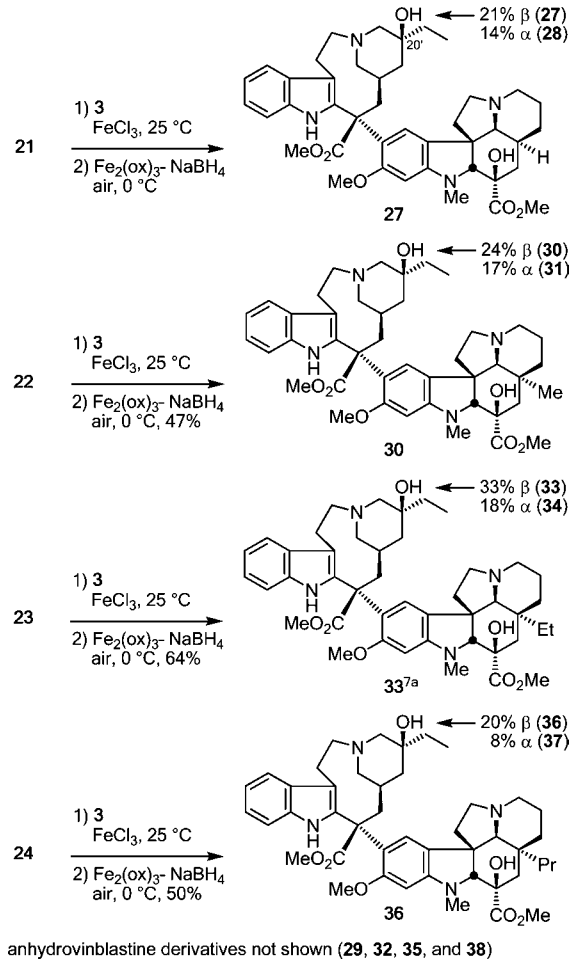
Attempts to prepare the C5 alkynyl (**39**) and vinyl (**40**) vinblastine analogues by direct coupling of the C5 alkynyl (**25**) and vinyl (**26**) vindoline analogues, respectively, were not successful, in part because of competitive oxidation of the C5 unsaturation during the oxidative stage of the biomimetic coupling (Scheme 4). However, omitting the oxidant [air, $Fe_2(ox)_3$] and the second step of the direct coupling afforded the C5 vinyl anhydrovinblastine analogue **41** in good yield

(12) Gigant, B.; Wang, C.; Ravelli, R. B. G.; Roussi, F.; Steinmetz, M. O.; Curmi, P. A.; Sobel, A.; Knossow, M. *Nature* **2005**, *435*, 519.

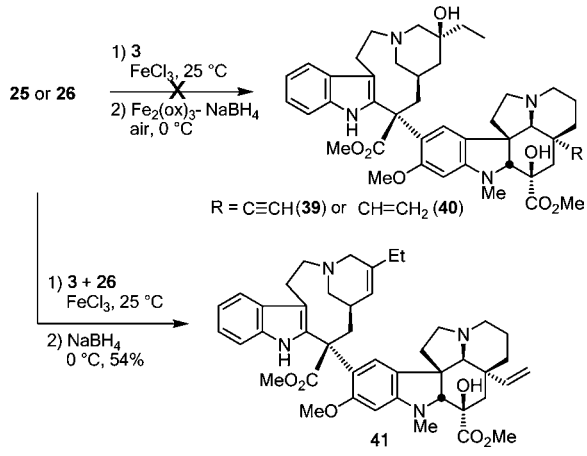
(13) A single-crystal X-ray structure determination of **16** (CCDC 755749) confirmed the structural and stereochemical assignment.

(14) In addition to the confirmation of the structural and relative stereochemical assignments, the absolute configurations were assigned by X-ray structure determinations of a heavy-atom derivative of a synthetic intermediate (CCDC 609613) leading to 4-desacetoxy-4-desethylvindoline (R = H),[9a] a heavy-atom (S) derivative disclosed earlier (R = Et, natural enantiomer of **19**, CCDC 295590),[7a] and the heavy-atom derivatives prepared herein: R = Me (unnatural enantiomer of **18**, CCDC 755748) and R = Pr (unnatural enantiomer of **20**, CCDC 755747). The absolute configurations of **25** (R = C≡CH), **26** (R = CH=CH_2), and their derivatives were established by conversion of the unnatural enantiomer of **16** (R = C≡CH) into **14** (R = Et) by reduction with Raney Ni (THF, 25 °C, 15 h, 60%) and correlation of the optical rotation.

