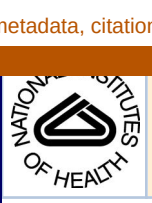

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Total Synthesis of (+)-11,11'-Dideoxyverticillin A

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Abstract

The fungal metabolite (+)-11,11'-dideoxyverticillin A, a cytotoxic alkaloid isolated from a marine *Penicillium* sp., belongs to a fascinating family of densely functionalized, stereochemically complex, and intricate dimeric epidithiodiketopiperazine natural products. Although the dimeric epidithiodiketopiperazines have been known for nearly four decades, none has succumbed to total synthesis. We report a concise enantioselective total synthesis of (+)-11,11'-dideoxyverticillin A via a strategy inspired by our biosynthetic hypothesis for this alkaloid. Highly stereo- and chemoselective advanced stage tetrahydroxylation and tetrathiolation reactions, as well as a mild strategy for the introduction of the epidithiodiketopiperazine core in the final step were developed to address this highly sensitive substructure. Our rapid functionalization of the advanced molecular framework aims to mimic plausible biosynthetic steps and offers an effective strategy for the chemical synthesis of other members of this family of alkaloids.

The fungal metabolite (+)-11,11'- dideoxyverticillin A (**1**, Fig. 1) (1) is a member of the epidithiodiketopiperazine alkaloids, a large family of natural products that has received significant attention from the scientific community for its rich biological activity and complex molecular architecture (2–7). The dimeric subset of alkaloids to which the title compound belongs has been known for nearly four decades with the isolation of (+)-chaetocin A (**2**, 8) and (+)-verticillin A (**3**, 9). Reflective of the daunting challenges posed by molecular structures replete with sterically congested stereogenic centers, and highly acid-, base-, and redoxsensitive functional groupings (5), no dimeric epidithiodiketopiperazine alkaloid has yet succumbed to total synthesis. Herein we describe a concise strategy for the enantioselective total synthesis of the dimeric epidithiodiketopiperazine alkaloid (+)-**1**. Our biosynthetically inspired synthesis features stereo- and chemoselective advanced stage tetrahydroxylation and tetrathiolation reactions, providing a generalizable solution to the epidithiodiketopiperazine substructure found in the broader family of these alkaloids.

At the outset of our synthetic studies, structural similarities among members of the alkaloid family combined with related feeding experiments and biosynthetic hypotheses by Kirby (10) prompted us to consider the possibility that the epidisulfides are assembled by enzymes

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Supporting Online Material
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SOM Text, X-ray structures of (+)-**1** and (+)-**14**, and Figs. S1 to S5.

that exploit the inherent chemistry of dimeric diketopiperazines. Our retrosynthetic analysis of (+)-**1** imitates a plausible biosynthetic sequence of events linking dimeric epidithiodiketopiperazines to common α-amino acid precursors (Fig. 2). We envisioned preparation of (+)-**1** by mild oxidation of the tetrathiol **5**, which could be accessed via stereoselective tetrathiolation of an octacyclic tetraol **6**. In contrast to a previous biosynthetic hypothesis for monomeric epidithiodiketopiperazines invoking thiolation via an *N*-hydroxylation– dehydration sequence (11), we speculated that a post-dimerization C_α-hydroxylation would enable substrate directed hemiaminal thiolation through the intermediacy of an acyliminium ion. Intermediate **7**, which we hypothesized to arise from a dimerization event, could be assembled from the readily available *cyclo*-dipeptide **8** using the concise cobalt-mediated dimerization strategy reported from our laboratories (12–13). The imposing challenges associated with quadruple C_α-methine hydroxylation of **7** and the tetrathiolation of intermediate **6** notwithstanding, the need for absolute and relative stereochemical control (Fig. 2) of the six tetrasubstituted carbons of (+)-**1** posed noteworthy strategic concerns. We envisioned the introduction of C3 and C3' vicinal quaternary stereocenters as a prelude to stereochemical control of the four thiolated– carbon stereogenic centers of (+)-**1**. Such a final stage tetrathiolation followed by immediate disulfide formation would obviate the need to mask the notoriously sensitive epidithiodiketopiperazine functional grouping in the early stages of the synthesis.

