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Total Synthesis of Vinblastine, Related Natural Products, and Key Analogues and Development of Inspired Methodology Suitable for the Systematic Study of Their Structure–Function Properties

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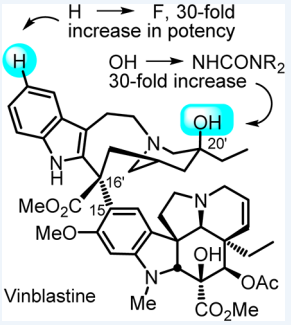
Justin E. Sears and Dale L. Boger*

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

CONSPECTUS: Biologically active natural products composed of fascinatingly complex structures are often regarded as not amenable to traditional systematic structure–function studies enlisted in medicinal chemistry for the optimization of their properties beyond what might be accomplished by semisynthetic modification. Herein, we summarize our recent studies on the *Vinca* alkaloids vinblastine and vincristine, often considered as prototypical members of such natural products, that not only inspired the development of powerful new synthetic methodology designed to expedite their total synthesis but have subsequently led to the discovery of several distinct classes of new, more potent, and previously inaccessible analogues.

With use of the newly developed methodology and in addition to ongoing efforts to systematically define the importance of each embedded structural feature of vinblastine, two classes of analogues already have been discovered that enhance the potency of the natural products >10-fold. In one instance, remarkable progress has also been made on the refractory problem of reducing Pgp transport responsible for clinical resistance with a series of derivatives made accessible only using the newly developed synthetic methodology. Unlike the removal of vinblastine structural features or substituents, which typically has a detrimental impact, the additions of new structural features have been found that can enhance target tubulin binding affinity and functional activity while simultaneously disrupting Pgp binding, transport, and functional resistance. Already analogues are in hand that are deserving of full preclinical development, and it is a tribute to the advances in organic synthesis that they are readily accessible even on a natural product of a complexity once thought refractory to such an approach.



Originally isolated from *Catharanthus roseus* (L.) G. Don,[1−3] vinblastine (**1**) and vincristine (**2**) not only represent the most widely recognized members of the *Vinca* alkaloids but also one of the most important contributions that plant-derived natural products have made to cancer chemotherapy (Figure 1).[4−6] First introduced into the clinic over 50 years ago, their biological properties were among the first to be shown to arise from perturbations in microtubule dynamics that lead to inhibition of mitosis. Even by today's standards, both vinblastine and vincristine are efficacious clinical drugs and are used in combination therapies for treatment of Hodgkin's disease, testicular cancer, ovarian cancer, breast cancer, head and neck cancer, and non-Hodgkin's lymphoma (vinblastine) or in the curative treatment regimens for childhood lymphocytic leukemia and Hodgkin's disease (vincristine). They, as well as their biological target tubulin, remain the subject of extensive and continuing biological and synthetic investigations because of their clinical importance in modern medicine, low natural abundance, and fascinatingly complex dimeric alkaloid structure.[7−16]

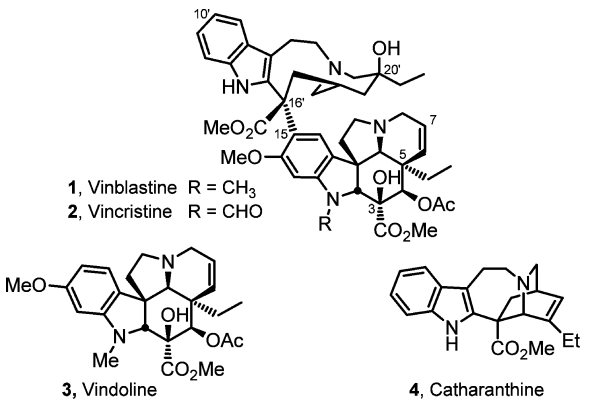


Figure 1. Natural product structures.

Vinblastine and vincristine share an identical upper velbanamine subunit and contain nearly identical vindoline-derived lower subunits that differ only in the nature of the indoline N-substituent, bearing either a methyl (vinblastine) or formyl (vincristine) group (Figure 1). We reported the development of concise total syntheses of (−)- and *ent*-(+)-vindoline[17,18] enlisting a tandem intramolecular [4 + 2]/[3 + 2] cycloaddition cascade of 1,3,4-oxadiazoles[19−25] in which the fully functionalized pentacyclic ring system is constructed in a single step.

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This occurs with formation of three rings and four C−C bonds and sets all six stereocenters within the central ring of vindoline, including its three quaternary centers (Figure 2). Moreover, the

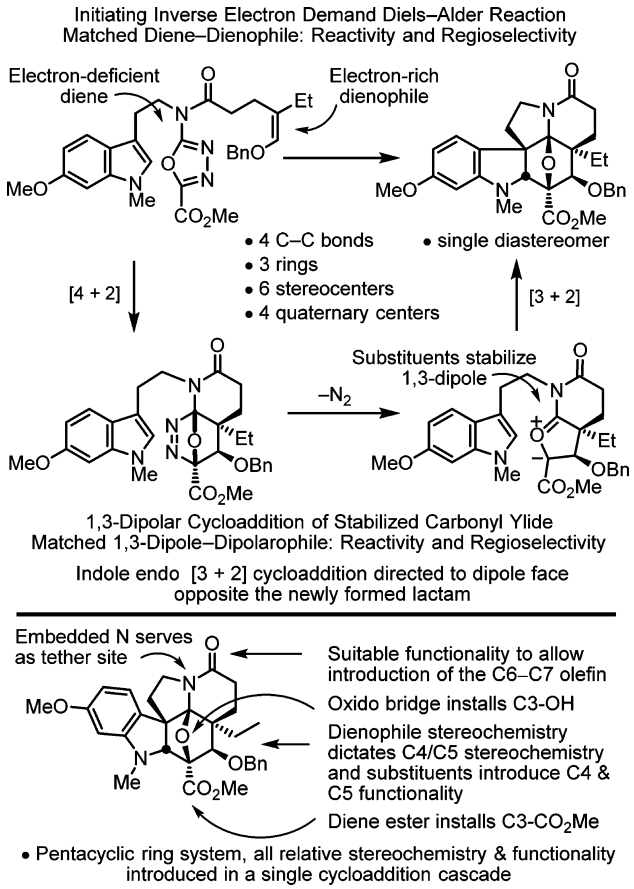

Figure 2. Key cycloaddition cascade.

