An improved synthesis of α,β-unsaturated nitrones relevant to the stephacidins and analogs thereof

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ABSTRACT

A new method is reported for the synthesis of the α,β-unsaturated nitrone moiety characteristic of the stephacidin/avrainvillamide family of bioactive prenylated indole alkaloids. Application to the synthesis of stephacidin analogs and a potential biological probe are showcased.

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The stephacidins and related family members (Fig. 1) are complex natural products of the alkaloid class broadly defined as containing a prenyl unit appended to an indole framework.[1] The combination of structural intrigue and biological potential has stirred significant interest in both the synthesis[2] and the study of fundamental biochemical mechanisms[3] that are operative in the anti-cancer activity they exhibit. In 2005 we reported a synthesis of **1–3** by an approach that was notably inefficient in the final steps (Fig. 1).[4]

Specifically, the conversion of stephacidin A (**1**) to avrainvillamide (**2**) was stymied by chemoselectivity issues that prevented clean and efficient oxidation. As shown in Figure 2, the general strategy of Somei[5] was utilized to construct the characteristic α,β-unsaturated nitrone system. Thus, the indole (**A**) is first reduced to the indoline oxidation state (**B**) followed by a reoxidation that commences with N-oxidation (to *N*-hydroxyindoline **C**) and two sequential dehydrogenation events to deliver **E** via **D** in a single step. Whereas the Gribble reduction[6] (**A–B**) proceeds in high yield, the Somei oxidation (**B–E**) in the stephacidin series is low yielding. In this Letter an interim solution to this problem is put forth that utilizes dimethyldioxirane (DMDO) to chemoselectively access *N*-hydroxyindoles (isolable) followed by a separate dehydrogenation step. The overall efficiency and scalability of this protocol is superior to those previously employed, permitting the synthesis of stephacidin analogs and biological probes.

During our investigations into the synthesis of a biological probe, it became apparent that our method for generating the α,β-unsaturated nitrone via Gribble reduction and subsequent Somei oxidation was insufficient.

Reasoning that the indoline nitrogen should be susceptible to hydroxylation with a highly electrophilic oxidant, indoline **6** was treated with 1.1 equiv of DMDO at −40 °C to furnish the *N*-hydroxyindole **7** in nearly quantitative yield. The intermediate hydroxy indoline presumably oxidized spontaneously to furnish **7**. Mild dehydrogenation using *p*-chloranil cleanly led to the desired nitrone **8** in 82% overall isolated yield (Scheme 1).[7]

This two step procedure is simple to perform and proved critical for the preparation of several avrainvillamide analogs. The synthesis of one such analog, a biological probe specifically designed for Cravatt's ABPP method,[8] is delineated in Scheme 2.

Thus, we began with HATU mediated amide bond formation between acid **9** and amine **10** (Scheme 2). In contrast to our route to **1**, **2**, and **3** in which the benzopyran double bond poses chemoselectivity challenges to standard hydrogenolysis of the Cbz group, **11** can be treated with H_2/Pd/C followed by heating to effect deprotection and diketopiperazine formation. Treatment of the intermediate diketopiperazine with base followed by MOMCl protected the amide nitrogen and set the stage for oxidative coupling (57% over two steps). Unfortunately, tosyl protected derivative was not amenable to oxidative coupling presumably due to the acidic methyl protons of the tolyl group. Exchange of the tosyl group for a benzyl ether (**12**) alleviated this problem. Removal of the tosyl group with

