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Diversification of Monoterpene Indole Alkaloid Analogs through Cross-Coupling

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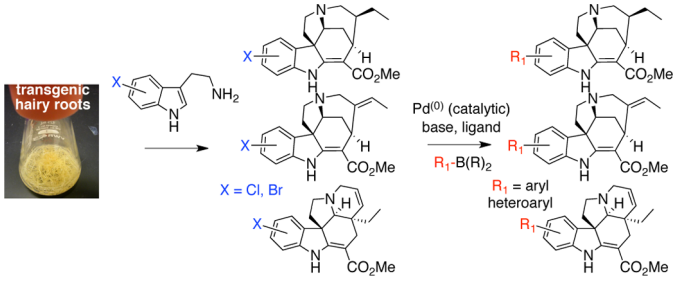
ABSTRACT



Catharanthus roseus **monoterpene indole alkaloid analogs have been produced via a combination of biosynthetic and chemical strategies. Specifically, introduction of a chemical handle—a chlorine or a bromine—into the target molecule by mutasynthesis, followed by postbiosynthetic chemical derivatization using Pd-catalyzed Suzuki-Miyaura cross-coupling reactions robustly afforded aryl and heteroaryl analogs of these alkaloids.**

Modification of natural products can yield analogs, or "unnatural products", with improved or novel medicinal properties.[1] Traditional approaches to generate natural product analogs include total and semisyntheses, precursor-directed biosynthesis, mutasynthesis, and combinatorial biosynthesis.[2] More recently, these strategies have been combined to yield an even broader array of compounds in a cost-effective and rapid manner. For example, analogs of the uridyl peptide antibiotic pacidamycin have been successfully generated by the genetic manipulation of a biosynthetic pathway to yield unnatural halogenated analogs, followed by postbiosynthetic chemical derivatization.[3]

Monoterpene indole alkaloid biosynthesis in the medicinal plant *Catharanthus roseus*, which is only partially characterized at the enzymatic level, produces a variety of alkaloids with diverse biological activities and complex molecular architecture. While numerous semi-[2a] and biosynthetic[4] methods have been applied to generate analogs of these alkaloids, new approaches to broaden the scope of modifications that can be made to these biologically important scaffolds would be advantageous. For example, while halogenation in and of itself often has profound effects on the bioactivity of natural products, the halides also serve as a useful handle for further chemical derivatization. Here we demonstrate that the introduction of a chemical handle, a halide, into the indole moiety of the monoterpene indole alkaloids by mutasynthesis or by introducing prokaryotic halogenases into plant cell cultures, followed by subsequent chemical derivatizations using Pd-catalyzed Suzuki−Miyaura cross-coupling reactions, robustly afforded aryl and heteroaryl analogs of monoterpene indole alkaloids.