cycloaddition cascade introduces each substituent, each functional group, each embedded heteroatom, and all the necessary stereochemistry for direct conversion of the cycloadduct to vindoline. The reaction cascade is initiated by a [4 + 2] cycloaddition reaction of a 1,3,4-oxadiazole with a tethered dienophile, which entailed the use of an electron-rich enol ether whose reactivity and regioselectivity were matched to react with the electron-deficient 1,3,4-oxadiazole in an inverse electron demand Diels–Alder reaction. Loss of N_2 from the initial cycloadduct provides a carbonyl ylide, which undergoes a subsequent 1,3-dipolar cycloaddition with a tethered indole.[16] For **3**, the diene and dienophile substituents complement and reinforce the [4 + 2] cycloaddition regioselectivity dictated by the linking tether, the intermediate 1,3-dipole is stabilized by the complementary substitution at the dipole termini, and the tethered dipolarophile (indole) complements the [3 + 2] cycloaddition regioselectivity that is set by the linking tether. The relative stereochemistry of the cascade cycloadduct is controlled by a combination of the dienophile geometry and an exclusive indole endo [3 + 2] cycloaddition dictated by the dipolarophile tether and sterically directed to the face opposite the newly formed fused lactam. This methodology provided the basis for an 11-step total synthesis of (−)- and *ent*-(+)-vindoline.[18]

Central to the further advancement of this work was the use of a biomimetic[9] Fe(III)-promoted coupling of vindoline with catharanthine[26,27] and the additional development of a subsequent in situ Fe(III)-mediated hydrogen-atom initiated free radical alkene oxidation for C20′-alcohol introduction[27] that allows for their single-step incorporation into total syntheses of vinblastine, related natural products including vincristine, and key analogues (Figure 3).[16]

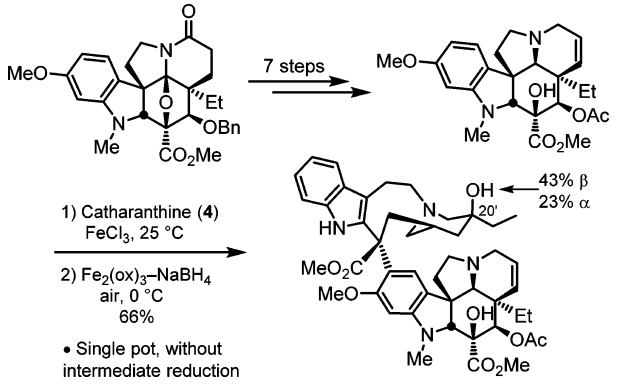

Figure 3. First generation total synthesis of vinblastine.

A subsequent asymmetric variant of this approach was developed in which the tether linking the initiating dienophile and 1,3,4-oxadiazole bears a chiral substituent that sets the absolute stereochemistry of the remaining six stereocenters in the cascade cycloadducts, providing two distinct and concise asymmetric total syntheses of vindoline (Figure 4).[28,29] Since the approach enlisted a shortened dienophile tether that resulted in both good facial control and milder reaction conditions for the cascade cycloaddition, its implementation for the synthesis of vindoline required the development of a ring expansion reaction to provide a six-membered ring suitably functionalized for introduction of the $\Delta^{6,7}$-double bond found in the core structure of the natural product. Two unique approaches were developed that defined our use of a protected hydroxymethyl group as the substituent that controls the stereochemical course of the cycloaddition cascade. The first entailed a facile tautomerization of a reactive α-amino aldehyde generated from the primary alcohol, which is trapped as a remarkable *N,O*-ketal that undergoes a subsequent hydrolytic ring expansion upon further activation of the primary alcohol.[28,29] The second approach relied upon a thermodynamically controlled reversible ring opening reaction of an intermediate aziridinium ion for which the stereochemical features of the reactions are under stereoelectronic control.[29] In the course of these studies, several analogues of vindoline were prepared containing deep-seated structural changes presently accessible only by total synthesis.[19−21]

Prior to these efforts, the majority of vinblastine analogues were prepared by semisynthetic modification of peripheral substituents (tailoring effects), with the disclosure of a limited number of analogues that contain deep-seated structural changes.[30−33] As detailed herein, the powerful intramolecular [4 + 2]/[3 + 2] cycloaddition cascade has provided access not only to a series of analogues bearing systematic changes to the C5 ethyl substituent[34] but to a series of analogues bearing deep-seated structural modifications to the vindoline core ring system.[35] These latter efforts represent one of few examples of productive core structure redesign of a natural product.

Similarly, detailed herein are efforts to address the major limitation to the continued clinical use of vinblastine and

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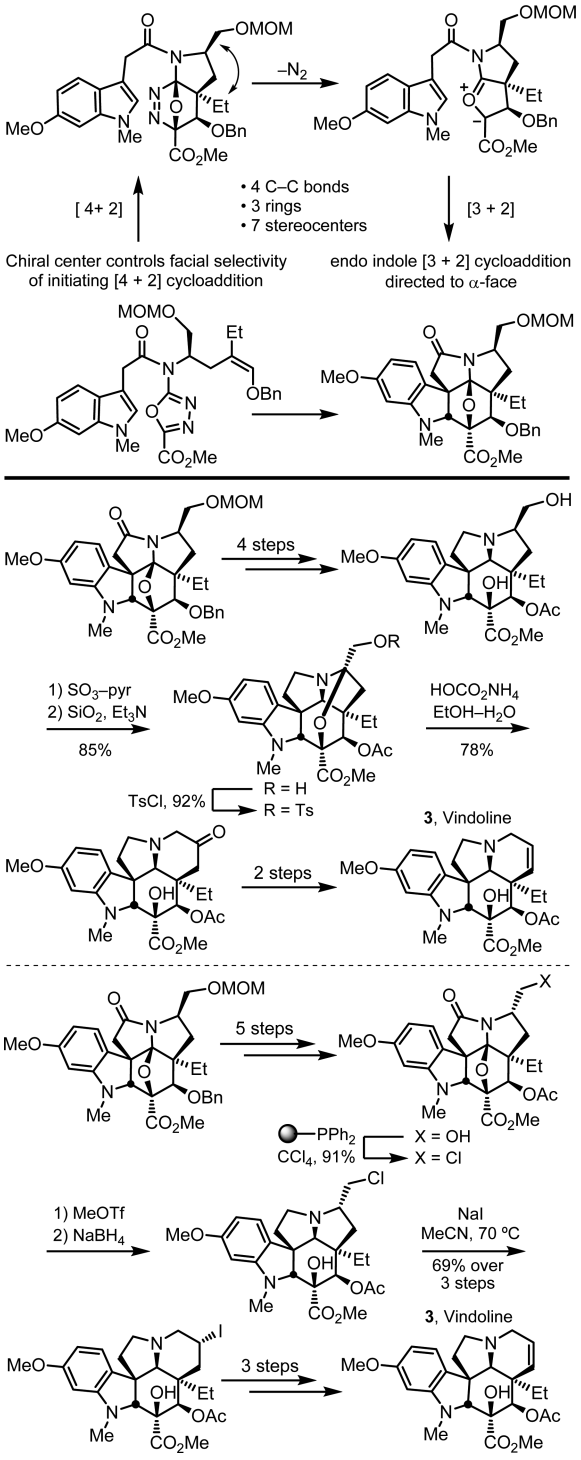

