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# Synthesis of (±)-Actinophyllic Acid and Analogs: Applications of Cascade Reactions and Diverted Total Synthesis

Brett A. Granger,[§,†] Ivan T. Jewett,[§,†,∥] Jeffrey D. Butler,[⊥,†] Bruce Hua,[†] Claire E. Knezevic,[‡] Elizabeth I. Parkinson,[‡] Paul J. Hergenrother,[‡] and Stephen F. Martin*[,†]

[†]Department of Chemistry and Biochemistry, The University of Texas at Austin, Austin, Texas 78712, United States

[‡]Roger Adams Laboratory, Department of Chemistry, University of Illinois at Urbana−Champaign, Urbana, Illinois 61801, United States

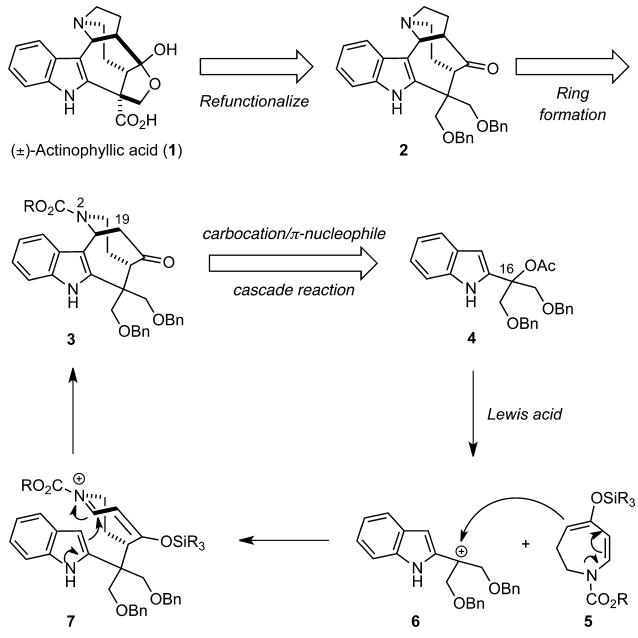
Supporting Information

**ABSTRACT:** Actinophyllic acid is a biologically active indole alkaloid with a unique structural framework that incorporates five contiguous stereocenters. A total synthesis of (±)-actinophyllic acid has been completed that proceeds in only 10 steps from readily available, known compounds and with the isolation of nine intermediates. The synthesis features a novel cascade of reactions of *N*-stabilized carbocations with π-nucleophiles to create the tetracyclic core of actinophyllic acid in a single chemical operation. This pivotal cascade sequence generates substructures of the actinophyllic acid core that are not otherwise accessible, and one key intermediate was modified to furnish several novel compounds having potentially promising anticancer activity, one of which induces cell death in a wide range of cancer cell lines.

Actinophyllic acid (**1**) is an indole alkaloid that was isolated in 2005 by Carroll and co-workers from the leaves of *Alstonia actinophylla*.[1] Initially isolated on the basis of its biological activity as a potent inhibitor of carboxypeptidase U (CPU), **1** has possible therapeutic indications for the treatment of thrombotic diseases. We were first attracted to actinophyllic acid by its unique skeleton, which afforded an opportunity to develop novel chemistry.[2] When we initiated our work toward **1**, we were aware of no other synthetic efforts in the area. However, since that time Overman and co-workers have published an elegant approach that culminated in syntheses of racemic and enantiomerically pure actinophyllic acid.[3] Furthermore the groups of Wood,[4] Taniguchi,[5] and Maldonado[6] have revealed alternate entries to this novel alkaloid.

Our approach to actinophyllic acid (**1**) was predicated upon a novel cascade of transformations that we envisioned would generate the key tetracyclic intermediate **3** in a single chemical operation that merged the indole **4** with the diene **5** (Scheme 1). The inspiration for this sequence of reactions owed its origin to a discovery that we made during our successful work directed toward the synthesis of the welwitindolinones[7] and that we applied to developing a general entry to β-heteroaryl carbonyl compounds.[8] In particular, we found that benzylic-like cations, including those generated from 2-hydroxymethyl indole derivatives related to **4**, could be captured by π-nucleophiles such as enol ethers to provide β-heteroaryl propionates.

