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Design and total synthesis of a fluorescent phorboxazole a analog for cellular studies

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Abstract—To enable studies to elucidate the intracellular processing and targeting of the potent cytostatic/apoptotic anticancer natural products phorboxazoles A and B, a fluorescent derivative has been developed. This involved the total syntheses of the terminal alkyne 33-*O*-Me-45,46-dehydrobromophorboxazole A (MDHBPA) and a terminal vinyl iodide derivative of the blue fluorescent dye *N*,*N*,-dimethyl-7-aminocoumarin (DMC). Sonogashira coupling of these partners provided enyne DMC–MDHBPA in high yield.

Natural products phorboxazole A (**1**, Fig. 1) and its C13 epimer display potent cytostatic activity against many human cancer cell lines.[1] Synthetic **1** was also found to induce apoptosis.[2] The phorboxazoles arrest human cancer cell growth during S phase, but their undefined mechanism of action does not correlate with other known cytostatic/cytotoxic agents. Hence, their anticancer activities may illuminate cellular targets that have not previously been explicitly exploited. As such, the phorboxazoles remain active candidates for further exploration and therapeutic development.

The isolation of phorboxazoles from sponges of two different genera collected in the Indian Ocean off western Australia[1a,3] suggests that these natural products may be biosynthesized by a common symbiotic microorganism that has yet to be identified.[4] To date, laboratory synthesis remains the most reliable option to provide sufficient quantities of the phorboxazoles[5] and more importantly, designed structural variants[6,7] to enable continued exploration of their scientific and biomedical potential. Accordingly, we have described a total synthesis,[5a] structure–activity relationship study,[6a] and the preparation of a biotinylated phorboxazole derivative for target identification.[6b] Reported here are the design and synthesis of a fluorescent derivative for in vivo biophotonic studies to elucidate intracellular processing and targeting of the natural product.

Our initial structure–activity-relationship study identified the C33 mixed methyl ketal (**2**) and the terminal alkyne 45,46-dehydrobromophorboxazole A (DHBPA, **3**) as synthetic analogs that maintained the anticancer activities of **1** (Fig. 1). Accordingly, we had previously prepared a biotinylated derivative of **3** from synthetic DHBPA and a biotin-bearing polyethylene glycol linker for affinity localization and isolation studies.[6b] This involved Sonogashira coupling[8] between the terminal alkyne of **3** and a vinyl iodide terminated linker bearing a biotin moiety. It was recognized that this reliable, late stage Sonogashira coupling approach for the generation of phorboxazole analogs could be extended to incorporate fluorescently labeled probes, such as **4** (Fig. 1).

Derivatives of the blue fluorescent dye *N*,*N*-dimethyl-7-aminocoumarin-4-acetic acid have been demonstrated

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to display an ideal profile of abiotic non-interference upon cellular uptake and protein binding.[9] Hence, the *N*,*N*-dimethyl-7-aminocoumarin (DMC) moiety was selected as the fluorophore.

To merge coumarin fluorophore and bioactive phorboxazole derivatives via the mild Sonogashira coupling reaction requiring no protective groups on either partner, one could consider coupling **1** via its vinyl bromide terminus with a terminal alkyne derivative of DMC. However, the terminal alkyne of **3** (Fig. 1) is obtained more efficiently than the (*E*)-vinyl bromide of **1** via total synthesis. Furthermore, a terminal alkyne is a more robust and versatile functional group for derivatization. Thus, the obvious roles of vinyl halide and terminal alkyne in the Sonogashira coupling process were reversed. The alkyne would be assigned to the phorboxazole chemophore and the vinyl halide to the DMC fluorophore. The vinyl halide moiety would reside at the terminus of a polar diether linker to enhance aqueous solubility. This linker would be attached to the readily functionalizable 4-position of DMC. The immediate synthetic precursor to **3** is the corresponding C33 mixed methyl ketal **5** (Scheme 1). The culmination of these design considerations resulted in the targeting of *N*,*N*-dimethyl-7-aminocoumarin–MDHBPA conjugate **4** (DMC–MDHBPA, Fig. 1) obtained from phorboxazole derivative **5** and DMC analog **6** (Scheme 1).

