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Article

Synthesis of (−)-Melodinine K: A Case Study of Efficiency in Natural Product Synthesis

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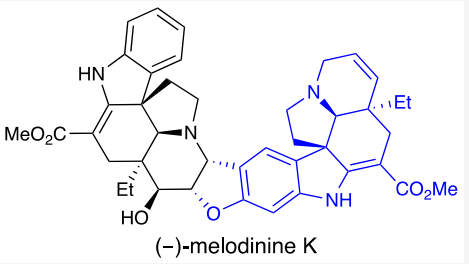
ABSTRACT: Efficiency is a key organizing principle in modern natural product synthesis. Practical criteria include time, cost, and effort expended to synthesize the target, which tracks with step-count and scale. The execution of a natural product synthesis, that is, the sum and identity of each reaction employed therein, falls along a continuum of chemical (abiotic) synthesis on one extreme, followed by the hybrid chemoenzymatic approach, and ultimately biological (biosynthesis) on the other, acknowledging the *first synthesis* belongs to Nature. Starting materials also span a continuum of structural complexity approaching the target with constituent elements on one extreme, followed by petroleum-derived and "chiral pool" building blocks, and complex natural products (i.e., semisynthesis) on the other. Herein, we detail our approach toward realizing the first synthesis of (−)-melodinine K, a complex bis-indole alkaloid. The total syntheses of monomers (−)-tabersonine and (−)-16-methoxytabersonine employing our domino Michael/Mannich annulation is described. Isolation of (−)-tabersonine from *Voacanga africana* and strategic biotransformation with tabersonine 16-hydroxylase for site-specific C−H oxidation enabled a scalable route. The Polonovski−Potier reaction was employed in biomimetic fragment coupling. Subsequent manipulations delivered the target. We conclude with a discussion of efficiency in natural products synthesis and how chemical and biological technologies define the synthetic frontier.



(−)-melodinine K

Synthesis must always be carried out by plan, and the synthetic frontier can be defined only in terms of the degree to which realistic planning is possible, utilizing all of the intellectual and physical tools available. − R. B. Woodward (1956)[1]

What is the most efficient way of synthesizing a natural product? Synthetic efficiency—as described by the concepts of selectivity,[2] green chemistry,[3] and the atom,[4] step,[5] and redox[6] economies[7]—has been both an organizing principle and a key driver of strategic and tactical innovation in modern organic synthesis.[8] The significance of Corey's contributions—retrosynthetic analysis, computer-aided synthesis design, and the overarching logic of chemical synthesis—in the quest for efficiency cannot be overstated.[9] Regarding the logic of synthesis design, Hendrickson argued that time, cost, and effort (TCE) associated with a synthetic campaign represent pragmatic criteria of economy for evaluating efficiency.[10] To select the optimal synthesis plan derived from his SYNGEN (synthesis generation) computer program and benchmark against published routes, Hendrickson employed step-count as the defining figure of merit on the premise that the length of a synthesis (i.e., *reaction distance*) is directly proportional to the TCE required for its realization.[11] All prospective and retrospective synthetic campaigns—academic or industrial—carry a TCE component, which sharply comes into focus when the goal is to procure material on a large (e.g., multigram or kilogram plus) scale.[12]

Natural products are secondary metabolites of myriad diversity and complexity whose biosynthesis and chemical logic are genetically encoded.[13] Organic chemists have long recognized that structurally complex natural products are synthetic targets *par excellence*,[14] but we must not forget that the *first synthesis* belongs to Nature.[8b] Viewed from this perspective, the execution of any natural product synthesis, that is, the sum and identity of *each reaction* employed in a campaign, falls along a continuum of purely chemical synthesis at one extreme and biological synthesis at the other (Figure 1A). The former employs abiotic catalysts and/or reagents, whereas the latter employs enzymes—*in vivo*, *ex vivo*, or *in vitro*—that have evolved over millions of years. Along this continuum lies the hybrid chemoenzymatic approach,[15] which includes enzymes (i.e., from the cognate biosynthetic pathway or not) to effect biotransformations.[16]

The concept of a "synthesis tree", that is, the graphical representation of all possible synthesis plans derived from iterative retrosynthetic analysis of a natural product target

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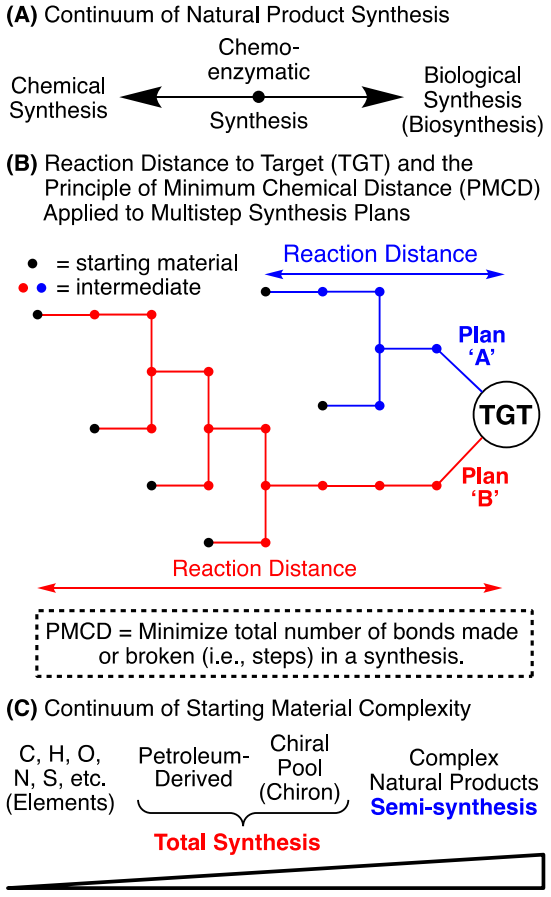
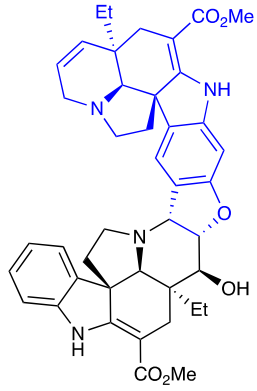


Figure 1. (A) Chemical–biological synthesis continuum of natural products as targets (TGTs); (B) reaction distance to TGT and the principle of minimum chemical distance applied to multistep synthesis plans; and (C) the continuum of starting material complexity.

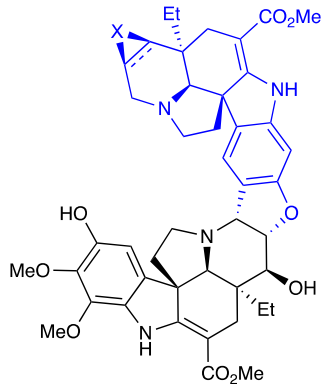
(TGT) and consequent intermediates that ultimately lead back to commercial starting materials, can be traced to the seminal 1969 *Science* paper by Corey and Wipke entitled "Computer-Assisted Design of Complex Organic Synthesis".[9b] When evaluating multiple synthesis plans within a synthesis tree, Hendrickson coined the expression "reaction distance" as a visual proxy for step-count, either prospectively or retrospectively (e.g., plan A versus plan B in Figure 1B). Therein, the superior efficiency of *convergent* versus *linear* synthesis plans can be fully appreciated.[17]

