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Total Synthesis of a Biotinylated Derivative of Phorboxazole A Via Sonogashira Coupling

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Abstract—The C46 terminus of phorboxazole A has been modified to incorporate a biotin-terminated linker via direct palladium-mediated Sonogashira reaction conditions. Synthetic 45,46-dehydrobromophorboxazole A was joined with a *tris*-(polyethyleneglycol)vinyl iodide-biotin ester using catalytic $PdCl_2(PPh_3)_2$, CuI, and Et_3N in THF. This process demonstrates the utility of mild carbon-carbon bond formation in the context of phorboxazoles' architecture and provides a potential affinity probe.

The phorboxazoles are natural products reported by Searle and Molinski in 1995. These compounds were isolated from an Indian Ocean sponge *Phorbas* sp. collected off Muiron Island, Western Australia.[1] The phorboxazoles showed potent antifungal activity against *Candida albicans* and *Saccharomyces carlsbergensis*. In addition, phorboxazole A (Fig. 1) showed remarkably potent levels of cytostatic activity against the National Cancer Institute's 60 tumor cell line with a mean $GI_{50} = 1.58 \times 10^{-9}$ M. Therefore, the phorboxazoles may be considered to be among the most potent cytostatic agents yet discovered. There has been intense synthetic interest in the phorboxazoles, resulting in five published total syntheses to date.[2] However, their cellular targets and mode of action remain unknown.

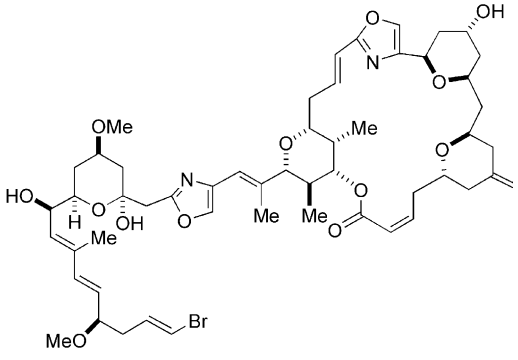

Figure 1. Structure of phorboxazole A.

As part of an on-going research program aimed at evaluating the potential of the phorboxazoles as new leads for therapeutic development, the total synthesis and deployment of affinity derivatives is being pursued. The total synthesis of a biotinylated derivative of phorboxazole A (**1**, Scheme 1) designed as a potential affinity probe for the isolation of cellular targets of the phorboxazoles is described here.

Several biologically active, totally synthetic analogues of phorboxazole A have been reported.[3] Among these is 45,46-dehydrobromo-phorboxazole A (**2**)[4] which bears a terminal alkyne in place of the natural product's (*E*)-vinyl bromide (Scheme 1). Because the terminal vinyl bromide appears to contribute little to the biological activities of the phorboxazoles,[3c] the C46 position emerged as a prime candidate for derivatization.

The natural product's vinyl bromide could be considered as a potential participant in a Sonogashira coupling reaction[5] with an alkynyl terminated tether to install an affinity linker. However, omission of the C46-vinyl bromide substantially enhances the overall synthetic access to phorboxazole derivatives via our synthetic approaches.[2,4] Therefore, the roles of the Sonogashira coupling partners were reversed in functionalizing the alkyne of **2** with a vinyl halide-linked biotin moiety (**3**, Scheme 1).