Figure 4. Key elements of the asymmetric total synthesis approaches.

vincristine, which is the emergence of resistance mediated by overexpression of the drug efflux pump phosphoglycoprotein (Pgp). The identification of structural analogues that might address such resistance has remained a major focus of the field and would represent a major advance for oncology therapeutics. With this objective in mind and by virtue of the unique methodology developed, we disclosed a series of C20′ urea derivatives[36−38] that not only possess extraordinary potency but also exhibit further improved activity against a Pgp over-expressing vinblastine-resistant human tumor cell line, displaying improved potency (10−30-fold) and a reduced difference in activity against a matched sensitive and resistant human tumor cell line (HCT116 vs HCT116/VM46, 10−20-fold vs 100-fold difference for vinblastine).[38] Although this site is known to be critical to the biological properties of vinblastine and is found to be deeply embedded in the tubulin bound complex, this study represented the first systematic examination of analogues bearing alternative C20′ functionality.

As highlighted earlier, a powerful Fe(III)-promoted coupling of catharanthine with vindoline generating anhydrovinblastine[26] was enlisted and combined with a newly developed in situ Fe(III)/$NaBH_4$-promoted oxidation to provide vinblastine in a single operation (Figure 5).[27] This development not only converted the synthetic efforts into one capable of use for the systematic exploration of the vinblastine structure but also assured that supplies of any analogue needed for preclinical studies or clinical introduction could be accessed by total synthesis. Consequently, in addition to its use in the completion of the total syntheses of vinblastine (12 steps), vincristine, and a series of additional naturally occurring *Vinca* alkaloids,[16] the approach also permits the incorporation of vindoline analogues containing single site peripheral changes to the structure as well as more deep-seated changes to the vindoline core accessible only by total synthesis.

An early example of the power of the approach entailed systematic replacements of the vindoline C5 ethyl group, which were introduced as alternative substituents on the oxadiazole tethered dienophile in the cascade cycloaddition substrate.[34] Their examination revealed the surprising importance of the C5 ethyl substituent where even conservative methyl (10-fold), hydrogen (100-fold), or propyl replacements (10-fold) led to significant reductions in activity (Figure 6).[34]

Further representative of the opportunities the work has provided and complementary to the vindoline 6,5-DE ring system, 5,5-, 6,6-, and the reversed 5,6-membered DE ring system analogues that contain deep-seated changes to the core structure were prepared (Figure 5).[35] Preparation of these analogues, not accessible from natural product sources, further demonstrates the versatility of the intramolecular [4 + 2]/[3 + 2] cycloaddition cascade. Both the natural *cis* and unnatural *trans* 6,6-membered DE ring systems proved accessible, with the latter unnatural stereochemistry representing a surprisingly effective class for analogue design. After 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 possessing two different unprecedented modifications to the vindoline subunit core architecture were discovered that matched the potency of the comparison natural products. Remarkably, both lack the 6,7-double bond whose removal in vinblastine leads to a 100-fold loss in activity, and both represent the most dramatic departures from the structure of vindoline of those examined to date. Thus, although single functional group removals from the vinblastine lower subunit typically result in pronounced and additive losses in activity,[28] two unprecedented deep-seated structural changes to the core structure were found that maintain potent activity. Significantly and unlike modifications of peripheral substituents (tailoring effects), core structure redesign in such complex natural products is rarely explored, and the results in this series indicate this may offer far more opportunities than most might anticipate.[35]

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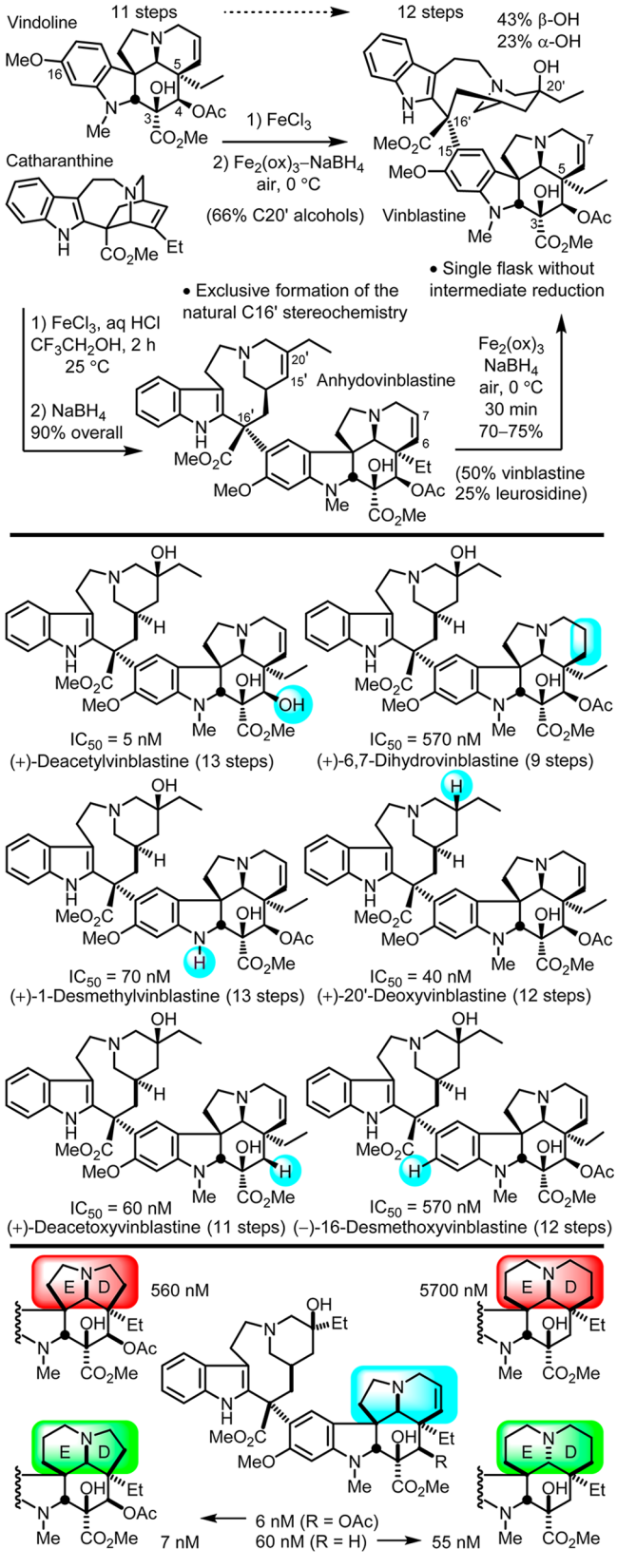