Hendrickson's discussion of "reaction distance" in the context of efficiency dovetails nicely with Ugi's heuristic *principle of minimum chemical distance* to the extent that they focus on reducing step-count.[18] In a treatise on convergence and complexity in synthesis, Bertz noted "the *principle of minimum chemical distance,* by which the total number of bonds made and broken is minimized, is successful in synthetic analysis because it tends to keep the complexities of the intermediates as close as possible to those of the starting material and the target, thus tending to minimize the excess complexity".[19] From this viewpoint (Figure 1C), it follows that starting materials employed in a natural product synthesis—chemical or biological—span a continuum of structural complexity approaching that of the target with its constituent elements on one extreme (e.g., C, H, N, O, etc.) and complex natural product precursors (i.e., semisynthesis) on the other. Petroleum-derived feedstocks employed in *bona fide* total syntheses lie along this continuum, as do the more complex "chiral pool" building blocks featured in the Chiron approach.[14c] A caveat of employing starting materials further along the continuum (e.g., carbohydrates) is that structural complexity and synthetic efficiency can be mutually exclusive, particularly when excessive functional group transformations (i.e., concession steps) are required.[8e,17,19] Taken together, optimal leveraging of the *principle of minimum chemical distance* to shorten the "reaction distance" to a TGT requires pattern recognition to identify readily available natural product precursors whose complexities most closely match. In other words, strategically developed total or semisynthesis design plans enable highly efficient and scalable routes to complex natural products.[20]

Monoterpene indole alkaloids and their dimeric congeners, which include nearly 3000 unique members all derived from tryptophan, have commanded the attention of both organic and natural products chemists over the past six decades.[21] In 2010, Luo and co-workers isolated (−)-melodinine K (**1**), a bis-indole alkaloid of the *Aspidosperma*–*Aspidosperma* class, from the plant *Melodinus tenuicaudatus* in 0.001% yield.[22] The authors evaluated the cytotoxicity of **1** and found it to be more potent than the controls cisplatin and vinorelbine in four of the five tumor cell lines tested (e.g., against the MCF-7 breast cancer cell line, **1** displayed IC_{50} (μM) values of 2.7 versus 18.7 for cisplatin and 17.2 for vinorelbine). Accordingly, (−)-melodinine K (**1**) represents a promising antitumor lead whose mechanism of action is currently unknown. Owing to the architectural complexity manifest in **1**, combined with poor isolation yields from the producing plant, we set out to develop an efficient synthesis of (−)-melodinine K (**1**) for future studies regarding its function in biological systems. In addition, a successful synthesis of **1** could be readily applied to oxygenated congeners **2** and **3**.



(−)-melodinine K (**1**)



2: X=O = (−)-conophylline
3: X=H,H = (−)-conophyllidine

In 1993, Kam and co-workers isolated bis-indole alkaloids (−)-conophylline (**2**) and (−)-conophyllidine (**3**) from the plant *Tabernaemontana divaricata*.[23] In 2011, Fukuyama reported elegant *tour de force* asymmetric total syntheses of both **2** and **3** in 28 steps (longest linear sequence) and 61 overall steps, which remain the only reported syntheses of these target molecules to date.[24] While **1**–**3** share the same fused polycyclic framework, they are differentiated by levels of oxidation at the southern indoline nucleus and at the northern dehydropiperidine heterocycle.

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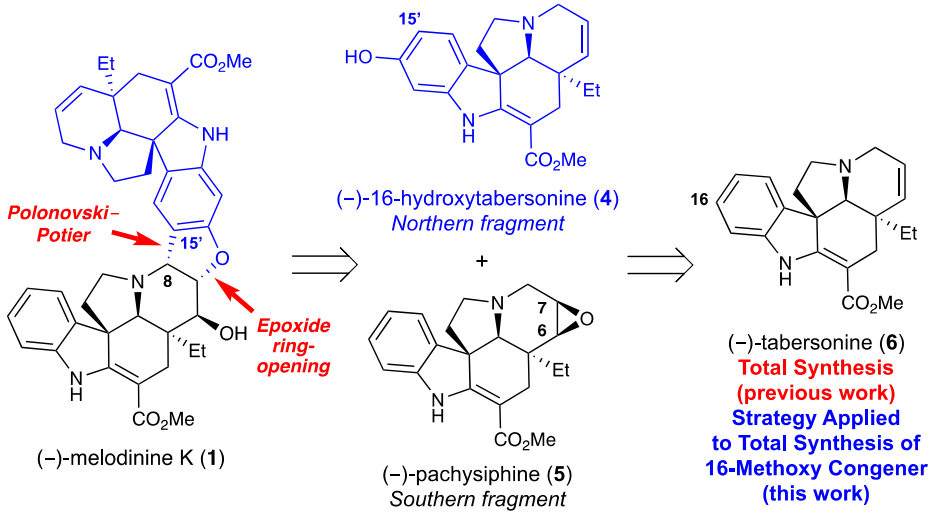
Scheme 1. Retrosynthetic Analysis of (−)-Melodinine K (1) Leading to Two Fragments, (−)-16-Hydroxytabersonine (4) and (−)-Pachysiphine (5), Which Are Biosynthetically Derived from a Common Precursor, (−)-Tabersonine (6)



RESULTS AND DISCUSSION

The retrosynthetic analysis of (−)-melodinine K (**1**) shown in Scheme 1 reveals it to be a heterodimeric union of two known *Aspidosperma* monoterpene indole alkaloids—a northern (−)-16-hydroxytabersonine (**4**) fragment shown in blue and a southern *β*-6,7-epoxytabersonine (i.e., pachysiphine, **5**) fragment—both of which are derived from common precursor (−)-tabersonine (**6**), which our lab previously prepared by total synthesis in 2013.[25] In a biomimetic fashion, we selected the venerable Polonovski−Potier reaction to join both fragments at the C-8−C-15′ bond.[26] The dihydrofuran heterocycle embedded in **1** would be derived from stereospecific epoxide ring-opening of a pendant O-16′ functionality. Importantly, Fukuyama validated these tactics in his total syntheses of bis-indoles **2** and **3**.[24]

Our laboratory has had a long-standing interest in the development of novel synthetic methods and their application toward the asymmetric syntheses of bioactive indole alkaloids.[25,27] To be sure, natural products are unrivaled in their ability to stimulate the creation of novel synthetic methodology. In 2013, we reported a novel annulation reaction featuring a domino Michael/Mannich/*N*-allylation sequence to rapidly access multigram quantities of tetrahydrocarbazole **11**, which makes up a tricyclic portion of the *Aspidosperma* framework (Scheme 2A).[25] Our approach was inspired by Magnus's efficient indole-2,3-quinodimethane strategy[28] to these alkaloids, in addition to Ellman's use of chiral *N*-*tert*-butanesulfinyl metalloenamines for 1,2-additions to aldehydes[29] and 1,4-additions to conjugated enones.[30] Thus, we found that treatment of *N*-sulfinyl imine **7** with LHMDS generated *N*-sulfinyl metallodienamine (NSMD) **8** *in situ*. Addition of methyl ethacrylate (**9**) at −78 °C effected a vinylogous Michael addition whose attendant enolate cyclized stereoselectively to give tricycle **11** after trapping the *N*-sulfinamide anion with allyl bromide in DMF (90% yield, dr = 11:1). Significantly, **11** possesses the hallmark relative and absolute *Aspidosperma* configurations, and we invoke the Davis−Ellman transition state **10** to rationalize selectivity.[31] Tricycle **11** was elaborated to (−)-tabersonine (**6**) in seven additional steps (*vide infra*). Overall, our synthesis required 10 steps (total) and furnished **6** in 26% yield.[25]

The synthesis of northern fragment (−)-16-hydroxytabersonine (**4**) required both a suitable protecting group for O-16 and facile access to material for our annulation protocol. To save a step, we targeted the 16-methoxy congener **26**, which is a biosynthetic precursor of vindoline and various *Vinca* alkaloids, in addition to being a classic target for total synthesis.[32] After surveying a variety of tactics for preparing the requisite starting material, we recruited the Glorius indole synthesis (Scheme 2B) to prepare ethyl 6-methoxy-2-methylindole-3-carboxylate (**15**).[33] Thus, Lewis acid-mediated condensation of *m*-anisidine (**12**) and ethyl acetoacetate (**13**) gave *N*-aryl enamide **14** in 80% yield. Treatment of **14** with 10 mol % $Pd(OAc)_2$, three equivalents of $Cu(OAc)_2$, and K_2CO_3 in DMF at 85 °C for 15 h furnished 6-methoxyindole **15** in 53% yield via Pd-mediated C−H functionalization.