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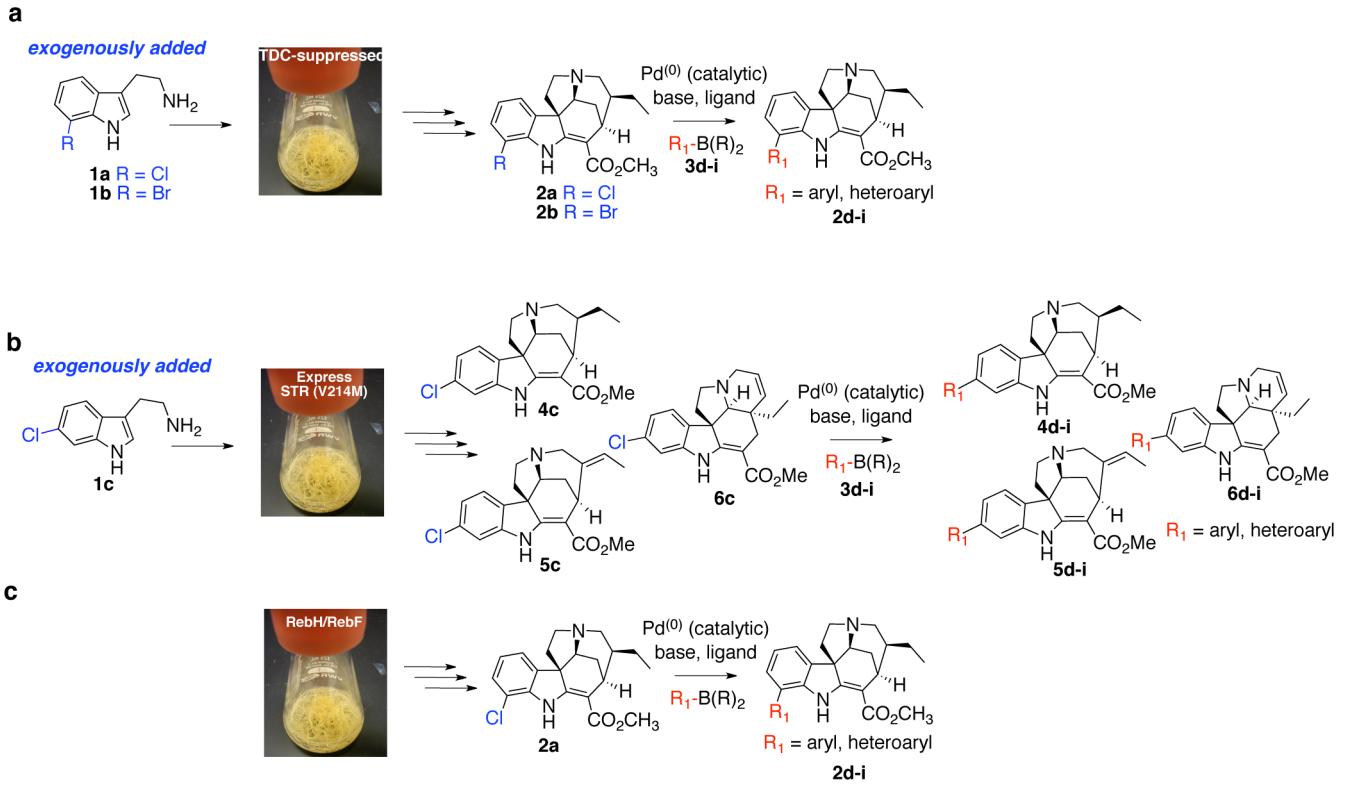

Figure 1. Strategy for synthetic diversification of monoterpene indole alkaloids using Pd-catalyzed Suzuki–Miyaura coupling reaction. Alkaloid analogs with chlorine and bromine at the 7-position of the indole ring were obtained by culturing transgenic tryptophan decarboxylase-suppressed hairy root cultures in the presence of 7-chlorotryptamine **1a** or 7-bromotryptamine **1b** (a). Alkaloid analogs with chlorine at the 6-position of the indole ring were obtained by culturing hairy root cultures that express a mutant strictosidine synthase enzyme (V214M) in the presence of 6-chlorotryptamine **1c** (b). Alkaloid analogs with chlorine at the 7-position of the indole ring could also be obtained from hairy root cultures that express prokaryotic halogenases, RebH and RebF (c).

Monoterpene indole alkaloids are derived from tryptamine **1**. Endogenous tryptamine biosynthesis can be suppressed by silencing the enzyme tryptophan decarboxylase in *C. roseus* hairy root culture. This silenced culture can then be subjected to precursor directed feeding, in which halogenated tryptamine **1** precursors are cocultivated with the root tissue. This strategy, termed mutasynthesis, has allowed generation of chlorinated and brominated monoterpene indole analogs.[5] The desired, halogenated alkaloid profile is streamlined in these tissues since no natural, nonhalogenated alkaloids derived from endogenous tryptamine **1** are present. We opted to use these tryptophan decarboxylase-suppressed hairy root lines to obtain 7-halogenated analogs for subsequent cross-coupling derivatization. Alkaloid analogs with chlorine and bromine at the 7-position of the indole ring were obtained by culturing transgenic tryptophan decarboxylase-suppressed hairy root cultures in liquid Gamborg's B5 media in the presence of either 0.6 mM 7-chlorotryptamine **1a** or 7-bromotryptamine **1b** (Figure 1a). After one week of culture, the plant material was lysed and alkaloids were extracted into methanol. Liquid chromatography mass spectral (LC-MS) analysis of these extracts indicated accumulation of one major product, 12-chloro-19,20-dihydroakuammicine **2a** or 12-bromo-19,20-dihydroakuammicine **2b**, as previously demonstrated.[7]