Figure 5. Single-step coupling and in situ oxidation and representative vinblastine analogues prepared by total synthesis.

Ongoing efforts have also begun to define catharanthine substituents that are required for the Fe(III)-promoted biomimetic coupling with vindoline, and these include (1) a probe of the importance of the C16′ methyl ester and

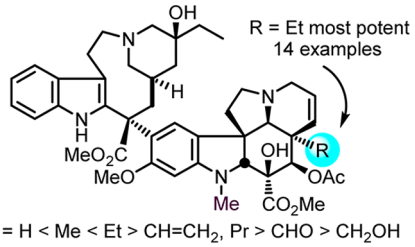

Figure 6. Probing the importance of the C5 ethyl group.

requirement for a C16′ electron-withdrawing group for coupling,[39] and (2) a Hammett series of C10′ indole substituents where electron-donating substituents were found to maintain and electron-withdrawing substituents to progressively slow the rate and efficiency of coupling.[40] The former unexpectedly revealed that even conservative replacements of the vinblastine C16′ methyl ester with an ethyl ester (10-fold), a cyano group (100-fold), an aldehyde (100-fold), a hydroxymethyl group (1000-fold), or a primary carboxamide (>1000-fold) led to surprisingly large reductions in biological activity, indicating that the C16′ methyl ester is not only required for the biosynthetic coupling but uniquely integral to the expression of vinblastine's biological properties (Scheme 1).[39]

The latter study, which included an examination of the impact of catharanthine C10′ and C12′ indole substituents on the Fe(III)-mediated coupling with vindoline, led to the discovery and characterization of two new exciting derivatives, 10′-fluorovinblastine and 10′-fluorovincristine (Figure 7).[40] In addition to defining a pronounced unanticipated substituent effect on the biomimetic coupling that helped refine its mechanism,[41] fluorine substitution at C10′ was found to uniquely enhance the activity 8-fold against both a sensitive (IC_{50} = 800 pM, HCT116) and a vinblastine-resistant tumor cell line (IC_{50} = 80 nM, HCT116/VM46). As depicted in the X-ray structure of vinblastine bound to tubulin, this site resides at one end of the upper portion of the T-shaped conformation of the tubulin-bound molecule, suggesting that the 10′-fluorine substituent makes critical contacts with the protein at a hydrophobic site uniquely sensitive to steric interactions. With the development of an effective single-step coupling and in situ oxidation protocol for convergent analogue assemblage, the synthesis and evaluation of a systematic series of C10′ and C12′ analogues were possible. Although C12′ substituents were not well tolerated, C10′ substituents were. Moreover and with the provision that C10′ polar substituents are not well tolerated, the activity of the vinblastine C10′ derivatives in cell-based assays exhibited no apparent relationship to the electronic character of the substituents but rather exhibited activity that correlates with their size and shape [activity for analogue R = H < F > Cl > Me, Br ≫ I, SMe (10-fold) ≫ CN (100-fold)]. Thus, small hydrophobic C10′ substituents are tolerated with one derivative exceeding (R = F) and several matching the potency of vinblastine (R = Cl, Me, Br vs H), whereas those bearing the larger (R = I, SMe) or rigidly extended (R = CN) substituents proved to be 10−100-fold less potent. With a recognition that the 10′-fluoro substitution conveys uniquely potent activity to such *Vinca* alkaloids, 10′-fluorovincristine was also prepared. Thus, Fe(III)-promoted coupling (70%) of synthetic 10-fluorocatharantine with synthetic *N*-desmethylvindoline[16] and subsequent in situ Fe(III)-mediated oxidation provided 10′-fluoro-N^1-desmethylvinblastine, which was for-

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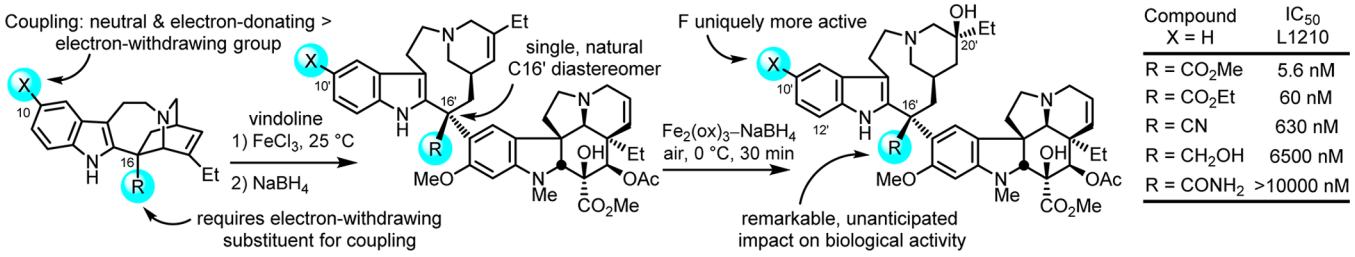
Scheme 1



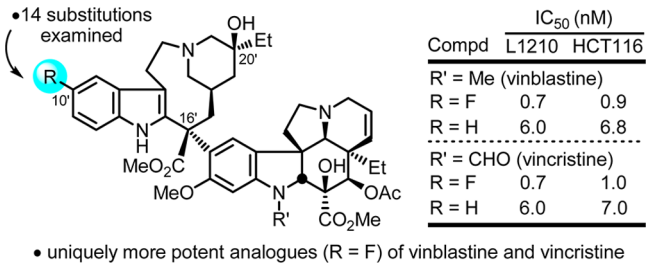


Figure 7. 10′-Fluorovinblastine and 10′-fluorovincristine.