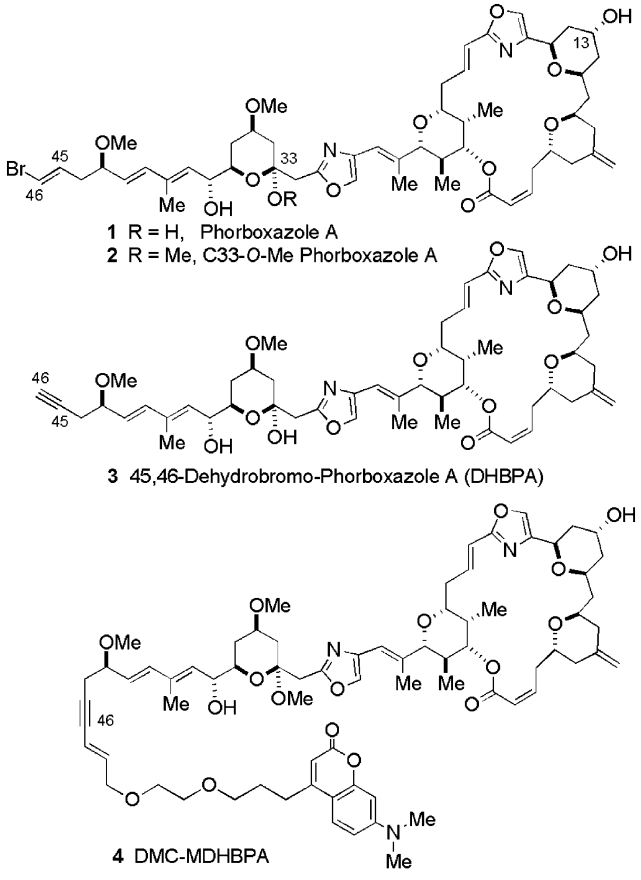


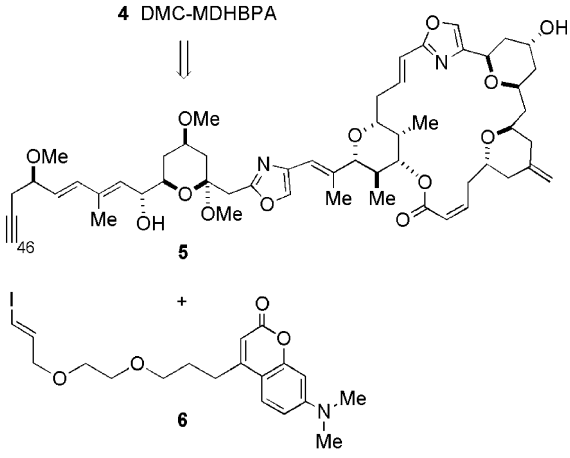
Figure 1. Structures of phorboxazole A and analogs. Natural **1** bears a C33 hemiketal and an (*E*)-vinyl bromide. Synthetic C33 mixed methyl ketal **2** and terminal alkyne **3** maintain the cytostatic activity of **1**. Coumarin conjugate **4** joins *N*,*N*-dimethyl-7-amino-coumarin (DMC) and 33-*O*-Me-**3** (MDHBPA).

The analog 33-*O*-methyl-45,46-dehydrobromo-phorboxazole A (**5**) is obtainable via our established tri-component coupling entry to the phorboxazole architecture.[5a,10] A C31–C46 fragment bearing a carboxylate at C31 and an alkyne at C45,46[11] was joined with a C1–C30 amino-alcohol via sequential amide and oxazole formation. Fluoride-induced cleavage of C13 and C38 silyl ethers gave terminal alkyne **5**.

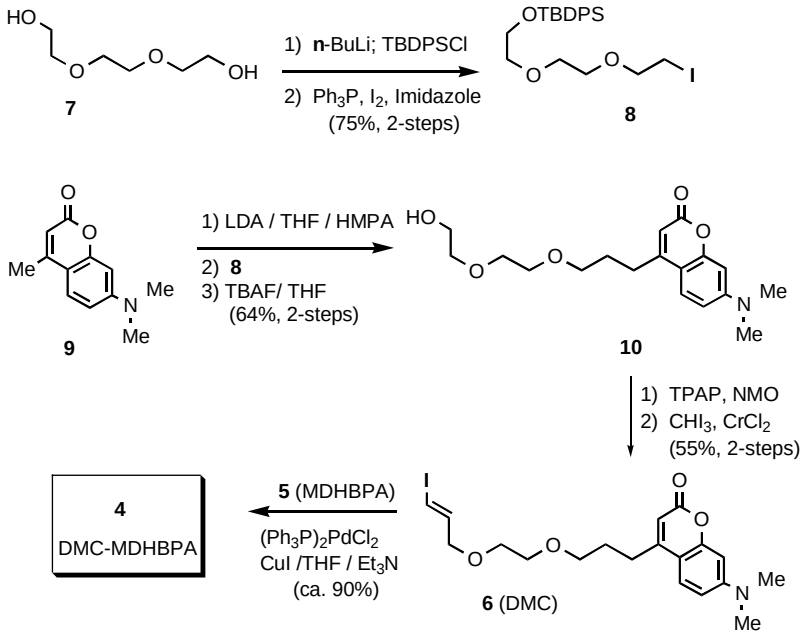
The complementary vinyl iodide **6** (Scheme 1) was prepared from triethylene glycol (**7**) and *N*,*N*-dimethyl-7-amino-4-methyl-coumarin (**9**, Scheme 2). Differential terminal functionalization of **7** involved mono-silylation followed by reciprocal iodination to yield silyl ether-iodide **8**. Commercially available **9** was converted into the extended enolate with LDA/HMPA. Alkylation with iodide **8** followed by desilylation yielded primary alcohol **10**. Oxidation[12] to the corresponding aldehyde followed by Takai iodo-olefination[13] yielded a 3:1 *E*/*Z* mixture of vinyl iodides, from which the (*E*)-isomer **6** was chromatographically isolated.

