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Transannular Diels–Alder/1,3-Dipolar Cycloaddition Cascade of 1,3,4-Oxadiazoles: Total Synthesis of a Unique Set of Vinblastine Analogues

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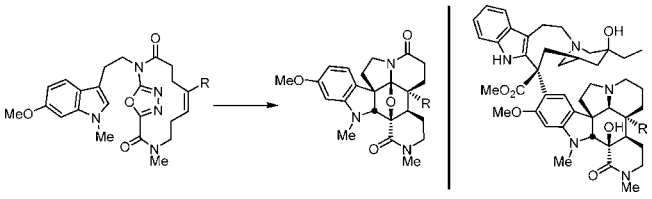
ABSTRACT



A powerful tandem [4 + 2]/[3 + 2] cycloaddition cascade of 1,3,4-oxadiazoles initiated by a transannular [4 + 2] cycloaddition is detailed. An impressive four rings, four carbon–carbon bonds, and six stereocenters are set on each site of the newly formed central six-membered ring in a cascade thermal reaction that proceeds at temperatures as low as 80 °C. The resulting cycloadducts provide the basis for the synthesis of unique analogues of vinblastine containing metabolically benign deep-seated cyclic modifications at the C3/C4 centers of the vindoline-derived subunit of the natural product.

Vinblastine (**1**) and vincristine (**2**) are the most widely recognized members of the Vinca alkaloid family and have been extensively used as antitumor drugs (Figure 1).[1] Originally isolated in trace quantities from the leaves of *Catharanthus roseus* (L.) G. Don.,[2] **1** and **2** were among the first chemotherapeutic agents shown to block mitosis through inhibition of microtubule formation that is still regarded as an especially attractive oncology drug target.[1,3] They 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. Despite this minor structural difference, vinblastine and vincristine display distinct clinical and toxicological profiles.[1,3] Both compounds have enjoyed extensive use and success as antitumor drugs, however the major limitation to their continued clinical use is the emergence of resistance mediated primarily by overexpression of the drug efflux pump phosphoglycoprotein (Pgp).[4] The identification of structural analogues that might address such resistance would constitute a major advance, but has largely remained an elusive goal. The exception to this generalization is the recent disclosure of a series of key C20′ urea derivatives that simultaneously improve potency (ca. 30-fold) and reduce the difference in activity against a vinblastine sensitive versus resistant cell line from 100-fold to 10-fold,[5] indicating that such key modifications to vinblastine may be possible.

Recently, we reported concise total syntheses of vindoline[6] enlisting two variations on a tandem intramolecular [4 + 2]/[3 + 2] cycloaddition cascade of 1,3,4-oxadiazoles[7] that

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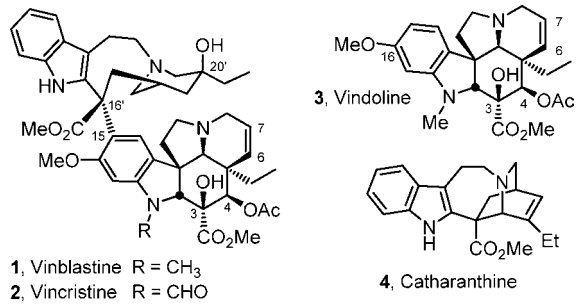

Figure 1. Vinca alkaloids and key natural products.

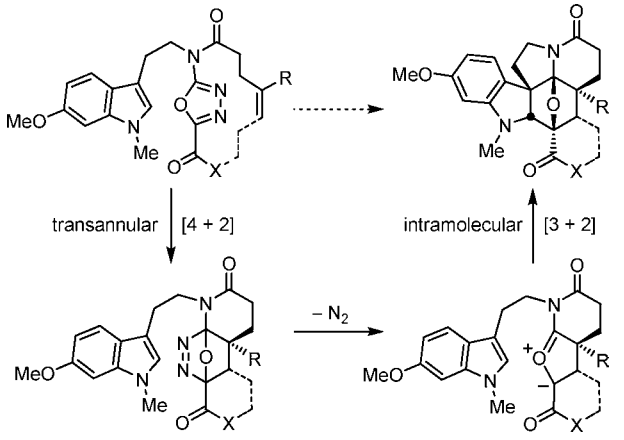

Figure 2. Transannular [4 + 2]/[3 + 2] cycloaddition cascade.

form the fused pentacyclic core of the natural product in one step and as a single diastereomer. The development of a single flask Fe(III)-promoted coupling with catharanthine[8] and subsequent in situ Fe(III)/$NaBH_4$ air oxidation[9] provided the basis for a 12-step total synthesis of vinblastine and related natural products.[10,11] Significantly, this approach has proved sufficiently concise and general as to allow the preparation of numerous analogues[12] that systematically explore many of the key structural features of vinblastine (substituents and core redesign) that are otherwise inaccessible.[3] Moreover, it assures that efficacious analogues can be accessed as needed for preclinical or clinical investigation.

