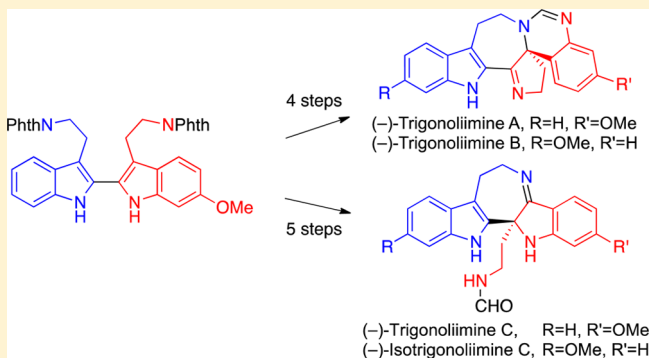


Total Synthesis, Stereochemical Assignment, and Biological Activity of All Known (–)-Trigonoliimines

Sunkyu Han,[†] Karen C. Morrison,[‡] Paul J. Hergenrother,[‡] and Mohammad Movassaghi^{*,†}[†]Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, United States[‡]Department of Chemistry, University of Illinois at Urbana–Champaign, Urbana, Illinois 61801, United States

S Supporting Information

ABSTRACT: A full account of our concise and enantioselective total syntheses of all known (–)-trigonoliimine alkaloids is described. Our retrosynthetic analysis of these natural products enabled identification of a single bistryptamine precursor as a precursor to all known trigonoliimines through a sequence of transformations involving asymmetric oxidation and reorganization. Our enantioselective syntheses of these alkaloids enabled the revision of the absolute stereochemistry of (–)-trigonoliimines A, B, and C. We report that trigonoliimines A, B, C and structurally related compounds showed weak anticancer activities against HeLa and U-937 cells.



INTRODUCTION

Bisindole alkaloids containing 2,2'-connectivity constitute a large number of natural products which includes fascaplycins and indolocarbazole glycopeptides.^{1,2} The potent antitumor activity of indolocarbazole glycopeptides such as rebeccamycin³ and staurosporine⁴ have stimulated the development of synthetic analogs of this family of natural products for the development of anticancer agents.⁵ Additionally, recent studies have shown possible application of these natural products as selective and sensitive chemical sensors based on their unique chemiluminescence properties.⁶

In 2010, Hao and co-workers reported the isolation of 2,2'-linked bistryptamine alkaloids (+)-trigonoliimines A (**1**) and B (**2**) along with (–)-trigonoliimine C (**3**) from the leaves of *Trigonostemon lii* Y. T. Chang collected in the Yunnan province of China.⁷ From their biological studies of these alkaloids, **1** showed modest activity in an anti-HIV assay (EC₅₀ = 0.95 μg/mL, TI = 7.9). Fascinated by their unique molecular architecture, we initiated a synthetic program aimed to provide a general solution to trigonoliimine alkaloids in enantiomerically enriched form. In 2011, we completed the first total synthesis of all known (–)-trigonoliimines A (**1**), B (**2**), and C (**3**) using a synthetic strategy based on asymmetric oxidation and reorganization of a single heterodimeric bistryptamine and revised the absolute stereochemistry of all (–)-trigonoliimines.⁸ In a contemporaneous study, Tambar and co-workers reported the total synthesis of (±)-trigonoliimine C,⁹ and shortly after, Hao and co-workers published the synthesis of the pentacyclic skeleton of (±)-trigonoliimine C using a similar oxidation and 1,2-alkyl shift strategy.¹⁰ Interest toward these fascinating natural products has continued and Shi and co-workers reported the synthesis of the desmethoxy hexacyclic skeleton

of (±)-trigonoliimines A and B using carbanion mediated seven-membered ring formation as a key step.¹¹ In 2012, Zhu and co-workers reported the total synthesis of (±)-trigonoliimine B using a late-stage Bischler–Napieralski reaction.¹² This strategy was also applied in Hao and co-workers' synthesis of the hexacyclic skeleton of (±)-trigonoliimines A and B.¹³ Most recently, Hao and co-workers reported the total synthesis of (±)-trigonoliimine A using a Strecker reaction for the formation of the C20 tetrasubstituted carbon center and a Houben–Hoesch¹⁴ type reaction for the seven-membered ring forming cyclization.¹⁵ Herein, we detail the full account of our synthetic approach to the trigonoliimine alkaloids, which currently remains the only solution to access all known trigonoliimines in enantiomerically enriched form. We also report anticancer activities of trigonoliimines A–C and structurally related alkaloids against HeLa (human cervical cancer) and U-937 (human lymphoma) cell lines.

RESULTS AND DISCUSSION

Design Plan for the Total Synthesis. Oxidation and rearrangement of 2,3-disubstituted indoles has served as an efficient strategy to access the indoxyl moiety and has been applied in the total synthesis of various alkaloids.¹⁶ In 2008, our laboratory proposed a synthetic strategy to access calycanthaceous alkaloids through oxidation and rearrangement of a 2,2'-bistryptamine derivative.^{17,18} Our continued interest in this area yielded an efficient and stereocontrolled synthetic strategy involving oxidation and rearrangement of 2,3-disubstituted indoles, especially those with an aryl substituent at the 2-

Received: September 12, 2013

Published: October 15, 2013

position, for efficient access to oxindole products.¹⁹ Despite outstanding advancements in the area of asymmetric oxidation,²⁰ enantioselective oxidation of 2,3-disubstituted indoles remains a challenging problem.^{21,22} Recently, in collaboration with the Miller group, we reported an asymmetric oxidation of 2,3-disubstituted indole for the formation of enantiomerically enriched hydroxyindolenines using an aspartyl based peptide catalyst.^{23,24}

With these considerations in mind, the molecular structure of trigonoliimine alkaloids (Figure 1) drew our immediate

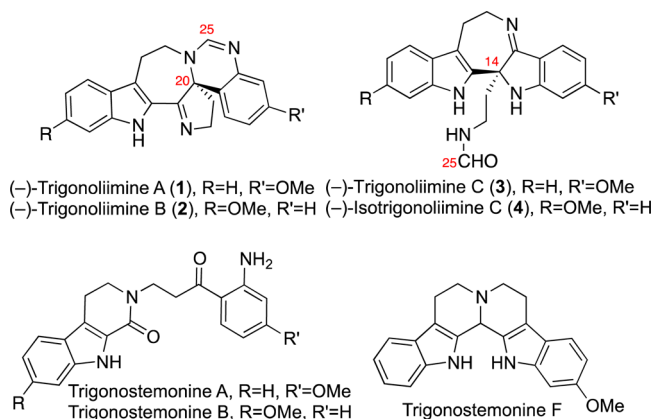


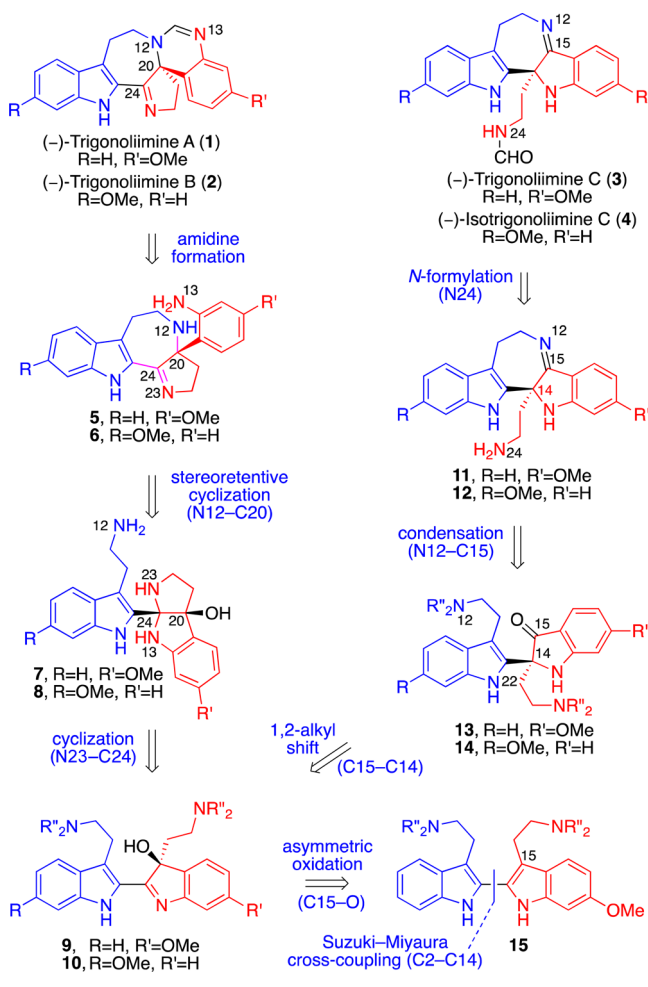
Figure 1. Structure of (-)-trigonoliimines A–C (1–3) with revised absolute stereochemistry (ref 8) and other representative trigonostemon alkaloids.

attention upon their isolation due to their possible structural relevance to intermediates in our primary investigations toward chimonanthine (S6, Scheme S1, Supporting Information).¹⁷ Our unified strategy for the enantioselective total synthesis of all known trigonoliimines was based on the hypothesis that bistryptamine heterodimer **15** may serve as a common biosynthetic precursor to these alkaloids (Scheme 1). We envisioned that mono-oxidation of bistryptamine **15** would generate hydroxyindolenines **9** and **10**, intermediates that could serve as a branching point from which to access the two distinct molecular architectures present in the trigonoliimine alkaloid family (Scheme 1).

To assemble the amidine moiety present in trigonoliimines A (1) and B (2), we designed a late stage *N*-formylation and intramolecular condensation of azepanes **5** and **6**. We planned to access azepanes **5** and **6** from animals **7** and **8** via ionization and *stereoretentive* intramolecular trapping of carbinol C20 by N12 and subsequent rearrangement. The requisite *cis*-fused animals **7** and **8** could result from intramolecular cyclization of hydroxyindolenines **9** and **10**, respectively. Retrosynthetic analysis of trigonoliimine C (3) and isotrigonoliimine C (4) commenced with formylations of N24 in imines **11** and **12**. The seven-membered imine moiety would be derived from an intramolecular condensation of N12 to C15 of indoxyls **13** and **14**, respectively. Indoxyls **13** and **14** would be accessible via a stereospecific Wagner–Meerwein type 1,2-alkyl shift²⁵ of hydroxyindolenines **9** and **10**, the common intermediates that serve as gateways to access all trigonoliimines.

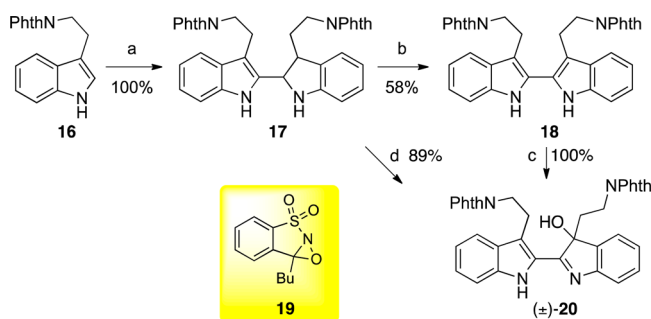
Our biosynthetically inspired retrosynthesis predicted the *S*-configuration on the stereogenic centers of trigonoliimines A–C (1–3) resulting from common hydroxyindolenines **9** and **10** with *S*-configuration at their stereogenic centers (Scheme 1). Interestingly, the isolation report predicted the *S*-configuration

Scheme 1. Retrosynthetic Analysis of (-)-Trigonoliimines A–C (1–3) and Isotrigonoliimine C (4)



at the stereogenic centers of (+)-trigonoliimines A and B (class A) and the *R*-configuration at the stereogenic center of (-)-trigonoliimine C (class B),⁷ suggesting that these two classes of trigonoliimines stem from enantiomeric hydroxyindolenine precursors. This provided further impetus to develop an enantioselective synthesis of these alkaloids as we could begin to address these questions by accessing synthetic samples of enantiomerically enriched trigonoliimines A–C.

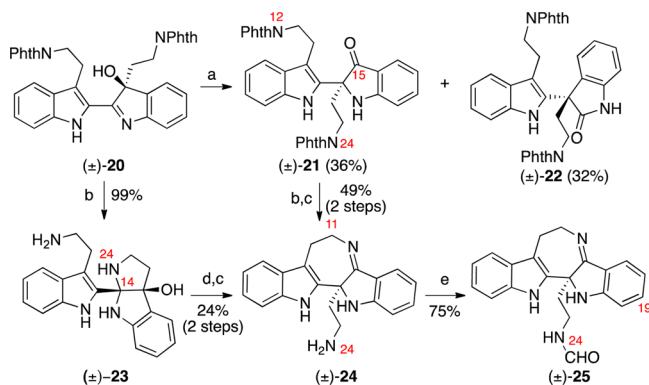
Synthesis of (±)-19-Desmethoxytrigonoliimine C. In the initial stages of our studies, in order to quickly evaluate the plausibility of our retrosynthetic analysis of trigonoliimines, we focused on a desmethoxytrigonoliimine model system starting from tryptamine **16** (Scheme 2).²⁶ Recently, Hao and Liu reported their synthetic studies of a similar desmethoxy model system.¹⁰ When phthalimide protected tryptamine **16** was treated with trifluoroacetic acid (TFA), the desired dimer **17** was formed in quantitative yield.²⁷ Heterodimer **17** could be oxidized to homodimeric 2,2'-bistryptamine **18** in the presence of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in tetrahydrofuran in 58% yield. Subsequent treatment of bistryptamine **18** with Davis' oxaziridine **19** afforded hydroxyindolenine (±)-**20** in quantitative yield. After further attempts to optimize this synthetic sequence, we found that treatment of heterodimer **17** with Davis' oxaziridine **19** (2.2 equiv) afforded the desired hydroxyindolenine (±)-**20** in 89% yield. Notably, treatment of **17** with excess Davis' oxaziridine **19** (4 equiv) did

Scheme 2. Synthesis of Hydroxyindolenine (\pm)-20^a

^aConditions: (a) TFA, 23 °C; (b) DDQ, THF, 23 °C; (c) **19** (1.1 equiv), CH₂Cl₂, 23 °C; (d) **19** (2.2 equiv), CH₂Cl₂, 23 °C.

not result in the formation of overoxidized products consistent with our original hypothesis.¹⁷

With ready access to hydroxyindolenine (\pm)-20, we investigated the Wagner–Meerwein type 1,2-alkyl shift²⁵ to form indoxyl (\pm)-21, which possesses the structural backbone of trigonoliimine C (**3**, Scheme 1). When hydroxyindolenine (\pm)-20 was treated with scandium trifluoromethanesulfonate [Sc(OTf)₃] in toluene at 85 °C,¹⁸ the desired indoxyl **21** was obtained in 36% yield along with oxindole **22** (32% yield, Scheme 3) as a byproduct. Interestingly, treatment of

Scheme 3. Synthesis of (\pm)-19-Desmethoxytrigonoliimine C (**25**)^a

^aConditions: (a) Sc(OTf)₃, PhMe, 85 °C; (b) NH₂NH₂·H₂O, MeOH, 80 °C; (c) Ti(OEt)₄, THF, 40 °C; (d) Sc(OTf)₃, MeCN, PhMe (2:1), 81 °C;²⁸ (e) *N*-formylimidazole, THF, 23 °C.