Elaboration of ester **15** to the annulation substrate **19** required four steps: (1) protection of indole nitrogen using NaH and $PhSO_2Cl$; (2) ester reduction to the alcohol with DIBAL-H; (3) Parikh−Doering oxidation to the aldehyde; and (4) condensation with (*R*)-*tert*-butanesulfinamide (**18**) in the presence of $Ti(OEt)_4$, which proceeded in 52% overall yield. Subjection of *N*-sulfinyl imine **19** to the domino Michael/Mannich/*N*-allylation protocol (Scheme 2C) delivered tricycle **20** in 96% yield (dr = 10:1). To prepare the dehydropiperidine heterocycle, we employed ring-closing metathesis, which Rawal and Kozmin demonstrated in their elegant total synthesis of (−)-tabersonine (**6**).[32a] Thus, the ester moiety in **20** was converted to the aldehyde by stepwise reduction with DIBAL-H and oxidation with the Dess−Martin periodinane to afford aldehyde **21** in 90% yield over two steps. Wittig methylenation furnished diene **22** in 80% yield. The structure of **22** was rigorously established by single-crystal X-ray analysis, which also served to unambiguously confirm the stereochemical course of our domino Michael/Mannich annulation (i.e., **19** → **20**). Ring-closing metathesis of diene **22** using 10 mol % of the second-generation Grubbs−Hoveyda catalyst (HG-II)[34] afforded tetracycle **23** in 99% yield. Installation of the final pyrrolidine ring of the pentacyclic *Aspidosperma* framework was realized with the Bosch−Rubiralta spirocyclization,[35] which required removal of the Ellman auxiliary and *N*-alkylation with 2-bromoethanol (49% over two steps). Treatment of alcohol **24** with two equivalents of *t*-BuOK in

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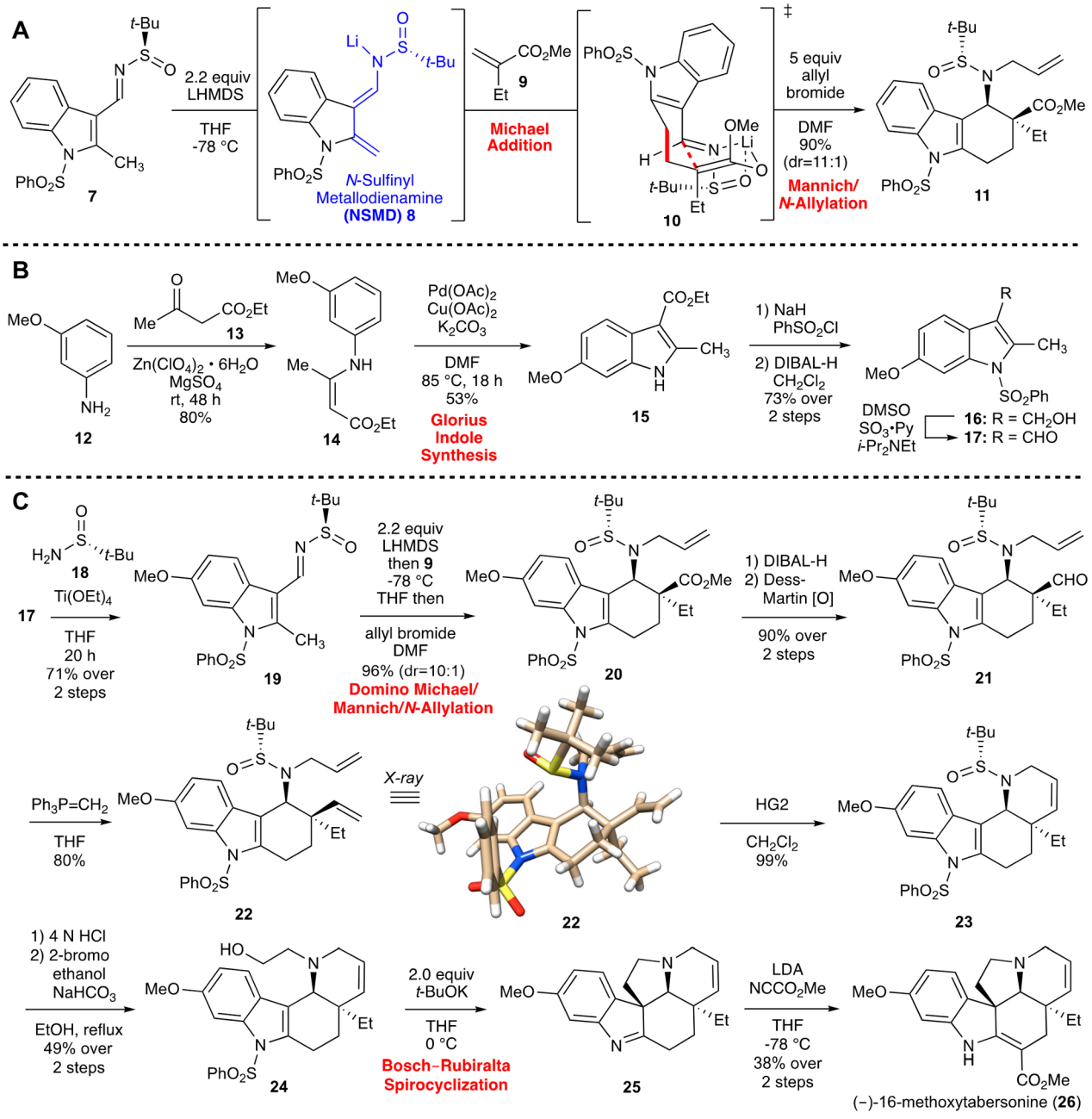
Scheme 2. (A) Novel Domino Michael/Mannich Annulation Methodology Employed in the Total Synthesis of (−)-Tabersonine (6); (B) Glorius Indole Synthesis of 6-Methoxyindole 17; (C) Total Synthesis of (−)-16-Methoxytabersonine (26) from *N*-Sulfinyl Imine 19



THF at 0 °C gave spiroindolenine **25**, which was subsequently reacted with LDA and Mander's reagent to afford (−)-16-methoxytabersonine (**26**) in 38% yield over two steps (Scheme 2C). Overall, our synthesis of northern fragment **26** required 15 steps (total) and proceeded in 3% overall yield. Spectroscopic data (i.e., ^{1}H and ^{13}C NMR, IR), HRMS, and specific rotation values for synthetic (−)-16-methoxytabersonine (**26**) were in complete agreement with those reported by Magnus,[32c] Overman,[32d] and Rawal.[32a]

At this stage of our synthetic campaign toward (−)-melodinine K (**1**), we had registered a total of 25 steps. While we had a suitably protected northern fragment in the form of (−)-16-methoxytabersonine (**26**), we required elaboration of (−)-tabersonine (**6**) into southern fragment (−)-pachysiphine (**5**), which necessitated stereo- and chemoselective oxidation of both alkene and basic nitrogen functionalities to enable fragment coupling. Acutely mindful of the time, cost, and effort associated with our approach, we reflected on the question, what is the *most efficient* way of preparing (−)-melodinine K (**1**)?

