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Total Synthesis and Evaluation of Vinblastine Analogues Containing Systematic Deep-Seated Modifications in the Vindoline Subunit Ring System: Core Redesign

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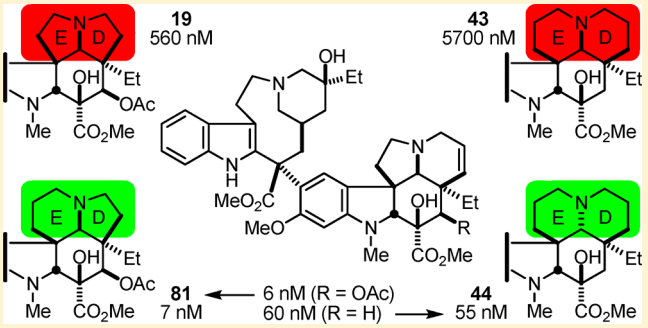
Supporting Information

ABSTRACT: The total synthesis of a systematic series of vinblastine analogues that contain deep-seated structural modifications to the core ring system of the lower vindoline subunit is described. Complementary to the vindoline 6,5 DE ring system, compounds with 5,5, 6,6, and the reversed 5,6 membered DE ring systems were prepared. Both the natural *cis* and unnatural *trans* 6,6-membered ring systems proved accessible, with the latter representing a surprisingly effective class for analogue design. Following Fe(III)-promoted coupling with catharanthine and in situ oxidation to provide the corresponding vinblastine analogues, their evaluation provided unanticipated insights into how the structure of the vindoline subunit contributes to activity. Two potent analogues (**81** and **44**) possessing two different unprecedented modifications to the vindoline subunit core architecture were discovered that matched the potency of the comparison natural products and both lack the 6,7-double bond whose removal in vinblastine leads to a 100-fold drop in activity.



INTRODUCTION

Vindoline (**1**),[1,2] a major alkaloid of *Catharanthus roseus*, comprises the more complex lower subunit of vinblastine (**4**)[2−5] and serves as both a biosynthetic[3,4] and synthetic[6] precursor to this natural product (Figure 1). Vinblastine and vincristine (**5**), originally isolated in trace quantities from *Catharanthus roseus* (L.) G. Don, inhibit assembly of tubulin into microtubules, arresting mitosis. Half a century after the introduction of *Vinca* alkaloids into the clinic and the seminal identification of their mechanism of action, tubulin remains among the more successful therapeutic targets for cancer chemotherapy.[7] Previously, we reported the development of a concise total synthesis of (−)- and *ent*-(+)-vindoline[8−10] enlisting a tandem intramolecular [4 + 2]/[3 + 2] cycloaddition cascade of 1,3,4-oxadiazoles,[11] the extension of this methodology to the preparation of a series of related natural products,[12] and the subsequent asymmetric total synthesis of vindoline and vindorosine (**2**).[13] Central to this work was the use of a biomimetic Fe(III)-promoted coupling[14] of vindoline with catharanthine (**3**) and the additional development of a subsequent in situ Fe(III)-mediated alkene oxidation[6i,j] for C20′-alcohol introduction that allows for their single-step incorporation into total syntheses of vinblastine, related natural products including vincristine, and key analogues in routes as short as 8−12 steps.[6j]

Prior to these efforts, the majority of vinblastine analogues have been prepared by modification of accessible peripheral sites on the natural products, with the disclosure of a limited number of analogues that contain deep-seated structural changes.[15] As a result, we expanded the scope of the Fe(III)-promoted biomimetic coupling reaction to prepare vinblastine

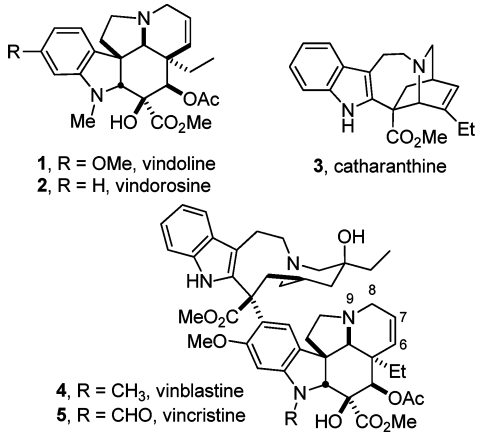


Figure 1. Natural product structures.

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analogues containing key modifications to the upper velbanamine subunit with replacements of the C16′ methyl ester,[16] incorporation of a systematic series of C10′ and C12′ substituents,[17] and introduction of previously inaccessible C20′ functionality.[18] In addition to serving to probe and redefine the mechanistic details of the Fe(III)-promoted coupling reaction,[14b] the studies have identified unappreciated structural features of the natural products contributing to their target binding and functional activity[16] and have provided altered structures that match or surpass[17,18] the potency of the clinical drugs.

With respect to modifications to the lower vindoline subunit, including systematic changes to the C5 ethyl substituent,[19] one of the most remarkable observations confirmed in our initial studies was the impact of removing the C6−C7 double bond.[6j] $\Delta^{6,7}$-Dihydrovinblastine was found to be nearly 100-fold less active than vinblastine, indicating that this region of the natural product has a pronounced impact on its biological properties. Because the X-ray crystal structure of vinblastine bound to tubulin[20] does not reveal an obvious stabilizing interaction with the olefin that might account for the difference, we have begun to explore its origin. In conjunction with our studies on the asymmetric total synthesis of vindoline, analogues **6**−**9** containing a heteroatom β to the basic amine, including several compounds in which the olefin-containing six-membered ring was replaced with a saturated five-membered ring, were prepared and coupled with catharanthine to afford the corresponding vinblastine analogues (Figure 2).[13b] These C7-

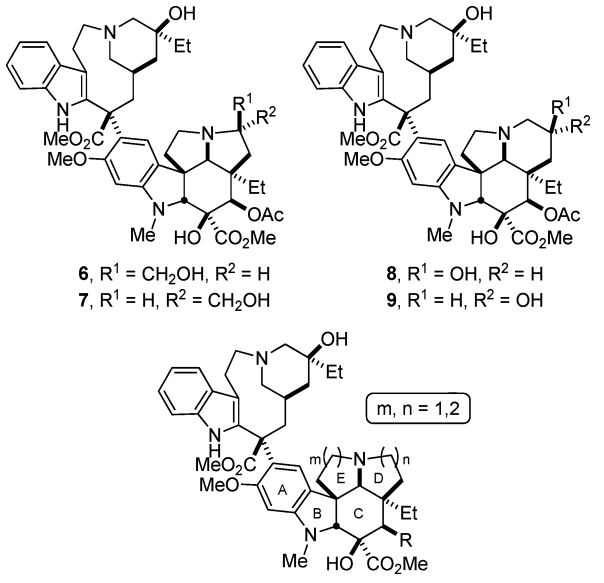

Figure 2. Compounds used to probe changes to the C6−C7 region of the vindoline subunit.

substituted six-membered or five-membered analogues only matched or were a further 10-fold less potent than $\Delta^{6,7}$-dihydrovinblastine, being 100- to 1000-fold less active than vinblastine itself and indicating that the olefin impact may not be due simply to its influence on the N9 pK_a. In light of this unappreciated major role for the C6−C7 region in establishing functional activity and its unknown origin, we elected to probe the steric and conformational features of the vindoline ring system that might be responsible for these effects.

Herein, we report the synthesis of a series of analogues bearing deep-seated structural modifications to the vindoline core ring system. Complementary to the 6,5 DE ring system of vindoline, compounds with 5,5, 6,6, and the reversed 5,6 membered DE ring systems were targeted to systematically address the impact of ring size and conformation on activity. Preparation of these analogues, not accessible from natural product sources, further demonstrates the versatility of the intramolecular [4 + 2]/[3 + 2] cycloaddition cascade. The cumulative examination of the vinblastine analogues bearing the key vindoline ring system modifications not only provided analogues containing two different unprecedented deep-seated changes to the core of the lower vindoline subunit whose activity matched that of vinblastine, but it also revealed that the impact of the vinblastine C6−C7 double bond is not the result of a pronounced π-stabilizing interaction with tubulin and that it does not appear to be related to its impact on the N9 pK_a, its lone pair orientation, or even the precise spatial location of N9. In addition to discovering that alternative lower subunit ring systems without the key double bond may be equally effective even though the double bond removal in vinblastine results in a 100-fold loss in activity, the results of the studies suggest that such deep-seated core redesign of biologically active natural products should be more frequently explored than is presently considered.