Scheme 3



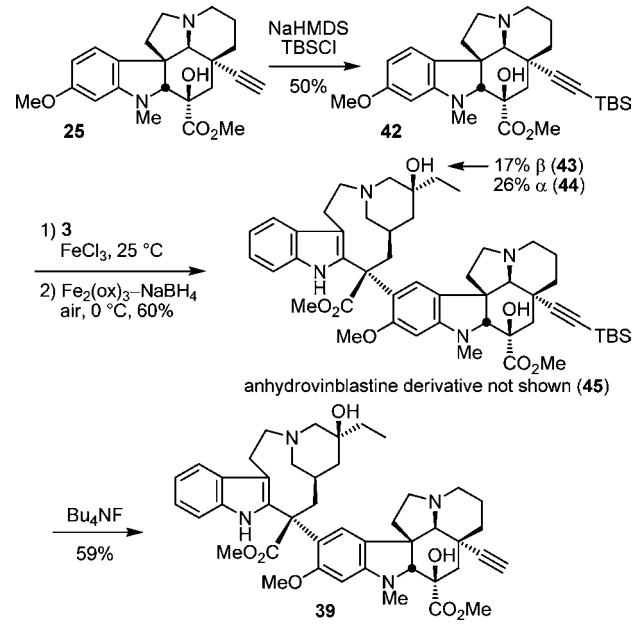
Scheme 4



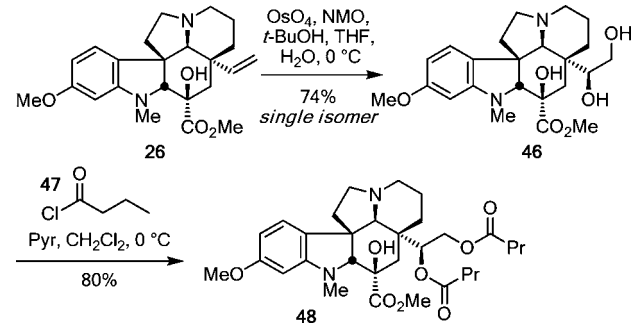
(54%). Alternatively, protection of the alkyne of **25** as the *tert*-butyldimethylsilyl (TBS) alkyne **42** enabled the two-step coupling with catharanthine and subsequent oxidation to afford the corresponding analogue **43** (Scheme 5). Treatment of **43** with Bu_4NF dried over 4 Å molecular sieves afforded the C5 alkyne vinblastine analogue **39**.

The vinyl substituent of the vindoline analogue **26** was converted into a series of additional key functional groups with which we could probe the introduction of polar functionalities and explore a unique series of conformational constraints. Treatment of **26** with osmium tetroxide afforded diol **46** as a

Scheme 5



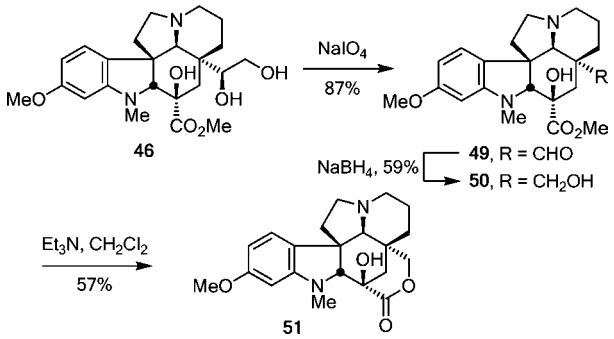
Scheme 6



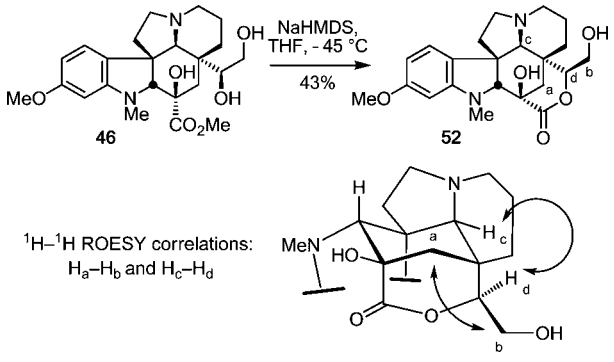
single diastereomer (Scheme 6). To explore the size of the tubulin binding site and because the incorporation of hydrophobic groups has been shown to overcome Pgp-mediated multidrug resistance with other classes of antitumor drugs,[15] diol **46** was also converted to the dibutyrate **48** for incorporation into a vinblastine analogue. Alternatively, treatment of C5 diol **46** with sodium periodate gave aldehyde **49**, and its reduction afforded the primary alcohol **50** (Scheme 7). The transformation of **49** and **50** into the corresponding vinblastine analogues bearing C5 formyl and hydroxymethyl substituents (R = CHO or CH_2OH, respectively, vs Et) was anticipated to address directly the ability of the tubulin C5 ethyl binding site to accommodate polar functionalities and the potential accessibility of solvent to this site. Just as interesting, the primary alcohol **50** could be converted to lactone **51** by a facile base-catalyzed intramolecular transesterification with the C3 methyl ester. This unique lactonization alters the conformational state of the vindoline subunit. The central six-membered ring characteristically adopts a boat conformation stabilized by a transannular H bond from the C3 hydroxyl group to N9, placing the C5 ethyl