In 2016, our lab reported the first chemical syntheses of bis-*Strychnos* alkaloids (−)-sungucine and (−)-isosungucine in nine steps (longest linear sequence) and 11 steps (total) from commercially available (−)-strychnine.[27b,36] Application of the *principle of minimum chemical distance* was key to recognizing that both monoterpene indole fragments comprising the sungucines could be readily prepared from commercially available (−)-strychnine. Accordingly, we looked for natural

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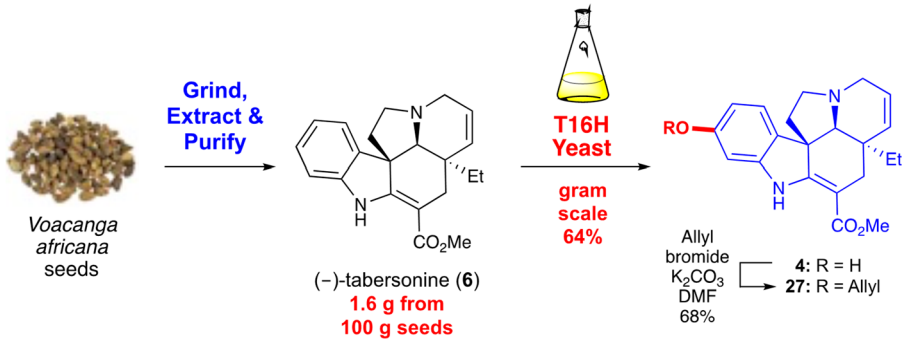
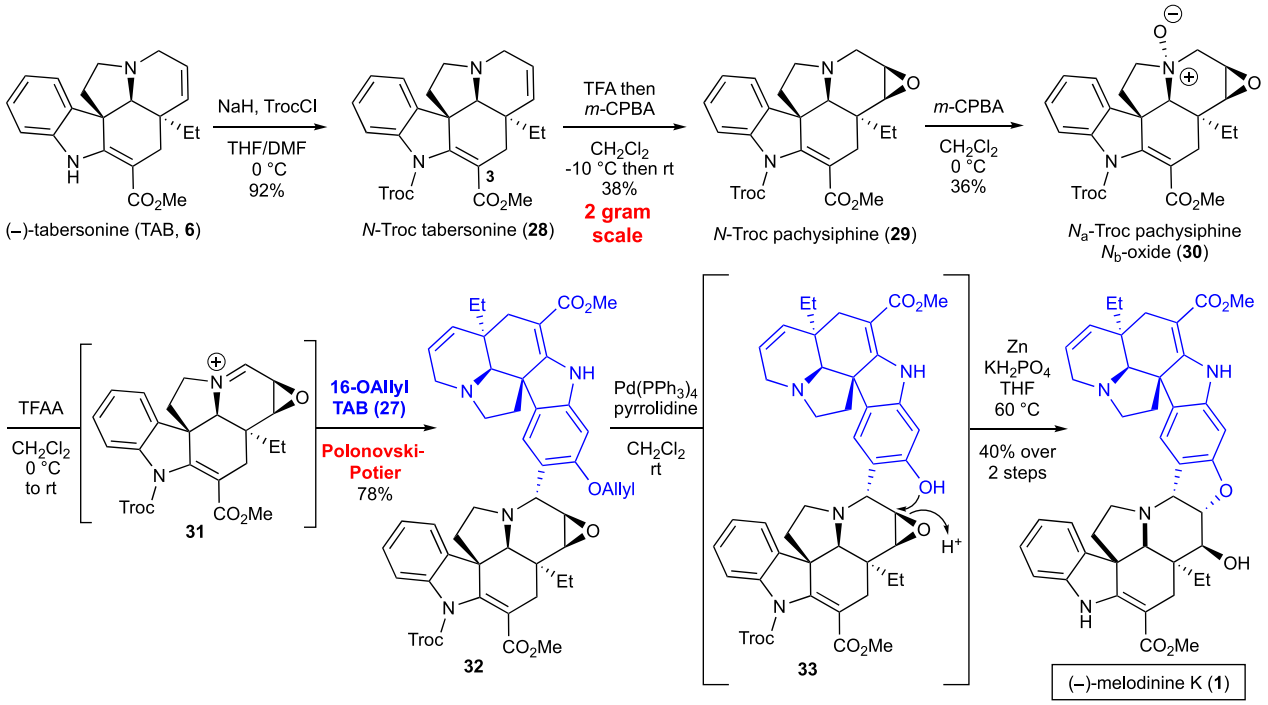
Scheme 3. Isolation of (−)-Tabersonine (6) from *Voacanga africana* Seeds, Biotransformation into (−)-16-Hydroxytabersonine (4) with T16H-Modified Yeast, and *O*-Allylation to Furnish the Protected Northern Fragment 27 for Polonovski−Potier Coupling



Scheme 4. Synthesis of (−)-Melodinine K (1)



and sustainable sources of (−)-tabersonine (**6**) for our synthesis of **1**.

Voacanga africana is a small African tree (Apocynaceae family) used in folkloric medicine.[37] The seeds are a rich source of indole alkaloids, including (−)-tabersonine (**6**), which can range from 1−2% isolation yield by weight! We ultimately identified a supplier of *V. africana* seeds, namely, the Agribusiness in Sustainable Natural African Plant Products (ASNAPP) cooperative in Ghana,[38] and developed a 5-day isolation procedure where we routinely isolated multigram amounts of (−)-tabersonine (**6**) by extraction and chromatographic purification.

With ready access to the putative biosynthetic precursor of (−)-melodinine K (**1**) on a multigram scale, we reevaluated our approach to northern fragment (−)-16-methoxytabersonine (**26**) with an eye toward efficiency. To be sure, site-selective oxidation of (−)-tabersonine (**6**) at the C-16 position is chemically challenging. In addition to not being innately the most reactive site on the indoline nucleus (i.e., C-15 is the most reactive), there are three additional functional groups—the basic tertiary amine, Δ^2- and Δ^6-alkenes—that readily compete for oxidation. While we initially considered two chemical approaches, namely, Ir-catalyzed C−H borylation[39] and Pd-catalyzed acetoxylation,[40] the need to deploy protecting and/or directing groups and concerns with the reactivity of nitrogen and alkene functionalities in (−)-tabersonine (**6**) led us to explore alternatives.

The Madagascar periwinkle (*Catharanthus roseus*) is the source of the antitumor drugs vincristine and vinblastine. Elucidation of the complete biosynthesis of these fascinating *Vinca* alkaloids *in planta* was recently accomplished.[41] Reconstitution of the seven-step biosynthetic pathway of monoterpene indole precursor vindoline has been realized in yeast.[16d] The first step features site-specific oxidation of (−)-tabersonine (**6**) to (−)-16-hydroxytabersonine (**4**) by tabersonine 16-hydroxylase (T16H), which is a cytochrome P450 monooxygenase.[42]

We recognized that biotransformation of readily sourced (−)-tabersonine (**6**) from *V. africana* to (−)-16-hydroxytabersonine (**4**) with T16H would represent the most efficient

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route to the northern fragment of (−)-melodinine K (**1**). Fortuitously, we obtained genetically modified yeast (*Saccharomyces cerevisiae* WAT11 strain) bearing T16H (Scheme 3). Systematic optimization and scale-up of T16H-mediated oxidations of **6** on a milligram scale are now routinely carried out on a gram scale currently at 64% yield. Protection of the 16-hydroxy functionality as its allyl ether was accomplished by treatment of **4** with allyl bromide and K_2CO_3 in DMF to afford (−)-16-allyloxytabersonine (**27**) in 68% yield (Scheme 3).

At this stage, efforts were directed toward preparing the southern fragment, (−)-pachysiphine (**5**), in protected form. Accordingly, (−)-tabersonine (**6**) from *V. africana* was reacted sequentially with NaH and 2,2,2-trichloroethoxycarbonyl chloride (TrocCl) in THF/DMF (3:1) to furnish *N*-Troc tabersonine (**28**) in 92% yield on a multigram scale (Scheme 4). Protection of the indoline nitrogen was critical to avoid competitive oxidation at the C-3 position.[43] Stereoselective oxidation of the disubstituted Δ^6-alkene in **28** was accomplished by stepwise treatment with trifluoroacetic acid (TFA) to protect the basic tertiary amine (N_b) and *m*-CPBA at −10 °C then rt to give protected southern fragment *N*-Troc pachysiphine (**29**) in 38% yield on a 2 g scale. The diastereomeric α-epoxide *N*-Troc lochnericine was isolated in 15% yield.[44] Attempts at effecting this oxidation with other reagents (e.g., F_3CCO_3H, dimethyldioxirane, Davis oxaziridine) gave inferior results.

