JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Communication

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New Insights into the Mechanism and an Expanded Scope of the Fe(III)-Mediated Vinblastine Coupling Reaction

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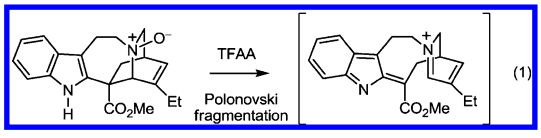
Supporting Information

ABSTRACT: A definition of the scope of aromatic substrates that participate with catharanthine in an Fe(III)-mediated coupling reaction, an examination of the key structural features of catharanthine required for participation in the reaction, and the development of a generalized indole functionalization reaction that bears little structural relationship to catharanthine itself are detailed. In addition to providing insights into the mechanism of the Fe(III)-mediated coupling reaction of catharanthine with vindoline suggesting the reaction conducted in acidic aqueous buffer may be radical mediated, the studies provide new opportunities for the preparation of previously inaccessible vinblastine analogs and define powerful new methodology for the synthesis of indole-containing natural and unnatural products.

Vinblastine (**1**) is the most widely recognized member of the Vinca alkaloids as a result of its clinical use as an antitumor drug (Figure 1).[1,2] Originally isolated in trace amounts from *Cantharanthus roseus*,[1] its biological activity was among the first to be shown to arise from inhibition of microtubule formation and mitosis that is still regarded as one of the more successful oncology drug targets.[2−4] Complementary to earlier efforts,[4] we recently reported the total synthesis of vinblastine and its unnatural enantiomer[5] enlisting a biomimetic Fe(III)-promoted coupling of vindoline (**3**) with catharanthine (**4**) and its extension to the total synthesis of a series of related natural products including vincristine (**2**) and key analogs,[6,7] Figure 1.

Although insights into this coupling reaction have been disclosed in earlier studies since its introduction by Kutney,[8] there are features of its mechanism that are not well understood. The Fe(III)-promoted coupling reaction displays a superb stereochemical selectivity, producing exclusively the natural C16′ diastereomer at 25 °C (90%, Figure 1), compared to the traditional Polonovski fragmentation[9−11] (5:1, −78 °C and 1:1, 0 °C, eq 1) or chloroindolenine-based couplings.[12,13] The lack of a



nucleophilic solvent (H_2O) trap of reactive intermediates under the aqueous buffer conditions of the Fe(III)-promoted reaction,

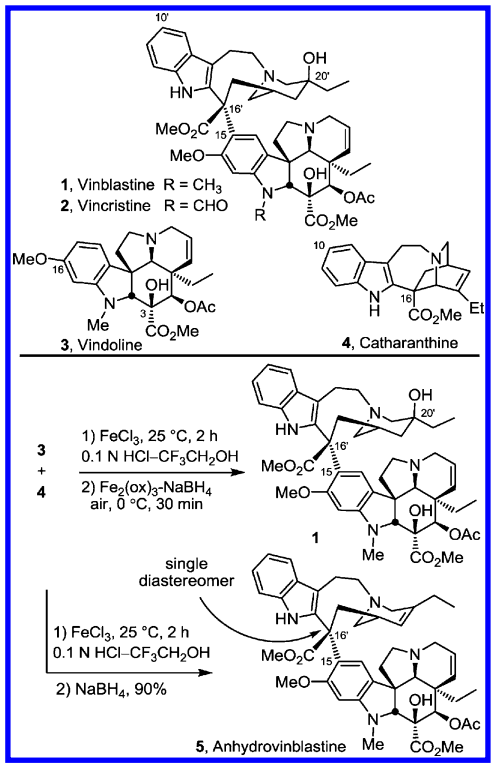


Figure 1. Natural products and Fe(III)-promoted coupling.

the recovery of catharanthine from the reaction mixture in the absence of vindoline (2 h, $NaBH_4$ workup), and the different substrate scope of the reactions[6,9] indicate that the azabenzfulvene intermediate central to the Polonovski fragmentation and related couplings may not be a participant in the Fe(III)-promoted coupling reaction as previously assumed[8] (eq 1).