With halogenated analogs readily available as a component of crude plant extracts, we then set out to explore chemical functionalization reactions that would derivatize halogenated analogs under mild reaction conditions. While cross-coupling reactions of aryl iodides and bromides with arylboronic acids are well precedented, reactions involving aryl chlorides require the use of catalyst systems that employ highly active and sterically demanding ligands, such as Sphos.[6] Therefore, we turned our attention to the Pd-catalyzed Suzuki–Miyaura cross-coupling reaction conditions refined by Goss et al. to derivatize chlorinated padamycin analogs.[3] Since the nonhalogenated metabolites that are present in crude extracts are unlikely to interfere with the progression of the cross-coupling reactions, crude plant extracts containing chlorinated or brominated alkaloids were used directly in the cross-coupling reactions without any purification.

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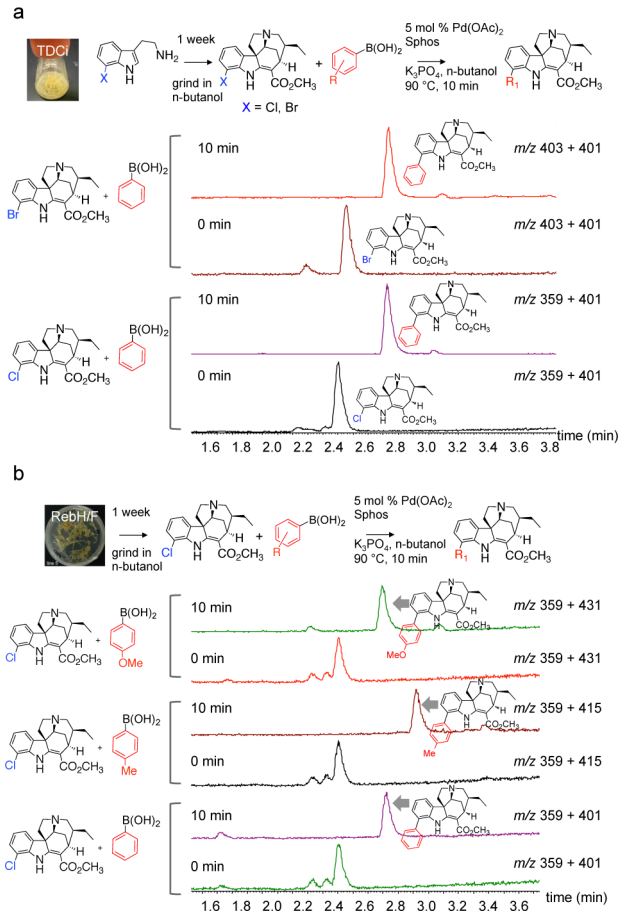
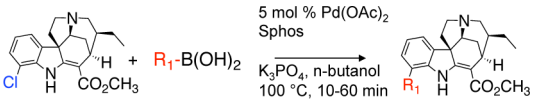


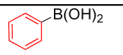
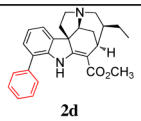
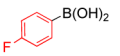
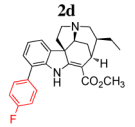
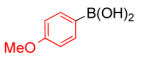
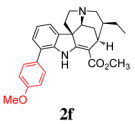
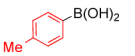
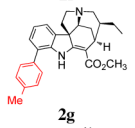
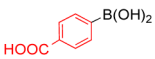
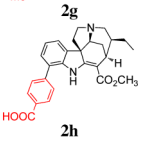
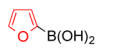
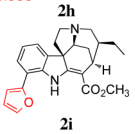
Figure 2. LC-MS traces showing the progression of Suzuki–Miyaura cross-coupling reactions of TDC-suppressed hairy root extracts containing 12-chloro-19,20-dihydroakuammicine **2a** (*m*/*z* 359, bottom) and 12-bromo-19,20-dihydroakuammicine **2b** (*m*/*z* 403, top) with phenylboronic acid **3d** to form 12-phenyl-19,20-dihydroakuammicine **2d** (*m*/*z* 401) (a). LC-MS traces showing the progression of Suzuki–Miyaura cross-coupling reactions of RebH/F hairy root extracts containing **2a** (*m*/*z* 359) with phenylboronic acid **3d** (bottom), 4-methylphenylboronic acid **3g** (middle), and 4-methoxyphenylboronic acid **3f** (top). Expected products were 12-phenyl-19,20-dihydroakuammicine **2d** (*m*/*z* 401), 12-(4-methylphenyl)-19,20-dihydroakuammicine **2g** (*m*/*z* 415), and 12-(4-methoxyphenyl)-19,20-dihydroakuammicine **2f** (*m*/*z* 431) (b).