The dimeric diketopiperazine (+)-**13** was assembled in six steps from commercially available amino acid derivatives (Fig. 3) (14). The sequential treatment of amide (–)-**9** with trifluoroacetic acid followed by cyclization with morpholine readily afforded access to the desired *cis*-diketopiperazine (–)-**10** in 84% yield (>20g). Exposure of *cyclo*-Ltryptophan-L-alanine (–)-**10** to molecular bromine in acetonitrile at 0 °C furnished the desired monomeric tetracyclic bromide (+)-**11** in 76% isolated yield (14). Treatment of tetracyclic bromide (+)-**11** with methyl iodide and potassium carbonate gave the base-sensitive dimerization precursor (+)-**12** in 77% yield (15). Reductive dimerization of the tertiary benzylic bromide (+)-**12** with tris(triphenylphosphine)cobalt(I) chloride in acetone provided the key dimeric octacyclic intermediate (+)-**13** in 46% yield (16). Preference for *cis*-fusion on 5,5-ring systems made this an effective strategy for simultaneously securing the two vicinal C3 and C3' quaternary stereocenters (12). This chemistry is amenable to multi-gram scale synthesis of (+)-**13** (e.g., 43% yield on 8g-scale).

Guided by our biosynthetic hypothesis for late stage functionalization of the diketopiperazines (i.e., **7**→**5**, Fig. 2), we sought methods for C_α-oxidation of the dimeric octacycle (+)-**13**. Initially, we focused on the oxidation of the readily accessible enol tautomers or corresponding enolates of (+)-**13** (14). Unfortunately, these strategies were plagued by formation of partially oxidized and diastereomeric products in addition to significant competing decomposition. Likewise, a variety of softenolization and electrophilic amide activation strategies failed to provide the necessary C_α-methine oxidation. Although we ultimately developed conditions for dihydroxylation (or didehydrogenation) of a model monomeric tetracyclic diketopiperazine **21** (Fig. 4A) along with its conversion to the corresponding monomeric epidithiodiketopiperazine **23** (14), none of these methodologies proved effective when applied to the more challenging dimeric octacyclic

bisdiketopiperazine (+)-**13**, likely due to additional modes of C3-C3' bond fragmentation and/or unfavorable interactions between the tetracyclic subunits.

Careful analysis of the bond dissociation energies (17) involved in our successful radical based abstraction of C_α-methines in the model tetracycle **21** (14) suggested weak C_α–H bonds due to stabilization of the ensuing C_α-radicals in diketopiperazines. Thus, the use of mild oxidants typically reserved for hydrogen atom abstraction from formyl groups became a focus of our efforts in pursuit of an effective strategy for single-step tetrahydroxylation of dimeric octacycle (+)-**13**. After extensive experimentation, we discovered that the treatment of the diketopiperazine **21** with tetra-*n*-butylammonium permanganate (3.0 equiv) (18) in pyridine at 23 °C for 2 h provided the desired tetracyclic diol **22** in 78% yield primarily as one diastereomer. Application of these conditions to the oxidation of the more challenging dimeric octacycle (+)-**13** resulted in 40% yield of the desired tetraol as a complex mixture of hemiaminal diastereomers. As this tetrahydroxylation was fraught with competing epimerization of C_α-methines and incomplete oxidation leading to complex product mixtures (14), we sought to refine this reaction.