Herein, we disclose studies conducted to probe the scope and reaction mechanism of the Fe(III)-promoted coupling reaction. They were undertaken inspired by the hypothesis that the Fe(III)-promoted coupling reaction conducted in aqueous buffer may be radical mediated. In addition to new experimental observations that may be interpreted to support such a view, the studies demonstrate new opportunities for the preparation of previously inaccessible vinblastine analogs, represent powerful

Received: June 26, 2012
Published: August 2, 2012

   dx.doi.org/10.1021/ja306229x | J. Am. Chem. Soc. 2012, 134, 13240−13243

methodology for the synthesis of indole-containing natural products, and provide new insights into the biosynthesis of the natural products.

To our knowledge, no systematic study of the Fe(III)-promoted coupling of catharanthine with simpler substrates has been reported.[14] As a result, the Fe(III)-promoted reaction of catharanthine (**4**) with candidate aromatic substrates was examined (Figure 2). Whereas strongly electron-rich aromatic

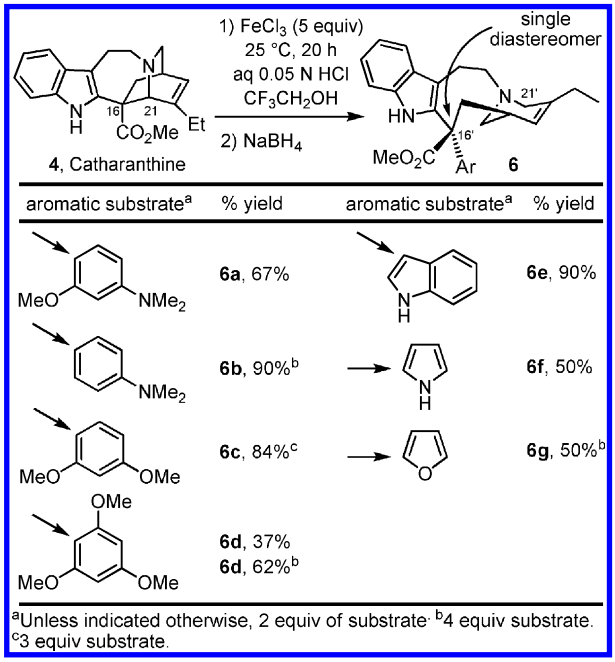


Figure 2. Catharanthine coupling with aromatic substrates.

substrates were found to participate in the coupling reaction effectively, less electron-rich substrates (anisole, thioanisole, 3-methoxythioanisole), neutral aromatic substrates (benzene and thiophene), and a series of electron-deficient aromatic substrates (e.g., methyl benzoate) failed to couple with catharanthine. In each instance of a reaction, the coupling provided a single diastereomer, possessing the natural C16′ stereochemistry found in vinblastine. This observation with simple substrates indicates that the stereochemistry of the Fe(III)-promoted coupling reaction can be controlled by catharanthine alone and may be independent of the structure of its reaction partner (e.g., vindoline). Moreover, each successful reaction produced a single product regioisomer that corresponds to the site of aromatic substitution by electrophiles or radicals. Although the scope of the more traditional Potier/Kutney–Polonovski fragmentation coupling has not been as extensively examined, it is reported not only to display an analogous regioselectivity and a similar requirement for electron-rich aromatic substrates but also to exhibit a temperature-dependent stereochemical outcome, typically producing predominately the natural C16′ stereochemistry at low temperatures (−40 °C) and the unnatural C16′ stereochemistry at higher temperatures (0 °C).[9−11]

In order to probe whether the initial oxidation of catharanthine might occur within the indole[15] and not require oxidation of the tertiary amino group as initially suggested by Kutney and subsequently by others,[8] we first found that tertiary amine additives to the coupling reaction, including *N*-Boc-catharanthine (5 equiv) and quinuclidine (5 equiv), do not impact the conversions. As a result, a series of simplified substrates were examined to establish whether the tertiary amino group was in fact required, as well as probe whether the reportedly important indole free NH[8c] was critical for the coupling reaction (Figure 3).