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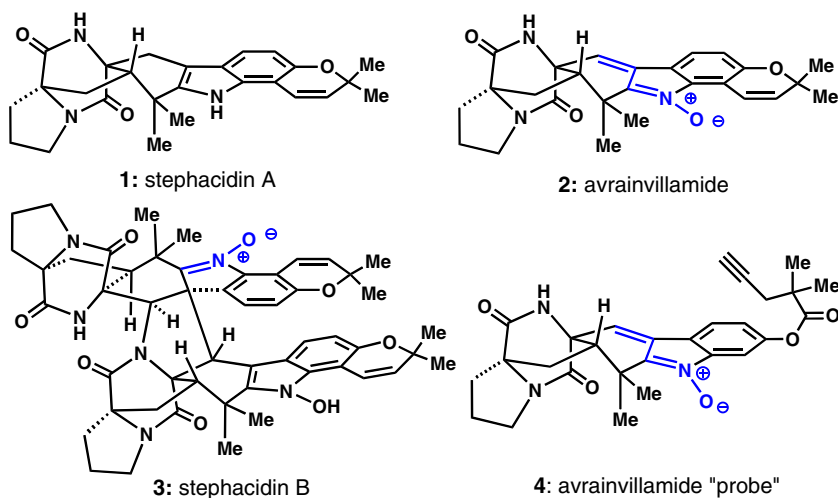*E-mail address:* pbaran@scripps.edu (P.S. Baran).

Figure 1. Selected stephacidin family members (**1–3**) and target 'probe' **4**.
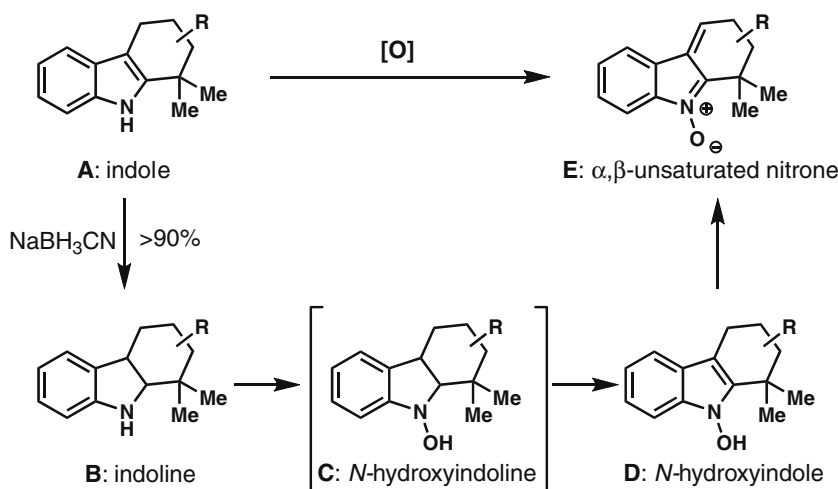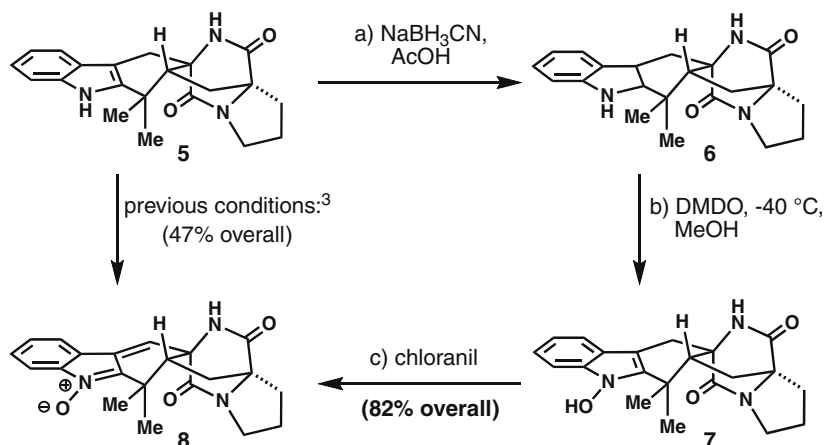

Figure 2. The general method for conversion of indoles to α,β-unsaturated nitrones.