In our efforts to examine such key analogues of vinblastine and in extensions of such cycloaddition methodology[13] to additional natural products,[14] we have conducted studies directed at expanding the substrate scope of the key [4 + 2]/[3 + 2] cycloaddition cascade leading to vindoline and related structures. Herein, we report the examination of cycloaddition substrates in which the initiating dienophile is constrained within a macrocycle, rendering the initiating [4 + 2] reaction of the cycloaddition cascade transannular in nature (Figure 2).[15] The expectation was not only that this would provide a cycloaddition reaction that proceeds at relatively low reaction temperatures but also that the cycloadducts would provide the basis for unique vinblastine analogues that incorporate an additional fused ring at the C3/C4 positions.

In these initial efforts, cycloaddition cascade precursors exemplified by **6** were examined (Scheme 1), in which the initiating dienophile is linked to the 1,3,4-oxadiazole by two amide groups (X = NMe). It was anticipated that the requisite macrocycles could be accessed by ring closing metathesis (RCM) of diene **7**, although several alternative approaches can be envisioned, and **7** would be derived from the oxadiazole **8** available from our prior studies.[6]

The addition of 4-amino-1-butene (**9**) to **8** provided amide **10** (Scheme 1). Acylation of **10** with 4-pentenoic acid (**11**) followed by *N*-methylation of the secondary amide **12** provided the RCM precursor **13** in excellent yield. Treating compound **13** with Grubbs second generation catalyst[16] in refluxing CH_2Cl_2 (0.002 M) provided the desired macrocyclic product **14** as a mixture of isomers favoring *Z*-**14** by a factor of ca. 3:1. The modest yield of **14** results from competitive formation of dimeric cyclic olefin metathesis products. Interestingly, alternative attractive

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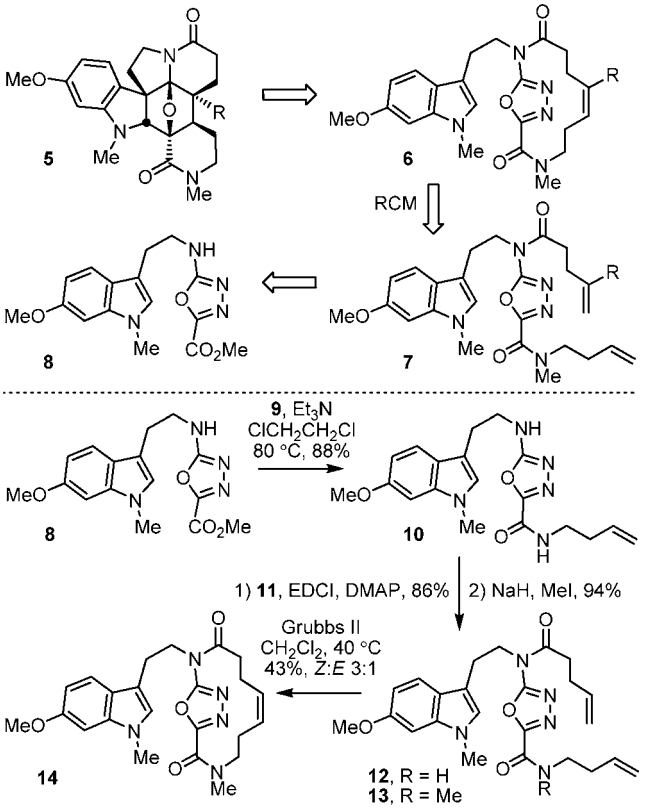
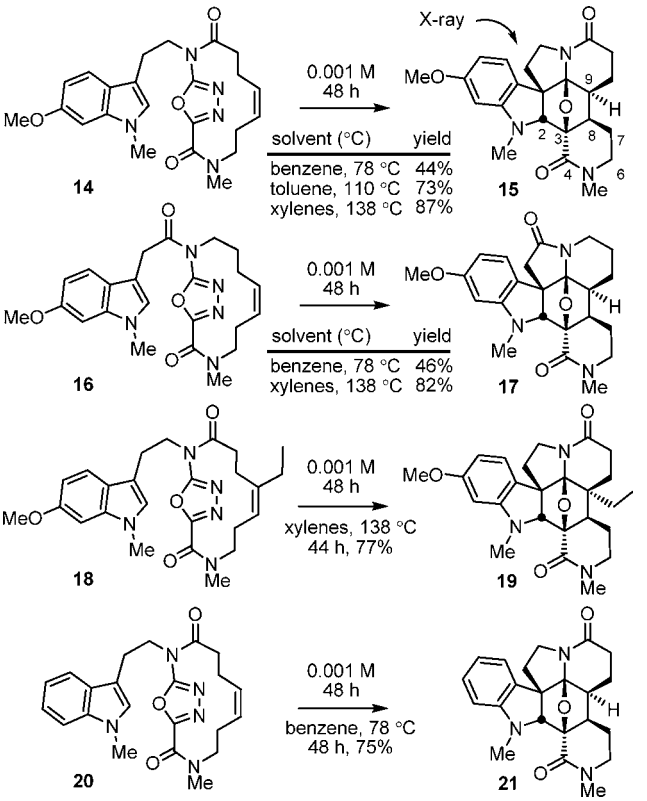
Scheme 1