mylated to provide synthetic 10′-fluorovincristine. Like 10′-fluorovinblastine, 10′-fluorovincristine exhibited exceptional activity in the cell-based functional assays, inhibiting tumor cell growth 8-fold more potently than vincristine itself (Figure 7).[40]

Although the enhanced oxidative metabolic stability of the 10′-fluoro derivatives may contribute to the increased potency, the lack of similar effects with closely related substituents indicate that an effect unique to the fluorine substitution is responsible. We have suggested that this is derived from the interaction of a perfectly sized hydrophobic substituent further stabilizing the compound binding with tubulin at a site exquisitely sensitive to steric interactions. Comparison models of the 10′ substituent analogues built from the X-ray structure of tubulin-bound vinblastine[39] illustrated a unique fit for 10′-fluorovinblastine (Figure 8).[40]

In order to confirm that the exceptional activity observed in our lab would be observed elsewhere, we had vinblastine and 10′-fluorovinblastine examined in a more comprehensive human tumor cell line panel including cell lines of clinical interest from breast, lung, colon, prostate, and ovary tissue (Figure 9) graciously conducted at Bristol-Myers Squibb.[42] 10′-Fluorovinblastine exhibited remarkable potency (avg IC_{50} = 300 pM), being on average 30-fold more potent than vinblastine (avg IC_{50} = 10 nM) and exceeding our more conservative initial observations.

A further delineation of the scope of aromatic substrates that participate with catharanthine in the Fe(III)-mediated coupling reaction, the definition of its key structural features required for participation in the reaction, and its extension to a generalized indole functionalization reaction that bears little structural relationship to catharanthine were defined.[41] In addition to revealing that the exclusive diastereoselectivity of the coupling reaction that installs the key C16′ center is controlled by the catharanthine-derived coupling intermediate, the studies provided key insights into the mechanism of the Fe(III)-mediated coupling reaction of catharanthine, suggesting that the reaction conducted in acidic aqueous buffer may result from a single-electron indole oxidation and may be radical mediated. Just as importantly, the studies provide new opportunities for

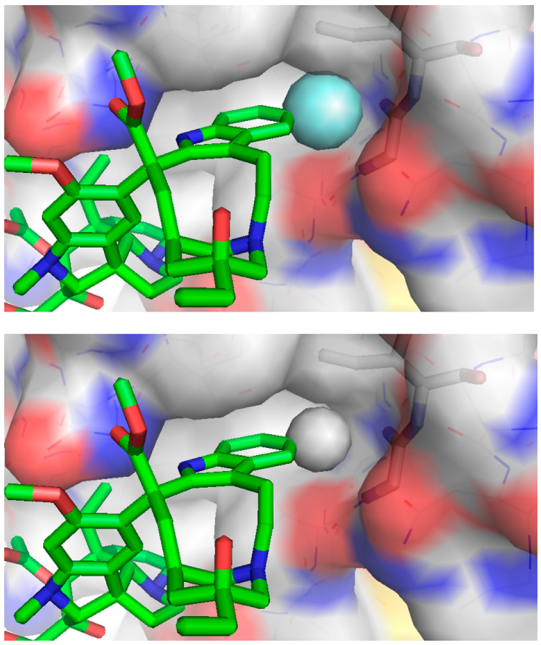


Figure 8. Space filling model of the 10′-fluoro binding site of 10′-fluorovinblastine (R = F, top) generated by adding the fluorine substituent to the X-ray structure of tubulin-bound vinblastine[43] (R = H, bottom).[40] Comparison models where R = Cl, Br, and I illustrated the unique fit for F, and the complexes exhibited increasingly larger destabilizing steric interactions as the substituent size progressively increased.

Human tumor cell line[a]	IC_{50} 10'-Fluorovinblastine	IC_{50} Vinblastine
AU565	0.17 nM	4.6 nM
DMS114	0.20 nM	7.5 nM
HCC15	0.21 nM	4.7 nM
HCC44	0.91 nM	22 nM
HCT116	0.29 nM	10 nM
LK2	0.20 nM	6.7 nM
LNCaP-FGC	0.33 nM	9.2 nM
PA-1	0.15 nM	4.5 nM
SW403	0.50 nM	21 nM
NCI-H1623	0.06 nM	1.3 nM
NCI-H1762	0.26 nM	9.3 nM
NIH-H2030	0.55 nM	20 nM
NCI-H23	0.25 nM	9.5 nM

[a]AU565 (Breast, over expression of her2/neu), DMS114 (small cell Lung cancer), HCC15 (non small cell Lung cancer), HCC44 (non small cell Lung cancer), HCT116 (Colon), LK2 (non small cell Lung cancer), LNCaP-FGC (Prostate), PA-1 (Ovary, over expression of AIB1), SW403 (Colon, KRASG12V mutation), NIH-H1623 (non small cell Lung cancer), NIH-H1762 (non small cell Lung cancer), NIH-H2030 (non small cell Lung cancer), NIH-H23 (non small cell Lung cancer).

Figure 9. 10′-Fluorovinblastine human tumor cell growth inhibition.

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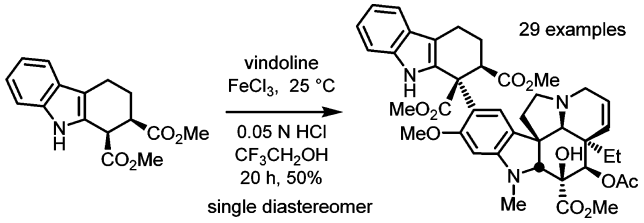
the synthesis of previously inaccessible *Vinca* alkaloid analogues (Scheme 2) and defined powerful new methodology for the synthesis of indole-containing natural products.[41]