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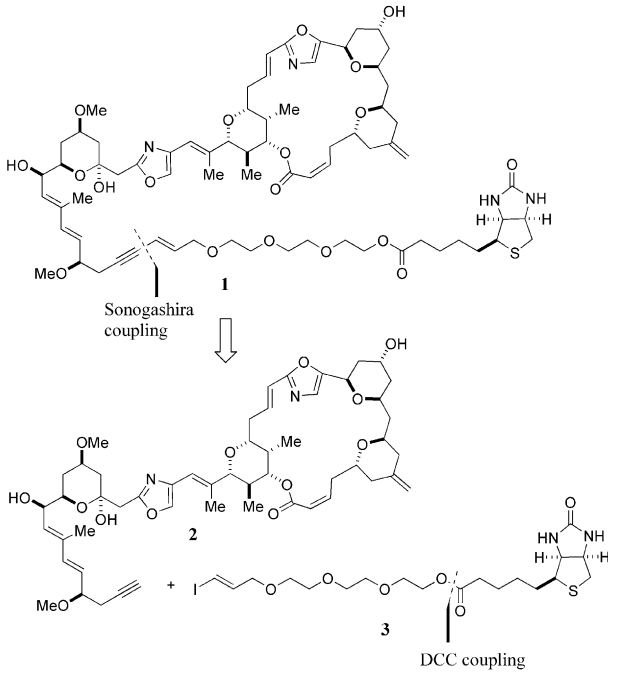
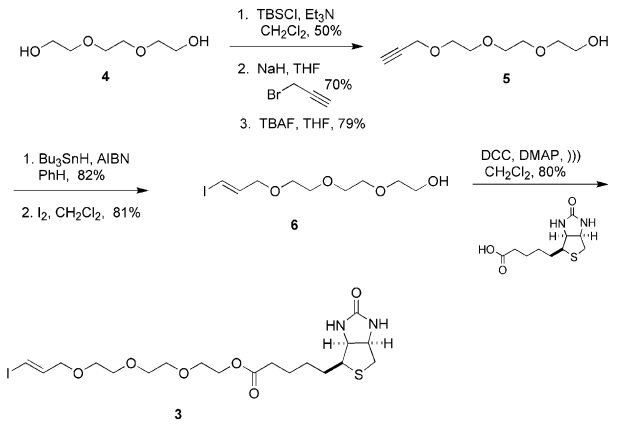


Scheme 1. Synthetic design.

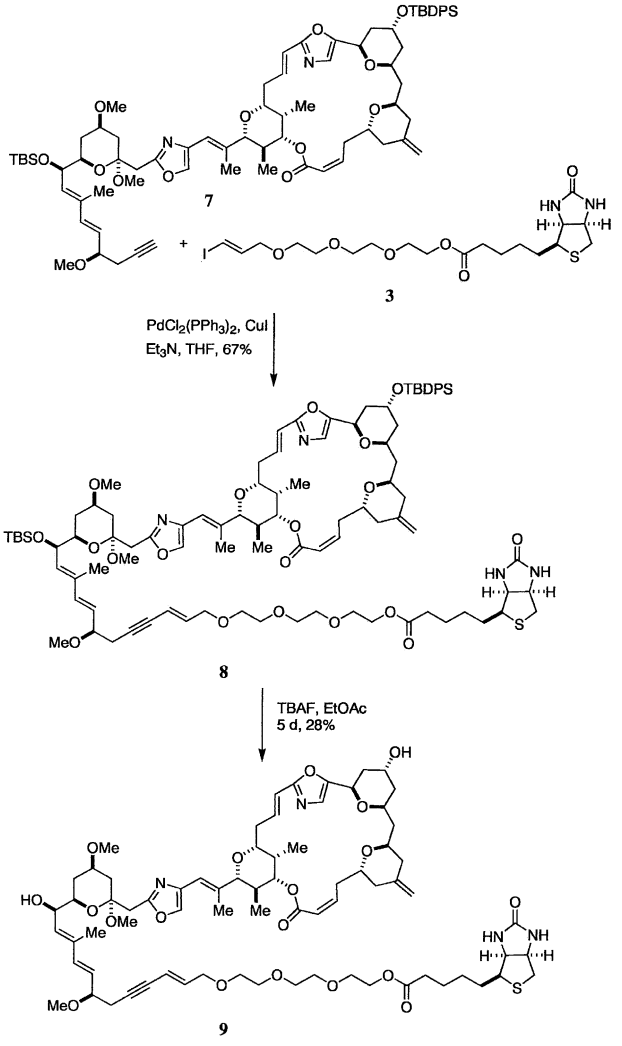
This strategy relies upon synthetic access to **2**, the generation of a unique biotinylated linker **3**, and a Sonogashira coupling of molecules of considerable complexity.

The synthesis of the biotinylated linker **3** is shown in Scheme 2. The linker was designed with two major features. The polyethylene glycol can be modified in length, and the terminus can be easily functionalized. For this, triethylene glycol was temporarily monosilylated, the remaining hydroxyl group was etherified with propargyl bromide, and the silyl ether cleaved to give the known alcohol **5**.[6] The alkyne was then subjected to hydrostannation and iodination under standard conditions to provide the (*E*)-vinyl iodide **6**. The hydroxyl moiety was esterified with (+)-biotin using DCC, DMAP, and sonication to provide **3** in 80% yield (Scheme 2).