RESULTS AND DISCUSSION

Synthesis of the Vindoline Analogue Containing a 5,5 DE Ring System and Incorporation into the Corresponding Vinblastine Analogue. Because **6** and **7** contained an extraneous C7 hydroxymethyl substituent that made the interpretation of their properties unclear, we targeted the C7 unsubstituted system **19**. Relative to vinblastine, this entails removal of the 6,7 double bond and ring contraction of the six-membered D ring to provide the vinblastine analogue containing a modified vindoline core 5,5 DE ring system.

A unique synthesis of the vindoline analogue **18** containing this alternative 5,5 DE ring system was developed (Scheme 1). In the course of studies to clarify the mechanism of a key ring expansion reaction used in our asymmetric synthesis of vindoline,[13] we observed that warming a solution of the primary alcohol **10** (vs tosylate **11**) predictably did not lead to ring expansion but did provide the unanticipated products **13** and **14**[21] derived from oxidative cleavage of the hydroxymethyl substituent. Although the mechanistic details of this unusual transformation are not yet fully defined, the conversion was improved by conducting the reaction in acetonitrile (50 °C, 24 h, and 70 °C, 48 h) versus water−tetrahydrofuran (THF), providing **13** (51%) in surprisingly good conversion. Consistent with studies that indicated the reaction was relatively insensitive to the presence of O_2, that it was less effective if O_2 was deliberately introduced, and that it was more effective with its careful exclusion, a plausible mechanism for the generation of **13** entails reversible iminium ion generation followed by elimination of formaldehyde to generate a stabilized singlet carbene that undergoes a subsequent insertion with the proximal C3 alcohol for reclosure of the *N*,*O*-acetal (eq 1). Without further optimization, extending this reaction to **15**, bearing the C16-methoxy substituent, provided **16** in comparable conversion (48%, CH_3CN, 50 °C, 72 h). Reductive cleavage of the cyclic *N*,*O*-acetal ($NaCNBH_3$, 10% *i*-PrOH−THF, 3 equiv of HCl, 0 °C) afforded the key vindoline analogue **18** (84%). Without optimization, **18** was coupled with catharanthine (**3**) using the Fe(III)-promoted coupling and

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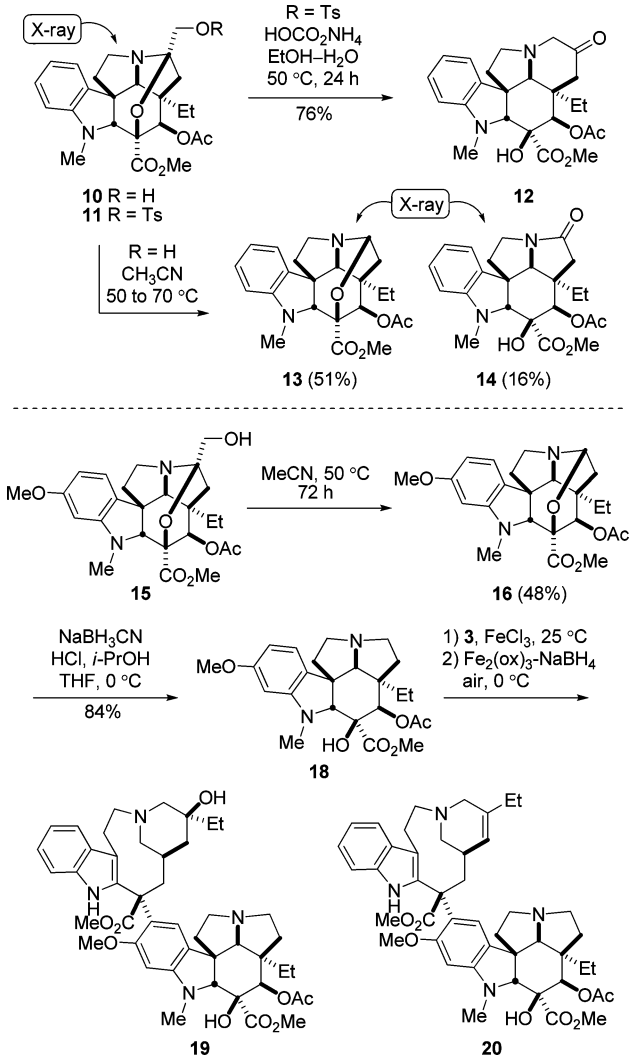
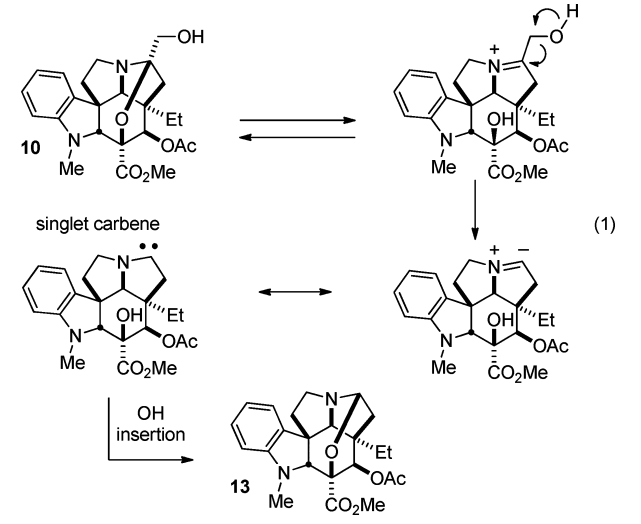
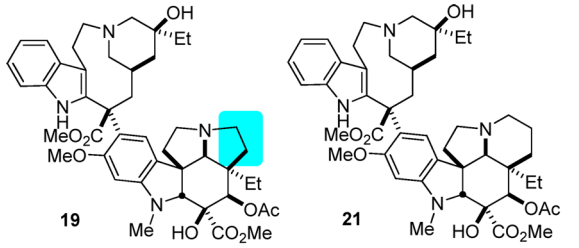
Scheme 1



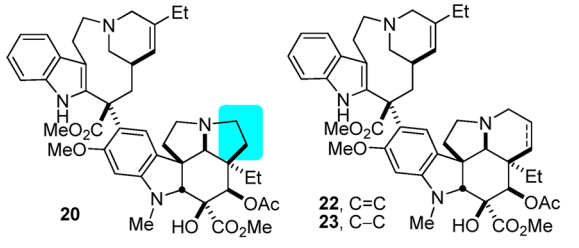


subsequent oxidation to afford the vinblastine analogue **19**, as well as the anhydrovinblastine **20**.

As in related studies,[6j,16−19] the compounds were examined for inhibition of cell growth against a mouse leukemia cell line (L1210), a human colon cancer cell line (HCT116), and a matched human colon cancer cell line (HCT116/VM46) that is resistant to vinblastine by virtue of overexpression of the drug exporter Pgp. The vinblastine analogue **19** containing the ring contracted D ring in the vindoline core proved to be 10-fold more potent than **6** and **7**, indicating that the C7 hydroxymethyl groups of the latter compounds were indeed negatively impacting their properties (Figure 3).[22] However,



Compound	IC_{50} (nM)		
	L1210	HCT116	HCT116/VM46
4, vinblastine	6.0	6.8	600
21, $\Delta^{6,7}$-dihydrovinblastine	570	370	4500
19	560	80	7800



Compound	IC_{50} (nM)		
	L1210	HCT116	HCT116/VM46
22, anhydrovinblastine	65	75	600
23	740	890	3500
20	700	630	7000

Figure 3. Activity of 5,5 DE ring system analogues.

unlike derivatives that follow, **19** remained 10- to 100-fold less potent than vinblastine, displaying activity that was essentially equivalent to 6,7-dihydrovinblastine (**21**) lacking the C6−C7 double bond. Similarly, although the anhydrovinblastine series is 10-fold less potent, identical trends were observed. The anhydrovinblastine analogue **20** was 10-fold less active than anhydrovinblastine (**22**) itself, and **20** proved to be equipotent to 6,7-dihydroanhydrovinblastine (**23**). In essence, the D ring contraction in going from **21** to **19** or **23** to **20** had no impact on the biological properties, but it also did not regain the activity lost with removal of the C6−C7 double bond.