RCM substrates in which the linking *N*-methyl amide was replaced by a secondary amide (X = NH), ester (X = O), or ketone (X = CH_2) were found to undergo preferential dimerization nearly exclusively under these conditions.

Using a defined reaction time (48 h), compound **14** was found to undergo the key cascade cycloaddition reaction at temperatures as low as 78 °C (refluxing benzene, 44%, 48 h) to provide **15**, whose structure and relative stereochemistry were confirmed by X-ray[17a] (Scheme 2). The rates of reaction were faster and the yields of product were higher when the 48 h reaction was conducted in refluxing toluene (110 °C, 73%; 73–91%) or xylenes (138 °C, 85%; 75–87%), incrementally improving with the higher reaction temperatures. Even at the higher reaction temperatures, the conditions are milder than those required for the initiating intramolecular [4 + 2] cycloaddition reactions. Compound **16**, where the upper amide carbonyl is moved to the dipolarophile tether (Supporting Information), participated in the cascade cycloaddition reaction with near equal facility. Remarkably, **18**, which was prepared by a route analogous to **14** (Supporting Information) and features a trisubstituted alkene dienophile expected to participate more slowly in the initiating [4 + 2] reaction, undergoes the cycloaddition cascade to provide **19** (77%) with similar ease. Impressively, **20** provided the cascade cycloadduct **21** in 75% yield in refluxing benzene (78 °C, 48 h).[7]

Scheme 2



In each instance, a single diastereomer of the product is generated in which the C8/C9 relative stereochemistry is inherent in the dienophile alkene *cis*-geometry with the remaining four stereocenters arising from a subsequent indole endo [3 + 2] cycloaddition directed to the face of the intermediate 1,3-dipole opposite the newly formed six-membered lactams. An impressive four rings, four carbon–carbon bonds, and six stereocenters, of which three or four are quaternary, are set in a single reaction that proceeds at temperatures as low as 80 °C.

With the cycloaddition products **15** and **19** in hand, their incorporation into novel vinblastine analogues was examined. An X-ray crystal structure of vinblastine bound to tubulin revealed that this region of the natural product extends into and interfaces largely with solvent, indicating that alterations made to the functionality at C3/C4 may be well tolerated (Figure S1, Supporting Information).[18]

The enantiomers of **15** were separated by chiral phase HPLC (Daicel ChiralCel OD column, 2 cm × 25 cm, 40% EtOH/hexanes, 7 mL/min, α = 1.20) (Scheme 3). Their absolute configuration was unambiguously assigned based on a single crystal X-ray structure determination of the

(17) (a) The structure and relative stereochemistry of **15** were confirmed by X-ray (CCDC 956386). (b) The structure and absolute stereochemistry of **22** (unnatural enantiomer) were established by X-ray (CCDC 956388). (c) The structure and absolute stereochemistry of **23** (natural enantiomer) were established by X-ray (CCDC 956389). (d) The structure and absolute stereochemistry of **25** (unnatural enantiomer) were established by X-ray (CCDC 956387).