**Scheme 1. Proposed Retrosynthesis**




We thus reasoned that reaction of the carbocation **6**, which would be generated by Lewis acid mediated ionization of the tertiary acetate at C(16) of **4**, with the nucleophilic enamido diene **5** would furnish the *N*-acyliminium ion **7**, spontaneous cyclization of which by a Mannich-like reaction would deliver the tetracyclic intermediate **3**. The geometric constraints associated with **7** guaranteed formation of a single stereoisomeric product. Following *N*-deprotection of **3**, our plan called for the formation of the pyrrolidine ring by sequential alkylations at N(2) and C(19) to give **2**. Subsequent deprotection of **2** followed by cyclization of the intermediate keto diol would give a hemiketal array and a primary alcohol that could be oxidized to deliver actinophyllic acid (**1**).

In order to set the stage for the pivotal cascade sequence, it was necessary to prepare the requisite indole **4** and a dihydroazepine related to **5**. In the event, carboxylate directed lithiation of indole (**8**) at C(2),[9] followed by reaction of the

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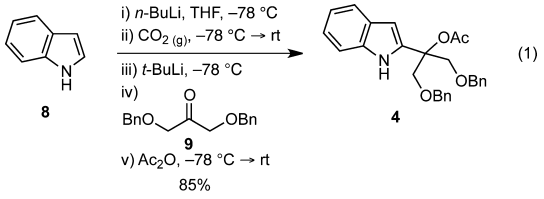
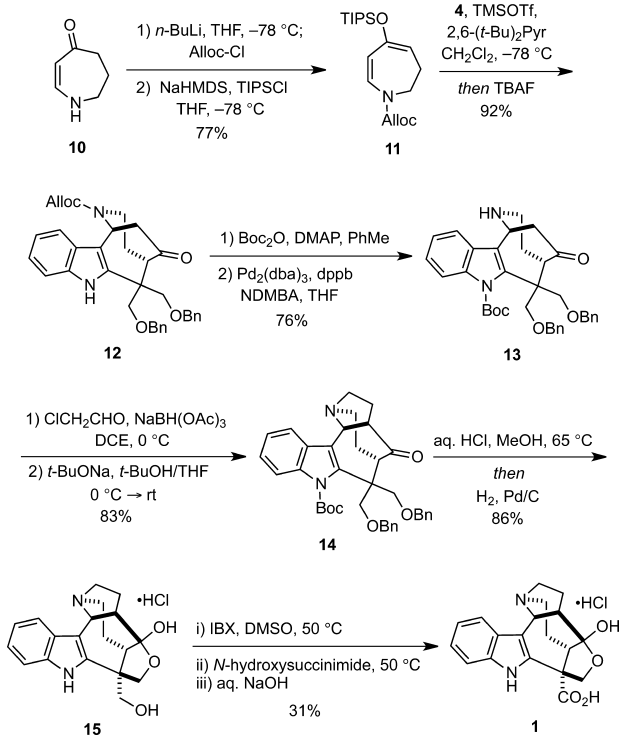
resultant dianion with 1,3-dibenzyloxyacetone (**9**), a known compound that was easily obtained by a Swern oxidation of the commercially available alcohol,[10] gave an intermediate alkoxide that was acetylated with acetic anhydride to furnish the indolyl acetate **4** in 85% yield (eq 1).




