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Synthesis and Assignment of the Absolute Configuration of Indenotryptoline Bisindole Alkaloid BE-54017

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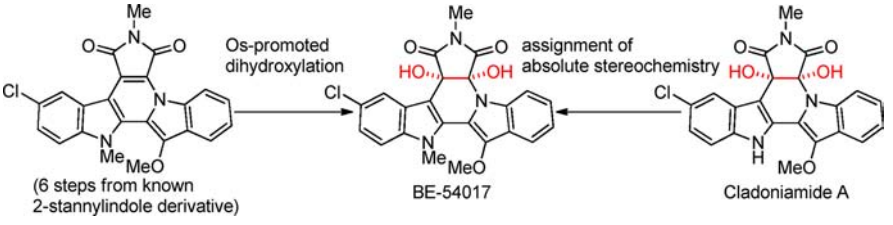
ABSTRACT



Synthesis of the indenotryptoline bisindole alkaloid, BE-54017, was accomplished using osmium-promoted *cis*-dihydroxylation of maleimide as a key step. After optical resolution, the absolute configuration of this molecule was determined by comparing its optical rotation and HPLC profile to those obtained for BE-54017 derived from enantiopure cladoniamide A, whose stereochemistry has been reported previously. BE-54017 with the correct absolute stereochemistry induced apoptosis of epidermal growth factor (EGF)-stimulated EGF receptor overexpressing A431 cells and inhibited vacuolar-type H^+-ATPase (V-ATPase).

The bisindole alkaloid family of natural products is a rich source of bioactive substances,[1,2] including potential lead compounds of medicines for clinical use. Among them, indolocarbazole-type compounds,[3] represented by rebeccamycin (**3**)[4] and staurosporine (**4**),[5] occur widely in nature. Typically, the carbon at the 3-position of each indole subunit connects to a characteristic five-membered ring heterocycle such as maleimide or γ-lactam. Less commonly observed, however, is another subfamily of bisindole alkaloids, the indenotryotoline-type compounds, in which one of the indole subunits of the indolocarbazole scaffold is flipped so that the nitrogen atom at the 1-position forms a bond with the 5-membered heterocycle, creating an asymmetrical overall framework (Figure 1). BE-54017 (**1**), an indenotryptoline-type bisindole alkaloid, was first reported as a natural cytotoxic product (i.e., IC_{50} for P388: 0.11 μg/mL) isolated from *Streptomyces* sp. A54017 by the Banyu group in 2000.[6] A recent study to screen for selective inducers of epidermal growth factor (EGF)-dependent apoptosis in EGF receptor (EGFR)-overexpressing tumor cells revealed that BE-54017 exhibited the desired activity. Overexpression of EGFR is observed in many tumor types and seems to enhance tumor development and malignancy; therefore, the development of EGFR-targeting drugs has been anticipated for their therapeutic efficiency.[7] In fact, BE-54017 dose-dependently induced apoptosis in EGF-stimulated A431 cells that overexpress EGFR, but not in other types of human tumor cells that do not overexpress the EGFR. Because of the unusual structure and biological

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activity of BE-54017, synthetic studies were performed to assign its relative and absolute configurations.

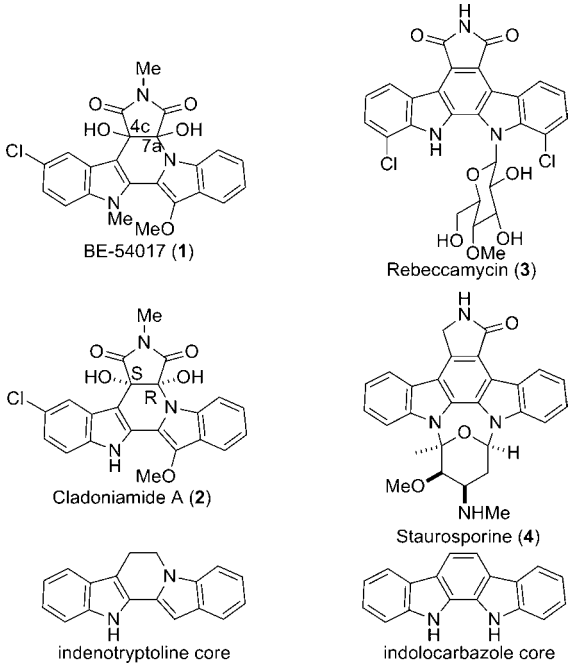
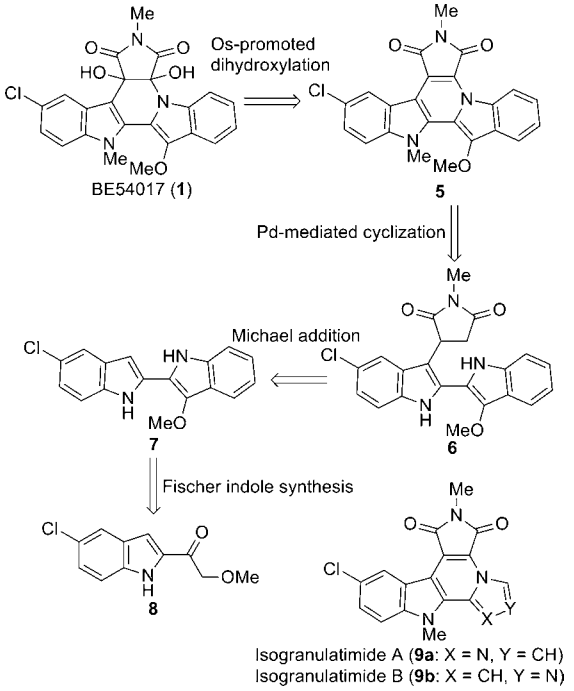