To couple the northern and southern fragments using the Polonovski−Potier reaction, which was used to advantage by Fukuyama in his syntheses of (−)-conophylline (**2**) and (−)-conophyllidine (**3**),[24] we required the *N*-oxide of **29**. To this end, we treated *N*-Troc pachysiphine (**29**) with *m*-CPBA at 0 °C and isolated N_a-Troc pachysiphine N_b-oxide (**30**) in 36% yield after chromatographic purification. Attempts at consolidating the previous two oxidation steps by reacting *N*-Troc tabersonine (**28**) with excess *m*-CPBA to directly access **30** resulted in oxidation exclusively at the tertiary amine (N_b). Heating the reaction mixture to coax epoxidation led to product decomposition. Ultimately, activation of *N*-oxide **30** with trifluoroacetic anhydride (TFAA) regioselectively generated iminium ion **31** owing to the presence of the adjacent epoxide that can stabilize the carbenium species by hyperconjugation. Regio- and stereoselective attack of iminium **31** by (−)-16-allyloxytabersonine (**27**) at the C-15 position via electrophilic aromatic substitution gave dimer **32** in 78% yield. Notably, the direct coupling of **31** with (−)-16-hydroxytabersonine (**4**), which obviates the need for a protecting group at O-16 and is thus more step-efficient, while successful only proceeded in poor (10%) yield. Of the various oxidizing agents tested, *m*-CPBA performed the best on a multigram scale to access both *N*-Troc pachysiphine (**29**) and *N*-oxide **30**, despite modest yields owing to the formation of polar byproducts.

The endgame for (−)-melodinine K (**1**) required removal of protecting groups at oxygen and nitrogen, in addition to cyclization to form the central dihydrofuran ring. Thus, bis-indoline **32** was first treated with $Pd(PPh_3)_4$ and pyrrolidine to remove the *O*-allyl protecting group and reveal the intermediary O-16′ alcohol **33**, which spontaneously triggered a cyclization event to install the dihydrofuran *in situ*.[24] Finally, reductive removal of the *N*-Troc protecting group using Poisson's conditions (i.e., Zn, KH_2PO_4 in THF at 60 °C)[45] delivered (−)-**1** in 40% yield over two steps. Spectroscopic data (i.e., ^1H and ^{13}C NMR, IR), HRMS, and specific rotation values for synthetic (−)-melodinine K (**1**) were in complete agreement with those reported for natural (−)-**1** by Luo and co-workers.[22,46]

CONCLUSION

The synthesis of natural products has made staggering progress since Wöhler's conversion of ammonium cyanate into urea in 1828.[8b−d,14a] Woodward's visionary statement on synthesis planning—encouraging practitioners to utilize "all intellectual and physical tools available" to define the synthetic frontier—is eminently relevant to efficiency. While the total chemical synthesis of natural products is a uniquely human activity, biological synthesis (i.e., biosynthesis) has been and continues to be practiced by living organisms from all kingdoms of life since the dawn of time.[47] The advent of disruptive technological innovations spanning biology and chemistry—biocatalysis,[15] metabolic engineering,[48] synthetic biology,[49] directed evolution,[50] CRISPR-mediated gene editing,[51] asymmetric synthesis,[14a,c] photochemistry,[52] radical chemistry,[53] organo-[54] and transition metal-mediated catalysis,[55] and flow chemistries,[56] photoredox catalysis,[57] and electrochemistry,[58] among others—have directly impacted efficiency in academia and industry and will certainly continue to shape the synthetic frontier in the foreseeable future.[8b,13b]

We have framed the issue of efficiency in natural products synthesis by considering two continuums—one pertaining to the chemical−biological nature of reactions employed en route and the other to starting material complexity—and the linchpin *principle of minimum chemical distance* that mandates the economic making and breaking of chemical bonds. The answer to the question "what is the most efficient way of synthesizing a natural product?" clearly depends on the target in question, the scale of production, and the project time scale (i.e., deadline). To be sure, judicious consideration of *all* possibilities—chemical and biological—when planning a natural product synthesis is *essential* for maximizing efficiency.

In summary, we have completed the first synthesis of bis-indole alkaloid (−)-melodinine K (**1**) in six steps (longest linear sequence) and eight total steps from (−)-tabersonine (**6**) in 4% yield. The evolution of our approach began with the asymmetric total synthesis of two constituent *Aspidosperma* alkaloids, (−)-tabersonine (**6**) and (−)-16-methoxytabersonine (**26**), which nobly served as inspiration for novel methodology development (i.e., domino Michael/Mannich annulation). Critical to the efficiency of our approach were (1) the identification and sourcing of putative biosynthetic precursor (−)-tabersonine (**6**) from readily available *V. africana* and (2) its strategic biotransformation to (−)-16-hydroxytabersonine (**4**) using T16H-modified yeast for site-specific C−H oxidation on multigram scales. The venerable Polonovski−Potier reaction was employed in a biomimetic fragment-coupling key step, and additional functional group manipulation delivered the bis-indole natural product target, (−)-melodinine K (**1**). The strategic combination of semisynthesis to expedite procurement and biotransformation using an enzyme in the target's biosynthetic pathway to execute a chemically challenging C−H oxidation represents a sustainable route to **1** and its congeners for biological studies, particularly as isolation from the producing plant *Melodinus tenuicaudatus* proceeds in such low yield.

EXPERIMENTAL SECTION

General Experimental Procedures. Specific rotation values were measured using a PerkinElmer model 341 polarimeter. Infrared

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(IR) spectra were recorded on a Thermo Scientific Nicolet iS5 FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were run on a Bruker AV-III 500, Bruker AVIIIHD 500, or Bruker Avance 400 spectrometer in $CDCl_3$ at 298 K. High-resolution mass spectra were obtained on a Waters LCT Premier XE LC/MS system. Single-crystal X-ray diffraction was performed using a Bruker KAPPA APEX II DUO diffractometer with Mo Kα radiation obtained from a sealed molybdenum tube with a TRIUMPH monochromator. The sample was mounted on a MiTeGen loop with Paratone N oil and cooled to 173 K using an Oxford Cryostream low-temperature device. The structure was solved using direct methods and refined using full-matrix least-squares (SHELXTL). Additional experimental and sample details are given in the crystallographic tables (see Supporting Information). Chemical shifts are indicated in ppm relative to tetramethylsilane (TMS, δ = 0.00) and referenced to residual solvent signals (^{1}H, 7.26 ppm, ^{13}C centerline of $CDCl_3$ triplet, 77.0 ppm). Flash column chromatography was performed according to the procedure of Still using ICN Silitech 32−63 D 60 Å silica gel with the indicated solvents.[59] Thin layer chromatography was performed on Merck 60 F254 silica gel plates. Detection was performed using UV light, $KMnO_4$ stain, and an iodine chamber, or PMA stain and subsequent heating.

Ester 20. To a solution of **19** (1.10 g, 2.56 mmol) in THF (10 mL) was added LHMDS (1.0 M in THF, 3.8, 3.8 mmol) at −78 °C, and the mixture stirred for 1 h. A solution of ethyl methacrolein (**9**) (0.430 g, 3.81 mmol) in THF (2 mL) was added at −78 °C and stirred for 2 h. The dry ice cooling bath was replaced with an ice−water cooling bath, and a solution of allyl bromide (1.56 g, 12.7 mmol) in DMF (60 mL) was added. The ice bath was removed, and the reaction was stirred for 14 h. The reaction was quenched with saturated aqueous NH_4Cl and diluted with H_2O (10 mL), followed by extraction with EtOAc (3 × 20 mL). The combined organic layers were washed with brine (5 × 20 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography eluting with EtOAc/hexane (0:100 → 40:60) to afford 1.43 g of **20** as a white foam (96%, dr = 10:1).