(15) (a) Lampidis, T. J.; Kolonias, D.; Podona, T.; Israel, M.; Safa, A. R.; Lothstein, L.; Savaraj, N.; Tapiero, H.; Priebe, W. *Biochemistry* **1997**, *36*, 2679. (b) Perego, P.; De Cesare, M.; De Isabella, P.; Carenini, N.; Beggiolin, G.; Pezzoni, G.; Palumbo, M.; Tartaglia, L.; Pratesi, G.; Pisano, C.; Carminati, P.; Scheffer, G. L.; Zunino, F. *Cancer Res.* **2001**, *61*, 6034.

Scheme 7



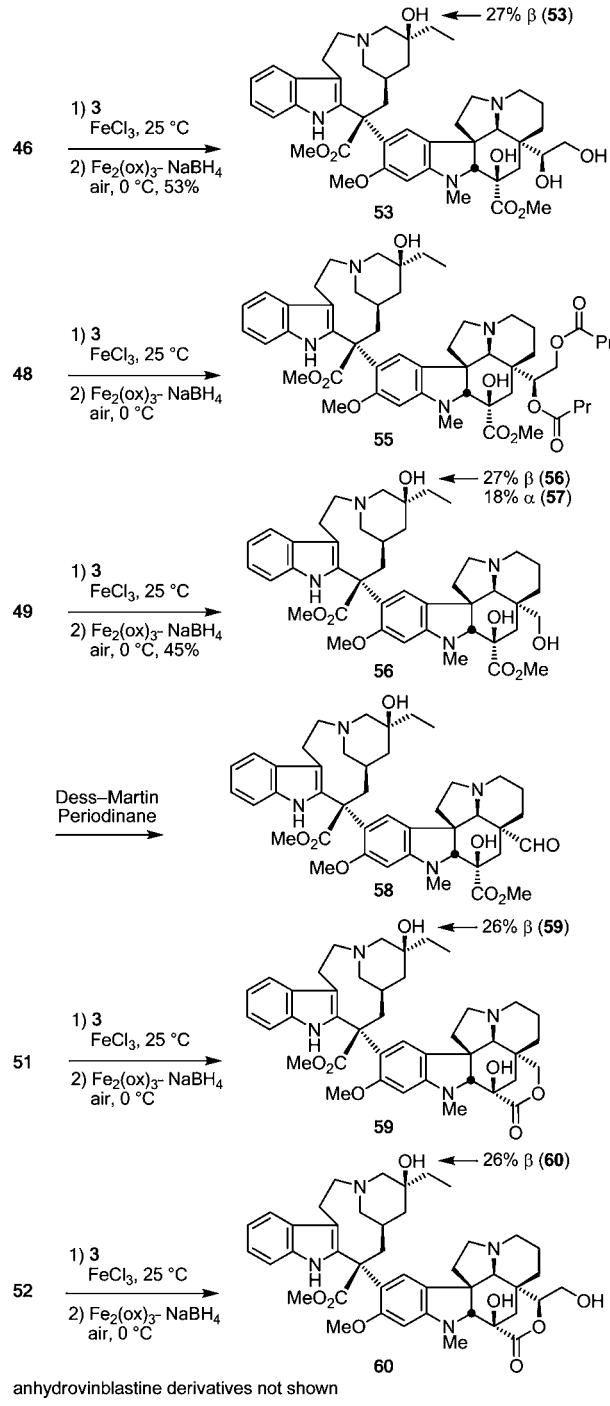
Scheme 8



group axial and the C3 methyl ester equatorial on this ring. This not only potentially attenuates the basicity of N9 but also strategically places the C3 methyl ester and C4 acetate of vinblastine at the interface of the solvent with the tubulin/vinblastine complex (Figure 2b). The lactonization to provide **51** requires the central six-membered ring of vindoline to adopt a chair (vs boat) conformation in which both the C3 and C5 substituents are axial, disrupting the C3 alcohol/N9 transannular H bond, and this would be expected to alter only the conformational features of vinblastine at the C3/C4 sites forming the interface with the solvent in the complex with tubulin without perturbing the relative location of the C5 substituent. Complementary to analogue **51**, the C5 diol **46** could also be closed to give lactone **52** (Scheme 8). Moreover, the detailed ^{1}H NMR characterization of **52** was utilized to confidently assign the C5 side-chain secondary alcohol stereochemistry obtained through OsO_4-catalyzed dihydroxylation of olefin **26**. The 2D ^{1}H−^{1}H rotating-frame Overhauser spectroscopy (ROESY) data revealed diagnostic nuclear Overhauser effects (NOEs) between H_a and H_b and between H_c and H_d, defining the stereochemistry shown. Significantly, this stereochemistry rigidly places the lactone hydroxymethyl substituent in a position to extend toward the solvent interface in the vinblastine/tubulin complex.

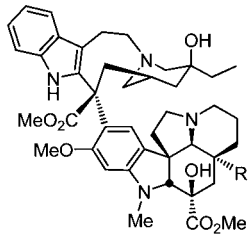