Further studies revealed that bis(pyridine)-silver(I) permanganate (Py_2AgMnO_4) (19) oxidized dimeric octacycle (+)-**13** selectively and efficiently. Under optimal conditions, treatment of dimer (+)-**13** with Py_2AgMnO_4 (4.8 equiv) in dichloromethane at 23 °C for 2 h afforded the desired dimeric octacyclic tetraol (+)-**14** in 63% yield as a single diastereomer (Fig. 3). The high level of diastereoselection (14) is consistent with a fast abstraction-rebound mechanism (20–21), as suggested by hydroxylation of the radical– clock hydantoin **24** (74%) (Fig. 4A) and X-ray diffraction analysis of tetraol (+)-**14**. Oxidation of the corresponding *cyclo*-D-Trp-L-Ala derivatives under these conditions resulted only in oxidation at the alanine C_α(Ala)-methines, leaving the C_α (D-Trp)-methines unchanged (14). This observation, which has important consequences for the choice of natural or unnatural amino acid precursors, is attributed to a non-optimal conformation of the C–H bond for abstraction and/or the sterically disfavored approach of the oxidant from the concave face of the 5,5- ring system.

The dimeric octacyclic tetraol (+)-**14** proved highly acid- and base-sensitive. Its treatment with BrØnsted acids led to formation of tetraene **26** (Fig. 4B) (22), whereas its exposure to base resulted in either decomposition or conversion to hemiaminal diastereomers (14). The high sensitivity of tetraol (+)-**14** to base may be attributed to reversible ring opening at the C15-aminal allowing deleterious side reactions of the alpha-keto amide derivative **27** (Fig. 4B). Surprisingly, even dissolution of (+)-**14** in methanol at ambient temperature led to slow decomposition.

With multi-gram access to dimeric octacyclic tetraol (+)-**14**, we focused on its conversion to alkaloid (+)-**1**. Removal of the benzenesulfonyl groups with sodium amalgam in methanol buffered with dibasic sodium phosphate unveiled an unstable diaminotetrahemiaminal **28** (Fig. 4C). Immediate exposure of this labile compound to condensed hydrogen sulfide at −78 °C with a Lewis acid (6), followed by warming, resulted in formation of the corresponding tetrathiol **29** as a mixture of hemithioaminal diastereomers. Oxidation of the

crude mixture of tetrathiols with potassium triiodide resulted in (+)-11,11'-dideoxyverticillin A (**1**), albeit in low overall yields (2–15%, 3-steps) from tetraol (+)-**14**.

The fragility of tetraol (+)-**14** and derivatives **28** and **29**, the poor mass balance of this capricious three-step sequence, and our preference to avoid the use of pressurized toxic hydrogen sulfide led us to seek a superior strategy for the synthesis of the epidithiodiketopiperazine substructure of this family of alkaloids. After substantial experimentation we realized that a simple tactical conversion of the tetraol (+)-**14** to the diol (+)-**15** (Fig. 3) imparted considerable stability to this structure, consistent with prevention of an undesired diketopiperazine ring-opening. The use of Fu's PPY-catalyst (5 mol%) (23) was optimal for the selective derivatization of both alanine-derived hemiaminals of (+)-**14** (14). Treatment of a methanolic solution of diol (+)-**15** containing monobasic sodium phosphate with sodium amalgam cleanly unveiled the stable diaminodiol (+)-**16** in 87% yield as a surrogate for our hypothetical biosynthetic intermediate **6** (Fig. 2).

At this juncture, we envisioned that coordinating the introduction of the two sulfur atoms on each diketopiperazine ring would provide greater stereochemical control and structural stability. Inspired by the Woodward-Prévost *cis*-dihydroxylation of alkenes with carboxylate ions (24), and cognizant of the observation from Kishi's seminal synthesis of gliotoxin that epidithiodiketopiperazines are acutely sensitive toward basic, reductive, oxidative and strongly acidic conditions (5), we reasoned that the use of a trithiocarbonate (25) would deliver a sulfurated product poised for mild unveiling of the targeted tetrathiol at an advanced stage. In the event, treatment of diaminodiol (+)-**16** with potassium trithiocarbonate and trifluoroacetic acid in dichloromethane resulted in rapid formation and isolation of the desired dimeric bisdithiepanethione (+)-**18** in 56% yield (14, 26), likely via kinetic trapping of iminium ion **17** followed by intramolecular dithiepanethione formation. In this single operation, four carbon–oxygen bonds are exchanged for four carbon–sulfur bonds, the stereochemistry at all four tertiary thiols is secured, and the targeted *cis*-dithiodiketopiperazine substructure of **5** is attained.