Figure 1. Structure of BE-54017 and related bisindole alkaloids.

In 2008, Davies and Andersen reported the isolation and structure determination of cladoniamide A (**2**), a desmethyl homologue of BE-54017, produced by *Streptomyces* uncialis.[8] In that study, the absolute stereochemistry of cladoniamide A was unequivocally assigned as shown in Figure 1 by X-ray crystallographic analysis; vicinal *cis*-diol in the 4c and 7a positions has the *S*- and *R*-configuration, respectively. Moreover, Chang and Brady recently proposed the biosynthetic pathway of BE-54017.[9] According to their report, the three methyltransferases (ie., Abe M1, Abe M2, and Abe M3) are responsible for the stepwise introduction of the three methyl groups at the late stage of the biosynthesis, which implies that cladoniamide A is a possible biosynthetic precursor of BE-54017. Although no stereochemical information on BE-54017 itself has been provided to date, it is likely that the diol moiety of this molecule also has a *cis* orientation and its absolute configuration should thus be 4c*S*,7a*R*. Herein, we report the first synthesis of indenotryptoline bisindole alkaloide BE-54017 and determination of the absolute stereochemistry of this molecule using the synthetic approach.

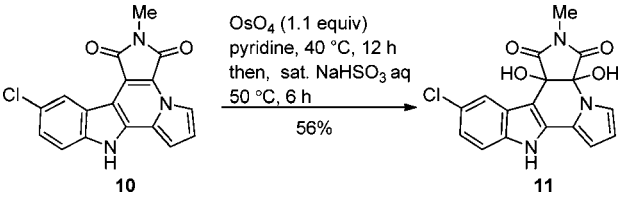
Scheme 1 depicts the synthetic design of BE-54017 in the present study. Because of the rarity of the indenotryptoline scaffold, only scattered reports describing synthetic efforts on structurally related compounds are found in the literature. The vicinal *cis*-diol was anticipated to be introduced

Scheme 1. Structure of BE-5417 and Related Bisindole Alkaloids



at the final stage of the synthesis by metal-mediated dihydroxylation of an unreactive double bond embedded in the maleimide moiety of **5**. This intermediate contains the entire hexacyclic system of BE-54017, which was planned to be constructed by the Michael addition of a bisindole **7** to maleimide, affording **6**, followed by Pd-promoted cyclization and subsequent methylation. This two-step protocol for the C−C and C−N bond formation was inspired by precedents in which a similar reaction sequence was examined with maleimide and indolylimidazoles or indolylpyrroles as the substrate in an effort to synthesize the analogues[10] of isogranulatimides A and B (**9a** and **9b**, respectively).[11] The bisindole building block **7** should be prepared by Fischer indole synthesis using properly functionalized 2-acylindole **8**.

Scheme 2. Model Study on Dihydroxylation of Maleimide Using an Isogranulatimide Analogue



Prior to our attempt to synthesize BE-54017, the conditions for *cis*-dihydroxylation of the maleimide moiety were