As a result of extensive experimentation, conditions for Sonogashira coupling applicable to phorboxazole analogues were determined. To this end, **3** and bis-silyl



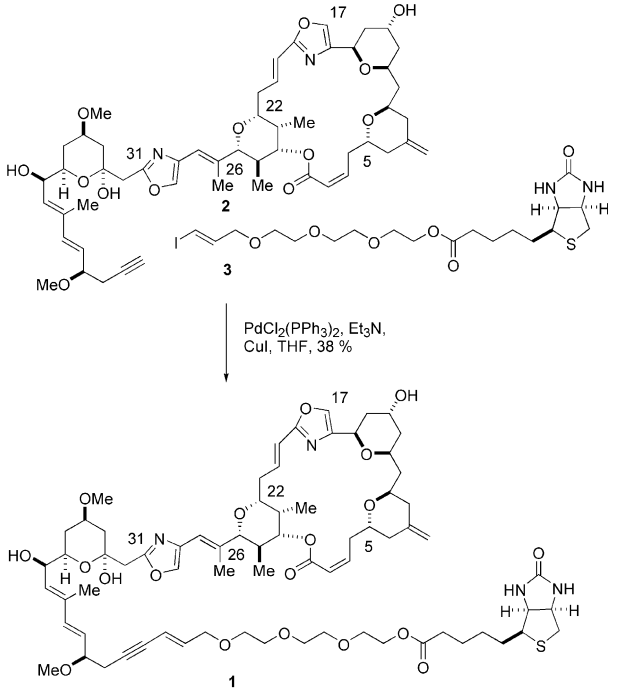
Scheme 2. Synthesis of a biotinylated triethylene glycol linker (**3**).



Scheme 3. Preliminary Sonogashira coupling-deprotection.

ether **7**[4] were subjected to catalytic $PdCl_2(PPh_3)_2$, CuI and Et_3N in THF to provide conjugated enyne **8** in moderate yield (Scheme 3).[7] With this intermediate in hand, desilylation with TBAF in ethyl acetate for 5 days[2a] provided diol **9**. Although carbon-carbon bond formation was successful, the low yield of the subsequent desilylation prompted a later stage Sonogashira coupling.

To this end, **2**,[4] *devoid of any protecting groups*, was subjected to the same coupling conditions with biotin linker **3** to rapidly provide a fully functionalized and deprotected biotinylated derivative of phorboxazole A (**1**, Scheme 4).[8] Although the isolated yield was modest, this result suggests that the phorboxazole natural products should be amenable to a similar palladium-mediated Sonogashira coupling at the C46 position with complementary terminal alkynes. Such direct covalent modification of the natural products or their dehydrobromo analogues may be useful for a range of purposes, including immobilization. The incorporation of a biotin linker chosen here provides both a proof of concept and a tool for the reversible immobilization of phorboxazole-like ligands via non-covalent avidin/streptavidin-biotin binding. The utility of these types of



Scheme 4. Sonogashira coupling of **2** and **3**.

affinity probes for elucidating the molecular basis of phorboxazoles' remarkable biological activities is currently being evaluated in our laboratories.

Acknowledgements

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4. Compounds **2** and **7** were first synthesized as described in: C. S. Lee, PhD. Thesis, University of Minnesota, 1999. An enhanced synthetic route is described in ref 3a, and will be detailed further in due course.