Scheme 1. Development of an improved protocol for indole oxidation. Reagents and conditions: (a) NaBH₃CN (40 equiv), AcOH, 20 °C, 24 h, >95%; (b) DMDO (1.1 equiv), MeOH/AcOEt (1:1), −40 °C, 1 h; (c) chloranil (2 equiv), THF, 70 °C, 40 min, 82% over two steps.[7]

Mg⁰ in MeOH required slightly elevated temperatures (50 °C) but proceeded in good yield (85%). Benzyl protection was effected using benzyl bromide and DBU to give **12** in 59% yield. With **12** in hand the key oxidative coupling was studied and after several attempts the use of LDA and Fe(acac)₃ followed by MeOH quench at low tem-

perature was found to produce the expected cyclic compound in 47% yield. As observed in our other substrates, MOM removal was capricious under all conditions examined; PPh₃·Br₂ gave the best results (43–70%). With the MOM group removed, Grignard addition followed by dehydration with Burgess reagent gave the corre-
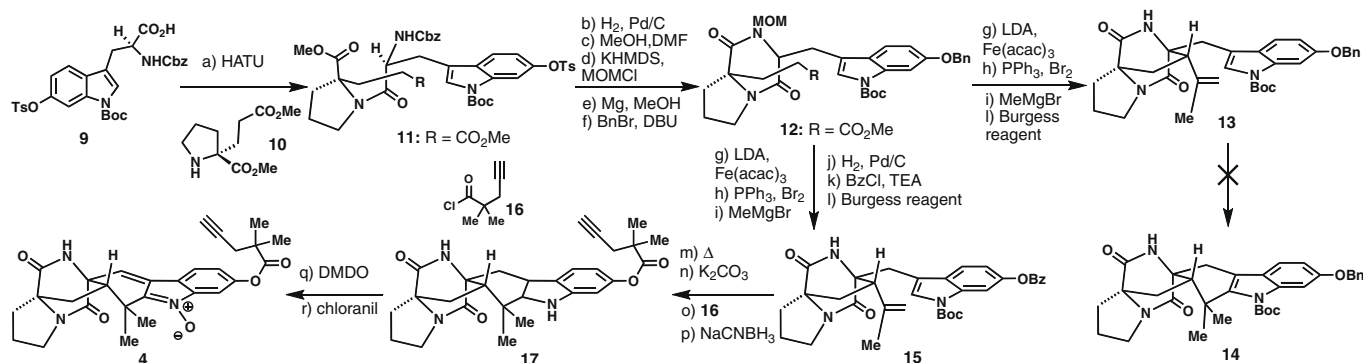
Scheme 2. Synthesis of avrainvillamide probe **4**. Reagents and conditions: (a) proline **10** (1 equiv), HATU (1.1 equiv), DIPEA (3 equiv), DMF, 20 °C, 12 h, 74%; (b) 10% Pd/C (20% w/w), H$_2$, MeOH, 20 °C, 18 h; (c) MeOH/DMF (1:1), 100 °C, 4 h, 85%; (d) KHMDS (1.1 equiv), MOMCl (1.2 equiv), THF, −78 °C to 20 °C, 2.5 h, 57%; (e) Mg^0, MeOH, 50 °C, 4 h (85%); (f) BnBr (3 equiv), DBU (3 equiv), CH$_3$CN/CH$_2$Cl$_2$ (1:1), 20 °C, 3 h, 59%; (g) LDA (2.2 equiv), Fe(acac)$_3$ (2.2 equiv), −78 °C to 20 °C, 47%; (h) PPh$_3$ (5 equiv), Br$_2$ (5 equiv), CH$_2$Cl$_2$, 0 °C, 18 h, 43–70%; (i) MeMgBr (10.0 equiv), toluene, 20 °C, 10 min; (j) 10% Pd/C (20% w/w), H$_2$, MeOH, 20 °C, 18 h; (k) BzCl (3 equiv), TEA (3 equiv), CH$_2$Cl$_2$, 20 °C, 12 h; (l) Burgess reagent (10 equiv), benzene, 50 °C, 5 min, 74% over four steps; (m) 190 °C, 30 min, 59%; (n) K$_2$CO$_3$, MeOH/ CH$_2$Cl$_2$ (1:1), 20 °C, 5 h, 81%; (o) **16** (10 equiv), TEA (3 equiv), acetone, 20 °C, 12 h 70%; (p) NaBH$_3$CN (40 equiv), AcOH, 20 °C, 24 h, >95%; (q) DMDO (1.1 equiv), MeOH/AcOEt (1:1), −40 °C, 1 h; (r) chloranil (2 equiv), THF, 70 °C, 40 min, 98% over two steps.