Synthesis of Vindoline Analogues Containing a 6,6 DE Ring System and Incorporation into the Corresponding Vinblastine Analogues. The vinblastine analogues containing modified vindoline subunits bearing a ring expanded 6,6 DE ring system while not yet being the most potent variation, proved to be among the most interesting and revealing. The syntheses of the 6,6 DE ring system vindoline analogues closely parallel our synthesis of vindoline itself, with the key difference being the use of a cascade cycloaddition substrate with a lengthened tether to the indole dipolarophile. Wittig olefination of aldehyde **24**[10] provided **25** smoothly in 88% yield (Scheme 2). Hydrogenation of the olefin required use of Raney nickel to

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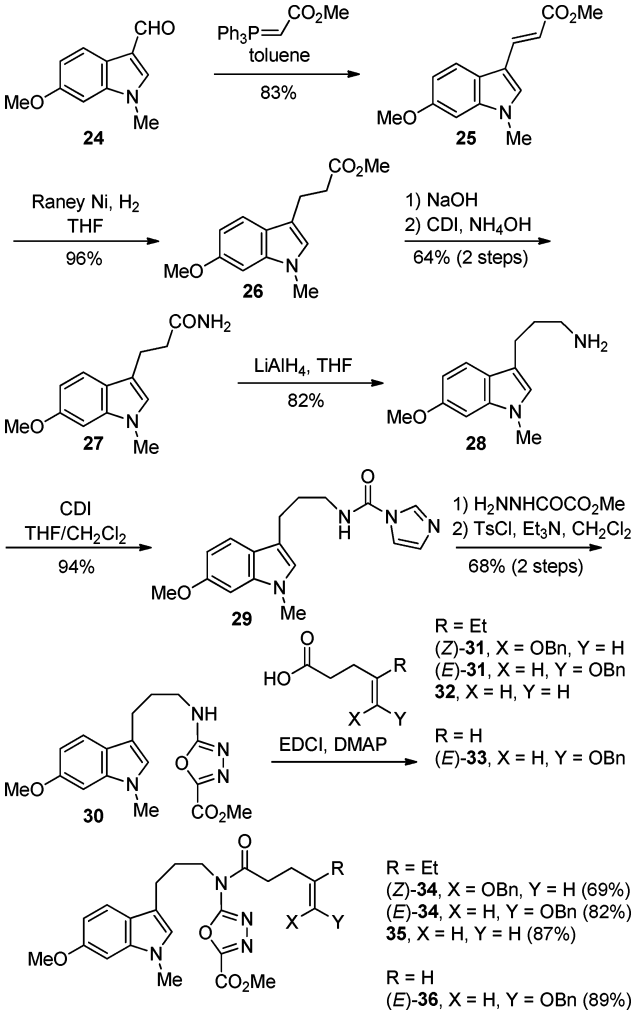
Scheme 2

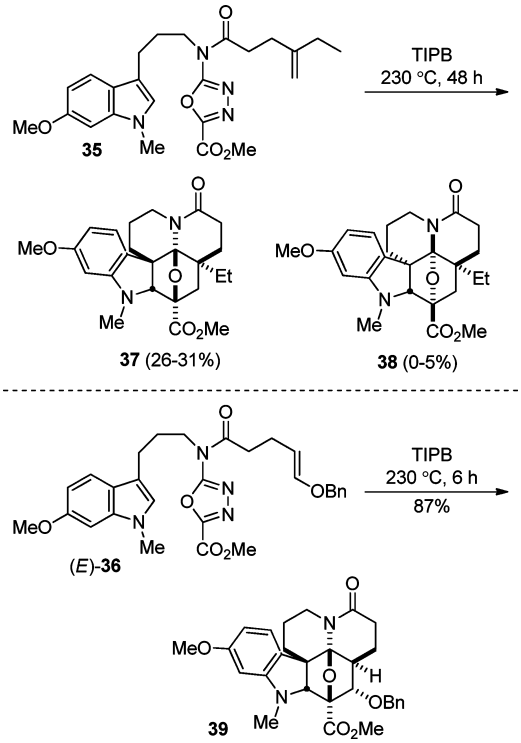


generate the saturated tether, as use of palladium on carbon provided a mixture of the desired product **26** and overreduction of the indole. Hydrolysis of the methyl ester cleanly provided the corresponding carboxylic acid, which was converted to the carboxamide **27** through reaction with 1,1′-carbonyldiimidazole (CDI) followed by addition of NH_4OH. Reduction of **27** by $LiAlH_4$ provided amine **28**. After coupling with CDI to give **29**, reaction with methyl oxalylhydrazide and subsequent dehydrative ring closure (TsCl, Et_3N) provided the 1,3,4-oxadiazole **30**. Coupling of **30** with a series of carboxylic acids tethered to candidate initiating dienophiles, including (*E*)-**31** or (*Z*)-**31**, generated the cycloaddition precursors **34**–**36**.

In prior studies, significant differences in the rate and facility of the cycloaddition cascade were observed in which either the initiating [4 + 2] or subsequent [3 + 2] cycloaddition was found to be rate limiting, depending on the substitution and stereochemistry of the initiating dienophile.[10,11] The (*Z*)-enol ether dienophiles permit the direct introduction of the naturally occurring C4 stereochemistry, while (*E*)-enol ether dienophiles provide the C4 isomer requiring subsequent inversion of configuration at this stereocenter. Typically, the reaction rate for the (*Z*)-isomer is slower, being limited by a rate determining [3 + 2] cycloaddition, and the reaction often requires additional dilution to maximize conversion to the desired cycloadduct. Unfortunately, neither (*E*)-**34** nor (*Z*)-**34** afforded the cascade cycloaddition product upon heating under a variety of conditions (*o*-$Cl_2C_6H_4$ or triisopropylbenzene, 0.5–2 mM, 180–230 °C, 24–72 h). Both provided lactone products derived from interception of the initial [4 + 2] cycloadditions that stall and fail to progress through the [3 + 2] cycloaddition.

In contrast, both **35** and (*E*)-**36**, containing less substituted initiating dienophiles, participated in the cycloaddition cascade effectively (Scheme 3). Especially notable was the reaction of

Scheme 3



(*E*)-**36**, lacking only the ethyl group of (*E*)-**34**, which provided a single diastereomer **39** of the cascade cycloadduct in superb conversion (68–87%, 2–0.1 mM) in relatively short reaction times (6 h, 230 °C). This reaction was found to proceed effectively even at the lower temperature of 180 °C in triisopropylbenzene (TIPB, 50%, 2 mM), albeit requiring longer reaction times (12 h), indicating that trisubstitution of the initiating dienophile slows and, in the case of (*E*)-**34**, ultimately precludes the subsequent [3 + 2] cycloaddition.

To initially assess the impact of a vindoline 6,6 DE ring system, we elected to carry the cycloadduct **37** forward. Although it was formed in lower conversions than **39**, it serves as a precursor to a series of vindoline analogues lacking only the C4 acetoxy group, a substituent whose impact on the properties of vinblastine are well established (10-fold reduction in potency).[6j] This decision was fortunate in that it led to the discovery of an unprecedented stereochemical impact on the properties of the resulting vinblastine analogues. The cycloadduct **37** could be resolved by chiral phase HPLC using a semipreparative Daicel Chiralcel OD column either at this stage or following conversion with Lawesson's reagent[23] to the thiolactam **40** (Scheme 4), the absolute stereochemistry of which was unambiguously established by X-ray crystallography.[24] Treatment of (+)-**40**, possessing the required absolute stereochemistry, with Me_3OBF_4 and subsequent reduction of the resulting *S*-methyl iminium ion ($NaBH_4$)[25] provided exclusively **42** in superb yield (86%), bearing the unnatural