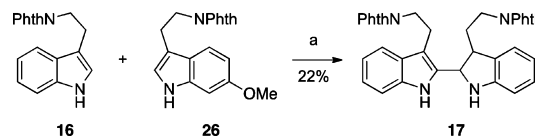
hydroxyindolenine **20** with 20 equiv of hydrazine in refluxing methanol afforded the cyclized hydroxyaminal **23** as a major isomer in 99% yield. While the ¹H NMR analysis of aminor **23** in deuterated chloroform was consistent with the structure shown in Scheme 3, ¹H NMR analysis of the same sample in deuterated methanol revealed the presence of multiple species consistent with structural flexibility of this intermediate (vide infra).

With synthetic access to indoxyl (\pm)-21, we were set to answer the question of whether the intramolecular condensation would occur between N12–C15 or N24–C15. Hydrazinolysis of indoxyl (\pm)-21 and subsequent treatment of the resulting crude mixture with titanium tetraethoxide yielded seven-membered imine (\pm)-24 in 49% yield over two steps (vide infra). Notably, we did not observe the formation of the 5-membered imine resulting from the condensation of N24

with the ketone moiety (vide infra), but noticed a broadening of ¹H NMR peaks at C11 possibly due to a rapid equilibration between imine and aminor structures. Interestingly, imine (\pm)-24 could also be accessed from hydroxyaminal (\pm)-23 upon heating with Sc(OTf)₃²⁸ followed by treatment of the resulting indoxyl intermediate with titanium ethoxide. When pentacycle **24** was treated with *N*-formylimidazole, N24 was formylated to give (\pm)-19-desmethoxy-trigonoliimine C (**25**) in 75% yield. ¹H NMR peaks of the formylated product (\pm)-25 were sharper and better resolved compared to intermediate **24**, consistent with the decreased nucleophilicity of N24.

Synthesis of the Heterodimeric 2,2'-Bistryptamine.

Promising results described above in the initial phase of this program prompted investigation of the synthesis of heterodimeric 2,2'-bistryptamine **15** (Scheme 1), which can be derived from the union of tryptamine **16** and 6-methoxytryptamine **26**.^{27b,29} Initial heterodimerization attempts with tryptamine **16** and 6-methoxytryptamine **26** under ionic conditions yielded dimeric tryptamine **17** as a single diastereomer in 22% yield (Scheme 4). However, no desired heterodimer could be

Scheme 4. Attempted Heterodimerization of Tryptamines **16** and **26** under Ionic Conditions^a

^aConditions: (a) TFA, 23 °C.

obtained in any synthetically useful amount. Hence, for the selective union of two tryptamine subunits, we envisioned the use of a metal-catalyzed and directed cross-coupling reaction.

The union of two indole subunits at their 2,2'-junction by virtue of a transition-metal-catalyzed cross-coupling reaction is well-precedented.^{30,31} However, to the best of our knowledge, there had been no reports that cross-couple two N1-unprotected indole subunits. Hence, we focused our attention on direct assembly of two N1-unprotected tryptamine subunits by application of the Suzuki–Miyaura cross-coupling reaction.³²

For the synthesis of the boronate coupling partner, we planned to achieve a direct C2 borylation of 6-methoxytryptamine **26** by using an iridium-based catalyst system developed by Ishiyama, Miyaura, and Hartwig (Table 1).³³ Initial attempts with pinacolborane (1 equiv) in the presence of 5 mol % of iridium catalyst and 10 mol % of bispyridine ligand in tetrahydrofuran at 70 °C did not afford any desired product (Table 1, entry 1). When 6-methoxytryptamine **26** was treated with pinacolborane (2.6 equiv) at 80 °C, only diborylated product **28** was obtained in 93% yield (Table 1, entry 2). After optimization (Table 1, entries 2–5), we found that 6-methoxytryptamine **26** could be converted selectively to monoborylated tryptamine **27** in 67% yield by exposure to pinacolborane (2.2 equiv), 10 mol % of iridium catalyst, and 10 mol % of bispyridine ligand in dichloromethane at 23 °C (Table 1, entry 6).

We next sought conditions that enabled the efficient union of two tryptamine subunits to form 2,2'-bistryptamine **32**. Initial cross-coupling attempts using various palladium sources in conjunction with XPhos³⁴ and potassium phosphate in toluene–water mixture at 110 °C provided the desired product

Table 1. Optimization of the Iridium-Catalyzed Borylation at C2 of 6-Methoxytryptamine 26

entry	HBPin (equiv)	Ir catalyst (mol %)	bipyridyl ligand (mol %)	temp (°C)	solvent	result ^a
1	1	5	10	70	THF	no reaction
2	2.6	3	6	80	THF	28: 93%
3	2	5	10	45	THF	27:28 = 2.2:1 ^b , 48%
4	2	5	10	45	CH ₂ Cl ₂	27: 55%
5	2.5	5	10	45	CH ₂ Cl ₂	27:28 = 4.5:1 ^b , 66%
6	2.2	10	10	23	CH ₂ Cl ₂	27: 67%

^aIsolated yield. ^bRatio of 27 to 28 was determined by ¹H NMR.

32, albeit in low and variable yields (7–44%). Hence, we sought to perform this carbon–carbon bond formation under milder conditions. When bromide **29** and boronic ester **31** were treated with the Buchwald biphenyl precatalyst **31** and potassium phosphate in toluene–water mixture at 23 °C,³⁵ the desired bistryptamine heterodimer **32** was formed in 40% yield (Table 2, entry 1). We discovered that the addition of a halophilic silver salt to the reaction mixture induced a notable increase in product yield (Table 2, entries 6 and 7).³⁶ Notably, the use of Ag₂CO₃ (2.0 equiv) as the sole base resulted in a lower yield (33%, Table 2, entry 8) of the desired product **32**. When we used Ag₃PO₄ (2.0 equiv) as the sole base additive, we were able to obtain the desired product in 63% yield (Table 2, entry 9), possibly due to the 2-fold role of this base as both a halophile and ideal base additive.

Oxidation of Bistryptamine 32. Unlike the oxidation of homodimeric bistryptamine **18** used in our model system (Scheme 2), **32** consists of two indole rings with different electronic properties, raising an interesting question regarding the regioselectivity of the following oxidation event. When bistryptamine **32** was treated with Davis' oxaziridine **19** at 0 °C, we obtained hydroxyindolenines (±)-**36** and (±)-**38** (36:38 = 1:1) in 91% combined yield (Table 3, entry 1). Interestingly, exposure of neat bistryptamine **32** to air for 12 d resulted in the formation of hydroxyindolenines (±)-**36** and (±)-**38** (36:38 = 1.5:1) in 27% combined yield along with 65% of unreacted starting material **32** (Table 3, entry 2). Notably, the presence of regioisomeric pairs is commonly observed in the trigonostemon alkaloids family (Figure 1).³⁷ Hence, we reasoned that the formation of both regioisomers during the oxidation of bistryptamine precursor might explain the biodiversity observed in the trigonostemon alkaloids family. Notably, modification of bistryptamine **32** through an N1-protection with various electron-withdrawing groups greatly altered their chemical properties toward oxidation. Phenylsulfonyl, methylsulfonyl, and *tert*-butoxycarbonyl protection of N1 of **32** completely suppressed their oxidation under the conditions previously

Table 2. Optimization of the Suzuki–Miyaura Cross-Coupling Reaction for the Formation of 2,2'-Bistryptamine 32

entry	halide	base	solvent	yield ^b (%)
1	29	K ₃ PO ₄	PhMe, H ₂ O (5:1)	40
2	29	KOH	PhMe, H ₂ O (5:1)	30
3	29	Tl ₂ CO ₃	THF, H ₂ O (5:1)	8
4	30	K ₃ PO ₄	PhMe, H ₂ O (5:1)	31
5	30	K ₂ CO ₃	PhMe, H ₂ O (5:1)	19
6 ^c	30	K ₃ PO ₄ + Ag(OTf)	PhMe, H ₂ O (5:1)	46
7 ^d	30	K ₃ PO ₄ + Ag ₂ CO ₃	PhMe, H ₂ O (5:1)	60
8	30	Ag ₂ CO ₃	PhMe, H ₂ O (5:1)	33
9	30	Ag ₃ PO ₄	PhMe, H ₂ O (5:1)	63
10	29	Ag ₃ PO ₄	PhMe, H ₂ O (5:1)	64
11 ^e	30	Ag ₃ PO ₄	PhMe, H ₂ O (5:1)	56

^aUniform conditions unless otherwise noted: precatalyst **31** (20 mol %), base (2.0 equiv), halide (1 equiv), boronate (1.2 equiv), 23 °C. ^bIsolated yield (%). ^cK₃PO₄ (2.0 equiv), Ag(OTf) (6.0 equiv). ^dK₃PO₄ (2.0 equiv), Ag₂CO₃ (1.0 equiv). ^ePrecatalyst **31** (10 mol %).

described using Davis' oxaziridine **19** (Table 3, entries 3, 4, and 6). When substrate **35** was treated with DMDO, the desired hydroxyindolenine could be formed as a single regioisomer, albeit in only 32% yield (Table 3, entry 7). However, substrate **35** was found to be inert under representative asymmetric oxidation conditions developed by Shi³⁸ and Sharpless³⁹ (Table 3, entries 8 and 9).

When unprotected bistryptamine **32** was oxidized under Sharpless³⁹ or Miller²³ asymmetric oxidation conditions, hydroxyindolenines (+)-**36** and (+)-**38** could be obtained albeit in low enantiomeric excess (Table 3, entries 10 and 11). Gratifyingly, we discovered that (+)-((8,8-dichlorocamphoryl)-sulfonyl) oxaziridine **41**, originally reported by the Davis research group,⁴⁰ afforded hydroxyindolenines (+)-**36** and (+)-**38** (36:38 = 2.2:1) in 95% yield and with an outstanding level of enantioselection for both regioisomers (96% ee, Table 3, entry 12).⁸ We envisioned that hydroxyindolenine (+)-**36** would serve as a synthetic precursor for trigonoliimines A (**1**) and C (**3**) and hydroxyindolenine (+)-**38** as a precursor for trigonoliimine B (**2**) and isotrigonoliimine C (**4**).

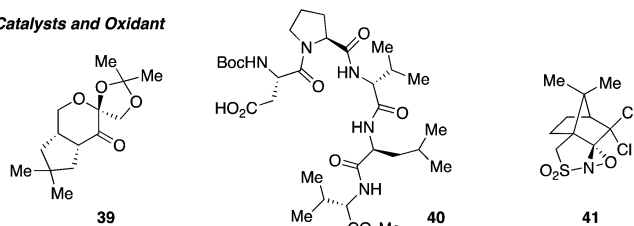
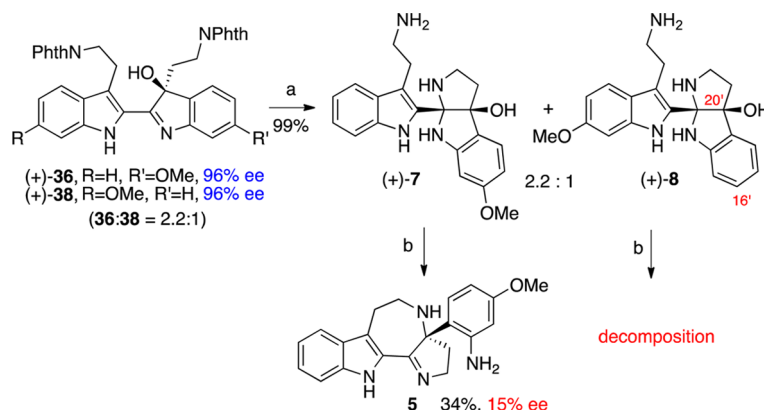
Total Synthesis of (–)-Trigonoliimines A and B. We next aimed to synthesize (–)-trigonoliimines A (**1**) and B (**2**) from hydroxyindolenines (+)-**36** and (+)-**38**. Consistent with our model studies (Scheme 3), hydrazinolysis of hydroxyindolenines (+)-**36** and (+)-**38** afforded the *cis*-fused amins (+)-**7** and (+)-**8** (7:8 = 2.2:1) in 99% combined yield through intramolecular cyclizations (Scheme 5). Amins (+)-**7** and (+)-**8** were separable at this stage to enable their chemical examination independently. However, during scale up they were easily processed together and separated at a more practical stage in the synthesis (vide infra, Scheme 7).