Scheme 2



Similarly, detailed investigations of the second stage of the coupling process, the Fe(III)-mediated free radical oxidation of the anhydrovinblastine trisubstituted alkene to introduce the vinblastine C20′ tertiary alcohol, revealed insights into not only its mechanism but also the synthesis of previously inaccessible vinblastine analogues. Initial studies revealed that it is a free radical mediated oxidation reaction, that the reaction is initiated by the addition of $NaBH_4$ to the Fe(III) salt, and that reactions in the absence of air (O_2) led to reduction of the double bond (Figure 10).[16] Subsequent studies provided additional details of the mechanism of the reaction, entailing a hydrogen atom transfer (HAT) initiated free radical reaction,[44] and defined a new method for the direct functionalization of unactivated alkenes.[36] Included in these studies was a definition of the alkene substrate broad scope, the reaction's extensive functional group tolerance, the establishment of exclusive Markovnikov addition regioselectivity, the use of a wide range of alternative free radical traps for O, N, S, C, and halide substitution, an examination of the Fe(III) salt and the hydride source best suited to initiate the reaction, the introduction of alternative reaction solvents beyond the water and aqueous buffer[27] originally disclosed, and the exploration of catalytic variants of the reactions.[36] The reaction was extended to a powerful Fe(III)/$NaBH_4$-mediated free radical hydrofluorination of unactivated alkenes using Selectfluor as the fluorine atom source.[45] Unlike the traditional and unmanageable free radical hydrofluorination of alkenes, the Fe(III)/$NaBH_4$-mediated reaction is conducted under exceptionally mild conditions (0 °C, 5 min, CH_3CN/H_2O), uses a technically nondemanding reaction protocol, is conducted open to the air with water as a cosolvent, demonstrates an outstanding substrate scope and functional group tolerance, and is suitable for ^{18}F introduction ($t_{1/2}$ =110 min) used in PET imaging.[45]

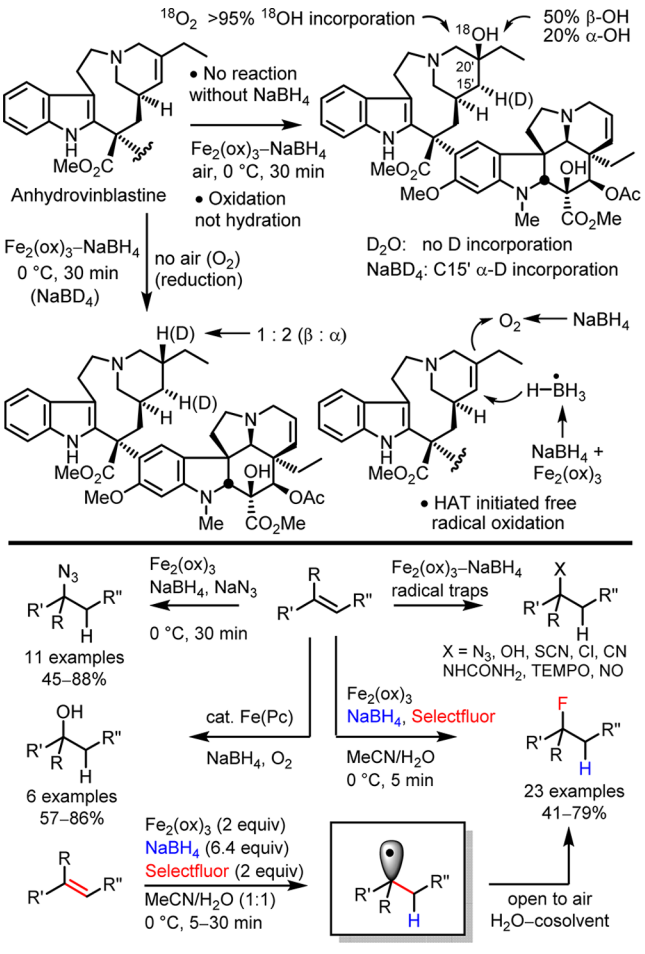


Figure 10. Hydrogen atom transfer (HAT) free radical oxidation of anhydrovinblastine and generalization of the methodology for unactivated alkene functionalization.

Our interest in this Fe(III)/$NaBH_4$-mediated reaction emerged not only from its use in accessing vinblastine but from the opportunity it presented for the late-stage, divergent[46] preparation of otherwise inaccessible vinblastine analogues incorporating alternative C20′ functionality. Although this site is known to be critical to the properties of vinblastine and is found deeply embedded in the tubulin bound complex (Figure 11),[43] prior exploration of C20′ substituent effects was limited to semisynthetic O-acylation of the C20′ alcohol, its elimination and subsequent alkene reduction, or superacid-catalyzed additions.[47] These invariably led to substantial reductions in biological potency of the resulting derivative, albeit with examination of only a limited number of analogues. Consequently and in the course of the development of the Fe(III)/$NaBH_4$-mediated alkene functionalization reaction, its use was extended to the preparation of a series of key vinblastine analogues bearing alternative C20′ functionality (e.g., N_3, NH_2, and SCN vs OH).[36] Those of initial interest included the C20′ azide and amine, both of which proved to be approximately 100-fold less potent than vinblastine and 10-fold less potent than 20′-deoxyvinblastine. However, acylation of the C20′ amine improved activity 10-fold[36] and installation of the unsubstituted C20′ urea or thiourea provided compounds that nearly matched the potency of vinblastine itself (Figure 11).[36,37] The requisite NH of the internal nitrogen of the latter series presumably best recapitulates the H-bond donor property of the vinblastine C20′ alcohol.

On the basis of these results and with the further observation that the site had an apparent pronounced impact on Pgp transport, we conducted a systematic exploration of C20′ amine, urea, and thiourea derivatives, which provided C20′ urea-based analogues that not only substantially exceed the potency of vinblastine but also exhibited good activity against a Pgp overexpressing, vinblastine-resistant tumor cell line. Thus, members in this group not only exceed the potency of vinblastine (10-fold) but exhibit even further improved activity against vinblastine-resistant cell lines (100-fold) in some cases, partially overcoming overexpressed Pgp transport.[37,38]

Just as remarkably and in contrast to expectations based on the steric constraints of the tubulin binding site surrounding the vinblastine C20′ center depicted in the X-ray cocrystal structure