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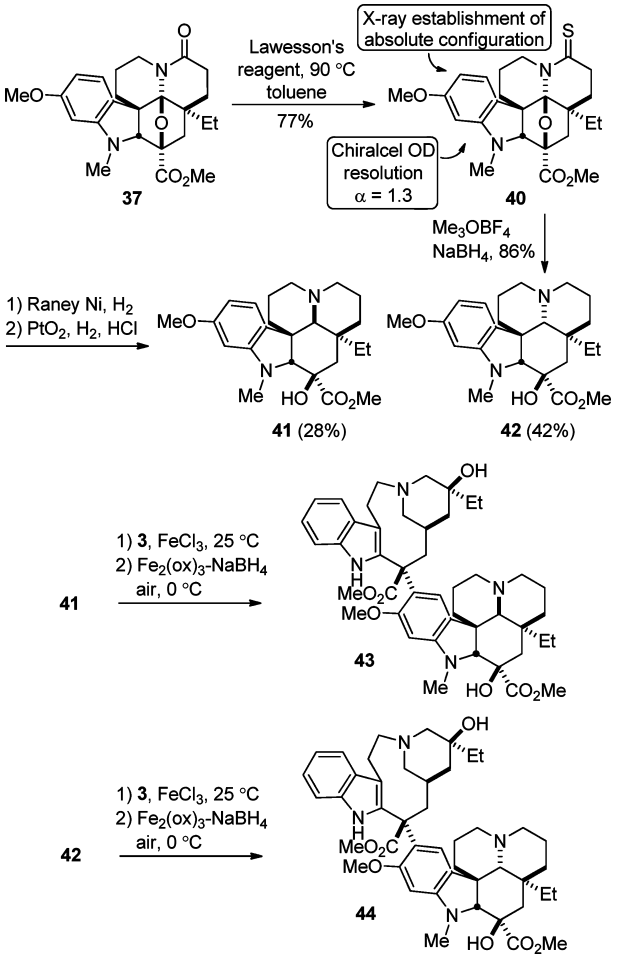
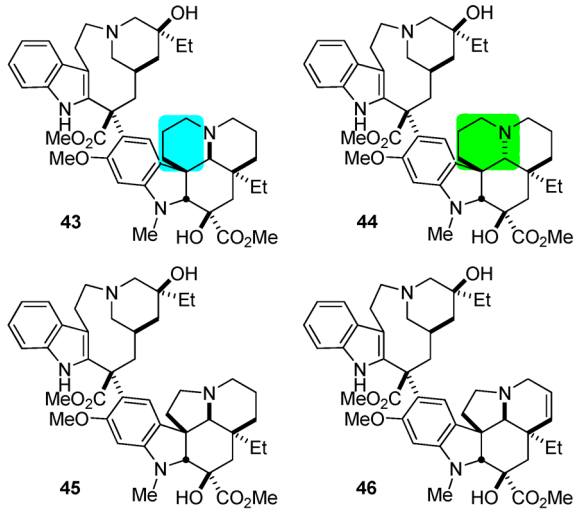
Scheme 4



stereochemistry at C9 and a *trans* ring structure. Mistakenly expecting that only the natural *cis* ring stereochemistry would be active when incorporated into a vinblastine analogue, considerable effort was spent searching for conditions that would effect reductive oxido bridge cleavage to produce the isomer **41**. In these efforts, we found that **41** could be obtained by a stepwise Raney nickel desulfurization of the thioamide followed by acid-catalyzed oxido bridge opening and in situ reduction of the resulting iminium ion with H_2/PtO_2. Satisfactory conversions were observed only at increased hydrogen pressures (250 psi) and upon prolonged reaction times (3.5 d), providing up to 28% of **41**, with competitive formation of its isomer **42** (42%). Without optimization, both **41** and the more readily accessible **42** were coupled with catharanthine (**3**) to assemble the respective vinblastine analogues **43** and **44** with an expanded E ring in the vindoline core.

Because the removal of the C4 acetoxy group has been established to contribute 10-fold to the potency of vinblastine (**4**),[6j] both **43** and **44** were anticipated to be less active than vinblastine. A more direct comparison of their biological activity can be made to 4-desacetoxy-6,7-dihydrovinblastine (**45**)[6j] and the additional natural product 4-desacetoxyvinblastine (**46**).[3h,26] Relative to **45**, the change to the *cis* 6,6 DE system in **43** resulted in a further 10-fold reduction in potency (Figure 4). Remarkably, the vinblastine analogue **44** incorporating the synthetically more accessible unnatural *trans* fused 6,6 DE ring system was found to be 100-fold more active than



Compound	IC_{50} (nM)		
	L1210	HCT116	HCT116/VM46
4, vinblastine	6.0	6.8	600
45	660	670	4000
46	60	60	660
43	5700	7400	> 10000
44	55	80	900

Figure 4. Activity of saturated 6,6 DE ring system analogues.

this *cis* isomer, 10-fold more potent than **45**, equipotent to the natural product **46**, and merely 10-fold less potent than vinblastine in spite of large structural differences in the vindoline portion of the molecule. This includes not only the incorporation of the unnatural *trans* fused and ring expanded 6,6 DE ring system but also the removal of both the C6−C7 double bond and the C4 acetoxy group.

In light of these observations, we elected to establish the impact of introducing the C6−C7 olefin into the analogues **43** and **44**. Accordingly, α-hydroxylation of **37** and subsequent acetylation of the secondary alcohol **47** afforded **48** (Scheme 5).[27] Treatment of **48** with Lawesson's reagent afforded thiolactam **49** in acceptable yield, albeit with significant amounts of recovered starting material. Efforts to drive the reaction to completion using more reagent, higher reaction temperatures, or longer reaction times simply led to full consumption of **48** and the generation of additional byproducts without improvements in the conversions to **49**. Desulfurization of **49** with Raney nickel and reductive cleavage of the oxido bridge with $NaBH_4$ provided a separable mixture of the diastereomers **50** and **51**. Removal of the acetyl groups in **50** and **51** was conducted by treatment with NaOMe to produce the corresponding secondary alcohols **52** and **53** in high yields. Subjection of **52** to Mitsunobu conditions for elimination (PPh_3, DEAD)[28] yielded the desired vindoline analogue **54**, whereas such treatment of **53** resulted in formation of the isomeric 7,8-anhydro product **55** instead of the desired 6,7-anhydro analogue. Alternative elimination reaction conditions (CCl_4, PPh_3) used by Kuehne[29] were also unsuccessful for the introduction of the required 6,7-olefin.

In light of the additional unsatisfactory, albeit unoptimized, elements of this route, we examined alternative stages and methods for the introduction of the C6−C7 double bond. Instead of performing a late-stage elimination, we explored