The known hydroazepinone derivative **10**, which was readily prepared by the photorearrangement of *N*-vinyl-pyrrolidone,[11] was then converted into the dihydroazepine **11** in 77% yield by sequential *N*-acylation using NaHMDS and allyl chloroformate followed by *O*-silylation with TIPSCl (Scheme 2). Having

**Scheme 2**

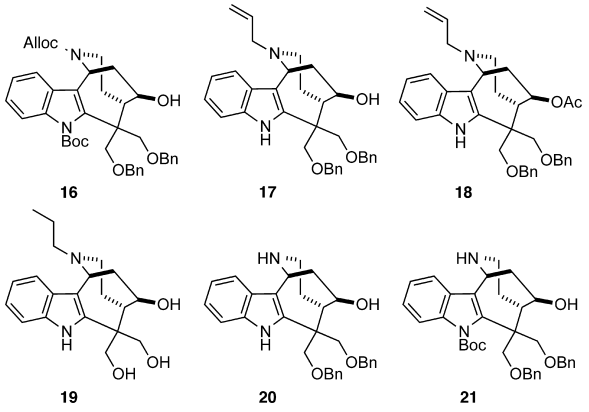



efficiently synthesized the necessary fragments, we turned our efforts toward examining the key cascade reaction. Early experiments directed toward inducing this series of transformations were plagued by side reactions because the initially formed tetracyclic product **12** was unstable toward facile carbon–nitrogen bond scission under the reaction conditions.[12] However, we eventually discovered that addition of TMSOTf to a mixture of indolyl acetate **4** and silyl enol ether **11** in the presence of 2,6-di-*tert*-butylpyridine followed by quenching the reaction using tetra-*n*-butylammonium fluoride delivered the desired product **12** in 92% yield. This remarkable cascade sequence thus proceeded precisely as planned and enabled the production of the tetracyclic core of actinophyllic acid in a single chemical operation from readily available starting materials. Moreover, the reaction could be performed on a multigram scale.

Because **12** was somewhat prone to fragmentation by carbon–nitrogen bond cleavage, we reasoned that the tetracyclic framework might be stabilized by introduction of an electron-withdrawing group on the indole nitrogen atom. After some experimentation, we found that treating **12** with Boc-anhydride under basic conditions followed by palladium-catalyzed removal of the Alloc protecting group in the presence of *N,N*-dimethylbarbituric acid (NDMBA) afforded amine **13** in 76% overall yield from **12**.[13] The pyrrolidine ring was formed by reductive alkylation of amine **13** with chloroacetaldehyde, followed by base-induced cyclization via an intramolecular enolate alkylation to furnish the pentacycle **14** in 82% yield over the two steps. Heating **14** under acidic conditions led to the removal of the Boc protecting group, whereupon Pd/C was simply added to the reaction, and the resulting mixture was stirred under an atmosphere of hydrogen to remove the benzyl ether protecting groups. The intermediate keto diol thus obtained cyclized spontaneously to give **15** in 86% yield. The structure and relative stereochemistry of **15** was unambiguously determined by single crystal X-ray analysis.

At this stage the seemingly straightforward oxidation of the neopentyl alcohol in **15** to the requisite carboxylic acid remained. This conversion proved, however, to be unexpectedly recalcitrant. After screening a number of reagents and conditions, we eventually discovered that the reaction of **15** with IBX in DMSO gave an intermediate aldehyde that could be treated directly with *N*-hydroxysuccinimide in the presence of excess IBX to give a succinimide ester that was readily saponified to deliver (±)-actinophyllic acid hydrochloride (**1**) in 31% yield.[14] The spectral data ($^1$H and $^{13}$C NMR) of the synthetic material thus isolated were identical with those obtained for an authentic sample kindly provided by Overman.

Diversion of synthetic intermediates encountered during total synthesis efforts has been shown to be an excellent strategy for generating unique compounds for biological screening and evaluation.[15] Given that the cascade sequence of reactions afforded facile access to **12**, which is not otherwise readily available, we queried whether analogs of **12** might have promising biological properties. Accordingly, compounds **16**–**21** were prepared from **12** by straightforward reactions. The bioactivity of racemic **13**, **14**, **16**–**21**, and actinophyllic acid (**1**)