We performed the Suzuki–Miyaura cross-coupling reactions using six aryl and heteroaryl boronic acid substrates **3d**–**i** (Table 1). Gratifyingly, small-scale coupling reactions of crude methanolic extracts containing either 12-chloro-19,20-dihydroakuammicine **2a** or 12-bromo-19,20-dihydroakuammicine **2b** from 100 mg of fresh hairy roots with each boronic acid went to completion in < 1 h at 90 °C, as monitored by LC-MS, to form products **2d**–**i** (Figure 2a). Product identity on this small scale was evidenced by high-resolution mass spectrometry, tandem MS/MS, and UV absorption spectra (Supporting Information (SI)).

Crude extracts from transgenic hairy roots that express prokaryotic 7-halogenases (RebF and RebH)[7] were also used as substrates for the Suzuki–Miyaura cross-coupling reactions with three representative boronic acids, phenylboronic acid **3d**, 4-methoxyphenylboronic acid **3f**, and 4-methylphenylboronic acid **3g**. LC-MS analysis of crude reaction mixtures demonstrated that the reactions went to completion in < 1 h at 90 °C (Figure 2b).

Table 1. Suzuki–Miyaura Cross-Coupling Reactions of 12-Chloro-19,20-dihydroakuammicine **2a** with Aryl and Heteroaryl Boronic Acids **3d**–**i**



boronic acid substrates	products	isolated yield (%)[a]
 **3d**	 **2d**	50
 **3e**	 **2e**	51
 **3f**	 **2f**	small-scale reaction product not isolated
 **3g**	 **2g**	small-scale reaction product not isolated
 **3h**	 **2h**	small-scale reaction product not isolated
 **3i**	 **2i**	25

[a] Compounds were purified using reversed-phase preparative HPLC.

We also wished to assess the potential for derivatization at an additional position at the indole ring. However, incorporation of tryptamine analogs with substituents at the 6-position present a challenge, since these substrate analogs are not accepted by an early biosynthetic enzyme, strictosidine synthase. Therefore, alkaloids incorporating 6-halo-tryptamine analogs cannot be produced using the tryprophan decarboxylase-silenced cultures described in the preceding section. Instead, we used hairy root cultures that express a mutant strictosidine synthase enzyme (V214M) that has been designed to turnover these tryptamine analogs.[8] These cultures were grown in liquid Gamborg's B5 media in the presence of 0.6 mM 6-chlorotryptamine **1c**, and after one week of culture, the plant

(8) Runguphan, W.; O'Connor, S. E. *Nat. Chem. Biol.* **2009**, *5*, 151.