Table 3. Oxidation of 2,2'-Bistryptamine Derivatives

entry	substrate	condition	yield (product)	% ee (product)
1	32	19, CH ₂ Cl ₂ , 0 °C	91% (36:38 = 1:1) ^a	
2	32	air, neat, 23 °C, 12 d	27% (36:38 = 1.5:1) ^{a,b}	
3	33	19, CH ₂ Cl ₂ , 23→50 °C	no reaction	
4	34	19, CH ₂ Cl ₂ , 23 °C	no reaction	
5	34	<i>m</i> -CPBA, CH ₂ Cl ₂ , 23 °C, 3 h	decomposition	
6	35	19, CH ₂ Cl ₂ , 23→65 °C	no reaction	
7	35	DMDO, acetone, 23 °C, 11 h	32% (37)	
8	35	Shi epoxidation ^c	no reaction	
9	35	Sharpless asymmetric dihydroxylation ^d	no reaction	
10	32	Sharpless asymmetric dihydroxylation ^d	39% (36:38 = 1.7:1) ^a	<3 (36), <3 (38)
11	32	40 (10 mol %), DMAP, H ₂ O ₂ , DIC, CHCl ₃ , 0 °C, 24.5 h	49% (36:38 = 1.3:1) ^a	20 (36), <5 (38)
12	32	41, CH ₂ Cl ₂ , 30→23 °C	95% (36:38 = 2.2:1) ^a	96 (36), 96 (38)

^aThe ratio of 36 to 38 was determined by ¹H NMR. ^b65% of unreacted starting material 33 was isolated. ^cShi catalyst (39), Na₂B₂O₇·H₂O, *n*-Bu₄NHSO₄, oxone, K₂CO₃, EDTA, H₂O, DMM, MeCN. ^dAD-mix- α , methanesulfonamide, *t*-BuOH, H₂O.

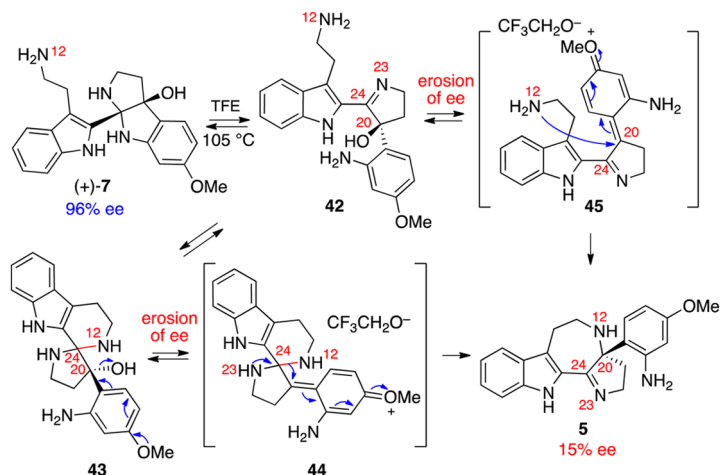
Catalysts and Oxidant

Scheme 5. Formation and Attempted Stereoselective Rearrangement of Hydroxyaminals (+)-7 and (+)-8^a

^aConditions: (a) NH₂NH₂·H₂O, MeOH, 80 °C, 99%; (b) TFE, 105 °C.

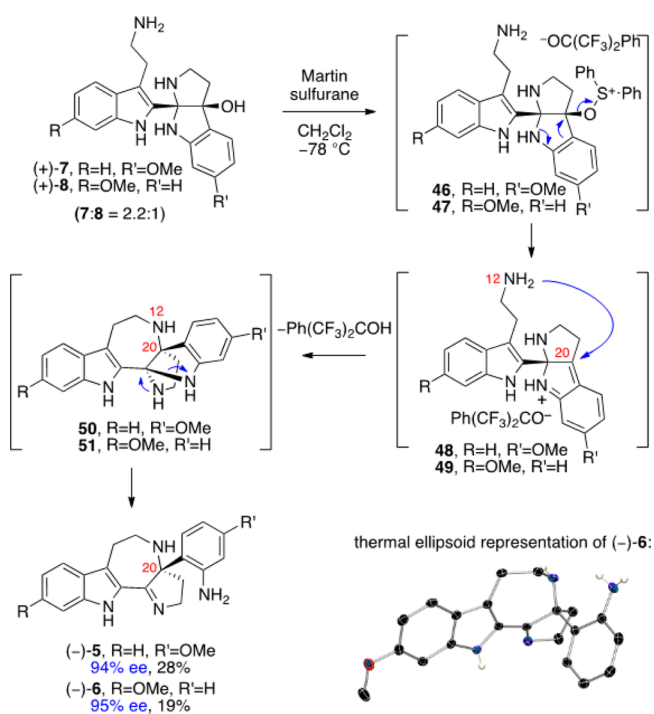
When aminal (+)-7 (96% ee) was heated at 105 °C in trifluoroethanol (TFE), rearranged azepane 5 was obtained in 34% yield, albeit with significantly eroded enantiomeric excess (15% ee). On the other hand, when aminal (+)-8 (96% ee) was subjected to identical reaction conditions, only decomposition of the starting material was observed. In the latter case, we reasoned that the absence of the electron donating methoxy group at C16' results in less efficient ionization of hydroxyl group at C20'. While the NMR analysis of aminals (+)-7 and

(+)-8 in deuterated chloroform was consistent with *cis*-fused pentacyclic structures, analysis of the same synthetic samples in deuterated methanol showed the presence of multiple species, consistent with formation of aminal and imine isomers (Scheme 6). We rationalized that the erosion of ee was due to a low level of diastereoselection during the N12–C24 bond formation followed by reversible ionization of C20 in the form of intermediate 44. Alternatively, the C20 dehydroxylation of intermediate 42, or the corresponding solvent adduct, would

Scheme 6. Plausible Mechanism for the Erosion of Enantiomeric Excess during the Formation of **5**

result in overall N12–C20 bond formation with low level of stereochemical control (Scheme 6).

Based on these observations, we reasoned that the activation of the hydroxyl groups of aminals (+)-**7** and (+)-**8** under mild conditions in an aprotic solvent would be ideal in order for this transformation to proceed with minimal erosion of enantiomeric excess. Hence, we treated a solution of aminals (+)-**7** and (+)-**8** (7:8 = 2.2:1) in dichloromethane with Martin sulfurane⁴¹ at -78°C , which provided the desired azepanes (–)-**5** and (–)-**6** in 47% combined yield (28% and 19% yield, respectively, Scheme 7). Azepanes (–)-**5** and (–)-**6** could be readily separated using flash column chromatography at this stage. Importantly, azepanes (–)-**5** and (–)-**6** were obtained with minimal erosion of enantiomeric excess (94% ee and 95% ee, respectively). A plausible mechanism for this transformation involves an activation of hydroxyl moiety of the *cis*-fused

Scheme 7. Stereoselective Formation of Azepanes (–)-**5** and (–)-**6**

aminals (+)-**7** and (+)-**8** upon treatment with Martin sulfurane to give iminium ions **48** and **49**. The fused bicyclic structure of intermediates **48** and **49** allows N12 to attack only from the α -face, resulting in a *stereoretentive* cyclization. The expulsion of the aniline nitrogen from aminal intermediates **50** and **51** would result in the formation of azepanes (–)-**5** and (–)-**6**. X-ray crystal structure analysis of pentacycle (–)-**6** (Scheme 7), the direct precursor of (–)-trigonoliimine B (**2**), unambiguously confirmed the molecular structure and assigned the *S*-configuration at C20, corroborating the stereoretentive mode of N12–C20 cyclization.

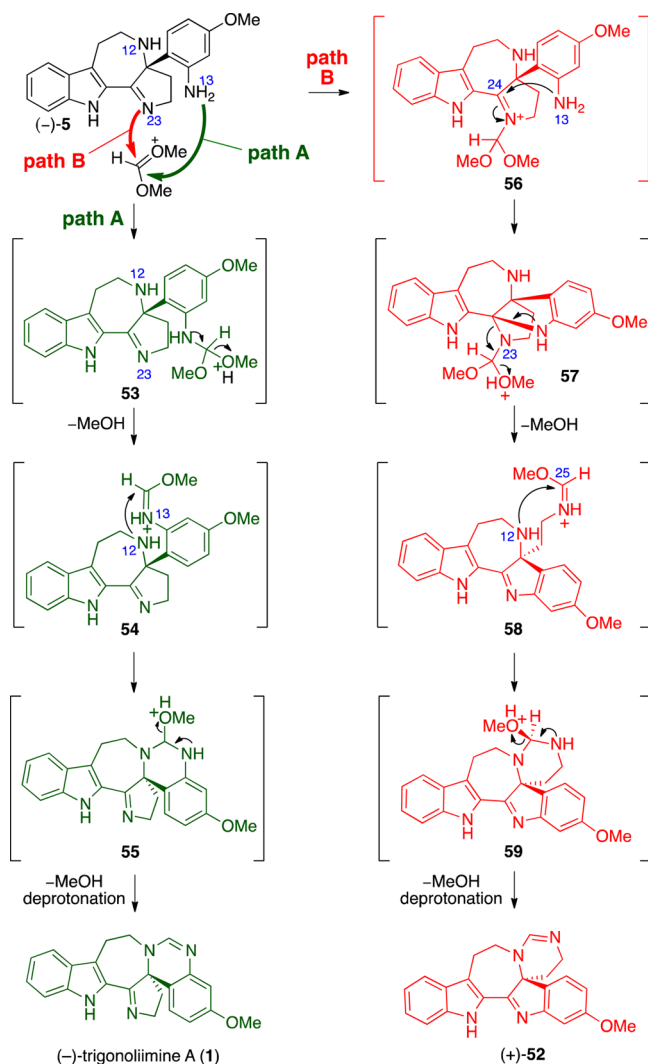
For the completion of the total synthesis of (–)-trigonoliimine A (**1**), we initially treated the azepane (–)-**5** with a catalytic amount of pyridinium *p*-toluenesulfonate (PPTS) in trimethylorthoformate at 50°C (Table 4, entry 1).⁴² Under

Table 4. Completion of the Total Synthesis of (–)-Trigonoliimine A (**1**)

entry	PPTS (equiv)	orthoformate + solvent	temp ($^{\circ}\text{C}$)	1 (%)	52 (%)
1	0.1	$\text{CH}(\text{OMe})_3$ (solvent)	50	16	61
2	2	$\text{CH}(\text{O-}i\text{-Pr})_3$, CH_2Cl_2 (1:1)	45	49	37
3	3	$\text{CH}(\text{O-}i\text{-Pr})_3$ (10 equiv), CH_2Cl_2	23	82	15

these conditions, we were able to obtain the first synthetic sample of (–)-trigonoliimine A (**1**), albeit in low yield (16%). A possible pathway for the formation of (–)-trigonoliimine A (**1**) (path A) and byproduct (+)-**52** (path B) from azepane (–)-**5** is shown in Scheme 8. Conversion of (–)-**5** to (–)-**1** involves imide formation at N13 followed by cyclization with N12.⁴³ Interestingly, the major product formed under these conditions was hexacyclic imine **52** (61%, Scheme 8). Initial reaction of N23 of azepane (–)-**5** with the oxocarbenium ion generated from trimethylorthoformate may form the iminium ion **56** (path B), and the resulting electrophilic C24 would be trapped with N13 to form intermediate **57**. The N23 released

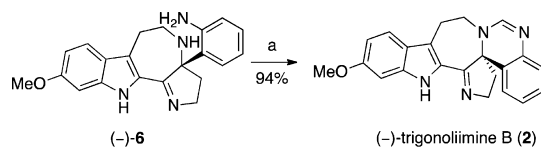
Scheme 8. Plausible Mechanism for the Formation of (–)-Trigonoliimine A (1) and Byproduct (+)-52



from the amination moiety of 57 will give the pentacyclic intermediate 58. Intramolecular cyclization resulting from the N12 attack to the imide moiety of 58 followed by methanol elimination would yield the isomeric hexacyclic byproduct (+)-52.

In order to suppress the undesired reactivity at N23 of (–)-5, we examined its pretreatment with PPTS (2 equiv) followed by exposure to triisopropyl orthoformate, a more sterically crowded reagent. As expected, the desired (–)-trigonoliimine A (1) was obtained as a major product (49%, Table 4, entry 2). After further optimization, we found that pretreatment of azepane (–)-5 with PPTS (3 equiv) in dichloromethane at 23 °C, followed by addition of triisopropylorthoformate (10 equiv) afforded the desired (–)-trigonoliimine A (1) in 82% yield $[[\alpha]^{24}_D = -294$ (c 0.24, CHCl_3)] (Table 4, entry 3). Under identical reaction conditions, azepane (–)-6 was converted to (–)-trigonoliimine B (2) in 94% yield $[[\alpha]^{24}_D = -352$ (c 0.32, CHCl_3), Scheme 9]. All ^1H and ^{13}C NMR data for our synthetic (–)-trigonoliimines A (1) and B (2) matched those provided in the isolation report,⁷ confirming the molecular structure of these alkaloids.

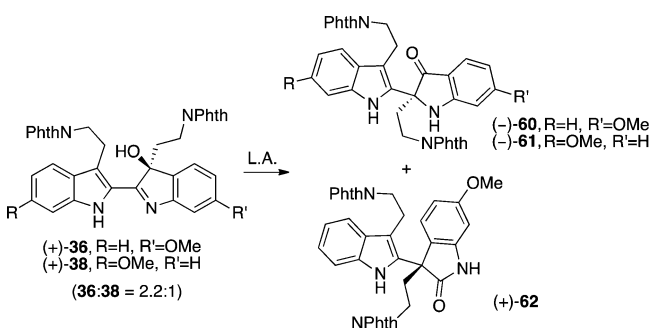
Total Synthesis of (–)-Trigonoliimine C and (–)-Iso-trigonoliimine C. We also wished to gain access to

Scheme 9. Total Synthesis of (–)-Trigonoliimine B (2)^a

^aConditions: (a) $\text{CH}(\text{O}-i\text{-Pr})_3$, PPTS, CH_2Cl_2 , 23 °C.