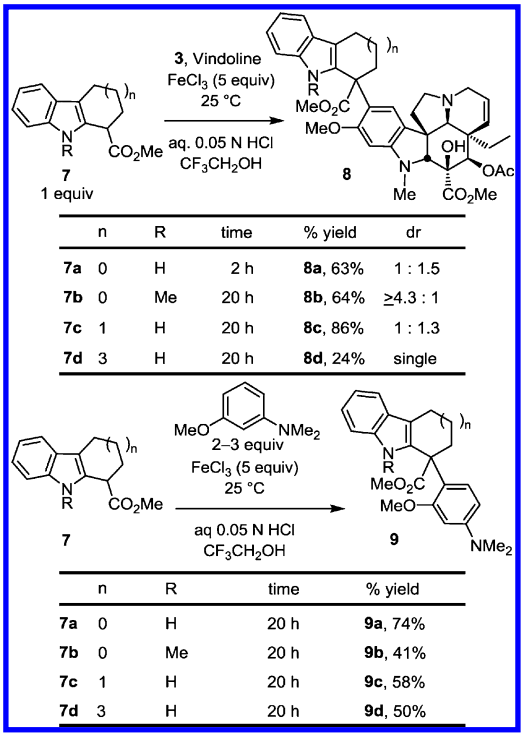


Figure 3. Fe(III)-promoted coupling reactions of simpler indoles.

Notably, the indole NH would be required of a reaction proceeding through a neutral azabenzfulvene and protonation of the tertiary amino group under the reaction conditions may preclude its otherwise competitive single electron oxidation. Impressively and contrary to the expectations based on prior mechanistic proposals, a range of simplified indoles were found to participate in an Fe(III)-mediated coupling reaction with vindoline (Figure 3). Moreover, each of the substrates **7a**−**7d** was also found to couple effectively with *N*,*N*-dimethyl-3-methoxyaniline, establishing the generality of the observations. Thus, the removal of the tertiary amino group as well as indole *N*-methylation did not preclude coupling with vindoline. In contrast, indole *N*-acylation (e.g., *N*-Boc and *N*-CO_2Me derivatives, not shown) did prevent participation in the coupling reaction. These results indicate that an Fe(III)-mediated indole oxidation[15] is sufficient for the coupling reaction to occur and reveal that the reaction does not require the presence of the indole free NH[8c] or the participation of the catharanthine tertiary amino group.[8] Since the reaction no longer requires the reduction of the catharanthine-derived iminium ion in the reaction product, $NaBH_4$ is no longer required for reductive workup of the reactions. In these cases, the reaction typically provided a mixture of diastereomers, confirming that C16′ stereochemistry set in the vinblastine Fe(III)-promoted coupling reaction is principally controlled by catharanthine.

The generality of the reaction was established further by examining the coupling of **7a** with the series of aromatic substrates that react with catharanthine (Figure 4). The indole **7a** displayed the identical reactivity pattern observed with catharanthine itself, coupling with the strongly electron-rich aromatic substrates, but failing to react with anisole, 3-methoxythioanisole, benzene, or methyl benzoate. Significantly,

dx.doi.org/10.1021/ja306229x | *J. Am. Chem. Soc.* 2012, 134, 13240−13243

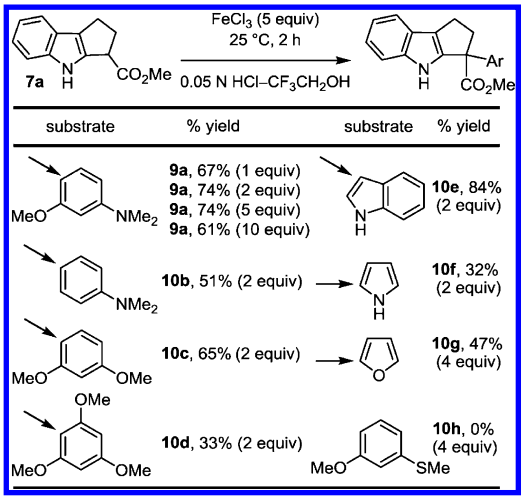
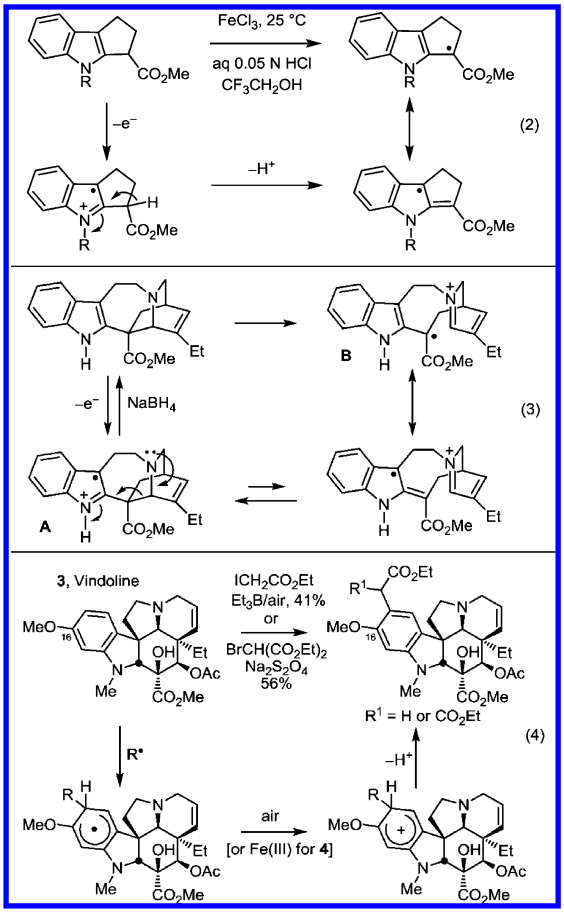