Addition of ethanolamine to a solution of bisdithiepanethione (+)-**18** at 23 °C rapidly afforded the proposed biosynthetic precursor diaminotetrathiol **5** (27), which is subject to mild oxidation to (+)-**1** upon exposure to air (14). Under optimized conditions, following the formation of diaminotetrathiol **5**, partitioning of the reaction mixture between aqueous hydrochloric acid and dichloromethane and immediate addition of potassium triiodide to the organic layer provided (+)-11,11'-dideoxyverticillin A (**1**, $[\alpha]^{21}_{D}$ = +590 (*c* 0.30, $CHCl_3$); lit. $[\alpha]^{21}_{D}$ = +624.1 (*c* 0.3, $CHCl_3$), 1) in 62% yield as a colorless solid. All spectroscopic data for (+)-**1** matched those reported in the literature (1). Furthermore, we unambiguously secured the structure of synthetic (+)-**1** by crystallographic analysis.

This concise strategy for the synthesis of (+)-**1** required a carefully choreographed sequence of events. In this sequence, the inherent chemistry of intermediates was maximally utilized in generation of chemical complexity and stereochemical control. For example, unveiling of the aniline nitrogen (N1) of (+)-**13** followed by attempted tetrahydroxylation led to complete decomposition under a variety of conditions. The challenges associated with the high sensitivity of (+)-**13** toward epimerization at the L-amino acid derived C_α-stereocenters was

compounded by the requirement for oxidation prior to epimerization (*vide supra*). Furthermore, thiolation of the oxidized diketopiperazines at an earlier stage led to significant reductive cleavage or elimination of the sensitive carbon–sulfur bonds during subsequent transformations. These key insights guided our described strategy, whereby the conversion of diaminodiol (+)-**16** to dimeric dithiepanethione (+)-**18** enabled tetrathiolation with concomitant inversion of all four Cα-stereocenters, allowing rapid epidithiodiketopiperazine formation.

Collectively, our observations on the inherent reactivity of these structures hint at a plausible biosynthetic sequence for alkaloid (+)-**1** (Fig. 2). While the viability of the proposed biosynthetic intermediates is supported through chemical synthesis, the successful implementation of our synthetic strategy offers a potential roadmap to the function of enzymes involved in the biosynthesis of epidithiodiketopiperazine alkaloids. For instance, Howlett's studies of the epidithiodiketopiperazine biosynthetic gene clusters (28) have identified genes with unassigned specific function bearing structural homology to the cytochrome P450 mono-oxygenase. The mechanistic semblance of our permanganate diketopiperazine hydroxylation to the well studied C–H abstraction–hydroxylation of substrates by P450 oxygenases (29–30) prompts consideration of the involvement of these unassigned genes in the C_α-oxidation of the diketopiperazine core.