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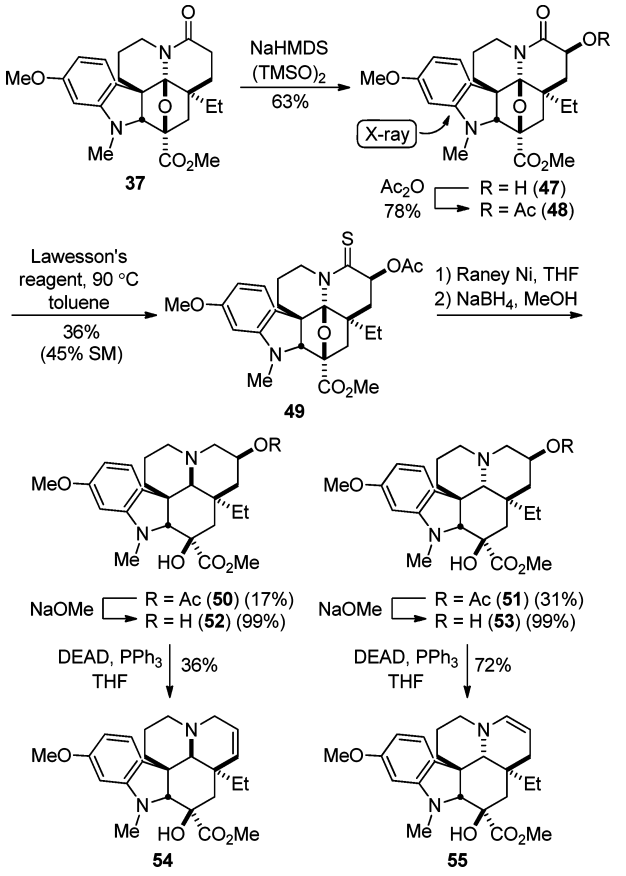
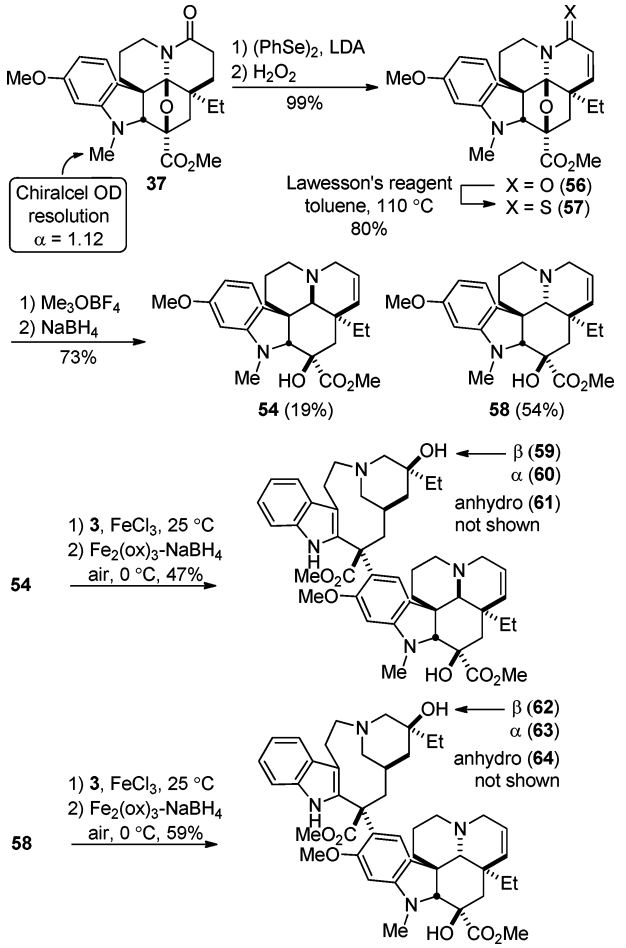
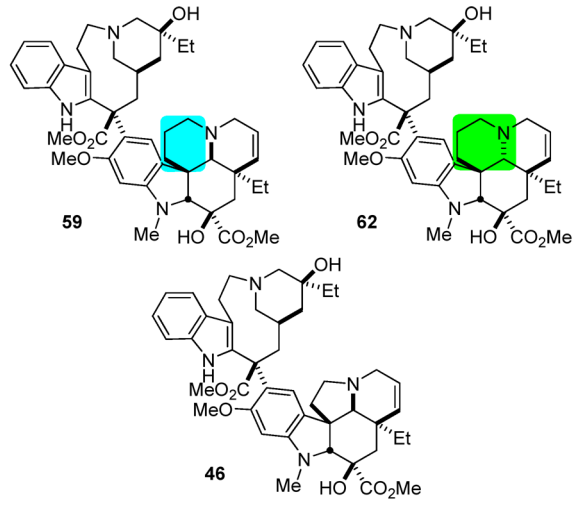
Scheme 5



Scheme 6





what proved to be a much more effective route where the olefin was inserted prior to removal of the lactam carbonyl oxygen and opening of the oxido bridge. Treatment of optically active **37** with $(PhSe)_2$ and LDA yielded the α-selenide product, which upon oxidation with H_2O_2 gave the α,β-unsaturated lactam **56** in exceptionally high yield (99%, Scheme 6). Thionation of **56**, carried out with Lawesson's reagent, provided **57** in excellent yield (80%). Reaction of **57** with Me_3OBF_4 followed by $NaBH_4$ reduction of the resulting *S*-methyl iminium ion under neutral conditions, afforded a diastereomeric mixture of the oxido bridge cleaved products **54** and **58** in good yield, again favoring formation of the more interesting unnatural *trans* fused isomer **58** (3:1) upon reduction of the intermediate iminium ion derived from oxido bridge cleavage. Without optimization, both **54** and **58** were coupled with catharanthine to provide the respective 4-desacetoxyvinblastine analogues **59** and **62**, along with the corresponding anhydrovinblastine and leurosidine analogues.

Like the observations made with vinblastine itself, the introduction of the C6−C7 double bond in **59** (**59** vs **43**) provided a >10-fold increase in activity (Figure 5), although it remained less active than 4-desacetoxyvinblastine (**46**). In contrast, introduction of the olefin into the already potent unnatural *trans* fused series with **62** (**62** vs **44**) resulted in no further gain in activity, and the compound remained equipotent to the comparison natural product 4-desacetoxyvinblastine (**46**). In essence, the combined studies comparing **43**, **44**, **59**, and **62** show that introduction of the saturated *trans* 6,6 DE ring system on **44** is functionally equivalent to the natural unsaturated *cis* 6,5 DE ring system found in vinblastine.

Compound	IC_{50} (nM)		
	L1210	HCT116	HCT116/VM46
4, vinblastine	6.0	6.8	600
46	60	60	660
59	370	430	5700
62	65	100	730

Figure 5. Activity of the unsaturated 6,6 DE ring system analogues.

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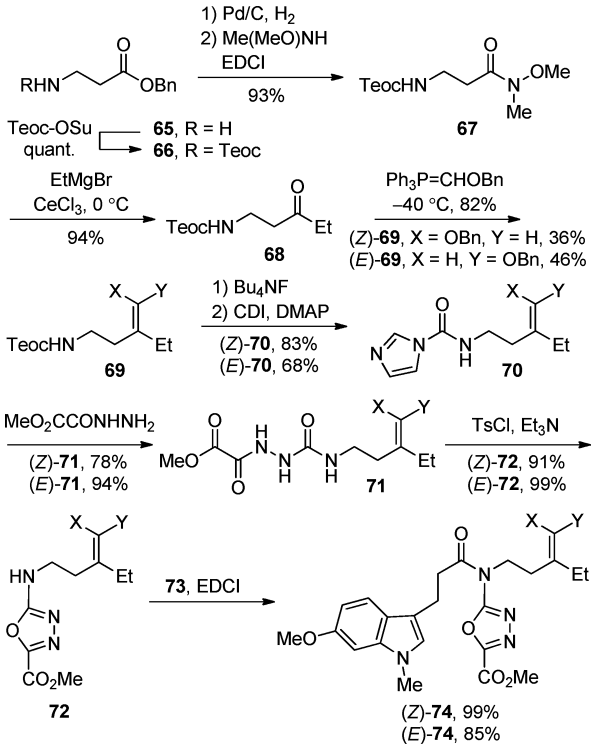
Synthesis of Vindoline Analogues Containing the Reversed 5,6 DE Ring System and Incorporation into the Corresponding Vinblastine Analogues. Among the most interesting and certainly the most surprising of the deep-seated structural modifications were those that contained the vindoline reversed 5,6 DE ring system. Here, not only is the vindoline C6−C7 double bond removed with the D ring contraction to a 5-membered ring, but the E ring is expanded from a five-membered to a six-membered ring. To access the targeted vindoline analogue **80** with this reversed 5,6 DE ring system, a shorter two-carbon tether to the initiating dienophile is required and was prepared starting from the commercially available β-alanine benzyl ester **65** (Scheme 7). Teoc protection

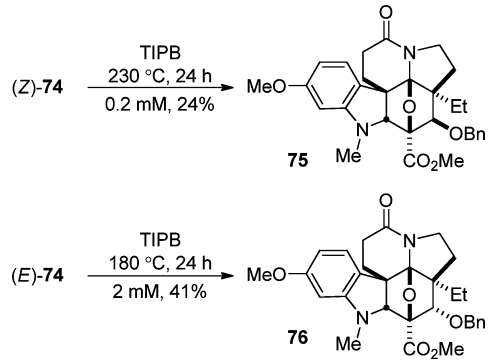
Scheme 7



of **65**, hydrogenolysis of the benzyl ether, and formation of the Weinreb amide[30] **67** set the stage for reaction with EtMgBr (3 equiv, 3 equiv of $CeCl_3$, THF, 0 °C, 94%) to afford the ethyl ketone **68**. Wittig olefination with $Ph_3P{=}CHOBn$[31] provided a separable 1:1.2 mixture of the (*Z*)- and (*E*)-enol ethers **69**, which were independently converted to the cycloaddition precursors **74**. Teoc deprotection (Bu_4NF) of (*Z*)- and (*E*)-**69** and treatment of the liberated amines with carbonyldiimidazole afforded (*Z*)- and (*E*)-**70**. Addition of methyl oxalylhydrazide afforded **71**, which underwent cyclization to the corresponding oxadiazoles **72** in the presence of TsCl and Et_3N. Coupling of (*Z*)-**72** and (*E*)-**72** with 3-(*N*-methyl-6-methoxyindol-3-yl)-propanoic acid (**73**), obtained from methyl ester **26** (2N NaOH, MeOH, 90%), provided the cycloaddition substrates (*Z*)-**74** and (*E*)-**74**, respectively.