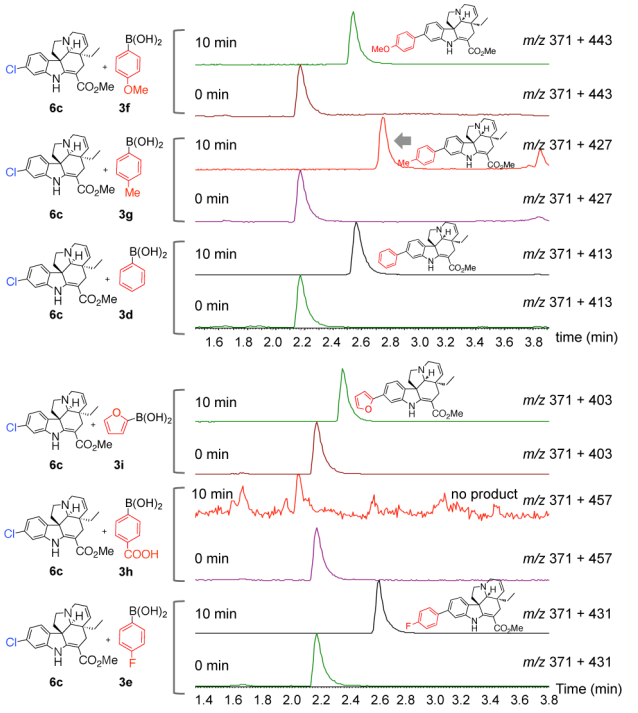


Figure 3. LC-MS traces showing the progression of Suzuki–Miyaura cross-coupling reactions of 11-chlorotabersonine **6c** (*m*/*z* 371) with phenylboronic acid **3d** (bottom), 4-methylphenylboronic acid **3g** (middle), and 4-methoxyphenylboronic acid **3f** (top). Expected products were 11-phenyltabersonine **6d** (*m*/*z* 413), 11-(4-methylphenyl)-tabersonine **6g** (*m*/*z* 427), and 11-(4-methoxyphenyl)-tabersonine **6f** (*m*/*z* 443). LC-MS traces showing the progression of Suzuki–Miyaura cross-coupling reactions of **6c** (*m*/*z* 371) with 4-fluorophenylboronic acid **3e** (bottom), 4-carboxyphenylboronic acid **3h** (middle), and furanylboronic acid **3i** (top). Expected products were 11-(4-fluorophenyl)-tabersonine **6e** (*m*/*z* 431), 11-(4-carboxyphenyl)-tabersonine **6h** (*m*/*z* 457), and 11-furanyl-tabersonine **6i** (*m*/*z* 403).

material was lysed and extracted (Figure 1b). LC-MS analysis of these extracts indicated accumulation of three major analogs: 11-chloro-19,20-dihydroakuammicine **4c**, 11-chloroakuammicine **5c**, and 11-chlorotabersonine **6c** (Figure S2, SI).

Crude extracts containing 11-chloro-19,20-dihydroakuammicine **4c**, 11-chloro-akuammicine **5c**, and 11-chlorotabersonine **6c** were good substrates for the Suzuki–Miyaura cross-coupling reactions using the same set of aryl and heteroaryl boronic acid substrates used to derivatize the 7-halogenated alkaloids (Figure 3). Of the six boronic acids tested, only the electron-deficient 4-carboxyphenyl boronic acid did not yield the desired analogs. High-resolution mass spectrometry, tandem MS/MS, and UV absorption spectra were used to confirm the identities of all alkaloid analogs (SI).

Encouraged by these results, we performed large-scale cross-coupling reactions to more rigorously structurally characterize several representative products. Crude extracts containing 12-chloro-19,20-dihydroakuammicine **2a** from 16 g of hairy roots were used as the substrate. Three representative boronic acids—phenyl, 4-fluorophenyl, and furanyl boronic acids (**1d**, **1e**, and **1i**, respectively)—were tested under these scaled-up conditions. Standard workup of the reaction followed by reversed-phase HPLC purification of the reaction mixture afforded milligram quantities of these analogs. ^{1}H, ^{13}C, and ^{19}F (when applicable) NMR spectroscopy, in addition to high-resolution mass spectrometry, tandem MS/MS, and UV absorption spectra, confirmed the structural identities of the resulting products (SI).

In summary, we have applied a chemogenetic approach—installation of a chemical handle onto the alkaloid framework via mutasynthesis or precursor directed biosynthesis, followed by chemical derivatization—to produce analogs of *C. roseus* monoterpene indole alkaloids. Using this strategy, we have successfully obtained milligram quantities of three alkaloid analogs. The effectiveness of this approach should facilitate rational modification of other classes of plant-derived natural products via cross-coupling for biological activity screening and drug development.

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Supporting Information Available. Experimental procedures and compound characterization. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.