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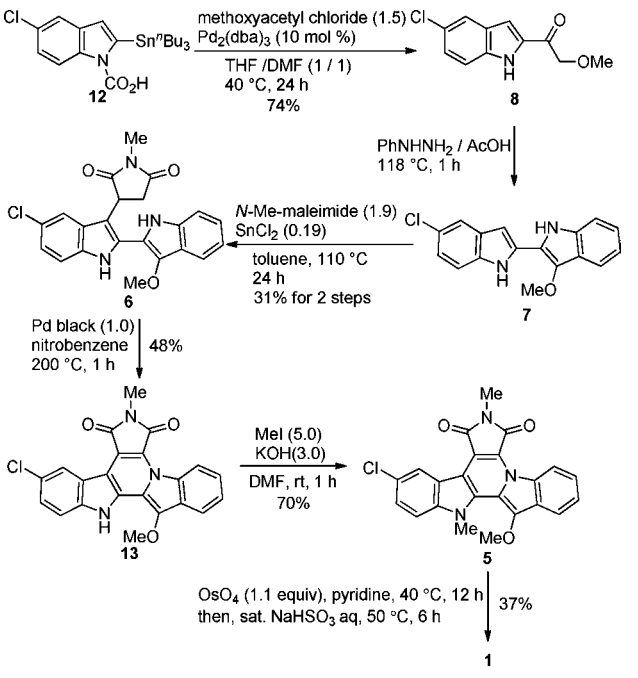
screened using a structurally simplified substrate **10** that can be prepared according to the reported procedure.[10] The standard conditions with a catalytic amount of OsO_4 in the presence of NMO as a co-oxidant did not work, and all of the starting material was recovered. This result is consistent with the previous report that dihydroxylation of *N*-phenylmaleimide under identical conditions resulted in an extremely low yield (4%).[12] No reaction occurred when the *N*-methylated congener of **10** was used. Addition of citric acid to the media showed no improvement in efficacy of the reaction.[13] Ru- and Mn-based oxidation was also not effective for this system, and no reaction took place ($RuCl_3$ (0.5 or 2.5 mol %), $NaIO_4$ (1.4 or 1.5 equiv), $CeCl_3$ (10 mol %), 0 °C for 18 h,[14] and $Mn(ClO_4)_2 \cdot 6H_2O$ (0.3 mol %), pyridine-2-COOH (1.8 mol %), NaOAc (3.0 mol %), H_2O_2, (2.0 equiv), 0 °C to rt for 18 h,[15] respectively). We then returned to osmium-mediated reactions and discovered that the reaction proceeded with reasonable efficacy when a stoichiometric amount of OsO_4 (1.1 equiv) was used, giving rise to the osmate ester of **10**, which could be converted to the free form using a reductive workup procedure (saturated aq $NaHSO_3$, 50 °C for 6 h) in 56% yield (Scheme 2). Encouraged by this result, we began the synthesis of BE-54017 as shown in Scheme 3.

Scheme 3. Synthetic Route to BE-54017 (**1**)



Synthesis commenced with Stille coupling between the known stannane **12**[16] and 1.2 equiv of methoxyacetyl chloride to afford **8**. The use of 10 mol % of $(Ph_3P)_2PdCl_2$ resulted in a poor yield even under reflux in toluene (4%). Changing the palladium source to 10 mol % of $Pd_2(dba)_3$ along with switching the solvent to THF or DMF slightly improved the yield even at ambient temperatures (11% and 16%, respectively). A mixed solvent system of equal volumes of THF and DMF dramatically increased the chemical yield to 46%. Finally, slightly increasing the reaction temperature to 40 °C afforded the desired coupling product in 74% yield. Upon reflux at 80 °C, however, decomposition of the stannane **12** predominated.

In the next step, the 2-acylindole derivative **8** was subjected to the standard Fischer indole synthesis protocol in the presence of phenylhydrazine to afford unstable bisindole intermediate **7**, which was immediately used without purification for the succeeding Michael addition to maleimide to give **6** in 31% over two steps. The cyclization of **6** was accomplished by treating a stoichiometric amount of Pd black in nitrobenzene under heating at 200 °C[10] leading to **13** in moderate yield (48%), and the whole BE-54017 framework was constructed at this stage. Subsequent methylation of the indenotryptoline intermediate **13** with excess MeI in the presence of 3.0 equiv of KOH as a base afforded **5** in 70% yield. The prolonged reaction time in this step resulted in opening the maleimide core, which substantially reduced the isolated yield. Finally, OsO_4-promoted dihydroxylation and a subsequent reductive workup as demonstrated in the model study completed the synthesis of BE-54017 (37%). All of the physicochemical data of the synthetic sample except for optical rotation were indistinguishable from those of the natural product.[6]

Synthetic BE-54017 was submitted to an optical resolution to define its absolute stereochemistry and elucidate a relationship between its stereochemistry and biological activity. To this end, an HPLC method using a chiral stationary phase (Daicel, CHIRALPAC IC 4.6 × 250 mm, 20% AcOEt in *n*-hexane, 2 mL/min) was effective. Two enantiomers were eluted at retention times of 8.0 and 10.0 min: the first enantiomer had a negative specific rotation value ($[\alpha]^{24}_D$ −347 (c = 0.075, DMSO)), whereas the second enantiomer had a positive specific rotation value ($[\alpha]^{24}_D$ +332 (c = 0.065, DMSO)). The value of the first enantiomer was consistent with the reported value for BE-54017 ($[\alpha]^{20}_D$ −428 (c = 0.5, DMSO)). With enantiopure BE-54017 in hand, the stage was set for determination of its absolute configuration. Toward this end, BE-54017 was independently prepared starting from enantiopure cladoniamide A whose absolute stereochemistry is known.[8]