Cyclization of (*Z*)-**74** proceeded in TIPB (230 °C, 24 h, 0.2 mM) to give the cycloadduct **75** possessing the natural C4 stereochemistry, albeit in modest yield and requiring dilution of the reaction mixture to 0.2 mM to afford **75** in conversions up to 24% (Scheme 8). Nevertheless, this reaction serves as an interesting contrast to both the unsuccessful behavior of (*E*)-**34**

Scheme 8



and (*Z*)-**34** and the more successful cascade cycloaddition of the substrate containing a tether substituted dienophile employed in the vindoline asymmetric total synthesis, which also incorporated a shortened dipolarophile tether.[13] While these differences may be attributed in part to reduced entropic costs encountered when initiating the reaction with the shorter and/or substituted dienophile tether, it is also clear that the further conformational restriction in the dipolarophile tether in **74** (**74** vs **34**) contributes to this success. Consistent with expectations, (*E*)-**74** underwent a more facile reaction (TIPB, 180 °C, 24 h, 41%, or xylene, 150 °C, 24 h, 43%), requiring lower reaction temperatures and accommodating higher reaction concentrations (2 mM) for the cycloaddition cascade to provide **76**.

Following resolution of the enantiomers of **75** on a Chiralcel OD column (30% *i*-PrOH−hexanes, 2 cm × 25 cm, α = 1.4), benzyl group removal ($Pd(OH)_2$, 45 psi H_2, MeOH, 16 h) provided the secondary alcohol **77** (Scheme 9). Acetylation of **77** to give **78**, followed by treatment with Lawesson's reagent, provided thiolactam **79**, the absolute stereochemistry of which was established by X-ray crystallography.[32] Reductive desulfurization and a final reductive oxido bridge cleavage cleanly afforded the desired vindoline analogue **80**. Without optimization, single-step Fe(III)-promoted coupling of **80** with catharanthine (**3**) and in situ oxidation produced the vinblastine analogue **81** along with the epimeric C20′ leurosidine analogue **82** and the anhydrovinblastine analogue **83**.

Remarkably, this vinblastine analogue **81** proved to be equipotent with vinblastine (Figure 6). Not only does **81** lack the critical C6−C7 double bond of vinblastine, but it incorporates both a ring contracted five-membered D ring and a ring-expanded six-membered E ring. Individually, these changes result in 100-fold (**21** vs **4**), 10- to 100-fold (**19** vs **4**), and nearly 10-fold (**59** vs **46**) losses in activity but reinstate full activity when combined into a single analogue containing the deep-seated core modifications. Most notably, the C6−C7 double bond is no longer required within **81**.

Because of the greater facility of the cycloaddition with (*E*)-**74**, a route to **80** proceeding through **76** and requiring inversion of the C4 stereocenter was also examined in studies that also provided the opportunity to examine the C4 diastereomer of vinblastine and its related analogues. The enantiomers of **76** were separated on a Chiralcel OD column (30% *i*-PrOH−hexanes, 2 cm × 25 cm, α = 1.4). The benzyl group of **76** was removed by hydrogenation ($Pd(OH)_2$, 1 atm H_2, MeOH, 16 h) to afford **84**, which proceeded under milder conditions than required of its C4 isomer **75** (Scheme 10). The

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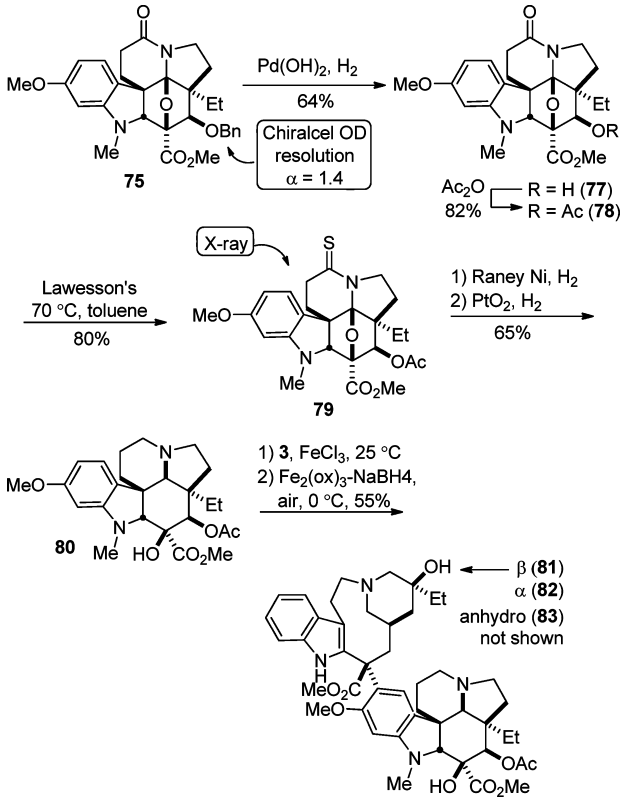
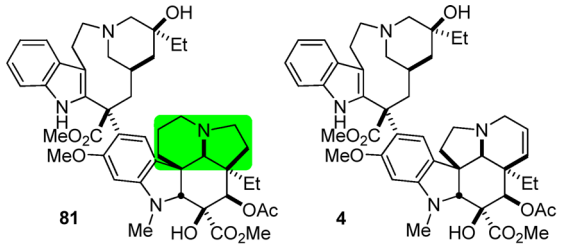
Scheme 9

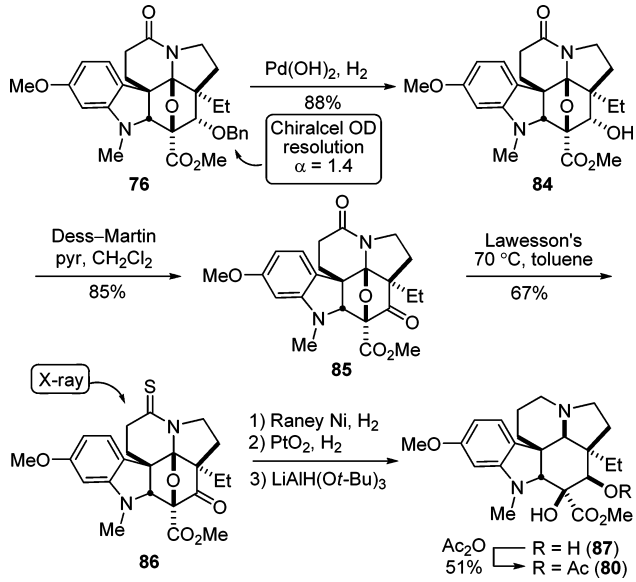




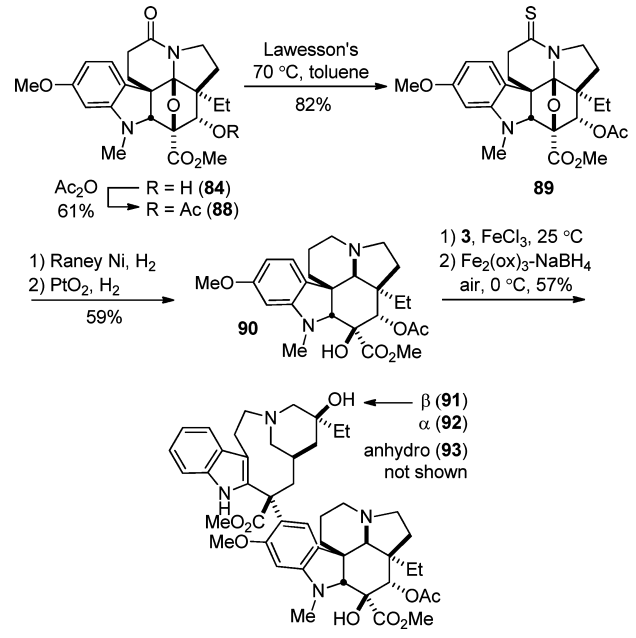
Compound	IC50 (nM)		
	L1210	HCT116	HCT116/VM46
4, vinblastine	6.0	6.8	600
81	7.2	6.5	450

Figure 6. Activity of the reversed 5,6 DE ring system analogue.