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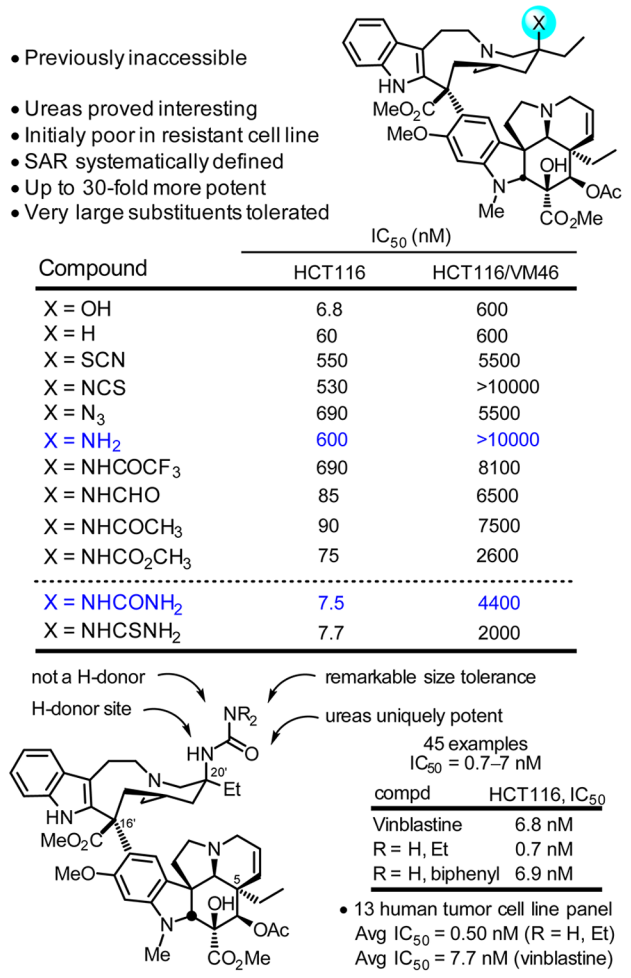

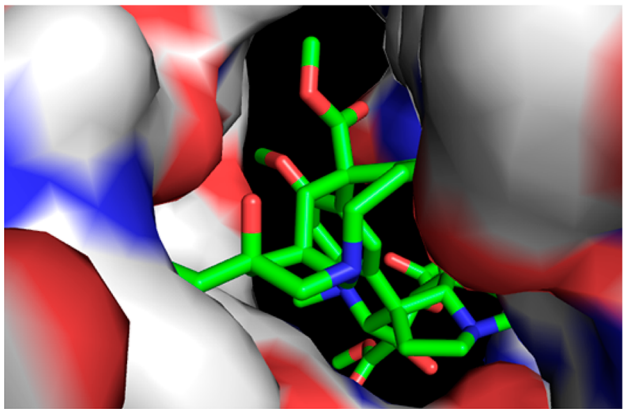

Figure 11. (top) Initial C20′ vinblastine analogues. (bottom) X-ray structure[43] of vinblastine bound to tubulin highlighting the region surrounding vinblastine C20′ site.

of a tubulin bound complex,[43] large C20′ urea derivatives are accommodated (e.g., biphenyl), exhibiting potent functional activity in cell-based proliferation assays and effectively binding tubulin.[37]

Continued and ongoing studies have provided even more potent analogues as superb candidate drugs, especially for vinblastine-resistant relapse tumors (Figure 12). Thus, a series of disubstituted C20′-urea derivatives of vinblastine were prepared from 20′-aminovinblastine, accessible through the unique Fe(III)/$NaBH_4$-mediated alkene functionalization re-

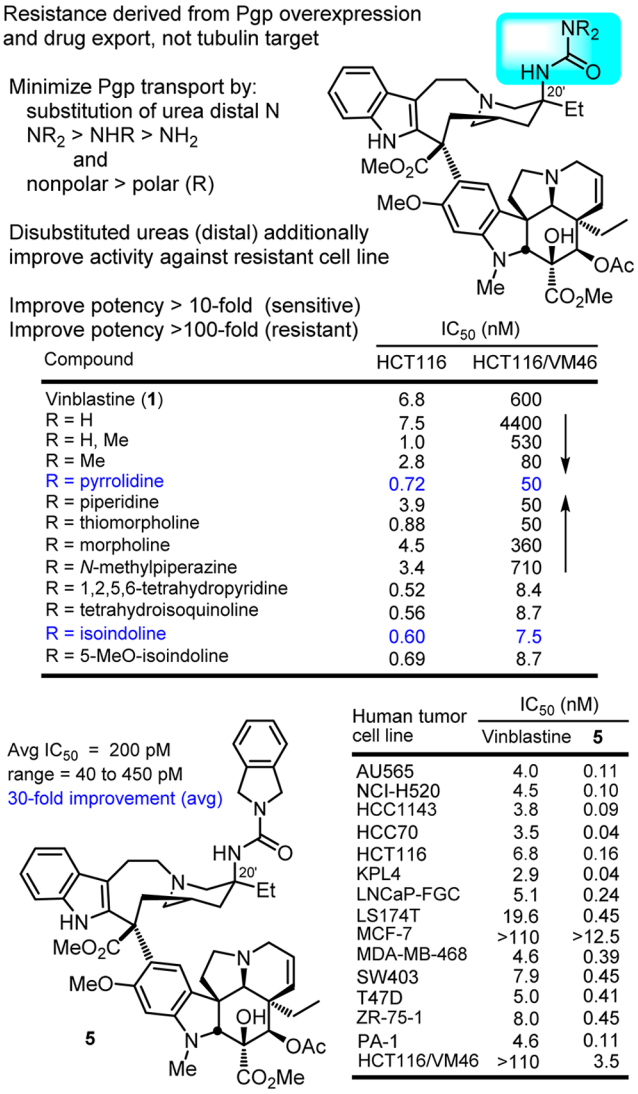

Figure 12. Disubstituted C20′ urea derivatives of vinblastine.

action of anhydrovinblastine. They were found to not only possess extraordinary potency (IC_{50} = 40−450 pM) but also exhibit further improved activity against a Pgp overexpressing vinblastine-resistant cell line.[38] Three such analogs were examined across a panel of 15 tumor cell lines graciously conducted at Bristol-Myers Squibb,[38,42] and each displayed remarkably potent cell growth inhibition activity (avg IC_{50} = 200−300 pM vs avg vinblastine IC_{50} = 6.1 nM) against a broad spectrum of clinically relevant human cancer cell lines, being on average 20−30-fold more potent than vinblastine (range of 10−200-fold more potent). Significantly, the analogues also display further improved activity against the vinblastine-resistant HCT116/VM46 cell line that bears the clinically relevant overexpression of Pgp, exhibiting IC_{50} values on par with that of vinblastine against the sensitive HCT116 cell line, 100−200-fold greater than the activity of vinblastine against the resistant HCT116/VM46 cell line, and display a reduced 10−20-fold activity differential between the matched sensitive and resistant cell lines (vs 100-fold for vinblastine).[38] Clearly, the C20′ position within vinblastine represents a key site amenable to functionalization capable of improving tubulin binding affinity, substantially enhancing biological potency, and simultaneously decreasing binding and relative Pgp transport central to clinical