Alkaloid (+)-**1** potently inhibits the tyrosine kinase activity of the epidermal growth factor receptor (IC_{50} = 0.14 nM), exhibits antiangiogenic activity, and has efficacy against several cancer cell lines (31–33). The strategy and methodologies described here are expected to yield ready access to related compounds and provide an inroad to further biological studies. In this report we have attempted to capture the power of biosynthetic considerations as a guiding principle for synthetic planning and as an inspiration for the development of new reactions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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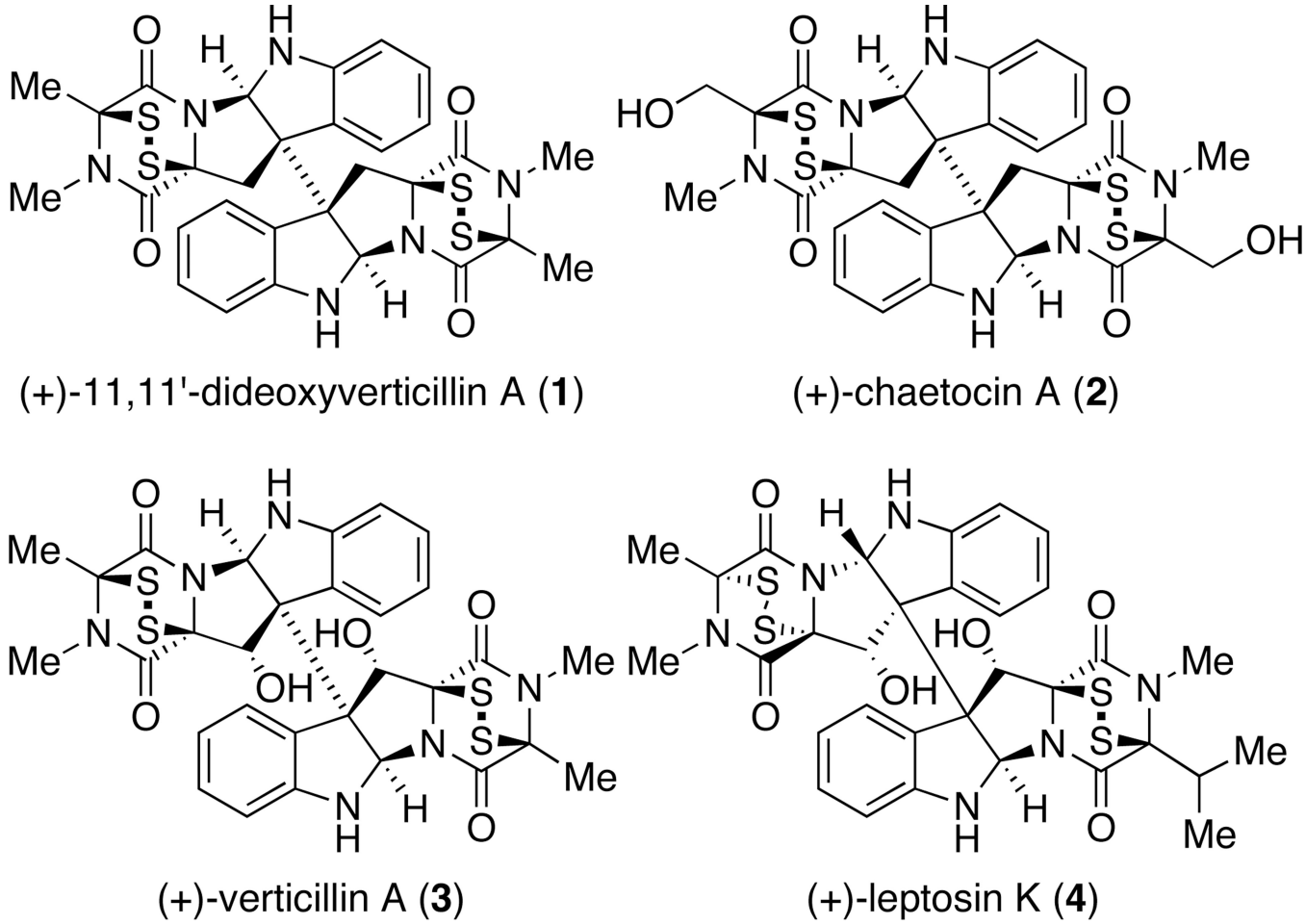

Fig. 1.