sponding olefin **13** in 97% yield over two steps. Several reaction conditions were examined for the cyclization of **13** but **14** was never observed due to the incompatibility of **13** with Brönsted acids. The benzyl group was removed to reveal the free phenol in hopes that it would provide a subtle modulation in reactivity and allow for successful ring closure. This was also unsuccessful. It was postulated that the electron donating effects of the oxygen substituent on the indole mired the cyclization. Our previous studies have shown this cyclization to be effective on unsubstituted indole substrates.[4a] In an attempt to approximate the electronics of an unsubstituted indole, the benzoyl ester was formed from the free phenol and olefin **15** efficiently cyclized when heated at 190 °C. The benzoyl moiety was easily cleaved by treatment with K$_2$CO$_3$ and the free phenol derivative was treated with acid chloride **16** in the presence of TEA. The resulting ester was then reduced under Gribble conditions to yield indoline **17**. Using previously reported oxidation conditions (SeO$_2$ or Na$_2$WO$_4$·2H$_2$O) the ester side chain was readily cleaved. Alternatively, using DMDO as an oxygen source at −40 °C produced the N-hydroxyindoline in good yield and direct treatment with chloranil installed the desired α,β-unsaturated nitrone (**4**) in 98% yield over two steps.

Initial testing in the Cravatt lab demonstrated that **4** showed high levels of protein labeling in breast cell (T47D) lysates at concentrations ranging from 1–200 μM upon visualization by in-gel fluorescence following click-chemistry with rhodamine-azide.

Subsequent competition studies were performed on avrainvillamide mimic **8**, in which cell lysates were incubated with 100 μM of **8** prior to the addition of 1–10 μM of **4**. These studies demonstrated the disappearance of a doublet around the 50 kD range upon pre-treatment with **8**, suggesting a possible selective protein target for avrainvillamide (**2**) in the 50 kD molecular weight region.

In summary an efficient two step protocol for the chemoselective preparation of avrainvillamide and stephacidin analogs containing the hallmark α,β-unsaturated nitrone has been developed. Application of this method to the improved synthesis of analog **8** and bioprobe **4** will enable further studies of the biochemical functions of this alkaloid family.

Acknowledgments

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7. General procedure for the synthesis of indoline derivatives: indole derivative (1 equiv) was dissolved in glacial AcOH (0.1 M). To the stirring solution was added NaCNBH$_3$ (40 equiv) and the reaction mixture stirred at room temperature for 24 h. The solution was then basified with 1 M aqueous NaOH and extracted repeatedly with AcOEt. The organics were combined and washed with satd aq NaCl solution, dried over MgSO$_4$, filtered and the solvent was removed under vacuum. The indoline derivative obtained was used directly in the next step. General procedure for the synthesis of N-hydroxy derivatives: indoline (1 equiv) was dissolved in a 1:1 mixture of MeOH/AcOEt (the solvent combination was used to insure complete dissolution of indoline). After the solution was cooled to −40 °C, dimethyldioxirane (1.1 equiv) was added and the reaction mixture was stirred at −40 °C for 1 h. Dimethylsulfide was added until its odor persisted and the reaction mixture was evaporated under high vacuum. The crude mixture was used directly in the next step.
General procedure for the synthesis of nitrone derivatives: N-hydroxy derivative (1 equiv) was dissolved in THF (0.01 M) and chloranil (2 equiv) was added. The resulting yellow solution was sealed with a plastic cap and heated in an oil bath preheated to 70 °C. After 40 min the reaction was removed from the heat and the solvent was removed under vacuum. The residue was purified by column chromatography.

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