Extraction and isolation of (−)-Tabersonine (6). To a ground powder of *Voacanga africana* seeds (100 g) was added 1% aqueous H_2SO_4 (1 L), and the mixture was stirred for 24 h at rt. The acidic solution was filtered over a pad of Celite. To this solution was added sodium chloride (100 g), and the mixture was aged for 4 h. The solution was filtered over another pad of Celite, extracted with $CHCl_3$ (2 × 500 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The residue was recrystallized from acetone at 0 °C, redissolved in chloroform, washed with 10% aqueous NaOH (40 mL), H_2O (20 mL), and brine (20 mL), extracted with $CHCl_3$, dried over Na_2SO_4, and concentrated under reduced pressure. The crude residue was purified by column chromatography eluting with EtOAc/hexanes (1:6) to afford **6** (1.30 g, 1%) as a white foam.

16-Hydroxytabersonine (4). A Petri plate with CSM-Ura-Ade medium was streaked with yeast (T16H in pYedp60) and incubated at 30 °C for 48 h. YPG medium (500 mL) containing yeast extract (1%, 5.0 g), peptone (2%, 10.0 g), and glucose (2%, 10.0 g) in H_2O was autoclaved. The media was inoculated with yeast, covered with cheesecloth, and incubated at 30 °C at 250 rpm for 36 h. The media was then poured into 16 Falcon tubes (30 mL each) and centrifuged at 10 °C at 3000 rpm for 10 min. The yeast pellets were then washed with H_2O (20 mL each) and centrifuged again at 10 °C at 3000 rpm for 10 min. Five 2 L round-bottom flasks each containing YPGal medium (1 L) containing yeast extract (1%, 10.0 g), peptone (2%, 20.0 g), and galactose (2%, 20.0 g) in H_2O were autoclaved. The yeast pellets were inoculated into each flask evenly, covered with cheesecloth, and incubated at 30 °C at 250 rpm for 16 h. Then, to each flask was added tabersonine hydrochloride salt (1.50 g, 4.03 mmol) in DMSO (10 mL), followed by the addition of galactose (2%, 20.0 g), covered with cheesecloth, and incubated at 30 °C at 250 rpm for 96 h. The media was then stirred with EtOAc (1 L) for 1 h, extracted with EtOAc (2 × 1 L), filtered, washed with brine (1 × 100 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash column chromatography eluting with EtOAc/hexanes (1:4) to afford 4.51 g (64%) of **4** as a white foam.

16-Allyloxytabersonine (27). To a solution of **4** (100 mg, 0.280 mmol) in DMF (2 mL) were added K_2CO_3 (157 mg, 1.14 mmol) and allyl bromide (0.074 mL, 0.85 mmol). The reaction mixture was heated to 60 °C and stirred for 1 h. The reaction mixture was cooled to rt, and aqueous $NaHCO_3$ (5 mL) was added. The reaction mixture was extracted with EtOAc (3 × 5 mL), washed with brine (3 × 5 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by flash column chromatography eluting with EtOAc/hexanes (1:20) to afford 76 mg (68%) of **27** as a white foam.

***N*-Troc-Tabersonine (28).** To a solution of tabersonine (**6**) (1.98 g, 5.90 mmol) and DMAP (1.44 g, 11.8 mmol) in THF (30 mL) and DMF (10 mL) was added NaH (1.17 g, 29.5 mmol). The reaction mixture was stirred for 1 h at 0 °C. TrocCl (2.43 mL, 17.7 mmol) was added dropwise, and the reaction mixture was stirred an additional 3 h at rt. The reaction mixture was poured into aqueous $NaHCO_3$ (24 mL), extracted with $CHCl_3$ (3 × 24 mL), washed with brine (3 × 24 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash column chromatography eluting with EtOAc/hexanes (1:6) to afford 2.80 g of **28** (92%) as a white foam.

***N*-Troc-Pachysiphine (29).** To a solution of *N*-Troc-tabersonine (**28**) (2.00 g, 3.91 mmol) in CH_2Cl_2 (14 mL) was added TFA (2.30 g, 19.5 mmol) at −10 °C. The reaction mixture was stirred for 15 min. *m*-CPBA (2.0 g, 13.3 mmol) dissolved in CH_2Cl_2 (10 mL) was added dropwise at −10 °C. The reaction mixture was gradually warmed to rt and stirred overnight. The reaction was quenched with aqueous Na_2SO_3 (24 mL) and aqueous $NaHCO_3$ (24 mL), extracted with CH_2Cl_2 (3 × 24 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash column chromatography eluting with EtOAc/hexanes (1:3) to afford 800 mg of *N*-Troc-pachysiphine (**29**) (38%) as a white foam.

***N*$_a$-Troc-Pachysiphine *N*$_b$-Oxide (30).** To a solution of *N*-Troc-pachysiphine (**29**) (655 mg, 1.24 mmol) in CH_2Cl_2 (2 mL) at 0 °C was added *m*-CPBA (643 mg, 3.73 mmol) dissolved in CH_2Cl_2 (2 mL) dropwise. The reaction mixture was stirred for 30 min. The reaction was quenched with aqueous $NaHCO_3$ (5 mL), extracted with CH_2Cl_2 (3 × 5 mL), washed with brine (3 × 5 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography in 5% MeOH/$CHCl_3$ to afford 240 mg (36%) of **30** as a white foam.

Dimer 32. To a solution of N_a-Troc-pachysiphine N_b-oxide (**30**) (85.0 mg, 0.156 mmol) in CH_2Cl_2 (2 mL) at 0 °C was added TFAA (0.22 mL, 1.6 mmol). The reaction mixture was stirred for 15 min, upon which a solution of 16-allyloxytabersonine (**27**) (22 mg, 0.055 mmol) in CH_2Cl_2 (2 mL) was added dropwise. The reaction mixture was stirred at 0 °C for 1 h, then warmed to rt and stirred an additional 16 h. Aqueous $NaHCO_3$ (5 mL) was added, and the reaction mixture was extracted with CH_2Cl_2 (3 × 5 mL), washed with brine (3 × 5 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash column chromatography eluting with EtOAc/hexanes (1:1) to afford 40 mg (78%) of **32** as a white foam.

***N*-Troc-Melodinine K (33).** Argon was bubbled through a solution of **32** (50 mg, 0.054 mmol) in CH_2Cl_2 (2 mL) at rt for 20 min. $Pd(PPh_3)_4$ (62 mg, 0.054 mmol) was added, and the reaction mixture was stirred at rt for 1 h. Pyrrolidine (0.02 mL, 0.27 mmol) was then added, and the reaction was stirred at rt for 1 h. The resulting mixture was passed through a short plug of silica in 100% EtOAc. Aqueous $NaHCO_3$ (5 mL) was added, and the reaction mixture was extracted with CH_2Cl_2 (3 × 5 mL), washed with brine (3 × 5 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude residue **33** was isolated as a brown solid and used directly in the next step.

(−)-Melodinine K (1). To a solution of crude *N*-Troc-melodinine K (**33**) from the previous step dissolved in THF (2 mL) were added activated zinc dust (105 mg, 1.61 mmol) and aqueous KH_2PO_4 solution (1.0 M, 0.09 mL). The reaction mixture was heated to 60 °C

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and stirred for 3 h. The reaction mixture was cooled to room temperature and passed through Celite, and aqueous $NaHCO_3$ (5 mL) was added. The organic layer was extracted with EtOAc (3 × 5 mL), washed with brine (3 × 5 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash column chromatography eluting with EtOAc/hexanes (1:1) to afford 15 mg of **1** (40% over two steps) as a yellow foam.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.0c00310.

Experimental procedures for **14**−**25**, (−)-16-methoxytabersonine (**26**); isolation of (−)-tabersonine (**6**) from *V. africana* seeds; biotransformation of **6** to (−)-16-hydroxytabersonine (**4**) with T16H-functionalized yeast; characterization of (−)-tabersonine (**6**), (−)-16-hydroxytabersonine (**4**), intermediates **14**−**23**, **27**−**29**, **32**, **33**, and (−)-melodinine K (**1**); X-ray structure determination of **22** (PDF)

X-ray crystallographic data (CIF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Woodward, R. B. *Perspectives in Organic Chemistry*; Interscience: New York, 1956.