(–)-trigonoliimine C (3) and (–)-isotrigonoliimine C (4) from the same versatile hydroxyindolenines (+)-36 and (+)-38 described above via a divergent synthetic path employing a Wagner–Meerwein-type 1,2-alkyl rearrangement.²⁵ Literature precedent and prior synthetic studies from our laboratory suggested that hydroxyindolenines derived from the oxidation of 2-aryl-3-alkyl-disubstituted indoles can rearrange to the corresponding indoxyls, oxindoles, or mixtures of both depending on the reaction conditions.^{19,44} Hence, we turned our attention to finding conditions that would favor the selective formation of indoxyls (–)-60 and (–)-61 over the oxindole byproducts (Table 5). Initially, we examined various lanthanide trifluoromethanesulfonates as additives for the rearrangement of hydroxyindolenines (+)-36 and (+)-38

Table 5. Rearrangement of Hydroxyindolenines (+)-36 and (+)-38 in the Presence of Various Lewis Acids



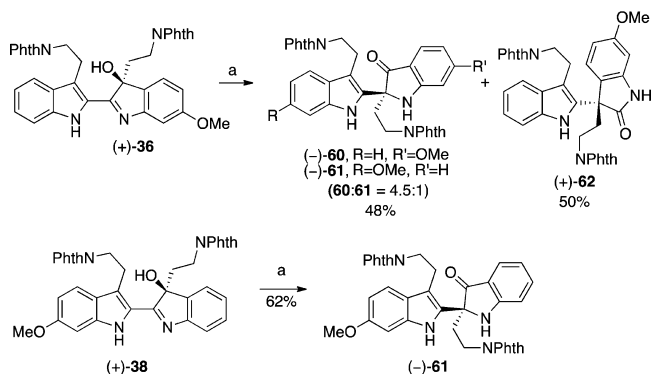
entry	L.A. (equiv)	solvent	temp (°C)	time (h)	% yield of indoxyls (60:61) ^a
1	Sc(OTf) ₃ (3)	MeCN	65	45	12 (60 only)
2	Dy(OTf) ₃ (3)	MeCN	65	45	35 (1.7:1)
3	La(OTf) ₃ (3)	MeCN	65	39	50 (1.6:1)
4	Pr(OTf) ₃ (3)	MeCN	65	14	42 (2:1)
5	Ho(OTf) ₃ (3)	MeCN	65	39	44 (2:1)
6	Eu(OTf) ₃ (3)	MeCN	65	14	50 (1.3:1)
7	Nd(OTf) ₃ (3)	MeCN	65	14	41 (1.4:1)
8	Gd(OTf) ₃ (3)	MeCN	65	14	45 (1.4:1)
9	Eu(OTf) ₃ (3)	PhMe, MeCN (1:1)	65	24	46 (1.3:1)
10	Eu(OTf) ₃ (3)	MeOH	65	45	no reaction
11	La(OTf) ₃ (3)	PhMe	90	14	40 (1:1.2)
12	La(OTf) ₃ (3)	THF	65	55	49 (1.3:1)
13	La(OTf) ₃ (3)	$\text{ClCH}_2\text{CH}_2\text{Cl}$	65	19	49 (1:1.2)
14	La(OTf) ₃ (1)	$\text{ClCH}_2\text{CH}_2\text{Cl}$	65	19	57 (1:1.1)
15	La(OTf) ₃ (0.5)	$\text{ClCH}_2\text{CH}_2\text{Cl}$	65	15	52 (1:1.1)
16	La(OTf) ₃ (0.5)	PhMe	80	19	56 (1:1.2)
17	La(OTf) ₃ (0.5)	$\text{ClCH}_2\text{CH}_2\text{Cl}$	23	72	no reaction
18	Eu(OTf) ₃ (0.5)	MeCN	65	18	42 (1.5:1)
19	La(OTf) ₃ (0.5)	MeCN	65	42	52 (1.3:1)
20	Eu(OTf) ₃ (0.5)	MeCN	72	38	48 (1.2:1)

^aThe ratio of 60 to 61 was determined by ^1H NMR.

(36:38 = 2.2:1, Table 5). In general, we were able to obtain indoxyls (–)-60 and (–)-61 in moderate yield (Table 5, entries 3–9, 11–16, 18–20). The use of methanol as solvent did not provide the desired indoxyl products in the presence of europium trifluoromethanesulfonate at 65 °C (Table 5, entry 10). We observed that the choice of solvent had a significant effect in products ratio. When acetonitrile, acetonitrile–toluene mixture, or tetrahydrofuran was used as solvent indoxyl (–)-60 was obtained as a major product. On the other hand, when toluene or 1,2-dichloroethane was used as solvent indoxyl (–)-61 was formed in slight excess (Table 5, entries 11, 13–16). The major byproduct observed in these reactions was oxindole (+)-62 (34% for Table 5, entry 16). Oxindole byproduct (+)-62 did not show any reactivity upon treatment with three equivalents of lanthanum trifluoromethanesulfonate in 1,2-dichloroethane at 65 °C. Similarly, when the indoxyl (–)-60 was treated with three equivalents of lanthanum trifluoromethanesulfonate in 1,2-dichloroethane at 65 °C, no reactivity was observed.

Due to the nontrivial separation of regioisomeric hydroxyindolenines (+)-36 and (+)-38 by flash column chromatography, prep-HPLC enabled us to access pure samples of both hydroxyindolenines (+)-36 and (+)-38 for their separate characterization and evaluation of chemical properties. When hydroxyindolenine (+)-36 was treated with three equivalents of europium trifluoromethanesulfonate in acetonitrile at 72 °C, indoxyls (–)-60 and (–)-61 (48%, 60:61 = 4.5:1) and oxindole (+)-62 (50%) were obtained (Scheme 10).⁴⁵ On the other

Scheme 10. Eu(OTf)₃-Mediated Rearrangement of (+)-Hydroxyindolenines (+)-36 and (+)-38^a

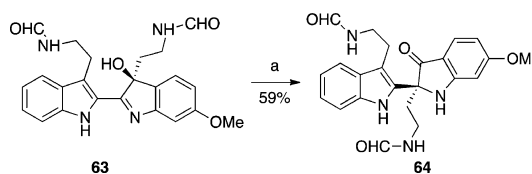


^aConditions: (a) Eu(OTf)₃, MeCN, 72 °C.

hand, upon treatment with three equivalents of europium trifluoromethanesulfonate in acetonitrile at 72 °C, hydroxyindolenine (+)-38 afforded indoxyl (–)-61 as the sole product in 62% yield (Scheme 10).

While most of the reaction conditions that utilized lanthanide Lewis acids produced oxindoles as byproducts during the rearrangement of hydroxyindolenines (+)-36 and (+)-38, promising results were obtained during our study directed toward the total synthesis of (–)-trigonoliimine A (1). Inspired by the transformation of aminal (+)-7 to azepane 5 upon heating in TFE (Scheme 5), we attempted to directly access trigonoliimine A (1) by exposing formylated hydroxyindolenine 63 under the same reaction conditions. Interestingly, when the formylated hydroxyindolenine 63 was heated in TFE at 105 °C, indoxyl 64 was selectively formed in 59% yield (Scheme 11).

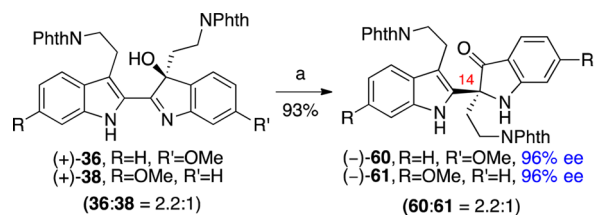
Scheme 11. Selective Rearrangement of Hydroxyindolenine 63 to Indoxyl 64^a



^aConditions: (a) TFE, 105 °C.

Based on these observations, we heated hydroxyindolenines (+)-36 and (+)-38 (36:38 = 2.2:1) in TFE at 102 °C and obtained indoxyls (–)-60 and (–)-61 (60:61 = 2.2:1) selectively in 93% yield without any formation of the undesired oxindole (Scheme 12). X-ray crystal structure analysis of the enantiomerically enriched (96% ee) C18-bromide derivative of (–)-60 enabled us to unequivocally assign the S-configuration at C14.⁸

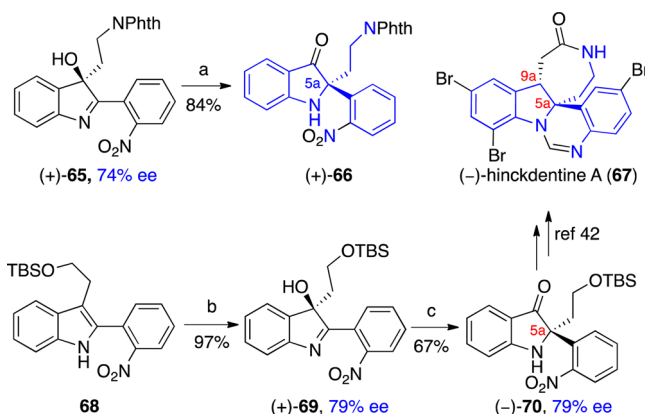
Scheme 12. Selective 1,2-Alkyl Shift of Hydroxyindolenines (+)-36 and (+)-38^a



^aConditions: (a) TFE, 102 °C.

The synthetic utility of asymmetric oxidation followed by stereospecific rearrangement of 2-aryl-3-alkyl-indole derivatives holds great promise for other applications as illustrated in Scheme 13. The 1,2-alkyl shift of hydroxyindolenine (+)-65, obtained through Miller's asymmetric oxidation of the corresponding 2-aryltryptamine,²³ gave indoxyl (+)-66, which constitutes the core structure of hinckdentine A (67).⁴⁶ Similarly, when tryptophol derivative 68 was subjected to Miller's asymmetric oxidation²³ followed by base mediated 1,2-

Scheme 13. Further Synthetic Application of Asymmetric Oxidation and 1,2-Alkyl Shift of 2,3-Disubstituted Indole Derivatives^a

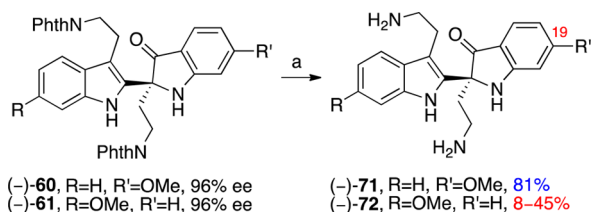


^aConditions: (a) HFIP, HCOOH (10:1), 90 °C; (b) 40, DMAP, H₂O₂, DIC, CHCl₃, 0 °C; (c) *t*-BuOK, *t*-BuOH, 23 °C.

alkyl shift sequence, indoxyl (–)-70 was obtained in 79% ee, providing a formal synthesis of (–)-hinckdentine A (67, Scheme 13).⁴² Notably, the indoxyl 70 in racemic form has served as a key intermediate in Kawasaki's total synthesis of (±)-hinckdentine A (67).⁴²

With robust access to indoxyls (–)-60 and (–)-61 secured, we next investigated their hydrazinolysis. When indoxyl (–)-60 was treated with 10 equiv of hydrazine hydrate in methanol at 80 °C, indoxyl 71 was obtained in 81% yield (Scheme 14).

Scheme 14. Hydrazinolysis of Indoxyls (–)-60 and (–)-61^a

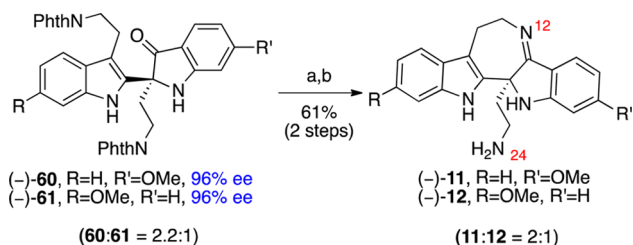


^aConditions: (a) $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$, MeOH, 90 °C.

However, when indoxyl (–)-61 was exposed to the same reaction conditions, lower and variable yields (8–45%) of the indoxyl 72 were obtained (Scheme 14). We reasoned that the absence of the methoxy group at C19 of indoxyl 72 rendered the carbonyl group more electrophilic, which resulted in the formation of polar hydrazone byproducts.

Hence, in order to achieve higher efficiency and practicality, we envisioned performing the hydrazinolysis of indoxyls (–)-60 and (–)-61 and the condensation of the resulting intermediates in a one-pot, two-step procedure. We predicted that indoxyls (–)-71, (–)-72, and hydrazone byproducts formed during the hydrazinolysis of indoxyls (–)-60 and (–)-61 would be viable substrates for the subsequent titanium ethoxide-mediated condensation reaction. Indeed, treatment of indoxyls (–)-60 and (–)-61 (60:61 = 2.2:1) with 10 equiv of hydrazine hydrate in methanol at 80 °C followed by concentration of the reaction mixture and addition of the tetrahydrofuran solution of titanium ethoxide and heating at 42 °C afforded the desired pentacyclic imines (–)-11 and (–)-12 (11:12 = 2:1) in 61% yield over two steps (Scheme 15).

Scheme 15. One-Pot, Two-Step Synthesis of Imines (–)-11 and (–)-12^a



^aConditions: (a) $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$, MeOH, 80 °C; (b) $\text{Ti}(\text{OEt})_4$, THF, 42 °C.