Sonogashira coupling of **5** and **6** to generate **4** was accomplished in 10 min and essentially quantitative yield on small scale using bis(triphenylphosphine)palladium(II) chloride and CuI in the presence of Et_3N and in THF (Scheme 2).[8,14] This remarkable reaction deserves comment. First, there are no protective groups in either coupling partner that required subsequent removal. Hence, this coupling culminated in the preparation of the targeted fluorescent phorboxazole probe. Second, the efficiency of coupling of **5** and **6** may be contrasted with the similar coupling of a biotinylated vinyl iodide linker with DHBPA (**3**). In the latter case, a considerably lower yield of enyne was obtained.[6b] This may be attributable to the presence of Pd-sequestering sulfur in the biotin moiety, whereas the vinyl iodide DMC derivative **6** participates in Pd/Cu-mediated enyne formation without complication.

This work provides a novel phorboxazole probe for in vivo intracellular localization and target elucidation



Scheme 1. Sonogashira disconnection of phorboxalog fluoroprobe **4**.



Scheme 2. Synthesis of coumarin label **6** and fluorescent conjugate **4**.

studies. Results derived from application of this probe for such studies will be reported in due course.

Acknowledgments

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14. Preparation of **4**: To a stirred, rt solution of vinyl iodide **6** (1.5 mg, 3.3 μmol) and C33-methoxy-45,46-dehydrobromophorboxazole A[10] (**5**, 0.5 mg, 0.5 μmol) in freshly distilled THF (0.5 mL) were added sequentially freshly distilled triethylamine (0.1 mL), bis(triphenylphosphine)palladium dichloride (0.5 mg, 0.7 μmol), and CuI (0.5 mg, 3 μmol). The resulting mixture was stirred under argon at rt. After 10 min, TLC showed no remaining **5**. Saturated aqueous NH_4Cl (0.5 mL) and ethyl acetate (1 mL) were added. The aqueous layer was separated and extracted with ethyl acetate (6× 1 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated under vacuum. The residue was purified by silica gel column chromatography (ethyl acetate/methanol, 20:1, v/v) followed by preparative thin layer chromatography (ethyl acetate/methanol, 10:1, v/v) to provide **4**

(0.6 mg, 0.5 μmol, ca. 90%) as a yellow amorphous solid: R_f 0.45 (ethyl acetate/methanol, 10:1, v/v); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.58 (s, 1H), 7.49 (d, J = 8.5 Hz, 1H), 7.43 (s, 1H), 6.69 (m, 1H), 6.63 (d, J = 9 Hz, 1H), 6.54 (s, 1H), 6.30 (d, J = 11 Hz, 1H), 6.28 (s, 1H), 6.16 (d, J = 12.5 Hz, 1H), 6.12 (dt, J = 5.5, 10.5 Hz, 1H), 5.99 (s, 1H), 5.92 (s, 1H), 5.73 (d, J = 16 Hz, 1H), 5.34 (dd, J = 9, 11.5 Hz, 1H), 5.30 (dd, J = 8, 11 Hz, 1H), 5.47 (d, J = 8.5, 1H), 5.00 (s, 1H), 4.75 (dd, J = 3.5, 10 Hz, 1H), 4.63 (s, 1H), 4.52 (dd, J = 4, 10.5 Hz, 1H), 4.41 (s, 1H), 4.33 (m, 1H), 4.18 (m, 1H), 4.10 (m, 1H), 4.06 (d, J = 5 Hz, 2H), 4.00 (m, 1H), 3.77 (m, 1H), 3.62 (m, 1H), 3.59 (s, 4H), 3.50–3.56 (m, 3H), 3.48 (m, 2H), 3.41 (m, 1H), 3.34 (s, 3H), 3.31 (s, 3H), 3.27 (s, 3H), 3.09 (m, 1H), 3.06 (s, 6H), 2.97 (s, 1H), 2.80 (t, J = 8.0 Hz, 2H), 2.72 (d, J = 13 Hz, 1H), 2.64 (m, 1H), 2.55 (m, 2H), 2.43 (m, 2H), 2.35 (m, 2H), 2.25 m, 1H), 2.02 (m, 2H), 1.99 (m, 2H), 1.98 (s, 3H), 1.97–1.90 (m, 5H), 1.86 (s, 3H), 1.73 (d, J = 14.5 Hz, 1H), 1.58 (m, 1H), 1.45 (m, 1H), 1.37 (m, 1H), 1.11 (m, 1H), 0.97 (d, J = 7.0 Hz, 3H), 0.77 (d, J = 6.5 Hz, 3H); HRMS (ESI): calcd for $[C_{74}H_{97}N_3O_{17}+2Na]^{2+}$ 1331.6446, found 1322.6512.