(2) Trost, B. M. *Science* **1983**, *219*, 245−250.

(3) Anastas, P. T.; Kirchhoff, M. M. *Acc. Chem. Res.* **2002**, *35*, 686−694.

(4) Trost, B. M. *Science* **1991**, *254*, 1471−1477.

(5) Wender, P. A.; Verma, V. A.; Paxton, T. J.; Pillow, T. H. *Acc. Chem. Res.* **2008**, *41*, 40−49.

(6) Burns, N. Z.; Baran, P. S.; Hoffmann, R. W. *Angew. Chem., Int. Ed.* **2009**, *48*, 2854−2867.

(7) Newhouse, T.; Baran, P. S.; Hoffmann, R. W. *Chem. Soc. Rev.* **2009**, *38*, 3010−3021.

(8) (a) Schwan, J.; Christmann, M. *Chem. Soc. Rev.* **2018**, *47*, 7985−7995. (b) Brocksom, T. J.; Desidera, A. L.; Alves, L. D.; de Oliveira, K. T. *Curr. Org. Synth.* **2015**, *12*, 496−522. (c) Breinbauer, R.; Pletz, J. In *From Biosynthesis to Total Synthesis: Strategies and Tactics for Natural Products*; Zografos, A. L., Ed.; John Wiley & Sons: New York, 2016; pp 519−547; . (d) Hoffmann, R. W. *Elements of Synthesis Planning*; Springer-Verlag: Heidelberg, 2009. (e) Gaich, T.; Baran, P. S. *J. Org. Chem.* **2010**, *75*, 4657−4673.

(9) (a) Corey, E. J.; Cheng, X. M. *The Logic of Chemical Synthesis*; John Wiley & Sons: New York, 1989. (b) Corey, E. J.; Wipke, W. T. *Science* **1969**, *166*, 178−192.

(10) Hendrickson, J. B.; Toczko, A. G. *Pure Appl. Chem.* **1988**, *60*, 1563−1572.

(11) Hendrickson, J. B. *Acc. Chem. Res.* **1986**, *19*, 274−281.

(12) Mulzer, J. *Nat. Prod. Rep.* **2014**, *31*, 595−603.

(13) (a) Walsh, C. T.; Tang, Y. *Natural Product Biosynthesis: Chemical Logic and Enzymatic Machinery*; RSC: London, 2017; . (b) Walsh, C. T.; Fischbach, M. A. *J. Am. Chem. Soc.* **2010**, *132*, 2469−2493.

(14) (a) Hudlicky, T.; Reed, J. W. *The Way of Synthesis: Evolution of Design and Methods for Natural Products*; VCH: Weinheim, Germany, 2007. (b) Nicolaou, K. C.; Sorensen, E. J. *Classics in Total Synthesis: Targets, Strategies, Methods*; VCH: Weinheim, Germany, 1996. (c) Hanessian, S.; Giroux, S.; Merner, B. L. *Design and Strategy in Organic Synthesis: From the Chiron Approach to Catalysis*; John Wiley & Sons: New York, 2013.

(15) (a) Honig, M.; Sondermann, P.; Turner, N. J.; Carreira, E. M. *Angew. Chem., Int. Ed.* **2017**, *56*, 8942−8973. (b) Hudlicky, T.; Reed, J. W. *Chem. Soc. Rev.* **2009**, *38*, 3117−3132.

(16) (a) Whitesides, G. M.; Wong, C. H. *Enzymes in Synthetic Organic Chemistry*; Pergamon Press: London, 1994; Vol. *12*. (b) Ro, D. K.; Paradise, E. M.; Ouellet, M.; Fisher, K. J.; Newman, K. L.; Ndungu, J. M.; Ho, K. A.; Eachus, R. A.; Ham, T. S.; Kirby, J.; Chang, M. C. Y.; Withers, S. T.; Shiba, Y.; Sarpong, R.; Keasling, J. D. *Nature* **2006**, *440*, 940−943. (c) Galanie, S.; Thodey, K.; Trenchard, I. J.; Interrante, M. F.; Smolke, C. D. *Science* **2015**, *349*, 1095−1100.