Consistent with our earlier observations in the model system (Scheme 3) and the molecular structure of the natural product trigonoliimine C (3), we did not observe the formation of the 5-membered imine resulting from the condensative cyclization of N24 onto the ketone moiety (Figure 2). Notably, DFT calculation (B3LYP, 6.31G*) of the equilibrium geometries and

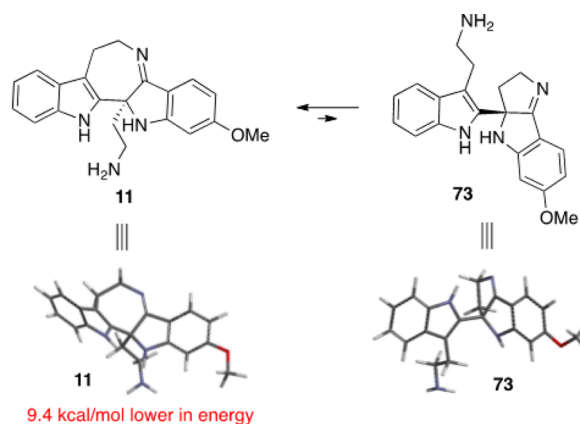
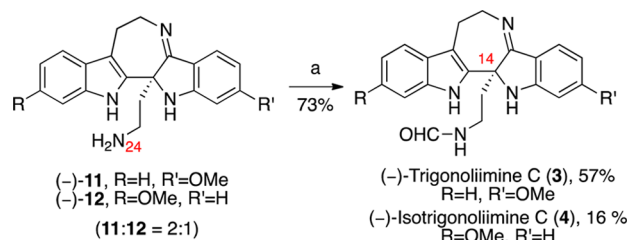


Figure 2. Calculated equilibrium geometries of 7-membered imine 11 and 5-membered imine 73.

energy of 7-membered imine 11 and 5-membered imine 73 predicted that imine 11 is 9.4 kcal/mol lower in energy (Figure 2).⁴⁷

Consistent with the model studies (Scheme 3), treatment of imines (–)-11 and (–)-12 (11:12 = 2:1) with freshly prepared *N*-formylimidazole afforded (–)-trigonoliimine C (3) $[[\alpha]_D^{24} = -147$ (c 0.12, CHCl_3)] and (–)-isotrigonoliimine C (4) $[[\alpha]_D^{24} = -220$ (c 0.10, CHCl_3)] in 57% and 16% yield, respectively (Scheme 16). All ^1H and ^{13}C NMR data of our

Scheme 16. Total Synthesis of (–)-Trigonoliimine C (3) and (–)-Isotrigonoliimine C (4)^a



^aConditions: (a) *N*-formylimidazole, THF, 23 °C.

synthetic (–)-trigonoliimines C (3) matched those provided in the isolation report.¹ While regioisomeric intermediates mentioned above could be separated earlier in the synthetic sequence for independent examination,⁸ the ease of separation of (–)-trigonoliimine C (3) and (–)-isotrigonoliimine C (4) by silica gel column chromatography allowed a practical processing of the earlier intermediates as mixtures until the final stage of the synthesis.⁴⁸

The sign and magnitude of specific rotation of our synthetic trigonoliimines in conjunction with our X-ray crystal structure data provided valuable information regarding the stereochemistry of these alkaloids. First, our synthetic trigonoliimines A–C (1–3) derived from hydroxyindolenines (+)-36 and (+)-38 exhibited negative signs in their specific rotation (Table 6). However, naturally occurring trigonoliimines A (1) and B (2) were reported to have a positive sign in their specific rotations whereas trigonoliimine C (3) was reported to have a negative sign in its specific rotation. These observations, in conjunction with our X-ray crystal structures, which unambiguously corroborated the absolute stereochemistry of our synthetic (–)-trigonoliimines A–C (1–3), enabled the revision of the absolute stereochemistry of all trigonoliimines (Figure

Table 6. Specific Rotation Values of Natural and Synthetic Trigonoliimines A–C (1–3)

entry	alkaloids	natural ($[\alpha]^{10}_D$)	synthetic ($[\alpha]^{10}_D$) ^a
1	trigonoliimine A	+13.3 (c 0.3, CHCl ₃)	–294 (c 0.24, CHCl ₃ , 94% ee)
2	trigonoliimine B	+5.0 (c 0.5, CHCl ₃)	–352 (c 0.32, CHCl ₃ , 95% ee)
3	trigonoliimine C	–4.8 (c 0.45, CHCl ₃)	–147 (c 0.12, CHCl ₃ , 96% ee)

^a% ee of the key precursor for the corresponding synthetic trigonoliimine.

1). Additionally, all of our synthetic (–)-trigonoliimines A–C (1–3) showed a significantly larger magnitude of specific rotations compared to those reported for the naturally isolated samples (Table 6). Importantly, the enantiomeric excess of our samples has been quantified by HPLC analysis of enantiomerically enriched samples of several intermediates against readily available racemic samples from our exploratory studies in this program. A potential explanation for the smaller magnitude of the specific rotations of the naturally occurring trigonoliimines is their possible natural formation via partial resolution after a nonselective oxidation of a precursor such as bistrryptamine **15** (Scheme 1). Our observations regarding the propensity of bistrryptamine **32** to undergo air oxidation (Table 3, entry 2) lends further credence to this hypothesis.^{8,49}

Testing of all known Trigonoliimines and Structurally Related Alkaloids for Their Anticancer Activity. Despite the numerous synthetic efforts targeting the trigonoliimine class of natural products, only one study has investigated their biological activity.⁷ This report identified modest anti-HIV activity for trigonoliimine A (0.95 μg/mL, 2.7 μM). Inspired by the potent anticancer activities of indolocarbazole glycopeptides with 2,2'-connectivity,² we set out to test the anticancer activities of trigonoliimines A–C and various synthetic intermediates generated from this synthetic program.

Our synthetic work has enabled a study of the anticancer activities of all known trigonoliimines as well as numerous advanced intermediates, many of which share similar cores to the natural products. Thirteen compounds were identified as preliminary hits for their ability to induce cell death and were carried on for further testing in two cancer cell lines (U-937, human lymphoma, and HeLa, human cervical cancer). The 72-h IC₅₀ values in both U-937 and HeLa cells for the lead compounds is shown in Table 7. In general, most of the compounds, including trigonoliimines A–C, showed weak activity (10–50 μM) in both cell lines.

TFA-*d*₁ salt of amine (±)-**24**, a precursor to trigonoliimine C (3), showed excellent activity in U-937 cells (730 nM). However, when comparing it to free amine of (±)-**24** and acetic acid-*d*₄ salt of (–)-**11** and (–)-**12**, which are all structurally related compounds, the TFA-*d*₁ salt of (±)-**24** was much more active than would be predicted from their subtle structural differences. As TFA is not able to induce cancer cell death on its own, this suggests that the activity of the TFA salt of amine (±)-**24** arises from a specific interaction resulting from the TFA salt. This is consistent with previous reports that TFA and TFA salts affect cell proliferation and protein synthesis in cells.⁵⁰ Tryptophol derivative **68** was also reasonably active against both U-937 and HeLa cells. However, **68** contains a nitroaromatic group which is known to behave promiscuously in cells.⁵¹ Hence, further biological studies

Table 7. Anticancer Activity of All Known Trigonoliimines and Structurally Related Alkaloids^a

entry	compd	HeLa (μM)	U-937 (μM)
1	(–)- 1	>50	44 ± 9
2	(±)- 1	>50	21.3 ± 4.5
3	(–)- 2	>50	34.6 ± 7.1
4	(–)- 3	>50	20.3 ± 3.1
5	(±)- 3	>100	29.6 ± 9.5
6	(–)- 4	>50	>35
7	(–)- 11 -CD ₃ CO ₂ D	29.7 ± 3.1	10.9 ± 0.2
8	(–)- 12 -CD ₃ CO ₂ D	>100	38.6 ± 9.5
9	(±)- 23	>50	19.2 ± 4.4
10	(±)- 24	>30	10.3 ± 3.0
11	(±)- 24 -CF ₃ CO ₂ D	8.6 ± 1.8	0.73 ± 0.05
12	(+)- 52	>100	35.6 ± 9.2
13	68	3.6 ± 1.1	7.9 ± 0.5
14	(±)- 69	13.6 ± 6.3	22.8 ± 2.2
15	(+)- 69 ^b	35.8 ± 8.6	>50

^a72 h IC₅₀ values in HeLa (human cervical cancer) or U-937 (human lymphoma) cell lines as monitored by SRB (HeLa) or MTS (U-937). Error is standard deviation of the mean, *n* = 3. SRB: sulforhodamine B. MTS: 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium. ^b43% ee.

would be necessary to unequivocally address anticancer activities of these compounds.

CONCLUSION

We have described the development of our synthetic strategy for the enantioselective total synthesis of all known (–)-trigonoliimines A (1), B (2), and C (3). Rapid access to the key hydroxyindolenines (+)-**36** and (+)-**38** was enabled by a Suzuki–Miyaura cross-coupling reaction using Buchwald's precatalyst **31** in conjunction with silver phosphate at ambient temperature, followed by a highly enantioselective oxidation at the enantiodetermining branching point of our syntheses. Stereoretentive and condensative cyclization of *cis*-fused amins (+)-**7** and (+)-**8**, obtained from the phthalimide deprotection of (+)-**36** and (+)-**38**, gave azepanes (–)-**5** and (–)-**6** as the direct precursors of (–)-trigonoliimines A (1) and B (2). Hence, the total synthesis of (–)-trigonoliimines A (1) and B (2) were achieved in seven steps from commercially available material. Our succinct enantioselective syntheses of (–)-trigonoliimines C (3) and (–)-isotrigonoliimine C (4) are each eight steps from commercially available material and draw on a selective Wagner–Meerwein-type 1,2-alkyl shift of hydroxyindolenines (+)-**36** and (+)-**38**, which gave indoxyls (–)-**60** and (–)-**61**.

Our enantioselective approach to these natural products allowed the revision of the absolute stereochemistry of alkaloids (–)-**1**–**3** and raised interesting questions related to the apparent low enantiomeric excess of the naturally occurring trigonoliimines. Furthermore, our synthetic efforts have enabled the testing of these alkaloids and several advanced intermediates in two cancer cell lines, revealing mild activity for certain members of this class. The concise total synthesis of (–)-trigonoliimines A–C (1–3) highlights the power of retrosynthetic analysis⁵² as a source of inspiration in antitoxic simplification of natural product targets.

EXPERIMENTAL SECTION

Experimental Procedures and Full Characterization Data for Previously Reported Key Compounds. For complete experimental procedures and full characterization data of all (–)-trigonoliimine alkaloids A–C (1–3, respectively), see the Supporting Information of our previous report of the total synthesis of all (–)-trigonoliimines.⁸ For complete experimental procedures and full characterization data of the key compounds (–)-4, (–)-5, (–)-6, (+)-7, (+)-8, (–)-11, (–)-12, 26, 27, 30, 32, (+)-36, (+)-38, (–)-60, and (–)-61 discussed in our fully optimized route, please see the Supporting Information of our previous report of the total synthesis of all (–)-trigonoliimines.⁸

(±)-2,2'-((3'-Hydroxy-1H,3'-H-[2,2'-biindole]-3,3'-diyl)bis(ethane-2,1-diyl))bis(isoindoline-1,3-dione) (20). A solution of Davis' oxaziridine **19** (145 mg, 0.606 mmol, 2.2 equiv) in anhydrous dichloromethane (2.5 mL) at 0 °C was transferred by cannula to a solution of indoline derivative **17** (164 mg, 0.276 mmol, 1 equiv) in anhydrous dichloromethane (9 mL) at 0 °C under an atmosphere of argon. The reaction mixture was allowed to gently warm to 23 °C. After 11 h, the reaction mixture was concentrated under reduced pressure and the residue was purified by flash column chromatography (silica gel: diameter 3 cm, ht 13 cm; eluent: 4% acetone in dichloromethane) to afford hydroxyindolenine (±)-**20** (121 mg, 73.6%) as a yellow solid. ¹H NMR (500.4 MHz, CDCl₃, 21 °C): δ 9.35 (s, 1H), 7.71 (dd, *J* = 5.5, 3.0 Hz, 2H), 7.61 (dd, *J* = 5.5, 3.0 Hz, 2H), 7.56 (dd, *J* = 5.6, 2.9 Hz, 2H), 7.51 (dd, *J* = 5.4, 3.2 Hz, 2H), 7.46 (d, *J* = 2.9 Hz, 1H), 7.44 (d, *J* = 4.0 Hz, 1H), 7.28 (d, *J* = 7.5 Hz, 1H), 7.11 (dt, *J* = 7.5, 1.2 Hz, 1H), 7.09 (d, *J* = 6.7 Hz, 1H), 7.02 (app-t, *J* = 7.5 Hz, 2H), 6.85 (ddd, *J* = 7.9, 7.0, 0.9 Hz, 1H), 4.08–4.02 (m, 2H), 3.64–3.58 (m, 2H), 3.54–3.48 (m, 1H), 3.46–3.39 (m, 2H), 2.68 (app-quintet, *J* = 7.1 Hz, 1H), 2.30 (app-quintet, *J* = 6.8 Hz, 1H). ¹H NMR (500.4 MHz, DMSO-*d*₆, 21 °C): δ 10.93 (s, 1H), 7.75–7.67 (m, 6H), 7.62 (dd, *J* = 5.5, 3.0 Hz, 2H), 7.60 (d, *J* = 8.3 Hz, 1H), 7.50 (d, *J* = 7.6 Hz, 2H), 7.44 (d, *J* = 7.6 Hz, 1H), 7.32 (dt, *J* = 7.6, 1.0 Hz, 1H), 7.18 (dt, *J* = 7.4, 0.7 Hz, 1H), 7.14 (app-t, *J* = 8.0 Hz, 1H), 6.92 (app-t, *J* = 7.8 Hz, 1H), 6.51 (s, 1H), 4.05–3.92 (m, 2H), 3.47 (t, *J* = 6.8 Hz, 2H), 3.38–3.23 (m, 2H), 2.55–2.49 (m, 1H), 2.33–2.27 (m, 1H). ¹³C NMR (125.8 MHz, CDCl₃, 21 °C): δ 173.2, 168.7, 168.0, 154.2, 138.3, 137.0, 133.8, 133.8, 132.4, 132.0, 130.4, 128.4, 127.8, 126.1, 124.9, 123.2, 123.0, 122.6, 121.4, 120.2, 119.8, 119.2, 112.0, 86.3, 38.6, 35.4, 33.4, 24.4. ¹³C NMR (100.6 MHz, DMSO-*d*₆, 21 °C): δ 174.1, 167.8, 167.2, 153.9, 139.8, 136.9, 134.0, 134.0, 131.7, 131.4, 129.3, 127.6, 127.6, 125.5, 123.6, 122.8, 122.6, 122.6, 120.4, 119.3, 118.8, 117.0, 113.0, 85.2, 37.9, 35.4, 32.8, 23.8. FTIR (neat) cm^{–1}: 3411 (br-s), 1771 (m), 1708 (s), 1548 (m), 1397 (s), 717 (s). HRMS (ESI, TOF) (*m/z*): calcd for C₃₆H₂₆N₄O₅, [M + H]⁺ 595.1976, found 595.1986. TLC (5% acetone in dichloromethane), *R*_f: 0.4 (CAM, UV).