The molecular structure of (+)-11,11'-dideoxyverticillin A (**1**) and representative dimeric epidithiodiketopiperazine alkaloids.

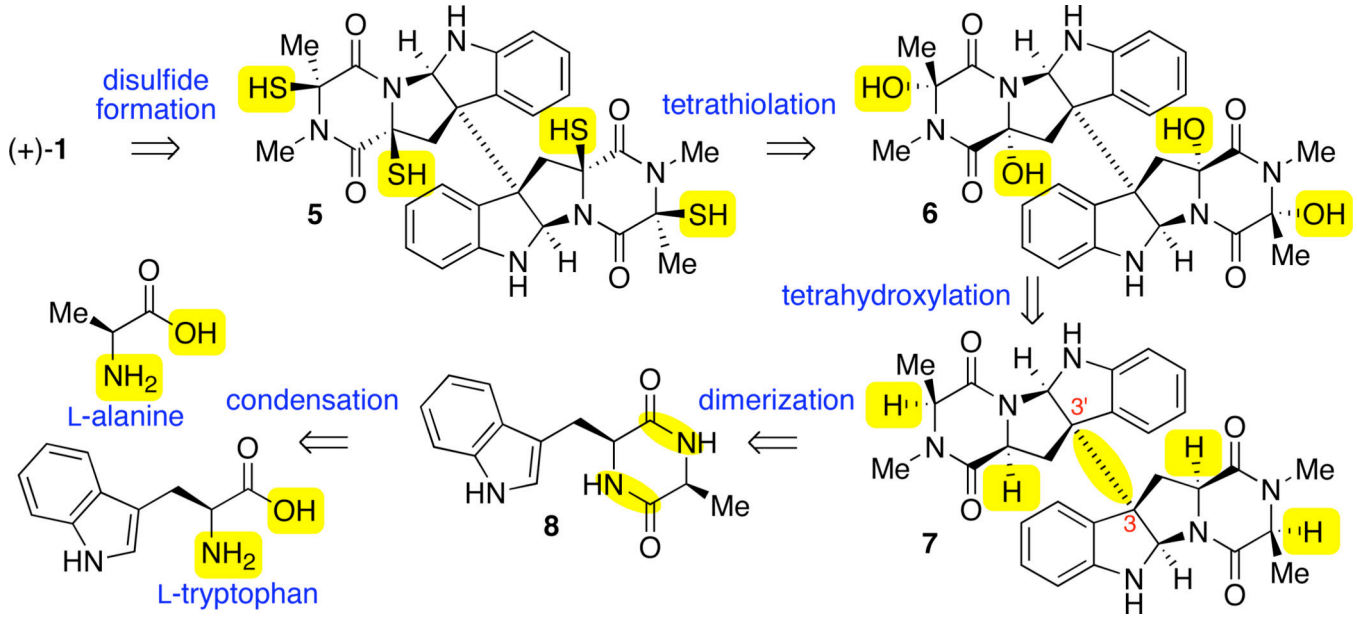

Fig. 2.

Retrosynthetic analysis of (+)-11,11'-dideoxyverticillin A (**1**) based on a biosynthetic hypothesis.