The reaction sequence shown in Scheme 3 could be also applied to the synthesis of cladoniamide A (**2**). In fact, the intermediate with the complete framework, **13**, was transformed into racemic cladoniamide A under the

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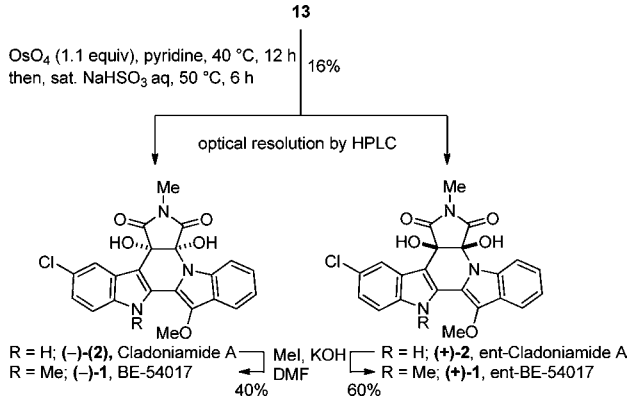
Scheme 4. Determination of the Absolute Stereochemistry of BE-54017 (**1**)



dihydroxylation conditions (Scheme 4). All of the physicochemical data other than optical rotation obtained from the synthetic cladoniamide A were confirmed to be identical to those of the natural sample. To our delight, the succeeding optical resolution could be accomplished uneventfully by HPLC under the same protocol used for the separation of BE-54017 (Daicel, CHIRALPAC IC 4.6 × 250 mm, 20% AcOEt in *n*-hexane, 2 mL/min). The fraction eluted out at 8.3 min showed a specific rotation of $[\alpha]^{22}_{D}$ −256 (c = 0.080, MeOH) that is consistent with the reported optical rotation for natural cladoniamide A ($[\alpha]^{22.5}_{D}$ −390.0 (c = 1.88, MeOH)).[8] This enantiomer (cladoniamide A, **(−)-2**) was subjected to *N*-methylation with MeI and KOH to give BE-54017 (**(−)-1**), confirming that the absolute configuration of the vicinal diols of BE-54017 was 4c*S*,7a*R*. Independently, the other enantiomer of cladoniamide A (**(+)-2**) was converted to **(+)-1** *ent*-BE-54017 using the same *N*-methylation procedure.

Each enantiomer was subjected to an assay to evaluate EGF-dependent apoptosis of human epidermal carcinoma A431 cells (see the Supporting Information). Synthetic BE-54017 (**(−)-1**) induced EGF-dependent apoptosis in a dose-dependent manner with an EC_{50} value of 0.16 μg/mL, consistent with that of natural BE-54017 (EC_{50} value of 0.23 μg/mL). In contrast, the antipode (**(+)-1**) was more than 10 times less potent (EC_{50} > 3.0 μg/mL). The results indicated that the stereochemistry of the diol moiety has a critical role in the observed capabililty to intervene in the EGF-mediated signaling pathway. The difference in biological activity supports the accuracy of the assignment of the absolute stereochemistry.

We previously reported that vacuolar-type H^+-ATPase (V-ATPase) inhibitors, such as concanamycin B and destruxin E, exhibited inhibitory activity toward EGF-mediated signaling pathways.[17] Therefore, we next examined whether BE54017 inhibited V-ATPase. BE-54017 inhibited the acidification of acidic organelles maintained by V-ATPases, indicating that BE-54017 induced EGF-dependent apoptosis in EGFR-overexpressing A431 cells, possibly through inhibiting V-ATPase activity (see the Supporting Information).

In summary, we synthesized BE-54017, a rare member of the indenotryptoline subfamily of bisindole alkaloid natural products, using osmium-promoted dihydroxylation of the maleimide core as the key step. After optical resolution of the synthetic sample by HPLC, the absolute configuration of BE-54017 was identified as 4c*S*,7a*R* by comparison of its specific rotation and HPLC profile to those obtained from independently prepared BE-54017 derived from enantiopure cladoniamide A, whose stereochemistry was reported previously. Further studies of the structure−activity relationship of the compounds in this class are underway.

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Supporting Information Available. Experimental procedures, detail in biological study, and spectroscopic data for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.