(±)-2-(2-(3-(2-(1,3-Dioxoisindolin-2-yl)ethyl)-1H-indol-2-yl)-3-oxoindolin-2-yl)ethyl)isoindoline-1,3-dione (21). Scandium(III) trifluoromethanesulfonate (11.6 mg, 0.0230 mmol, 1.50 equiv) was added to a solution of hydroxyindolenine (±)-**20** (9.1 mg, 15 μmol, 1 equiv) in toluene (1.5 mL) at 23 °C, and the resulting reaction mixture was warmed to 85 °C. After 18 h, the reaction mixture was allowed to cool to 23 °C. The reaction mixture was diluted with dichloromethane (8 mL), saturated aqueous sodium bicarbonate solution (8 mL) was added to the reaction mixture, and the layers were separated. The aqueous layer was extracted with dichloromethane (8 mL), and the combined organic layers were dried over anhydrous sodium sulfate and concentrated under reduced pressure. The sample of the crude residue was purified by flash column chromatography (silica gel: diameter 1.5 cm, ht 10 cm; eluent: 1.3% dichloromethane in acetone to 10% dichloromethane in acetone) to afford indoxyl (±)-**21** (3.3 mg, 36%) and oxindole (±)-**22** (2.9 mg, 32%) as pale yellow solids. ¹H NMR (500.4 MHz, CDCl₃, 21 °C): δ 9.08 (s, 1H), 7.87 (dd, *J* = 5.5, 3.0 Hz, 2H), 7.73 (dd, *J* = 5.5, 3.0 Hz, 2H), 7.61 (dd, *J* = 5.5, 2.9 Hz, 2H), 7.54–7.47 (m, 5H), 7.22 (app-t, *J* = 8.9 Hz, 2H), 7.05 (ddd, *J* = 8.1, 7.0, 1.1 Hz, 1H), 6.97 (ddd, *J* = 7.9, 7.0, 1.0 Hz, 1H), 6.79 (s, 1H), 6.77 (ddd, *J* = 7.8, 7.1, 0.8 Hz, 1H), 3.89–3.74 (m, 4H), 3.13 (ddd, *J* = 10.3, 6.8, 3.1 Hz, 2H), 2.69 (app-quintet, *J* = 7.4 Hz, 1H), 2.43–2.38 (m, 1H). ¹³C NMR (125.8 MHz, CDCl₃, 21 °C): δ 201.0, 168.8, 168.3, 161.1, 138.5, 135.5, 134.3, 134.0,

132.4, 131.7, 130.5, 128.6, 125.4, 123.6, 123.2, 122.6, 119.8, 119.3, 118.4, 118.2, 113.1, 111.2, 109.1, 67.8, 39.0, 37.0, 33.7, 24.3. FTIR (neat) cm^{–1}: 3381 (br-s), 1770 (s), 1719 (s), 1616 (s), 1397 (s), 1103 (w), 751 (s). HRMS (ESI, TOF) (*m/z*): calcd for C₃₆H₂₆N₄O₅, [M + H]⁺ 595.1976, found 595.1987. TLC (5% acetone in dichloromethane), *R*_f: 0.66 (CAM, UV).

(±)-2-(7,12,12b,13-Tetrahydro-6H-azepino[3,2-*b*:4,5-*b'*]-diindol-12b-yl)ethanamine (24). Hydrazine monohydrate (33.0 μL, 0.669 mmol, 15.0 equiv) was added via syringe to a solution of indoxyls (±)-**21** (26.5 mg, 0.0446 mmol, 1 equiv) in methanol (5 mL) under an atmosphere of argon at 23 °C, the reaction flask was equipped with a reflux condenser, and the reaction setup was sealed under an atmosphere of argon and heated to 80 °C. After 90 min, the pale yellow homogeneous reaction mixture was allowed to cool to 23 °C, and the volatiles were removed under reduced pressure to afford a pale yellow solid. A solution of titanium ethoxide (41.5 μL, 0.178 mmol, 4.00 equiv) in anhydrous tetrahydrofuran (5 mL) was added via syringe to the yellow solid under an atmosphere of argon, and the resulting mixture was warmed to 40 °C. After 3 h, the reaction mixture was concentrated under reduced pressure. The crude residue adsorbed onto silica gel (2.5 g) was dry loaded and purified by flash column chromatography (silica gel: diameter 2 cm, ht. 10 cm; eluent: 23% chloroform, 5.1% methanol, 0.6% ammonium hydroxide in dichloromethane) to afford imine (±)-**24** (6.9 mg, 49%, 2 steps) as a yellow solid. ¹H NMR (500.4 MHz, CD₃OD, 21 °C):⁵³ δ 7.83 (ddd, *J* = 8.3, 1.2, 0.8 Hz, 1H), 7.71 (ddd, *J* = 8.6, 7.0, 1.2 Hz, 1H), 7.51 (td, *J* = 7.9, 1.0 Hz, 1H), 7.42 (td, *J* = 8.2, 0.9 Hz, 1H), 7.21 (ddd, *J* = 8.2, 7.0, 1.2 Hz, 1H), 7.11 (td, *J* = 8.6, 0.8 Hz, 1H), 7.08 (ddd, *J* = 8.0, 7.0, 1.0 Hz, 1H), 6.97 (ddd, *J* = 8.4, 6.9, 0.8 Hz, 1H), 4.68 (td, *J* = 7.9, 1.0 Hz, 1H), 4.13 (ddd, *J* = 14.5, 4.1, 3.1 Hz, 1H), 3.38–3.24 (m, 2H), 3.17–3.14 (m, 1H), 3.01–2.92 (m, 2H), 2.88–2.82 (m, 1H). ¹³C NMR (125.8 MHz, CD₃OD, 21 °C):⁵³ δ 183.2, 162.3, 143.7, 137.5, 129.4, 126.3, 124.6, 124.6, 122.4, 121.1, 119.2, 115.1, 114.5, 112.5, 111.8, 70.0, 44.9, 37.7, 36.4, 24.7. FTIR (neat) cm^{–1}: 3272 (br-m), 1675 (s), 1636 (s), 1202 (s), 1134 (s), 723 (m). HRMS (ESI, TOF) (*m/z*): calcd for C₂₀H₂₀N₄, [M + H]⁺ 317.1761, found 317.1768. TLC (18% methanol, 2% ammonium hydroxide in chloroform), *R*_f: 0.59 (CAM, UV).

(±)-19-Desmethoxytrigonoliimine C (25). Freshly prepared *N*-formylimidazole⁵⁴ solution (0.058 M solution in tetrahydrofuran, 0.40 mL, 23 μmol, 1.1 equiv) was added dropwise via syringe to a solution of amine (±)-**24** (6.9 mg, 22 μmol, 1 equiv) in tetrahydrofuran (1 mL) at 23 °C and placed under an argon atmosphere. After 1.5 h, saturated aqueous sodium bicarbonate solution (4.5 mL) was added, and the resulting mixture was diluted with dichloromethane (4.5 mL), and the layers were separated. The aqueous layer was extracted with dichloromethane (4.5 mL), and the combined organic layers were dried over anhydrous sodium sulfate and concentrated under reduced pressure. The sample of the crude residue was purified by flash column chromatography (silica gel: diameter 2 cm, ht 10 cm; eluent: 4% methanol, 0.4% ammonium hydroxide in chloroform) to afford (±)-19-desmethoxytrigonoliimine C (**25**, 5.6 mg, 75%) as a yellow solid. ¹H NMR (500.4 MHz, CDCl₃, 21 °C): δ 7.94 (s, 1H), 7.59 (ddd, *J* = 7.8, 1.3, 0.7 Hz, 1H), 7.43 (td, *J* = 7.9, 1.0 Hz, 1H), 7.33–7.30 (m, 2H), 7.10 (ddd, *J* = 8.2, 7.0, 1.1 Hz, 1H), 7.00 (ddd, *J* = 7.9, 7.0, 1.0 Hz, 1H), 6.84 (td, *J* = 8.1, 0.8 Hz, 1H), 6.77 (ddd, *J* = 7.9, 7.0, 0.8 Hz, 1H), 4.46 (ddd, *J* = 14.0, 11.9, 2.5 Hz, 1H), 4.09 (td, *J* = 12.1, 3.6 Hz, 1H), 3.29–3.23 (m, 2H), 3.16 (td, *J* = 16.9, 3.2 Hz, 1H), 2.75 (ddd, *J* = 15.3, 9.0, 5.1 Hz, 1H), 2.40 (ddd, *J* = 15.0, 9.1, 5.0 Hz, 1H). ¹³C NMR (125.8 MHz, CDCl₃, 21 °C): δ 176.5, 164.0, 157.6, 137.1, 135.5, 132.2, 129.9, 124.7, 124.2, 123.1, 120.2, 120.1, 118.9, 112.5, 111.9, 110.6, 68.0, 48.5, 40.7, 35.3, 24.4. FTIR (neat) cm^{–1}: 3285 (br-m), 2923 (m), 1642 (s), 1611 (m), 1469 (m), 1375 (m), 1317 (m), 1141 (w), 743 (m). HRMS (ESI, TOF) (*m/z*): calcd for C₂₁H₂₁N₄O [M + H]⁺ 345.1710, found 345.1732. TLC (18% methanol, 2% ammonium hydroxide in chloroform), *R*_f: 0.69 (CAM, UV).

Hexacyclic Imine (+)-52. Anhydrous dichloromethane (4 mL) was added via syringe to a flask charged with pentacycle (–)-**5** (14.0 mg, 40.4 μmol, 1 equiv) and pyridinium *p*-toluenesulfonate (PPTS, 31.0 mg, 0.121 mmol, 3.00 equiv) at 23 °C under an atmosphere of argon to form a bright yellow solution. After 2 min, triisopropyl

orthoformate (93.0 μL , 0.404 mmol, 10.0 equiv) was added to the reaction mixture. After 1 h, saturated aqueous sodium bicarbonate solution (6 mL) was added, the resulting mixture was diluted with dichloromethane (2 mL), and the layers were separated. The aqueous layer was extracted with dichloromethane (2×6 mL), and the combined organic layers were dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (silica gel: diameter 2 cm, ht 12 cm; eluent: 1.8% methanol, 0.2% ammonium hydroxide in chloroform to 4.5% methanol, 0.5% ammonium hydroxide in chloroform) to afford (–)-trigonoliimine A (**1**, 12 mg, 82%) as a pale yellow solid. Hexacyclic imine (+)-**52** (2.2 mg, 15%) was also obtained as a yellow solid byproduct. Structural assignment of (+)-**52** utilized additional information from gCOSY, HSQC, and HMBC. ^1H NMR (500.4 MHz, CD_3OD , 21 $^\circ\text{C}$): δ 7.63 (s, 1H, C_{25}H), 7.58 (d, J = 8.1 Hz, 1H, C_7H), 7.46 (d, J = 8.3 Hz, 1H, C_4H), 7.29 (dt, J = 7.6, 0.8 Hz, 1H, C_6H), 7.24 (d, J = 8.2 Hz, 1H, C_{18}H), 7.15 (d, J = 2.3 Hz, 1H, C_{15}H), 7.11 (t, J = 7.5 Hz, 1H, C_5H), 6.86 (dd, J = 8.2, 2.3 Hz, 1H, C_{17}H), 3.95 (app-dd, J = 13.3, 4.0 Hz, 1H, C_{11}H), 3.85 (s, 3H, OMe), 3.64–3.53 (m, 2H, C_{22}H_2), 3.21 (app-t, J = 5.3 Hz, 2H, C_{10}H_2), 3.19–3.12 (m, 1H, C_{11}H_2), 1.82 (dt, J = 12.4, 5.1 Hz, 1H, C_{21}H), 1.36 (dd, J = 12.4, 3.4 Hz, 1H, C_{21}H), 2.96 (ddd, J = 16.9, 12.1, 4.3 Hz, 1H, C_{10}H), 2.19–2.13 (m, 1H, C_{21}H), 2.06 (dd, J = 12.0, 5.8 Hz, 1H, C_{21}H). ^{13}C NMR (125.8 MHz, CD_3OD , 21 $^\circ\text{C}$): δ 177.7 (C_{24}), 163.1 (C_{16}), 155.8 (C_{14}), 151.2 (C_{25}), 133.3 (C_8), 136.0 (C_{19}), 129.4 (C_9), 128.3 (C_2), 126.2 (C_{16}), 124.5 (C_{18}), 121.7 (C_3), 121.1 (C_5), 120.7 (C_7), 113.1 (C_4), 112.6 (C_{17}), 108.3 (C_{15}), 75.6 (C_{20}), 56.3 (C_{27}), 51.2 (C_{11}), 41.1 (C_{22}), 34.0 (C_{21}), 30.9 (C_{10}). FTIR (neat) cm^{-1} : 3057 (br-s), 2929 (br-s), 1632 (s), 1581 (s), 1483 (s), 1334 (s), 1147 (s), 1027 (m), 735 (s). HRMS (ESI, TOF) (m/z): calcd for $\text{C}_{22}\text{H}_{21}\text{N}_4\text{O}$, $[\text{M} + \text{H}]^+$: 357.1710, found: 357.1717. $[\alpha]_{\text{D}}^{24}$: +151 (c 0.43, MeOH). TLC (9% methanol, 1% ammonium hydroxide in chloroform), R_f : 0.15 (CAM, UV).