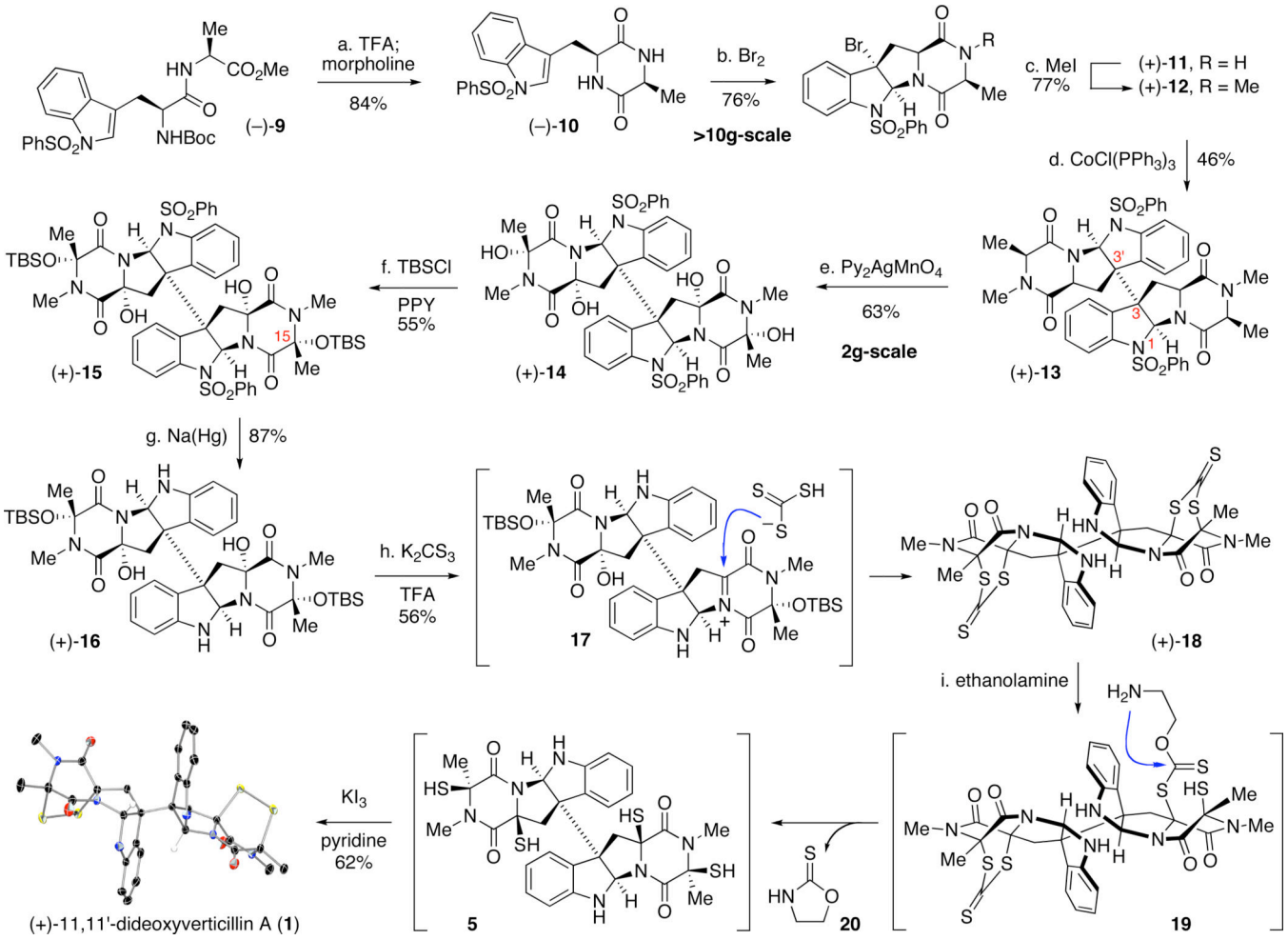

Fig. 3.