The vindoline analogues **46**, **48**, **49**, **51**, and **52** were coupled to catharanthine using the biomimetic Fe(III) coupling and oxidation[10] to afford the corresponding vinblastine analogues without optimization (Scheme 9). Coupling of the C5 aldehyde **49** occurred with concomitant reduction to afford the C5 hydroxymethyl analogue **56**, subsequent oxidation of which afforded the C5 aldehyde analogue **58**. Coupling of the conformationally constrained vindoline analogues **51** and **52** gave the corresponding vinblastine analogues **59** and **60** but interestingly failed to provide isolable quantities of the corresponding leurosidine (C20′ α-OH) products.

Scheme 9

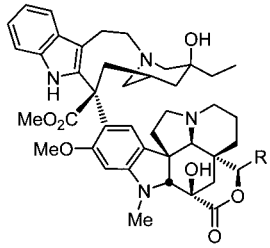


The biological activity of the vinblastine analogues was examined in three cytotoxic assays including the matched colon cancer cell lines HCT116 and HCT116/VM46, the latter of which is multidrug-resistant by virtue of Pgp overexpression.[15]

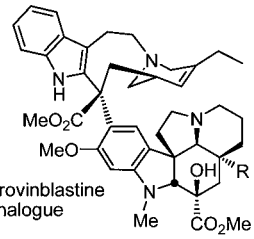
Consistent with expectations based on past observations, the vinblastine analogues (C20′ β-OH) proved more potent (~10-fold) than the corresponding anhydrovinblastine derivatives (C15′/C20′ double bond), which in turn were more potent than the corresponding leurosidine analogues (C20′ α-OH). The complete set of test results is provided in Table S1 in the Supporting Information and highlights such comparisons for the analogues prepared herein. A summary of the key results focusing on the impact of the C5 substituent is provided in



Compound	IC_{50} (nM)		
	L1210	HCT116	HCT116/VM46
27, R = H	5500	5600	>10000
30, R = Me	640	770	7100
33, R = Et	660	670	4000
36, R = Pr	5500	10000	9600
39, R = C≡CH	2500	9700	9800
56, R = CH_2OH	>10000	>10000	>10000
58, R = CHO	6200	9000	>10000
53, R = $CH(OH)CH_2OH$	>10000	>10000	>10000
55, R = $CH(OCOPr)CH_2OCOPr$	8000	>10000	>10000
43, R = C≡CTBS	>10000	>10000	>10000