were then assessed for their ability to cause cell death in Hs578t cells, a human breast cancer cell line (Figure 1). Of these, four compounds (**14**, **17**, **20**, and **21**) induced cell death, whereas the remaining compounds, including actinophyllic acid, were ineffective at concentrations up to 100 $\mu$M. The observed variations in potencies give some preliminary insights into the structure–activity relationships of this series. For example, the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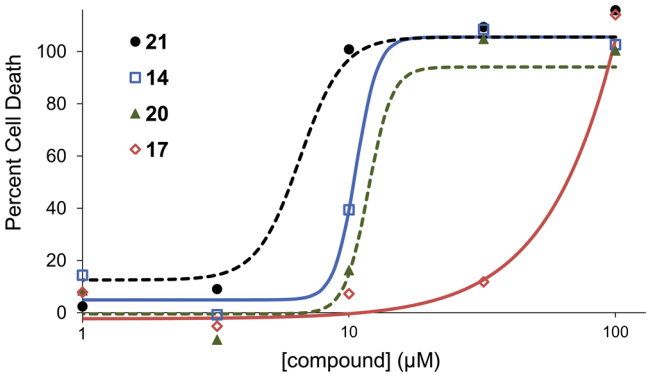


**Figure 1.** Ability of compounds to induce death of cancer cell lines in culture. Compounds (3 nM−100 $\mu$M) were coincubated for 48 h with Hs578t human breast cancer cells in 384-well plates (2,000 cells/well), and viability was assessed by Alamar Blue. Data are representative of triplicate data. $IC_{50}$ values were determined for **14** (11.2 ± 1.9 $\mu$M), **17** (63.1 ± 16.0 $\mu$M), **20** (14.9 ± 4.8 $\mu$M), and **21** (7.7 ± 2.8 $\mu$M). Compounds **1**, **13**, **16**, **18**, and **19** caused less than 20% cell death at 100 $\mu$M.

presence of an *N*-alkoxycarbonyl group on the secondary amine as in **16** appears detrimental, whereas such substitution on the indole nitrogen atom as found in **14** and **21** is tolerated. Removal of the *O*-benzyl protecting groups and *O*-acetylation of the secondary hydroxyl group lead to compounds **18** and **19**, which are also less active.

The $IC_{50}$ values of the active compounds range from ∼8 to 63 $\mu$M (Figure 1; Supporting Information, Table S1). The most potent compound, **21**, was tested against three additional cell lines, a human lymphoma (U937), a human lung cancer (A549), and a human glioblastoma cell line (U87), and it was found to have $IC_{50}$ values of ∼5−8 $\mu$M in these cells (Supporting Information, Table S2). The noteworthy persistence of potency across such diverse types of cancer suggests that **21** may have translational potential.

A concise synthesis of (±)-actinophyllic acid (**1**) was achieved by a route that required only 10 chemical operations and the isolation of nine intermediates starting with readily available, known compounds. The synthesis features a novel cascade of reactions of *N*-stabilized carbocations with $\pi$-nucleophiles to create the tetracyclic core of actinophyllic acid in a single operation. Notably, some synthetic intermediates were diverted by refunctionalization and derivatization to furnish novel compounds that induce death in several cancer cell lines, whereas actinophyllic acid itself is inactive. Further optimization of the potency of these small molecules and mode-of-action studies are a focus of current efforts. The discovery of actinophyllic acid analogs that exhibit potentially promising anticancer activity validates the importance of developing alternative entries to complex natural products as a critical strategy for identifying compounds that would not otherwise be accessible for biological screening and evaluation.

## ■ ASSOCIATED CONTENT

### Supporting Information

Experimental procedures for all intermediates in the synthesis of **1**, full characterization for all new compounds, and experimental procedures and data for cytotoxicity assays. This material is available free of charge via the Internet at http://pubs.acs.org.

## ■ AUTHOR INFORMATION


### Corresponding Author

sfmartin@mail.utexas.edu

### Present Addresses

[‖]The Broad Institute, 7 Cambridge Center, Cambridge, MA 02142, USA.

[⊥]AMPAC Fine Chemicals, Rancho Cordova, CA 95741, USA.

### Author Contributions

[§]These authors contributed equally.


### Notes

The authors declare no competing financial interest.

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