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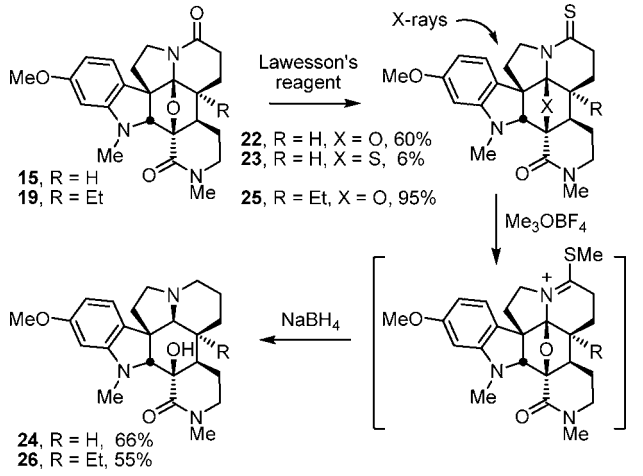
unnatural enantiomer of the thioamide derivative **22**,[17b] accessed by treating **15** with Lawesson's reagent (0.6 equiv, toluene, 75 °C). The thionation was completely selective for the C12 amide carbonyl; however a small amount of the side product **23**[17c] was isolated in which sulfur replaces oxygen as the bridging atom. *S*-Methylation of **22** with Me_3OBF_4 followed by reduction of the methylthioiminium ion with $NaBH_4$ and subsequent in situ oxido bridge cleavage and hydride reduction of the intermediate iminium ion from the convex face provided the vindoline analogue **24** as a single diastereomer in an unoptimized 66% yield. The same reaction sequence was applied to cycloadduct **19**, which incorporates the C5 ethyl substituent. Resolution of the enantiomers of **19** was achieved using conditions identical to those described for **15** (α = 1.23). Thionation of the natural enantiomer of **19** (1 equiv Lawesson's reagent, toluene, 110 °C) provided **25** as the sole product in superb yield. A single crystal X-ray crystal structure determination of **25** conducted on the unnatural enantiomer allowed the unambiguous assignment of the absolute configuration.[17d] Desulfurization and oxido bridge opening furnished the vindoline analogue **26**.

Scheme 3



Analogues **24** and **26** were coupled with catharanthine and subjected to a subsequent in situ oxidation in a one-pot reaction mediated by Fe(III) to provide the vinblastine analogues **27** and **28** in a single step (Scheme 4), completing their preparation in an eight-step total synthesis from **8** (available in two steps from 6-methoxytryptamine).[6,7] The analogues were examined for cell growth inhibitory activity against L1210 (mouse leukemia), HCT116 (human colon cancer), and HCT116/VM46 (resistant human colon cancer) tumor cell lines, the latter of which exhibits resistance (100-fold) to vinblastine through overexpression of Pgp.[19] The compound most comparable to **27** and **28** is 6,7-dihydrovinblastine. Prior studies have shown that reduction of the vinblastine C6–C7 double bond produces a

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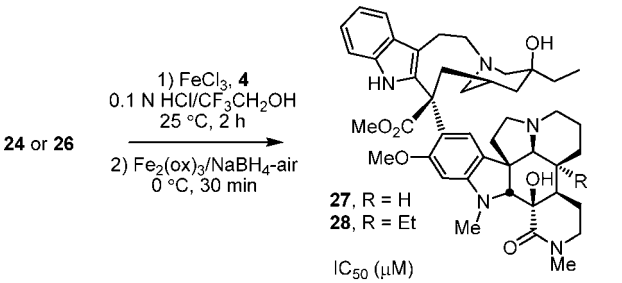
Scheme 4



IC_{50} (μM)

compound	L1210	HCT116	HCT116/VM46
vinblastine (**1**)	0.006	0.007	0.6
6,7-dihydro-vinblastine	0.57	0.37	4.5
27	0.57	0.52	6.6
28	8.4	7.9	>10

100-fold loss in activity.[10b] Compound **27**, which lacks the C5 ethyl substituent found in vinblastine, proved to be 10-fold more potent than **28**. Importantly, compound **27** was found to be equipotent with 6,7-dihydrovinblastine, indicating that vinblastine analogues incorporating the core structure of the vindoline analogue **24** produce analogues of significant interest. Notably, the vinblastine C3 and C4 substituents (CO_2Me and OAc) are metabolically labile and undergo in vivo hydrolysis that results in significant reductions in activity,[10b] whereas their replacement with a benign fused *N*-methyl lactam may provide equally effective, but metabolically stable analogues.

The macrocyclic oxadiazoles **14**, **16**, **18**, and **20** undergo a tandem [4 + 2]/[3 + 2] cycloaddition cascade initiated by a transannular [4 + 2] cycloaddition at temperatures substantially lower than those required for related intramolecular reactions. An impressive four rings, four carbon–carbon bonds, and six stereocenters are set on each site of the newly formed central six-membered ring in a single thermal reaction that proceeds at temperatures as low as 80 °C. The resulting cycloadducts provide the basis for the synthesis of unique analogues of vinblastine containing metabolically benign deep-seated modifications at the C3/C4 centers of the natural product. Such studies are in progress and will be reported in due course.

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Supporting Information Available. Full experimental details, compound characterization, and copies of ^1H NMR spectra are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.