(R)-2-(2-(3-(3-(2-(1,3-Dioxoisindolin-2-yl)ethyl)-1H-indol-2-yl)-6-methoxy-2-oxoindolin-3-yl)ethyl)isoindoline-1,3-dione (62). Lanthanum(III) trifluoromethanesulfonate (22.7 mg, 38.7 μmol , 0.500 equiv) was added to a solution of hydroxyindolenines (+)-**36** and (+)-**38** (36:38, 2.2:1, 48.5 mg, 77.6 μmol , 1 equiv) in toluene (7.7 mL) at 23 $^\circ\text{C}$, and the resulting reaction mixture was warmed to 80 $^\circ\text{C}$. After 19.5 h, the reaction mixture was allowed to cool to 23 $^\circ\text{C}$, saturated aqueous sodium bicarbonate solution (15 mL) and ethyl acetate (15 mL) were added and the layers were separated. The aqueous layer was extracted with dichloromethane (15 mL). The combined organic layers were dried over anhydrous sodium sulfate, were filtered, and were concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel: diameter 2 cm, ht 10 cm; eluent: 3.3% acetone in dichloromethane to 10% acetone in dichloromethane) to afford indoxyls (–)-**60** and (–)-**61** (60:61, 1:1.2, 27 mg, 56%) and oxindole (+)-**62** (17 mg, 34%) as a mixture of yellow solids. ^1H NMR (500.4 MHz, CDCl_3 , 21 $^\circ\text{C}$): δ 9.68 (s, 1H), 8.93 (s, 1H), 7.78 (dd, J = 5.3, 3.1 Hz, 2H), 7.70–7.65 (m, 5H), 7.61 (dd, J = 5.5, 3.1 Hz, 2H), 7.45 (d, J = 8.1 Hz, 1H), 7.16–7.05 (m, 3H), 6.60 (d, J = 2.3 Hz, 1H), 6.44 (dd, J = 8.2, 2.3 Hz, 1H), 4.06–4.00 (m, 1H), 3.97–3.91 (m, 1H), 3.69 (s, 3H), 3.64–3.51 (m, 2H), 2.83–2.76 (m, 2H), 2.70–2.64 (m, 1H), 2.57–2.51 (m, 1H), 2.71–2.66 (m, 1H), 2.37–2.32 (m, 1H). ^{13}C NMR (125.8 MHz, CDCl_3 , 21 $^\circ\text{C}$): δ 179.6, 168.6, 168.3, 160.6, 142.1, 135.4, 134.2, 133.9, 132.3, 132.1, 131.6, 129.2, 125.3, 123.5, 123.5, 123.1, 122.3, 119.8, 118.7, 111.4, 109.8, 108.2, 98.3, 55.6, 51.6, 37.7, 34.7, 33.9, 23.9. FTIR (neat) cm^{-1} : 3335 (br-s), 3020 (m), 2361 (w), 1771 (m), 1709 (s), 1633 (m), 1397 (s), 1032 (w), 718 (s). HRMS (DART, TOF) (m/z): calcd for $\text{C}_{37}\text{H}_{29}\text{N}_4\text{O}_6$, $[\text{M} + \text{Na}]^+$: calculated: 647.1901, found: 647.1914. $[\alpha]_{\text{D}}^{24}$: +93.6 (c 0.24, CHCl_3). TLC (14% acetone in dichloromethane), R_f : 0.34 (CAM, UV).

(R)-3-(2-(tert-Butyldimethylsilyloxy)ethyl)-2-(2-nitrophenyl)-3H-indol-3-ol (69). To a glass vial charged with tryptophol derivative **68** (22.6 mg, 57.0 μmol , 1 equiv) and catalyst **40** (3.7 mg, 5.7 μmol , 0.10 equiv) was added DMAP (0.35 mg, 2.9 μmol , 0.050 equiv) in chloroform (200 μL), and the reaction mixture was cooled to 0 $^\circ\text{C}$. After 3 min, hydrogen peroxide (30% w/w in water, 7.0 μL , 68

μmol , 1.2 equiv) was added to the reaction mixture. After 9 min, additional chloroform (80 μL) was added to the reaction mixture. After 4 min, DIC (10.7 μL , 68.4 μmol , 1.20 equiv) was added to the reaction mixture, and the resulting dark orange solution was placed in a cryogenic cooler adjusted to 0 $^\circ\text{C}$. After 22 h, the reaction mixture was diluted with chloroform (0.5 mL) and directly purified by flash column chromatography (silica gel: diameter 2.0 cm, ht. 11 cm; eluent: 13% ethyl acetate in hexanes) to afford hydroxyindolenine (+)-**69** (23 mg, 97%) as a colorless oil. Hydroxyindolenine (+)-**69** was found to have 89.5:10.5 er by chiral HPLC analysis [Chiralpak IC 0.5 mL/min; 80% hexanes, 20% 2-propanol; t_{R} (major) = 12.4 min, t_{R} (minor) = 10.1 min]. ^1H NMR (500.4 MHz, CDCl_3 , 21 $^\circ\text{C}$): δ 8.15 (dd, J = 7.7, 1.4 Hz, 1H), 7.93 (dd, J = 8.1, 1.1 Hz, 1H), 7.66 (dt, J = 7.6, 1.3 Hz, 1H), 7.58 (appt-dt, J = 7.7, 1.5 Hz, 1H), 7.51–7.49 (m, 1H), 7.46–7.45 (m, 1H), 7.35 (dt, J = 7.6, 1.4 Hz, 1H), 7.27 (dt, J = 7.4, 1.1 Hz, 1H), 5.11 (s, 1H), 3.98 (ddd, J = 10.6, 7.9, 4.1 Hz, 1H), 3.77 (ddd, J = 10.7, 6.1, 4.6 Hz, 1H), 2.21 (ddd, J = 12.6, 8.0, 4.6 Hz, 1H), 2.03 (ddd, J = 10.26, 6.1, 4.2 Hz, 1H), 0.88 (s, 9H), 0.04 (s, 3H), 0.04 (s, 3H). ^{13}C NMR (125.8 MHz, CDCl_3 , 21 $^\circ\text{C}$): δ 177.6, 152.9, 149.4, 140.5, 132.5, 130.9, 130.4, 129.8, 129.6, 127.1, 124.6, 123.0, 122.1, 89.1, 61.1, 37.4, 26.0, 18.2, –5.5, –5.6. FTIR (neat) cm^{-1} : 3406 (s), 2955 (s), 2857 (m), 1649 (w), 1538 (s), 1471 (m), 1361 (m), 1258 (m), 1984 (m), 838 (s). HRMS (DART, TOF) (m/z): calcd for $\text{C}_{22}\text{H}_{29}\text{N}_2\text{O}_4\text{Si}$, $[\text{M} + \text{H}]^+$: 413.1891, found: 413.1890. $[\alpha]_{\text{D}}^{24}$: +136.1 (c 0.49, CHCl_3). TLC (25% ethyl acetate in hexanes), R_f : 0.43 (CAM, UV).

(R)-2-(2-(tert-Butyldimethylsilyloxy)ethyl)-2-(2-nitrophenyl)indolin-3-one (70). To a solution of hydroxyindolenine (+)-**69** (26.2 mg, 63.5 μmol , 1 equiv) in freshly distilled *tert*-butanol (1.6 mL) under argon was added a solution of potassium *tert*-butoxide (3.8 mg, 32 μmol , 0.50 equiv) in *tert*-butanol (1.6 mL) via syringe at 23 $^\circ\text{C}$. After 16.5 h, saturated aqueous ammonium chloride solution (3 mL) was added and the resulting mixture was diluted with water (4.5 mL) and ethyl acetate (6 mL), and the layers were separated. The aqueous layer was extracted with ethyl acetate (2×6 mL), and the combined organic layers were dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The sample of the crude residue was purified by flash column chromatography (silica gel: diameter 2.0 cm, ht. 10 cm; eluent: 13% ethylacetate in hexanes) to afford indoxyl (–)-**70** (18 mg, 67%) as a yellow oil. Indoxyl (–)-**70** was found to have 89.5:10.5 er by chiral HPLC analysis [Chiralpak IC 0.5 mL/min; 80% hexanes, 20% isopropanol; t_{R} (major) = 14.9 min, t_{R} (minor) = 24.8 min]. ^1H NMR (500.4 MHz, CDCl_3 , 21 $^\circ\text{C}$): δ 7.79 (appt-d, J = 7.4 Hz, 1H), 7.61 (td, J = 7.7, 0.6 Hz, 1H), 7.53–7.50 (m, 1H), 7.48 (ddd, J = 8.3, 7.1, 1.3 Hz, 1H), 7.36–7.35 (m, 2H), 6.90 (appt-t, J = 7.8 Hz, 1H), 6.86 (td, J = 8.2, 0.7 Hz, 1H), 6.13 (s, 1H), 3.79–3.72 (m, 2H), 2.53 (ddd, J = 14.9, 6.8, 2.9 Hz, 1H), 2.08 (ddd, J = 14.8, 9.7, 4.9 Hz, 1H), 0.84 (s, 9H), –0.09 (s, 3H), –0.10 (s, 3H). ^{13}C NMR (125.8 MHz, CDCl_3 , 21 $^\circ\text{C}$): δ 201.5, 160.8, 150.9, 137.7, 131.0, 130.9, 129.7, 128.5, 125.3, 123.9, 120.6, 119.8, 113.7, 70.6, 60.1, 40.2, 26.0, 18.2, –5.7. FTIR (neat) cm^{-1} : 3350 (m), 2954 (m), 2929 (m), 1705 (s), 1617 (s), 1533 (s), 1484 (m), 1369 (m), 1324 (m), 1258 (m), 1096 (s), 836 (s), 778 (s). HRMS (DART, TOF) (m/z): calcd for $\text{C}_{22}\text{H}_{29}\text{N}_2\text{O}_4\text{Si}$, $[\text{M} + \text{H}]^+$: 413.1891, found: 413.1903. $[\alpha]_{\text{D}}^{24}$: –79.5 (c 0.15, CHCl_3). TLC (17% ethyl acetate in hexanes), R_f : 0.32 (CAM, UV).

Cell Culture Information. U-937 and HeLa cells were grown in RPMI-1640 supplemented with fetal bovine serum (10%, FBS) and antibiotics (100 $\mu\text{g}/\text{mL}$ penicillin and 100 U/mL streptomycin). Cells were incubated at 37 $^\circ\text{C}$ in a 5% CO_2 , 95% humidity atmosphere.

IC₅₀ Value Determination for HeLa Cells Using Sulforhodamine B (SRB). HeLa cells were added into 96-well plates (1,500 cells/well) in 100 μL media and were allowed to adhere for 2–3 h. Compounds were solubilized in DMSO as 100 \times stocks, added directly to the cells (100 μL final volume), and tested over a range of concentrations (0.25–100 μM) in triplicate (1% DMSO final) on a third-log scale. DMSO and cell-free wells served as the live and dead control, respectively. After 72 h of continuous exposure, the plates were evaluated using the SRB colorimetric assay as described previously.⁵⁵ Briefly, media was removed from the plate, and cells were fixed by the addition of 100 μL cold 10% trichloroacetic acid in

water. After being incubated at 4 °C for 1 h, the plates were washed in water and allowed to dry. Sulforhodamine B was added as a 0.057% solution in 1% acetic acid (100 μ L), and the plates were incubated at room temperature for 30 min, washed in 1% acetic acid, and allowed to dry. The dye was solubilized by adding 10 mM Tris base solution (pH 10.5, 200 μ L) and incubating at room temperature for 30 min. Plates were read at λ = 510 nm.

IC₅₀ Value Determination for U-937 Cells Using MTS. In a 96-well plate, compounds were preadded as DMSO stocks (1% final) in triplicate to achieve final concentrations of 0.25 μ M to 100 μ M on a third-log scale. DMSO and cell-free wells served as the live and dead control, respectively. U-937 (5,000 cells/well) cells were distributed in 100 μ L media to the compound-containing plate. After 72 h, cell viability was assessed by adding 20 μ L of a PMS/MTS solution⁵⁶ to each well, allowing the dye to develop at 37 °C until the live control had processed MTS, and reading the absorbance at λ = 490 nm.

■ ASSOCIATED CONTENT

● Supporting Information

General procedures, supplementary discussion, and copies of ¹H and ¹³C NMR spectra and HPLC data. This material is available free of charge via the Internet at <http://pubs.acs.org>.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: movassag@mit.edu.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We acknowledge financial support by NIH-NIGMS (GM074825) and the University of Illinois. S.H. acknowledges EMD Serono and Kenneth M. Gordon summer graduate fellowships. K.C.M. is a National Science Foundation predoctoral fellow and a Robert C. and Carolyn J. Springborn graduate fellow. We thank Changkyu Han for the sketch of the *Trigonostemon lii* Y. T. Chang used in the cover image.