59, R = H	>10000	>10000	>10000
60, R = CH_2OH	>10000	>10000	>10000

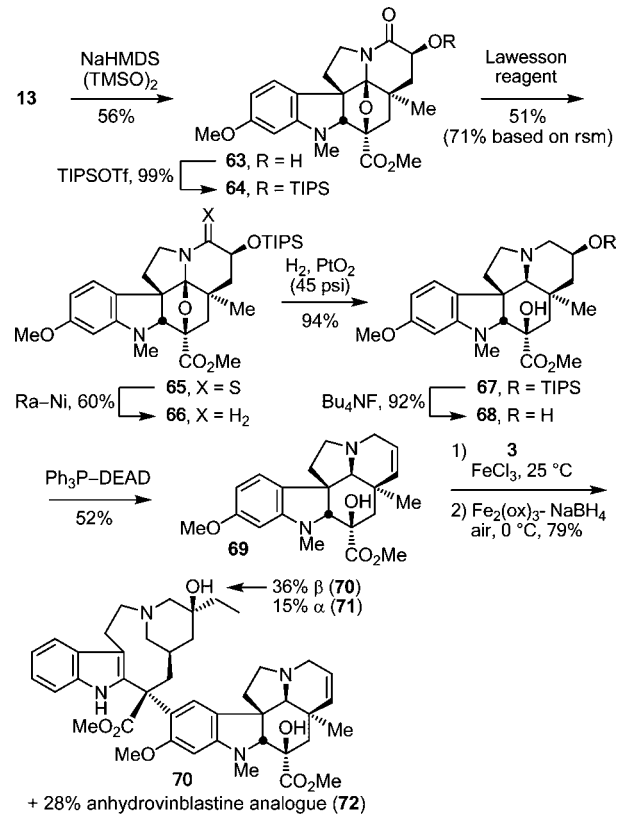


29, R = H	6700	>10000	>10000
32, R = Me	4800	6200	7500
35, R = Et	5400	5600	5300
38, R = Pr	6500	>10000	>10000
41, R = $CH=CH_2$	>10000	>10000	>10000

Figure 3. Cytotoxic activity.

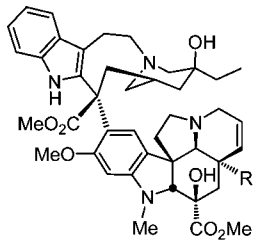
Figure 3. The activity proved to be remarkably sensitive to the presence, size, and polar nature of the C5 substituent. Whereas the analogues bearing the C5 methyl group (**30** and **32**) matched the potency of the corresponding analogues incorporating the natural C5 ethyl substituent (**33** and **35**), the analogues lacking a C5 substituent or bearing a C5 propyl group (**36**), which extends the length of the substituent by just one carbon relative to the natural ethyl substituent, led to ~10-fold losses in activity. Even more significant, the introduction of polar functionality with the C5 aldehyde group (**58**) or C5 hydroxymethyl group (**56**), substituents whose sizes closely approximate that of the ethyl group, led to progressively larger losses in activity of ~10-fold and >20-fold, respectively. This clearly indicates that the tubulin ethyl binding site is exclusively hydrophobic in nature and does not benefit from or permit an interaction with the

Scheme 10



solvent interface in the bound complex. More subtly, the introduction of unsaturation into the C5 substituent also led to significant losses in activity. The alkyne **39** was found to be >5-fold less active than **33**, indicating that either the rigidity of this altered C5 substituent or its π unsaturation reduces the tubulin binding affinity. Similarly, although we did not prepare the vinblastine analogue bearing a C5 vinyl substituent, comparison of the corresponding anhydrovinblastine analogues **35** and **41** indicates that even the substitution of a vinyl group for the C5 ethyl group reduces the activity, despite the fact that the two hydrophobic substituents would be expected to embody analogous size and conformational characteristics. Finally, the vinblastine analogues possessing even larger C5 substituents (**43**, **53**, and **55**) were inactive, as were the lactone derivatives **59** and **60** with altered conformational characteristics at C3/C4.

As a result of these observations, an analogous but smaller series of comparisons of the effect of replacing the C5 substituent on a more potent vinblastine scaffold was conducted. 4-Desacetoxyvinblastine (**61**) is a naturally occurring vinca alkaloid, but it is an even more minor (~10-fold) constituent of *C. roseus* than **1** itself (0.00025% of dry leaf weight).[2g] It has been reported to possess equally efficacious antitumor activity as **1**, albeit at higher doses, but was not pursued at the time of its isolation because of its even lower natural abundance.[16] Along with the efforts that provided **1** itself,[10] we recently reported the total synthesis of **61** and its extension to provide the vinblastine analogue **62** lacking the C5 ethyl substituent (R = H).[9a,10b] The preparation of the corresponding analogue **70** bearing a C5 methyl substituent was undertaken and required the additional steps needed to introduce the C6/C7 double bond utilizing the cycloadduct **13** (Scheme 10).[7] Thus, α-hydroxylation of lactam **13** and subsequent triisopropylsilyl (TIPS) ether protection of the free alcohol **63** provided **64**. Thiolactam formation and its reductive removal with Raney



Compound	IC_{50} (nM)		
	L1210	HCT116	HCT116/VM46
62, R = H	610	400	3500
70, R = Me	550	560	5300
61, R = Et	58	60	660
Anhydrovinblastine analogue			
R = H	>10000	>10000	>10000
72, R = Me	4800	7100	7900
R = Et	3900	4700	6000

Figure 4. Cytotoxic activity.