Scheme 10



Scheme 11



C4 alcohol in **84** was oxidized with Dess–Martin periodinane to afford ketone **85**. Selective reaction with Lawesson's reagent afforded the thiolactam **86**, for which the absolute stereochemistry was established by X-ray crystallography.[33] Reductive desulfurization with Raney nickel was followed by diastereoselective reductive cleavage of the oxido bridge (PtO_2, 45 psi H_2, 1:1 MeOH–EtOAc). This afforded oxido bridge cleavage product as an inconsequential mixture of C4 ketone and secondary alcohol **87**, which was further treated with $LiAlH(Ot\text{-}Bu)_3$ (0 °C, THF) to obtain clean **87**, with exclusive hydrogen delivery to the ketone from the α-face. Without optimization, acetylation of **87** also afforded the target vindoline analogue **80**.

Additionally, the secondary alcohol **84** was acetylated to give **88** (Scheme 11). Treatment with Lawesson's reagent afforded thiolactam **89**. Reductive removal of the thiolactam and a final reductive oxido bridge cleavage cleanly afforded **90**, the C4 isomer of the key vindoline analogue **80**. Without optimization, coupling of **90** with catharanthine (**3**) and in situ oxidation afforded the corresponding vinblastine analogue **91**.

For comparison purposes, the C4 epimer of vinblastine itself was prepared and used to determine the effect of this single point change in the natural product. This was accomplished by treatment of (−)-vindoline with NaOMe in MeOH to afford 4-desacetylvindoline (**94**, Scheme 12).[10a] Although SO_3–pyridine oxidation of **94** has been reported,[8f] oxidation with IBX (80 °C, EtOAc) afforded the ketone **95** in higher yield in our hands. Reduction of **89** with $NaBH(OAc)_3$ in 1,2-dichloroethane allowed for selective hydride delivery to the ketone from the β-face to give **96**, which was subsequently acetylated (48 h) to give 4-*epi*-vindoline **97**. Coupling with catharanthine (**3**) and in situ oxidation proceeded to give 4-*epi*-vinblastine (**98**) and 4-

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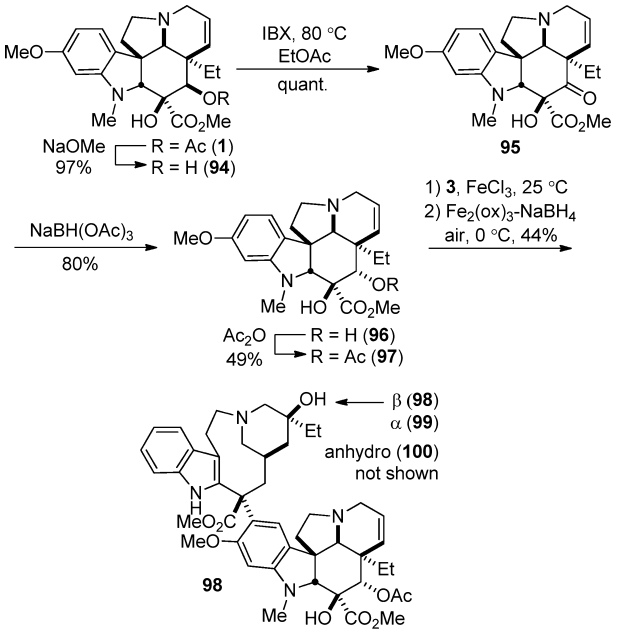
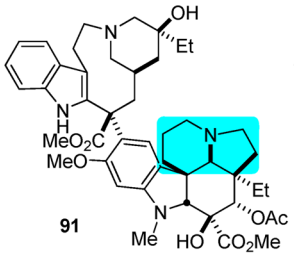
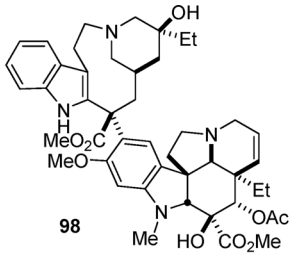
Scheme 12



epi-leurosidine (**99**) in the typical 2:1 β:α diastereoselectivity, along with 4-*epi*-anhydrovinblastine (**100**).

The C4 epimer of vinblastine (**98**) proved to be 10-fold less potent than the natural product, being essentially equipotent with 4-desacetoxyvinblastine, lacking the C4 acetoxy substituent (Figure 7). The analogue **91**, containing both the





Compound	IC_{50} (nM)		
	L1210	HCT116	HCT116/VM46
4, vinblastine	6.0	6.8	600
98, 4-*epi*-vinblastine	75	80	8400
91	600	680	7600

Figure 7. Activity of C4 epimeric analogues.

vindoline reversed 5,6 ring system as well as the C4 epimeric center, also displayed reduced cytotoxic activity, being approximately 100-fold less potent than vinblastine and **81** and 10-fold less potent than *epi*-4-vinblastine (**98**).

Origin of the Impact of the Core Modifications. The 50−100-fold reduction in the cytotoxic activity of $\Delta^{6,7}$-dihydrovinblastine (**21**) relative to vinblastine that we observe in our cell-based assays correlates well with its 30−50-fold reduction in affinity found in quantitative tubulin binding assays.[34] Thus, the diminished potency of **21** in our cell-based assays is the direct result of its reduced target binding affinity and not the result of other variables that conceivably could affect the activity (e.g., cell permeability, metabolism). Given the rather minor perturbations to the overall structure that the remaining analogues represent, it is reasonable to expect that they also accurately reflect qualitative and quantitative trends in target binding effects, especially those of **81** and **44** that match the activity of the corresponding natural products **4** and **46**.[6j] To confirm this assumption, tubulin binding affinities were assessed using a well-established tubulin binding assay[34] measuring the competitive displacement of ^{3}H-vinblastine from porcine tubulin. These studies, which are much more sensitive and quantitatively much more accurate than those using tubulin polymerization assays, established that **59** binds tubulin with a lower affinity than vinblastine and confirmed that **81** binds tubulin with an affinity matching that of vinblastine itself (Figure 8). These trends observed in the tubulin binding

Compound	[^{3}H] Vinblastine Competition (% remaining radioactivity)	L1210 IC_{50} (nM)
4, vinblastine	50.0†	6.0
81	48.4 ± 4.2‡	7.2
59	68.5 ± 5.7‡	370
21, $\Delta^{6,7}$-dihydrovinblastine	77.4 ± 3.3†	570

A 100 μL sample of tubulin solution diluted with 850 μL of buffer was incubated with 25 μL of 7.2 × 10^{-5} M [^{3}H]VLB for 15 min at 37 °C, after which 25 μL of 7.2 × 10^{-5} M unlabeled alkaloid was added and incubation continued for 60 min. Tubulin-bound [^{3}H]VLB was adsorbed onto DEAE filter paper and counted directly.
† Given in ref. 34.
‡ Average of 6 repeat determinations, ± 1 standard deviation, normalized to have dpm (25 μL VLB + 25 μL [^{3}H]VLB) = 50.0%.

Figure 8. Competition between ^{3}H-vinblastine and analogues for the porcine tubulin binding site.

assays follow those observed in the functional cell-based assays, further indicating that conclusions drawn from the latter assays accurately reflect tubulin binding effects.

Examination of the X-ray structure of vinblastine bound to tubulin[20] does not reveal a stabilizing π-interaction of the C6−C7 double bond with the protein, nor does it suggest that removal of the double bond with $\Delta^{6,7}$-dihydrovinblastine (**21**) introduces destabilizing steric interactions when modeled onto the crystal structure (Figure 9). With the caveat that the crystal structure is of a modest resolution (4.1 Å), there appears to be substantial and adequate space in the largely hydrophobic pocket that surrounds the DE ring system such that not only would **21** not be expected to suffer destabilizing steric interactions, but that the entire series of analogues may be accommodated. The fact that compounds so close in structure (**4** vs **21**) exhibit such disparate binding and functional activity while those so spatially or conformationally dissimilar (**81** vs **4** and **46** vs **44**) are essentially equally active suggests that the losses in binding affinity are not likely the result of sterically destabilized binding. In fact, it is the analogues that are most distinct from rather than similar to vinblastine that are most active.