(d) Qu, Y.; Easson, M.; Froese, J.; Simionescu, R.; Hudlicky, T.; De Luca, V. *Proc. Natl. Acad. Sci. U. S. A.* **2015**, *112*, 6224−6229.
(17) Bertz, S. H. *New J. Chem.* **2003**, *27*, 870−879.
(18) Jochum, C.; Gasteiger, J.; Ugi, I. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 495−505.
(19) Bertz, S. H. *J. Am. Chem. Soc.* **1982**, *104*, 5801−5803.
(20) Brill, Z. G.; Condakes, M. L.; Ting, C. P.; Maimone, T. J. *Chem. Rev.* **2017**, *117*, 11753−11795.
(21) Szabó, L. F. *Molecules* **2008**, *13*, 1875−1896.
(22) Feng, T.; Li, Y.; Wang, Y.-Y.; Cai, X.-H.; Liu, Y.-P.; Luo, X.-D. *J. Nat. Prod.* **2010**, *73*, 1075−1079.
(23) (a) Kam, T. S.; Loh, K. Y.; Wei, C. *J. Nat. Prod.* **1993**, *56*, 1865−1871. (b) Kam, T. S.; Loh, K. Y.; Lim, L. H.; Loong, W. L.; Chuah, C. H.; Wei, C. *Tetrahedron Lett.* **1992**, *33*, 969−972. (c) Kam, T. S.; Anuradha, S. *Phytochemistry* **1995**, *40*, 313−316.
(24) Han-ya, Y.; Tokuyama, H.; Fukuyama, T. *Angew. Chem., Int. Ed.* **2011**, *50*, 4884−4887.
(25) Zhao, S.; Andrade, R. B. *J. Am. Chem. Soc.* **2013**, *135*, 13334−13337.
(26) Cave, A.; Kan-Fan, C.; Potier, P.; Le Men, J. *Tetrahedron* **1967**, *23*, 4681−4689.
(27) (a) Zhao, S.; Andrade, R. B. *J. Org. Chem.* **2017**, *82*, 521−531. (b) Zhao, S.; Chen, H.; Sirasani, G.; Vaddypally, S.; Zdilla, M.; Dobereiner, G. E.; Andrade, R. B. *Chem. - Eur. J.* **2016**, *22*, 11593−11596. (c) Zhao, S.; Sirasani, G.; Vaddypally, S.; Zdilla, M. J.; Andrade, R. B. *Tetrahedron* **2016**, *72*, 6107−6112. (d) Zhao, S.; Sirasani, G.; Vaddypally, S.; Zdilla, M. J.; Andrade, R. B. *Angew. Chem., Int. Ed.* **2013**, *52*, 8309−8311. (e) Teijaro, C. N.; Zhao, S. Z.; Kokkonda, P.; Andrade, R. B. *Synthesis* **2015**, *47*, 1547−1556. (f) Teijaro, C. N.; Munagala, S.; Zhao, S. Z.; Sirasani, G.; Kokkonda, P.; Malofeeva, E. V.; Hopper-Borge, E.; Andrade, R. B. *J. Med. Chem.* **2014**, *57*, 10383−10390. (g) Sirasani, G.; Andrade, R. B. In *Strategies and Tactics in Organic Synthesis, Vol 9*; Harmata, M., Ed.; 2013; Vol. *9*, pp 1−44. (h) Sirasani, G.; Andrade, R. B. *Org. Lett.* **2011**, *13*, 4736−4737. (i) Sirasani, G.; Andrade, R. B. *Org. Lett.* **2009**, *11*, 2085−2088. (j) Kokkonda, P.; Brown, K. R.; Seguin, T. J.; Wheeler, S. E.; Vaddypally, S.; Zdilla, M. J.; Andrade, R. B. *Angew. Chem., Int. Ed.* **2015**, *54*, 12632−12635. (k) Andrade, R. B.; Kokkonda, P.; Munagala, S.; Zhao, S. Z.; Sirasani, G. *Abstracts of Papers of the American Chemical Society* **2012**, *244*, 1.
(28) Magnus, P.; Gallagher, T.; Brown, P.; Pappalardo, P. *Acc. Chem. Res.* **1984**, *17*, 35−41.
(29) Kochi, T.; Tang, T. P.; Ellman, J. A. *J. Am. Chem. Soc.* **2003**, *125*, 11276−11282.
(30) Peltier, H. M.; Ellman, J. A. *J. Org. Chem.* **2005**, *70*, 7342−7345.
(31) (a) Zhou, P.; Chen, B. C.; Davis, F. A. *Tetrahedron* **2004**, *60*, 8003−8030. (b) Robak, M. T.; Herbage, M. A.; Ellman, J. A. *Chem. Rev.* **2010**, *110*, 3600−3740.
(32) (a) Kozmin, S. A.; Iwama, T.; Huang, Y.; Rawal, V. H. *J. Am. Chem. Soc.* **2002**, *124*, 4628−4641. (b) Wang, N. Z.; Liu, J. R.; Wang, C.; Bai, L. Y.; Jiang, X. F. *Org. Lett.* **2018**, *20*, 292−295. (c) Cardwell, K.; Hewitt, B.; Ladlow, M.; Magnus, P. *J. Am. Chem. Soc.* **1988**, *110*, 2242−2248. (d) Overman, L. E.; Sworin, M.; Burk, R. M. *J. Org. Chem.* **1983**, *48*, 2685−2690.
(33) Wurtz, S.; Rakshit, S.; Neumann, J. J.; Droge, T.; Glorius, F. *Angew. Chem., Int. Ed.* **2008**, *47*, 7230−7233.
(34) Garber, S. B.; Kingsbury, J. S.; Gray, B. L.; Hoveyda, A. H. *J. Am. Chem. Soc.* **2000**, *122*, 8168−8179.
(35) Rubiralta, M.; Diez, A.; Bosch, J.; Solans, X. *J. Org. Chem.* **1989**, *54*, 5591−5597.
(36) Zhao, S.; Teijaro, C. N.; Chen, H.; Sirasani, G.; Vaddypally, S.; O'Sullivan, O.; Zdilla, M. J.; Dobereiner, G. E.; Andrade, R. B. *J. Braz. Chem. Soc.* **2018**, *30*, 436−453.
(37) Koroch, A. R.; Juliani, H. R.; Kulakowski, D.; Arthur, H.; Asante-Dartey, J.; Simon, J. E. In *African Natural Plant Products: New Discoveries and Challenges in Chemistry and Quality*; American Chemical Society, 2009; Vol. *1021*, pp 363−380.
(38) http://www.asnapp.org.za/.
(39) Feng, Y.; Holte, D.; Zoller, J.; Umemiya, S.; Simke, L. R.; Baran, P. S. *J. Am. Chem. Soc.* **2015**, *137*, 10160−10163.
(40) Yang, G. Q.; Lindovska, P.; Zhu, D. J.; Kim, J.; Wang, P.; Tang, R. Y.; Movassaghi, M.; Yu, J. Q. *J. Am. Chem. Soc.* **2014**, *136*, 10807−10813.
(41) (a) Caputi, L.; Franke, J.; Farrow, S. C.; Chung, K.; Payne, R. M. E.; Nguyen, T. D.; Dang, T. T. T.; Carqueijeiro, I. S. T.; Koudounas, K.; de Bernonville, T. D.; Ameyaw, B.; Jones, D. M.; Vieira, I. J. C.; Courdavault, V.; O'Connor, S. E. *Science* **2018**, *360*, 1235−1238. (b) Qu, Y.; Safonova, O.; De Luca, V. *Plant J.* **2019**, *97*, 257−266. (c) Tatsis, E. C.; Carqueijeiro, I.; Dugé de Bernonville, T.; Franke, J.; Dang, T.-T. T.; Oudin, A.; Lanoue, A.; Lafontaine, F.; Stavrinides, A. K.; Clastre, M.; Courdavault, V.; O'Connor, S. E. *Nat. Commun.* **2017**, *8*, 316.
(42) (a) Besseau, S.; Kellner, F.; Lanoue, A.; Thamm, A. M. K.; Salim, V.; Schneider, B.; Geu-Flores, F.; Höfer, R.; Guirimand, G.; Guihur, A.; Oudin, A.; Glevarec, G.; Foureau, E.; Papon, N.; Clastre, M.; Giglioli-Guivarc'h, N.; St-Pierre, B.; Werck-Reichhart, D.; Burlat, V.; De Luca, V.; O'Connor, S. E.; Courdavault, V. *Plant Physiol.* **2013**, *163*, 1792−1803. (b) St-Pierre, B.; De Luca, V. *Plant Physiol.* **1995**, *109*, 131−139. (c) Schroder, G.; Unterbusch, E.; Kaltenbach, M.; Schmidt, J.; Strack, D.; De Luca, V.; Schroder, J. *FEBS Lett.* **1999**, *458*, 97−102.
(43) Calabi, L.; Danieli, B.; Lesma, G.; Palmisano, G. *J. Chem. Soc., Perkin Trans. 1* **1982**, 1371−1379.
(44) Eles, J.; Kalaus, G.; Greiner, I.; Kajtar-Peredy, M.; Szabo, P.; Keseru, G. M.; Szabo, L.; Szantay, C. *J. Org. Chem.* **2002**, *67*, 7255−7260.
(45) Kunesch, N.; Miet, C.; Poisson, J. *Chem. Informationsdienst* **1983**, *14*, 286−287.
(46) Prof. Xiao-Dong Luo reported the optical rotation of natural (−)-melodinine K was −130 (*c* 0.24, $CHCl_3$) in ref 22, which has been replaced by −97 (*c* 0.10, DMSO) by Prof. Xiang-Hai Cai by personal communication. We obtained an optical rotation of −66 (*c* 0.10, DMSO) for semisynthetic (−)-melodinine K.
(47) Cornforth, J. W. *Aust. J. Chem.* **1993**, *46*, 157−170.
(48) Bernhardt, P.; O'Connor, S. E. *Curr. Opin. Chem. Biol.* **2009**, *13*, 35−42.
(49) (a) Keasling, J. D. *ACS Chem. Biol.* **2008**, *3*, 64−76. (b) Smanski, M. J.; Zhou, H.; Claesen, J.; Shen, B.; Fischbach, M. A.; Voigt, C. A. *Nat. Rev. Microbiol.* **2016**, *14*, 135−149.
(50) Arnold, F. H. *Acc. Chem. Res.* **1998**, *31*, 125−131.
(51) Jinek, M.; Chylinski, K.; Fonfara, I.; Hauer, M.; Doudna, J. A.; Charpentier, E. *Science* **2012**, *337*, 816−821.
(52) Kärkäs, M. D.; Porco, J. A.; Stephenson, C. R. J. *Chem. Rev.* **2016**, *116*, 9683−9747.
(53) Yan, M.; Lo, J. C.; Edwards, J. T.; Baran, P. S. *J. Am. Chem. Soc.* **2016**, *138*, 12692−12714.
(54) Dalko, P. I.; Moisan, L. *Angew. Chem., Int. Ed.* **2004**, *43*, 5138−5175.
(55) Hegedus, L. S. *Transition Metals in the Synthesis of Complex Organic Molecules*; University Science Books, 1999.
(56) McQuade, D. T.; Seeberger, P. H. *J. Org. Chem.* **2013**, *78*, 6384−6389.
(57) Prier, C. K.; Rankic, D. A.; MacMillan, D. W. C. *Chem. Rev.* **2013**, *113*, 5322−5363.
(58) Yan, M.; Kawamata, Y.; Baran, P. S. *Chem. Rev.* **2017**, *117*, 13230−13319.
(59) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923−2925.

https://dx.doi.org/10.1021/acs.jnatprod.0c00310
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