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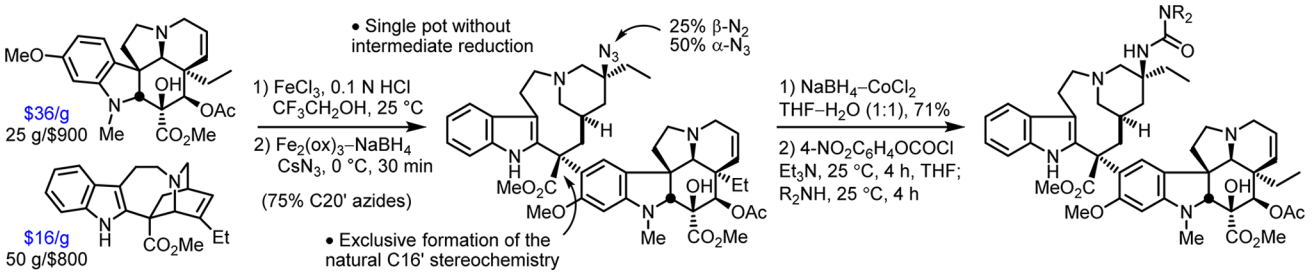
Scheme 3



resistance. Compound **5** was found to bind tubulin with a higher affinity than vinblastine, confirming that its enhanced potency observed in the cell growth functional assays correlates with its target tubulin binding affinity.[38] Remarkable in these developments is the observation that the activity against the vinblastine-resistant, Pgp overexpressing cell line uniquely and progressively improves as the terminal nitrogen of the urea is substituted (R = H, H < R = H, Me < R = Me, Me), diminishes smoothly with introduction of polarity, and smoothly increases with introduction of increasing π-hydrophobic substitution reflecting tunable and now predictable structural features that enhance tubulin binding and simultaneously diminish Pgp binding and transport.

What is most remarkable about these advances and although it could not have been imagined at the stage we initiated our efforts, the C20′ analogues of vinblastine are available in three steps from commercially available materials (Scheme 3). Although vinblastine is a true trace natural product, representing 0.00025% of the dried leaf weight of the periwinkle, its biosynthetic precursors catharanthine and vindoline are the major alkaloid components of the plant. They are readily available, relatively inexpensive starting materials. As a result, such C20′ analogues are not only readily accessible using the unique chemistry we introduced but also inexpensive to prepare on scales needed for preclinical development. Thus, the innovative chemistry developed in route to the total synthesis of vinblastine—the Fe(III)-mediated single-step coupling of catharanthine and vindoline that proceeds with complete control of the pivotal C16′ stereochemistry and the in situ Fe(III)-mediated hydrogen atom transfer free radical functionalization (Markovnikov hydroazidation)[36] of the key C20′ center—permits the exploration of exciting analogues previously unimaginable.[48]

Not only does this methodology mimic the biosynthetic pathway leading to vinblastine that has been presumed to be mediated by enzymes, but it is of special note that the diastereoselectivity of the Fe(III)-mediated coupling and subsequent oxidation reproduces the relative abundance of vinblastine and leurosidine (C20′ diastereomer) found in the plant. Moreover, it is known that catharanthine and vindoline are stored in plants spatially separated from one another.[49] This provocatively suggests that the low natural abundance of vinblastine (and leurosidine) may arise not from an orchestrated enzymatic coupling and subsequent functionalization of catharanthine and vindoline but rather by a stress-induced mixing of the two precursors in the presence of Fe(III) and air. Perhaps the chemistry we have developed is not simply biomimetic but constitutes nonenzymatic chemistry actually involved in the plant production of vinblastine.

CONCLUSIONS AND OUTLOOK

Those reading this Account may think the [4 + 2]/[3 + 2] cycloaddition cascade is powerful methodology and that vindoline and vinblastine represent perfect applications in which to showcase its potential. Truth is that it was the natural product targets vindoline and vinblastine, their importance in modern medicine, and the potential for their improvements that inspired the discovery of the synthetic methodology. Not only is the full pentacyclic skeleton of vindoline assembled in a single cycloaddition cascade, but each substituent, each functional group, each embedded heteroatom, and all the necessary stereochemistry are incorporated into the substrate and the cycloaddition cascade cycloadduct tailored for direct conversion to vindoline. It is methodology created for the intended target. Combined with the development of a powerful single-step Fe(III)-promoted coupling of catharanthine with vindoline and a newly developed in situ Fe(III)/$NaBH_4$-promoted C20′ oxidation, the approach provides vinblastine and its analogues in 8−13 steps. With use of the methodology and in addition to systematically defining the importance of each embedded structural feature of vinblastine, two classes of analogues have already been discovered that enhance the potency of the natural products >10-fold. In one instance, progress has also been made on the refractory problem of reducing Pgp transport responsible for resistance with a series of C20′ amine derivatives uniquely accessible using the newly developed methodology. Unlike the removal of vinblastine structural features or substituents, which typically has a detrimental impact, the addition of new features can enhance target tubulin binding affinity and functional activity and, in selected instances,[50] simultaneously disrupt Pgp binding, transport, and functional resistance. Already analogues are in hand that are deserving of preclinical development, and it is a tribute to the advances in organic synthesis that they are accessible even on a natural product of a complexity once thought refractory to such an approach.

AUTHOR INFORMATION

Corresponding Author

*Dale L. Boger. E-mail: boger@scripps.edu.

Notes

The authors declare no competing financial interest.

Biographies

Dale L. Boger is the Richard & Alice Cramer Chair in Chemistry at The Scripps Research Institute (1990-present) where he also serves as Chairman (2012−present) of the Department of Chemistry. He received his BS in Chemistry from the University of Kansas (1975) where he worked with Professor A. W. Burgstahler and his Ph.D. in Chemistry from Harvard University (1980) working with Professor E.

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J. Corey. He was on the faculty at the University of Kansas and Purdue University before joining TSRI in 1990.

Justin E. Sears received his BS in Chemistry from Haverford College (2010) where he worked with Professor Frances Blase. He is currently a Ph.D. student at The Scripps Research Institute (2010–present) with Professor Boger.

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