■ REFERENCES

- (1) Bergman, J.; Koch, E.; Pelcman, B. *Tetrahedron* **1995**, *51*, 5631–5642.
- (2) For a review on indolocarbazole natural products, see: Sánchez, C.; Méndez, C.; Salas, J. A. *Nat. Prod. Rep.* **2006**, *23*, 1007–1045.
- (3) Gallant, M.; Link, J. T.; Danishefsky, S. J. *J. Org. Chem.* **1993**, *58*, 343–349.
- (4) Link, J. T.; Raghavan, S.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1995**, *117*, 552–553.
- (5) (a) Davis, P. D.; Hallam, T. J.; Harris, W.; Hill, C. H.; Lawton, G.; Nixon, J. S.; Smith, J. L.; Vesey, D. R.; Wilkinson, S. E. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1303–1308. (b) Vice, S. F.; Bishop, W. R.; McCombie, S. W.; Dao, H.; Frank, E.; Ganguly, A. K. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1333–1338. (c) Animati, F.; Berettoni, M.; Bigioni, M.; Binaschi, M.; Cipollone, A.; Irrissuto, C.; Nardelli, F.; Olivieri, L. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 5013–5017.
- (6) Nakazono, M.; Nanbu, S.; Uesaki, A.; Kuwano, R.; Kashiwabara, M.; Kaitsu, K. *Org. Lett.* **2007**, *9*, 3583–3586.
- (7) Tan, C.-J.; Di, Y.-T.; Wang, Y.-H.; Zhang, Y.; Si, Y.-K.; Zhang, Q.; Gao, S.; Hu, X.-J.; Fang, X.; Li, S.-F.; Hao, X.-J. *Org. Lett.* **2010**, *12*, 2370–2373.
- (8) Han, S.; Movassaghi, M. *J. Am. Chem. Soc.* **2011**, *133*, 10768–10771.
- (9) Qi, X.; Bao, H.; Tambar, U. K. *J. Am. Chem. Soc.* **2011**, *133*, 10050–10053.
- (10) Liu, S.; Hao, X.-J. *Tetrahedron Lett.* **2011**, *52*, 5640–5642.
- (11) Feng, P.; Fan, Y.; Xue, F.; Liu, W.; Li, S.; Shi, Y. *Org. Lett.* **2011**, *13*, 5827–5829.
- (12) Buyck, T.; Wang, Q.; Zhu, J. *Org. Lett.* **2012**, *14*, 1338–1341.
- (13) Qiu, J.; Zhang, J.-X.; Liu, S.; Hao, X.-J. *Tetrahedron Lett.* **2013**, *54*, 300–302.
- (14) Gulati, K. C.; Seth, S. R.; Venkataraman, K. *Org. Synth.* **1935**, *15*, 70–71.
- (15) (a) Zhao, B.; Hao, X.-Y.; Zhang, J.-X.; Liu, S.; Hao, X.-J. *Org. Lett.* **2013**, *15*, 528–530. (b) For another recent total synthesis of (±)-trigonoliimine C, see: Reddy, B. N.; Ramana, C. V. *Chem. Commun.* **2013**, *49*, 9767–9769.
- (16) For elegant examples of the application of oxidation and rearrangement of 2,3-disubstituted indoles in complex synthesis, see: (a) Finch, N.; Taylor, W. I. *J. Am. Chem. Soc.* **1962**, *84*, 3871–3877. (b) Hutchison, A. J.; Kishi, Y. *J. Am. Chem. Soc.* **1979**, *101*, 6786–6788. (c) Williams, R. M.; Glinka, T.; Kwast, E.; Coffman, H.; Stille, J. K. *J. Am. Chem. Soc.* **1990**, *112*, 808–821. (d) Gueller, R.; Borschberg, H. *Helv. Chim. Acta* **1993**, *76*, 1847–1862. (e) Stoermer, D.; Heathcock, C. H. *J. Org. Chem.* **1993**, *58*, 564–568. (f) Cushing, T. D.; Sanz-Cervera, J. F.; Williams, R. M. *J. Am. Chem. Soc.* **1996**, *118*, 557–579. (g) Ito, M.; Clark, C. W.; Mortimore, M.; Goh, J. B.; Martin, S. F. *J. Am. Chem. Soc.* **2001**, *123*, 8003–8010. (h) Baran, P. S.; Corey, E. J. *J. Am. Chem. Soc.* **2002**, *124*, 7904–7905. (i) Liu, Y.; McWhorter, W. W., Jr. *J. Am. Chem. Soc.* **2003**, *125*, 4240–4252. (j) Williams, R. M.; Cox, R. J. *Acc. Chem. Res.* **2003**, *36*, 127–139 and references cited therein. (k) Liu, Y.; McWhorter, W. W., Jr.; Hadden, C. E. *Org. Lett.* **2003**, *5*, 333–335. (l) Baran, P. S.; Richter, J. M. *J. Am. Chem. Soc.* **2005**, *127*, 15394–15396. (m) Poriell, C.; Lachia, M.; Wilson, C.; Davies, J. R.; Moody, C. J. *J. Org. Chem.* **2007**, *72*, 2978–2987. (n) Miller, K. A.; Tsukamoto, S.; Williams, R. M. *Nature Chem* **2009**, *1*, 63–68.
- (17) Schmidt, M. A.; Movassaghi, M. *Synlett* **2008**, 313–324.
- (18) See the Supporting Information for details.
- (19) Movassaghi, M.; Schmidt, M. A.; Ashenhurst, J. A. *Org. Lett.* **2008**, *10*, 4009–4012.
- (20) (a) Hentges, S. G.; Sharpless, K. B. *J. Am. Chem. Soc.* **1980**, *102*, 4263–4264. (b) Katsuki, T. K.; Sharpless, K. B. *J. Am. Chem. Soc.* **1980**, *102*, 5974–5976. (c) Zhang, W.; Loebach, J. L.; Wilson, S. R.; Jacobsen, E. N. *J. Am. Chem. Soc.* **1990**, *112*, 2801–2803. (d) Irie, R.; Noda, K.; Ito, Y.; Katsuki, T. *Tetrahedron Lett.* **1991**, *32*, 1055–1058. (e) Tu, Y.; Wang, Z. X.; Shi, Y. *J. Am. Chem. Soc.* **1996**, *118*, 9806–9807. (f) Peris, G.; Jakobsche, C. E.; Miller, S. J. *J. Am. Chem. Soc.* **2007**, *129*, 8710–8711.
- (21) For an asymmetric oxidation of 2,3-disubstituted indole using chiral auxiliary, see: Pettersson, M.; Knueppel, D.; Martin, S. F. *Org. Lett.* **2007**, *9*, 4623–4626.
- (22) For biosynthetic studies of asymmetric oxidation of the 2,3-disubstituted indole moiety of prenylated indole natural products, see: Li, S.; Finefield, J. M.; Sunderhaus, J. D.; McAfoos, T. J.; Williams, R. M.; Sherman, D. H. *J. Am. Chem. Soc.* **2012**, *134*, 788–791 and references therein.
- (23) Kolundzic, F.; Noshi, M. N.; Tjandra, M.; Movassaghi, M.; Miller, S. J. *J. Am. Chem. Soc.* **2011**, *133*, 9104–9111.
- (24) For an application of aspartyl-based asymmetric oxidation in total synthesis, see: Sun, M.; Hao, X.-Y.; Liu, S.; Hao, X.-J. *Tetrahedron Lett.* **2013**, *54*, 692–694.
- (25) (a) Wagner, G.; Brickner, W. *Ber.* **1899**, *32*, 2302–2325. (b) Meerwein, H.; Van Emster, K.; Jousen, J. *Ber.* **1922**, *55*, 2500–2528.
- (26) We finished the synthesis of the (±)-19-desmethoxytrigonoliimine C (**33**) on June 2010 and focused on the enantioselective total synthesis of all trigonoliimines since then.
- (27) (a) Bergman, J.; Koch, E.; Pelcman, B. *Tetrahedron Lett.* **1995**, *36*, 3945–3948. (b) Gilbert, E. J.; Ziller, J. W.; Van Vranken, D. L. *Tetrahedron* **1997**, *53*, 16553–16564.
- (28) Treatment of hydroxyaminal **23** with 3 equiv of Sc(OTf)₃ in acetonitrile–toluene (2:1) at 81 °C provided a mixture of indoxyl and oxindole intermediates in good yield (indoxyl/oxindole = 4:1, >99%). However, nontrivial separation of these intermediates allowed only 31% isolated yield of a pure indoxyl intermediate.

- (29) For the synthesis of 6-methoxytryptamine, see: Woodward, R. B.; Bader, F. E.; Bickel, H.; Frey, A. J.; Kierstead, R. W. *Tetrahedron* **1958**, *2*, 1–57.
- (30) For examples of C2–C2' bond formation of two indoles using Stille cross-coupling reaction, see: (a) Palmisano, G.; Santagostino, M. *Helv. Chim. Acta* **1993**, *76*, 2356–2366. (b) Hudkins, R. L.; Diebold, J. L.; Marsh, F. D. *J. Org. Chem.* **1995**, *60*, 6218–6220. (c) Danieli, B.; Lesma, G.; Martinelli, M.; Passarella, D.; Peretto, I.; Silvani, A. *Tetrahedron* **1998**, *54*, 14081–14088. (d) Cai, X.; Snieckus, V. *Org. Lett.* **2004**, *6*, 2293–2295. (e) Selvi, S.; Pu, S.-C.; Cheng, Y.-M.; Fang, J.-M.; Chou, P.-T. *J. Org. Chem.* **2004**, *69*, 6674–6678. (f) La Regina, G.; Bai, R.; Maria Rensen, W.; Di Cesare, E.; Coluccia, A.; Piscitelli, F.; Famiglini, V.; Reggio, A.; Nalli, M.; Pelliccia, S.; Da Pozzo, E.; Costa, B.; Granata, I.; Porta, A.; Maresca, B.; Sorani, A.; Lisa Iannitto, M.; Santoni, A.; Li, J.; Miranda Cona, M.; Chen, F.; Ni, Y.; Brancale, A.; Dondio, G.; Vultaggio, S.; Varasi, M.; Mercurio, C.; Martini, C.; Hamel, E.; Lavia, P.; Novellino, E.; Silvestri, R. *J. Med. Chem.* **2013**, *56*, 123–149.
- (31) For examples of C2–C2' bond formation of two indoles using Suzuki–Miyaura cross-coupling reaction, see: (a) Merlic, C. A.; McInnes, D. M. *Tetrahedron Lett.* **1997**, *38*, 7661–7664. (b) Merlic, C. A.; You, Y.; McInnes, D. M.; Zechman, A. L.; Miller, M. M.; Deng, Q. *Tetrahedron* **2001**, *57*, 5199–5212. (c) Yamabuki, A.; Fujinawa, H.; Choshi, T.; Tohyama, S.; Matsumoto, K.; Ohmura, K.; Nobuhiro, J.; Hibino, S. *Tetrahedron Lett.* **2006**, *47*, 5859–5861. (d) Matsumoto, K.; Choshi, T.; Hourai, M.; Zamami, Y.; Sasaki, K.; Abe, T.; Ishikura, M.; Hatae, N.; Iwamura, T.; Tohyama, S.; Nobuhiro, J. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 4762–4764.
- (32) Miyaura, N.; Suzuki, A. *J. Chem. Soc. Chem. Commun.* **1979**, 866–867.
- (33) (a) Ishiyama, T.; Takagi, J.; Ishida, K.; Miyaura, N.; Anastasi, N. R.; Hartwig, J. F. *J. Am. Chem. Soc.* **2002**, *124*, 390–391. (b) Ishiyama, T.; Takagi, J.; Hartwig, J. F.; Miyaura, N. *Angew. Chem., Int. Ed.* **2002**, *41*, 3056–3058. (c) Boller, T. M.; Murphy, J. M.; Hapke, M.; Ishiyama, T.; Miyaura, N.; Harwig, J. F. *J. Am. Chem. Soc.* **2005**, *127*, 14263–14278.
- (34) Huang, X.; Anderson, K. W.; Zim, D.; Jiang, L.; Klapars, A.; Buchwald, S. L. *J. Am. Chem. Soc.* **2003**, *125*, 6653–6655.
- (35) Kinzel, T.; Zhang, Y.; Buchwald, S. L. *J. Am. Chem. Soc.* **2010**, *132*, 14073–14075.
- (36) Karabelas, K.; Hallberg, A. *J. Org. Chem.* **1986**, *51*, 5286–5290.
- (37) Hu, X.-J.; Di, Y.-T.; Wang, Y.-H.; Kong, S.-Y.; Gao, S.; Li, C.-S.; Liu, H.-Y.; He, H.-P.; Ding, J.; Xie, H.; Hao, X.-J. *Planta Med.* **2009**, *75*, 1157–1161.
- (38) Zhu, Y.; Tu, Y.; Yu, H.; Shi, Y. *Tetrahedron Lett.* **1998**, *39*, 7819–7822.
- (39) Sharpless, K. B.; Amberg, W.; Bennani, Y. L.; Crispino, G. A.; Hartung, J.; Jeong, K.-S.; Kwong, H.-L.; Morikawa, K.; Wang, Z.-M.; Xu, D.; Zhang, X.-L. *J. Org. Chem.* **1992**, *57*, 2768–2771.
- (40) (a) Davis, F. A.; Weismiller, M. C. *J. Org. Chem.* **1990**, *55*, 3715–3717. (b) Davis, F. A. *J. Org. Chem.* **2006**, *71*, 8993–9003.
- (41) Martin, J. C.; Arhart, R. J. *J. Am. Chem. Soc.* **1971**, *93*, 4327–4329.
- (42) Higuchi, K.; Sato, Y.; Tsuchinouchi, M.; Sugiura, K.; Hatori, M.; Kawasaki, T. *Org. Lett.* **2009**, *11*, 197–199.
- (43) An alternative reaction mechanism for the formation of (–)-trigonoliimine A (**1**) involves the initial imidate formation at N12 of (–)-**12** followed by condensation intramolecular condensation with N13.
- (44) Zhang, X.; Foote, C. S. *J. Am. Chem. Soc.* **1993**, *115*, 8867–8868.
- (45) For an excellent discussion of a similar scrambling of regioisomers during the 1,2-alkyl shift of a hydroxyindolenine, see ref 9.
- (46) Blackman, A. J.; Hambley, T. W.; Picker, K.; Taylor, W. C.; Thirasasana, N. *Tetrahedron Lett.* **1987**, *28*, 5561–5562.
- (47) Spartan '06 was used for the calculations.
- (48) For an X-ray crystal structure of (–)-trigonoliimine C (**3**), see ref 8.
- (49) For a report of similar air oxidation of a 2,2'-bistryptamine derivative, see ref 9.
- (50) (a) Ma, T. G.; Ling, Y. H.; McClure, G. D.; Tseng, M. T. *J. Toxicol. Environ. Health* **1990**, *31*, 147–158. (b) Cornish, J.; Callon, K. E.; Lin, C. Q.-X.; Xiao, C. L.; Mulvey, T. B.; Cooper, G. J. S.; Reid, I. R. *Am. J. Physiol. Endocrinol. Metab.* **1999**, *277*, E779–E783.
- (51) (a) Rishton, G. M. *Drug Discov. Today* **1997**, *2*, 382–384. (b) Walters, W. P.; Murcko, M. A. *Adv. Drug Delivery Rev.* **2002**, *54*, 255–271. (c) Baell, J. B.; Holloway, G. A. *J. Med. Chem.* **2010**, *53*, 2719–2740.
- (52) Movassaghi, M.; Siegel, D. S.; Han, S. *Chem. Sci.* **2010**, *1*, 561–566.
- (53) Acetic acid-*d*₄ (1.1 equiv) was added, resulting in sharpening of peaks.
- (54) Staab, H. A.; Polenski, B. *Liebigs Ann. Chem.* **1962**, *655*, 95–102.
- (55) Vichai, V.; Kirtikara, K. *Nature Prot.* **2006**, *1*, 1112–1116.
- (56) Cory, A. H.; Owen, T. C.; Barltrop, J. A.; Cory, J. G. *Cancer Commun.* **1991**, *3*, 207–212.