Ni followed by diastereoselective cleavage of the oxido bridge provided **67**. TIPS ether deprotection and subsequent regioselective alcohol elimination[7] provided the key 4-desacetoxyvindoline analogue **69** bearing the C5 methyl substituent. Without optimization, single-step Fe(III)-mediated coupling of **69** with **3** and its subsequent in situ oxidation provided vinblastine analogue **70** (36%), its leurosidine C20′ isomer **71** (15%), and the corresponding anhydrovinblastine analogue **72** (28%). Notably, this preparation of the synthetic analogue **70** of the complex natural product **61** containing a deep-seated single-site modification required only 10 steps from oxadiazole **5** with a resolution of intermediate **13**.

The results of the examination of **70** are presented in Figure 4 alongside those for the natural product **61**. Analogous to observations made with **27** versus **33**, removal of the C5 ethyl substituent (**62** vs **61**) resulted in a 10-fold loss in biological activity. However, and surprisingly distinct from the observations made with **30** versus **33**, the incorporation of a C5 methyl substituent also provided an analogue that was 10-fold less potent (**70** vs **61**) and roughly equipotent with **62** (R = H). Because of the unusual nature of these results, the activities of both **70** and **30** were examined on multiple occasions with independently prepared materials to ensure the accuracy of the results. As a result, we are confident that the comparisons indicate that while on occasion a methyl group may substitute effectively for a C5 ethyl group (e.g., **30** vs **33**), its incorporation may also lead to substantial (10-fold, **70** vs **61**) losses in activity, especially within the more potent analogue scaffolds. Thus, not only is the C5 ethyl group uniquely effective, but the magnitude of its effects on the properties of vinblastine is also surprisingly large (10-fold).

(16) Barnett, C. J.; Cullinan, G. J.; Gerzon, K.; Hoying, B. C.; Jones, W. E.; Newlon, W. M.; Poore, G. A.; Robison, R. L.; Sweeney, M. J.; Todd, G. C.; Dyke, R. W.; Nelson, R. L. *J. Med. Chem.* **1978**, *21*, 88.

Conclusions

A concise total synthesis of a series of key vinblastine analogues that systematically probe and define the importance of the C5 ethyl substituent has been detailed. The requisite deep-seated structural changes, which were accessible only by total synthesis, were accomplished by enlisting an intramolecular tandem [4 + 2]/[3 + 2] cycloaddition cascade of 1,3,4-oxadiazoles that is ideally suited for use in the preparation of the vindoline-derived lower subunit followed by its incorporation into the vinblastine analogues using a single-step biomimetic coupling with catharanthine. The evaluation of the key series of analogues revealed that this site is exquisitely sensitive to the presence (Et > H, 10-fold), size (Me ≤ Et > Pr, 10-fold), shape (Et > CH=CH_2 and C≡CH, >4-fold), and polarity (Et > CHO > CH_2OH, >10-fold) of the C5 substituent, with the corresponding analogues experiencing pronounced losses in activity even with such minor structural changes. The only exception to these observations is the selected equipotent activity observed with a C5 methyl analogue (**30** vs **33** but not **70** vs **61**), indicating that the C5 ethyl group is surprisingly important to the properties of vinblastine (≥10-fold) and that the tubulin binding site surrounding this ethyl substituent is remarkably important, exclusively hydrophobic in nature, and restricted in size and does not access the adjacent solvent interface of the complex. Studies targeting additional sites for single systematic changes enlisting the cycloaddition cascade synthetic strategy are in progress and will be disclosed in due course.

Acknowledgment. We gratefully acknowledge the financial support of the National Institutes of Health (CA115526 and CA042056) and the Skaggs Institute for Chemical Biology. We thank Dr. Raj Chadha for the X-ray structures disclosed herein, and we are especially grateful to Dr. Paul Hellier of Pierre Fabre for the generous gift of catharanthine. W.M.R. is a Skaggs Fellow.

Supporting Information Available: Full experimental details, complete ref 5a, and crystallographic data (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA1027748