Figure 4. Fe(III)-promoted couplings of **7a**.

these studies illustrate the unexpected generality of the reaction to substrates that bear no close structural resemblance to catharanthine, providing powerful methodology for the synthesis of indole-containing products structurally distinct from vinblastine. The identical substrate scope for the reactions of **7a** and catharanthine suggest that their couplings may proceed by analogous reaction mechanisms. Like those of catharanthine, the reactions of **7** are conducted at rt in acidic aqueous solution (0.05 N aq HCl/CF_3CH_2OH) without competitive nucleophilic solvent (H_2O) or counteranion (Cl^-) participation, indicating that the reactions are unlikely to proceed through an indole-derived, neutral, or cationic azabenzfulvene.

We suggest this entails coupling of the electrophilic radical generated by a single electron oxidation of the indole (eqs 2 and 3). Consistent with this formulation, we disclosed studies that established the required electron-withdrawing properties of the catharanthine C16′ substituent as well as the surprising impact of C10′ indole substituents on its participation in the Fe(III)-mediated coupling reaction.[16] Consistent with an initiating single electron indole oxidation, the latter studies revealed that catharanthine C10′ electron-donating and neutral substituents support the reaction, whereas electron-withdrawing substituents progressively diminish both the rate and yield of the coupling with vindoline. In further support of the direct coupling of the initial single electron indole oxidation intermediate, we have now confirmed that vindoline is susceptible to aromatic substitution by electrophilic carbon radicals. Treatment of vindoline with the radical derived from either ICH_2CO_2Et (Et_3B/air, DMSO or CF_3CH_2OH, 25 °C, 41%)[17] or $BrCH(CO_2Et)_2$ ($Na_2S_2O_4$, 1:1 CH_3CN/H_2O, 25 °C, 56%)[18] provided the aromatic substitution products arising from addition of the electrophilic radicals to the vindoline aromatic ring followed by air oxidation for rearomatization (eq 4). This electrophilic radical substitution reaction of vindoline in protic solvents suggests that the Fe(III)-promoted reactions of catharanthine conducted in water may be radical mediated, rather than involve an intermediate electrophilic azabenzfulvene as previously suggested for the Fe(III)-promoted reaction[8] in analogy to other coupling methods conducted in aprotic solvents.[9−13] The postulated coupling agent, the fragmented cation radical **B**, illustrated in eq 3 is both captodatively stabilized[19] and proposed to be reversibly generated to account for the recovery of catharanthine in the absence of vindoline (2 h) following a reductive workup. In contrast, a committed azabenzfulvene



intermediate (see eq 1) is not expected to revert back to catharanthine upon reductive workup. In the fragmented radical **B**, an intramolecular one-electron-two-center bonding interaction between the radical site α to the carbomethoxy group and the iminium carbon may stabilize a conformation in which the upper face of the radical is sterically blocked, thus accounting for the remarkably high stereoselectivity of the coupling reaction even when conducted at rt. Combined, this proposal suggests that the initial indole radical cation **A** and its fragmented cation radical **B** may possess a unique blend of stability, persistence, electrophilic character, and conformational properties to effectively react selectively with vindoline.