Concise enantioselective total synthesis of (+)-11,11'-dideoxyverticillin A (**1**). Isolated yields are given for each step. Conditions: (a) trifluoroacetic acid (TFA), dichloromethane (CH_2Cl_2), 23 °C, 4 h; *tert*-butanol (*t*BuOH), morpholine, 23 °C, 48 h. (b) Br_2, acetonitrile (MeCN), 0 °C, 5 min. (c) methyl iodide (MeI), K_2CO_3, acetone, 23 °C, 5 d. (d) tris(triphenylphosphine)cobalt(I) chloride ($CoCl(PPh_3)_3$), acetone, 23 °C, 30 min. (e) bis(pyridine)silver(I) permanganate (Py_2AgMnO_4), CH_2Cl_2, 23 °C, 2 h. (f) *tert*-butyl(chloro)dimethylsilane (TBSCl), (R)-(+)-4-pyrrolidinopyridinyl(pentamethylcyclopentadienyl)-iron (PPY) 5 mol%, triethylamine (Et_3N), *N*,*N*-dimethyl formamide (DMF), 23 °C. (g) 5% Na(Hg), NaH_2PO_4, methanol (MeOH), 23 °C. (h) K_2CS_3, TFA, CH_2Cl_2, 28 min. (i) ethanolamine, 23 °C; KI_3, pyridine, CH_2Cl_2, 23 °C. The thermal ellipsoid representation of synthetic (+)-**1** from X-ray crystallographic analysis is shown with most hydrogens omitted for clarity.

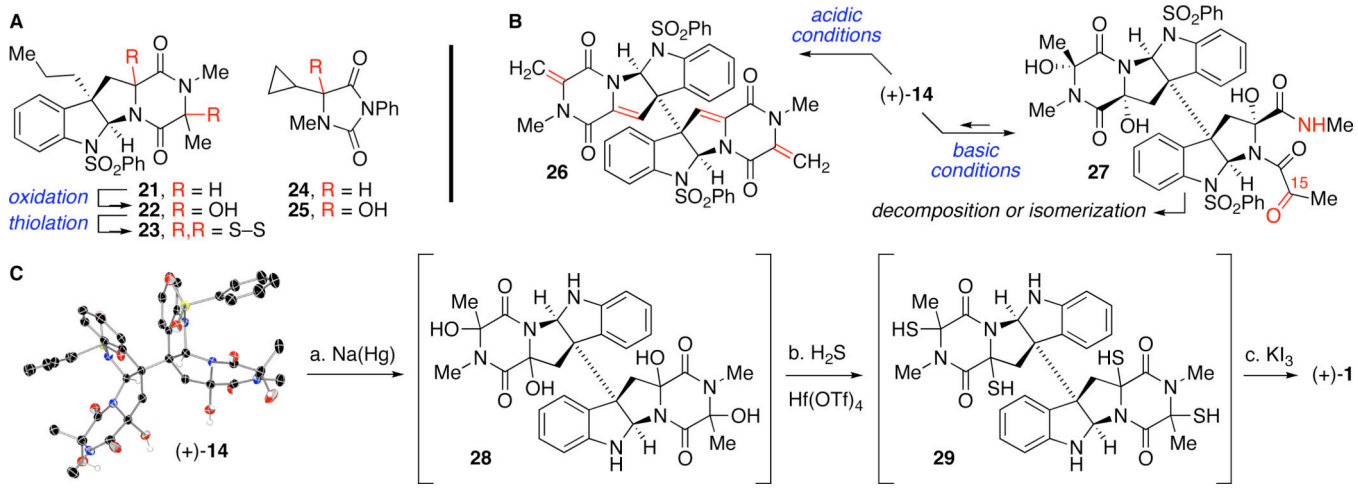

Fig. 4.

Key observations enabling our first-generation synthesis of (+)-11,11'-dideoxyverticillin A (**1**). **A**. Functionalization of exploratory models. **B**. Sensitivity of dimeric octacyclic tetraol (+)-**14** to both acidic and basic conditions. **C**. Thermal ellipsoid representation of (+)-**14**. Synthesis of alkaloid (+)-**1** from dimeric tetraol (+)-**14**. Conditions: (a) 5% Na(Hg), Na_2HPO_4, MeOH, 23 °C. (b) H_2S, CH_2Cl_2, hafnium(IV) trifluoromethanesulfonate ($Hf(OTf)_4$), –78→23 °C, 14 h. (c) KI_3, pyridine, CH_2Cl_2, 23 °C, 2–15% for 3-steps.