What does stand out is the potential impact that the conformational or structural changes may have on the location and orientation of the nitrogen (N9) lone pair as well as their impact on the N9 basicity. However, here again the expected impacts of the changes do not correlate with activity. Unlike vinblastine (**4**), dihydrovinblastine (**21**), and the even less active **43**/**59** series, where the N9 lone pair is oriented on the β-face, that of **19** and the active **44**/**62** series is oriented to the α-face. Similarly, even the spatial location of the N9 atom in the

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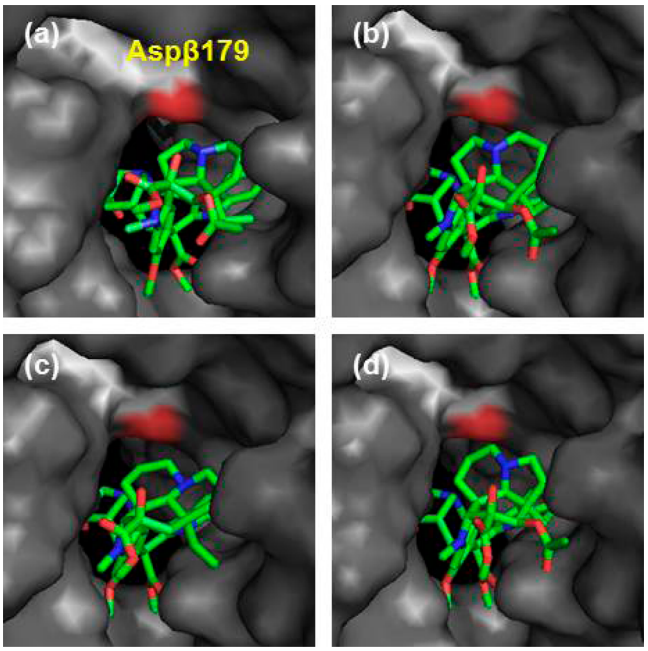

Figure 9. X-ray of vinblastine[20] ((a), PDB ID 1Z2B) and modeled binding of dihydrovinblastine (**21**) (b), **44** (c), and **81** (d) with tubulin viewed from the solvent accessible site into the binding pocket. The catharanthine-derived velbanamine subunit is buried deep in the binding pocket behind the vindoline subunit, which extends down the pocket to the solvent interface. Asp β179, as found in the vinblastine-tubulin X-ray structure, is highlighted (white, with the side chain oxygen in red) on the surface representation and labeled in (a).

active **44**/**62** series is farthest from that found in vinblastine (**4**) and, unlike all others, N9 in this series is not close enough to participate in H-bonding to the C3 OH irrespective of the lone pair orientation. Similarly, the projected basicity of N9 imposed by the structural and conformational changes do not seem to correlate with the observed changes in activity. Although **19**, like dihydrovinblastine (**21**), is expected to be more basic than vinblastine itself, **81** and **21** would be expected to be very similar yet display the most distinct activity. Similarly, **44** would be expected to be more basic than **43**, but it is the much more active of the two isomers. Thus, the N9 basicity, the lone pair orientation, and even the spatial location of the nitrogen do not seems to correlate with activity.

Finally, the only charged, polar residues near or in the tubulin binding pocket at the interface of the two tubulin heterodimers are Lys α336, Lys α352, Lys β176, and Asp β179. Lys α336 and Lys β176 are located too far from the N9 site to expect either to play a role and are shielded from participating by intervening atoms. While the full side chain of Lys α352 is not visible in the X-ray electron density, it appears to be oriented away from vinblastine. By contrast, Asp β179 is located ca. 4.3 Å away from N9 in vinblastine in the cocrystal structure, moving from a position close to a nucleotide binding site in the absence of vinblastine to the vicinity of the drug when bound,[20] and may contribute a stabilizing electrostatic interaction (Figure 9). It is conceivable that interactions with this mobile side chain residue influence the relative binding of the analogues in this series. If so, it is doing so in a way that is not dependent on the spatial site or orientation of N9 and its lone pair, likely with the adoption by Asp β179 itself of varied locations that are not easily anticipated or modeled. These questions and whether a basic N9 is even required are presently being examined.

When combined, the results of the evaluation of the modified vinblastines indicate that the role of the C6−C7 double bond is not derived from the π-unsaturation itself or its impact on the N9 pK_a or the nitrogen lone pair orientation. While many tailoring single-point peripheral changes, such as installation of the C4 acetoxy group, seem to be additive in their effects on the biological properties vinblastine, this need not be the case for structures containing more deep-seated changes to the core structure of the natural product. Herein, such deep-seated structural changes to the size and configuration of the vindoline core ring system of vinblastine, accessible only by total synthesis, provided exceptionally and even unexpectedly potent vinblastine analogues.

CONCLUSIONS

Full details of the synthesis of a systematic series of vinblastine analogues bearing deep-seated structural modifications to the vindoline core ring system are disclosed. Not accessible from natural product sources, analogues possessing 5,5, 6,6, and reversed 5,6 membered DE ring systems were assembled using a tandem intramolecular [4 + 2]/[3 + 2] cycloaddition cascade reaction of 1,3,4-oxadiazoles as the key step. In the course of converting the cycloadducts to the corresponding vindoline analogues, an oxido bridge reduction of the cycloadducts leading to 6,6 DE ring systems provided access to both the *cis* and *trans* ring systems, resulting in the first study of vindoline analogues with this structural feature and affording a surprisingly effective new stereochemical class for exploration. The vindoline analogues were found to be suitable substrates for a biomimetic Fe(III)-promoted coupling and subsequent in situ oxidation reaction, enabling access to vinblastine analogues containing key ring system modifications.

Although single functional group removals from the vinblastine lower subunit typically result in pronounced and additive losses in activity,[6j] two unprecedented deep-seated structural changes to the core structure were found that maintain potent activity. Striking among these is **81**, containing the vindoline reversed 5,6 DE ring system, which proved equipotent to vinblastine. Not only does **81** lack the critical C6−C7 double bond of vinblastine whose removal results in a 100-fold loss in activity, but it also incorporates a ring contracted five-membered D ring and a ring expanded six-membered E ring, representing changes that individually result in 10- to 100-fold reductions in activity. The behavior of the isomeric analogues with the expanded 6,6 DE ring systems was just as remarkable. Unlike the *cis* isomer **43** with the natural C9 stereochemistry that displayed diminished activity, the analogue **44** that not only lacks the C6−C7 double bond but also contains the ring expanded six-membered E ring and the unnatural (*trans*) stereochemistry was 100-fold more active, matching the activity of natural 4-desacetoxyvinblastine (**46**) and substantially exceeding that of the comparable 4-desacetoxy-6,7-dihydrovinblastine (**45**). Cumulatively, the studies demonstrate that the 100-fold impact of the vinblastine C6−C7 double bond is not the result of a unique stabilizing π-interaction with tubulin or its impact on the N9 pK_a, its lone pair orientation, or even the precise spatial location of N9. Just as significantly, unlike modifications of peripheral substituents or atom exchanges (tailoring effects), core structure redesign in natural products is rarely explored, and the results detailed herein with an important oncology drug indicate this may offer far more opportunities than anticipated.[35]

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■ ASSOCIATED CONTENT

Supporting Information

Full experimental details and compound characterizations are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

CDI, 1,1′-carbonyldiimidazole; DEAD, diethyl azodicarboxylate; DMAP, 4-dimethylaminopyridine; EDCI, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; HMDS, hexamethyldisilazide; IBX, 2-iodoxybenzoic acid; LDA, lithium diisopropylamide; pyr, pyridine; THF, tetrahydrofuran; TsCl, *p*-toluenesulfonyl chloride; Et_3N, triethylamine; TIPB, triisopropylbenzene; Teoc, 2-trimethylsilylethyl carbonyl; PPh_3, triphenylphosphine

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