In addition to the mechanistic implications on the Fe(III)-mediated vinblastine coupling reaction, the synthetic possibilities presented by the reactions of **7** are substantial, representing methodology for quaternary center generation adjacent to indole by a reaction mediated by Fe(III) and conducted in aqueous buffer at rt. The scope of the reaction is such that vinblastine analogs containing deep-seated changes to the upper velbanamine subunit not previously envisioned may now be accessed. To explore this possibility, the Fe(III)-mediated coupling of **7** was extended to the preparation of a series of increasingly more complex vinblastine analogs that contain changes in the upper velbanamine subunit (Figure 5). In each case, the coupling precursors **11a−17a** were prepared as racemic mixtures and chromatographically resolved (ChiralCel OD or AD) prior to coupling each individual enantiomer with vindoline (one enantiomer shown). Without optimization, the reaction of substrates that possess a substituent adjacent to the reacting center (**11a−15a**) proceeded selectively, generating a single diastereomer resulting from coupling on the sterically more

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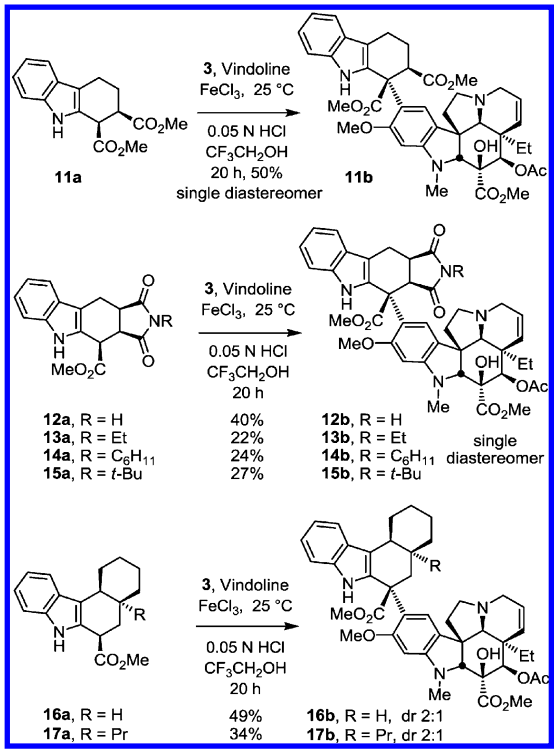

Figure 5. Previously inaccessible vinblastine analogs.

accessible face opposite the adjacent substituent. In contrast, substrates whose added substituents are more removed (**16a** and **17a**) typically provided two diastereomers in near equal amounts. Like the reactions of **7** and in these unoptimized reactions, recovered starting material (**3**) accounted for the material balance in the absence of coupling (e.g., **12**–**15a**). These key studies further define easily understood fundamental stereochemical features of the reaction, indicating that such non-catharanthine-derived indoles, like catharanthine itself, can participate in highly diastereoselective Fe(III)-promoted couplings with vindoline. These may be used in conjunction with the X-ray structure of vinblastine bound to tubulin to design previously inaccessible vinblastine analogs containing effective replacements for the upper velbanamine subunit.

Finally, in addition to a comparison of Fe(III) salts ($FeCl_3$, 90% > $Fe_2(SO_4)_3$, 71% > $Fe_2(ox)_3$, 0%), we examined additional oxidants in our studies.[8] Under near identical conditions (aq 0.05 N HCl/CF_3CH_2OH), $Mn(OAc)_3$ (2.1 equiv, 2 h, 25 °C, 79%) and $Ce(NH_4)_2(NO_3)_6$ (2 equiv, 16 h, 25 °C, 51%) were found to support the coupling of catharanthine and vindoline to provide **5**.

The extension of the studies to the preparation of such vinblastine analogs, the use of the single electron oxidative coupling in the synthesis of additional indole-containing natural products, further studies on the mechanism of the Fe(III)-promoted coupling of catharathine with vindoline and its analogs, and their implications on the biosynthesis of the natural products themselves are in progress and will be disclosed in due course.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Full experimental details and spectra 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.

■ ACKNOWLEDGMENTS

We gratefully acknowledge the support of the National Institute of Health (CA042056, CA115526). J.E.S. is a Skaggs Fellow.

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