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7. Experimental for **8**: A 1 dram vial was charged sequentially with alkyne **7** (3.0 mg, 2.3 μmol), vinyl iodide **3** (1.6 mg, 3.0 μmol) in freshly distilled and de-gassed THF (0.3 mL), freshly distilled and de-gassed Et_3N (0.1 mL, 702 μmol), $PdCl_2(PPh_3)_2$ (0.16 mg, 0.23 μmol), and anhydrous CuI (0.04 mg, 0.21 μmol). The vial was flushed with N_2 and capped. The reaction mixture was stirred vigorously for 3.5 h at which time, the reaction was judged to be complete by TLC. Saturated aqueous NH_4Cl and ethyl acetate were added. The layers were separated and the aqueous layer was extracted with ethyl acetate (5×4 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated by rotary evaporation. The residue was purified by preparative TLC (ethyl acetate→ethyl acetate/ethanol, 10:1, v/v) to provide **8** (2.6 mg, 1.5 μmol, 67%) as a white film: $R_f = 0.2$ (ethyl acetate/ethanol, 9:1, v/v); $[\alpha]_{23}^{D}$ +23.4 (*c* 2.95, $CHCl_3$); IR (neat, cm^{-1}) 3369, 2928, 1830, 1772, 1702, 1654; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.65 (t, $J = 6.5$ Hz, 4H), 7.56 (s, 1H), 7.45–7.36 (m, 7H), 6.72 (ddd, $J = 16.0$, 10.0, 6.5 Hz, 1H), 6.28 (m, 2H), 6.10 (dt, $J = 5.5$, 16.0 Hz, 1H), 5.93 (m, 2H), 5.70 (d, $J = 16.0$ Hz, 1H), 5.57 (dd, $J = 7.5$, 15.5 Hz, 1H), 5.40 (d, $J = 9.0$ Hz, 1H), 5.33 (m, 1H), 5.03–4.97 (m, 2H), 4.91 (d, $J = 12.0$ Hz, 1H), 4.82 (s, 1H), 4.66 (s, 1H), 4.52 (dd, $J = 3.5$, 9.5 Hz, 2H), 4.44 (t, $J = 7.0$ Hz, 1H), 4.33 (br s, 2H), 4.23 (d, $J = 3.0$ Hz, 2H), 4.19 (d, $J = 11.0$ Hz, 1H), 4.04 (d, $J = 5.5$ Hz, 2H), 3.99 (m, 1H), 3.79 (app q, $J = 5.0$ Hz, 1H), 3.70 (t, $J = 5.0$ Hz, 3H), 3.66 (m, 9H), 3.59 (m, 3H), 3.35 (s, 3H), 3.32 (s, 3H), 3.30 (s, 3H), 2.98 (d, $J = 15.5$ Hz, 1H), 2.93 (dd, $J = 5.5$, 13.0 Hz, 1H), 2.77 (d, $J = 12.5$ Hz, 1H), 2.73 (d, $J = 13.0$ Hz, 1H), 2.62 (d, $J = 4.5$ Hz, 1H), 2.58–2.43 (m, 7H), 2.38–2.29 (m, 3H), 2.11–2.01 (m, 1H), 1.99 (s, 3H), 1.96–1.81 (m, 4H), 1.80 (s, 3H), 1.77–1.63 (m, 3H), 1.58–1.53 (m, 6H), 1.43 (m, 9H), 0.99 (d, $J = 7.0$ Hz, 3H), 0.89 (s, 9H), 0.76 (d, $J = 6.5$ Hz, 3H), 0.07 (s, 3H), 0.03 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.4, 165.5, 162.7, 161.2, 159.1, 144.2, 142.1, 141.6, 138.2, 137.7, 137.2, 136.2, 135.6, 134.1, 133.7, 133.5, 132.2, 129.6, 129.5, 127.6, 120.9, 119.1, 119.0, 112.2, 110.0, 107.2, 105.3, 99.7, 96.0, 89.1, 87.0, 83.3, 80.5, 79.2, 77.8, 77.1, 73.3, 73.2, 72.0, 70.9, 70.5, 70.4, 69.4, 69.0, 68.7, 67.1, 65.8, 63.4, 61.7, 59.9, 56.5, 56.1, 55.5, 55.1, 47.8, 41.2, 40.4, 39.2, 39.1, 39.0, 36.9, 35.5, 35.1, 34.3, 33.6, 32.5, 31.6, 30.3, 29.6, 29.1, 28.1, 26.9, 26.6, 25.7, 24.5, 19.2, 18.1, 14.1, 13.4, 13.2, 5.9, 0.94,-4.6,-4.7; HRMS(ESI) *m/z* calcd for: $C_{95}H_{134}O_{19}N_4Si_2S$ [(M + H) +]: 1723.8902; found: 1723.8935.

8. Experimental for **1**: A 1 dram vial was charged sequentially with alkyne **2** (2.3 mg, 2.4 μmol), vinyl iodide **3** (4.1 mg, 7.7 μmol) in freshly distilled and de-gassed THF (0.5 mL), freshly distilled and de-gassed Et_3N (0.1 mL, 0.7 mmol), $PdCl_2(PPh_3)_2$ (0.16 mg, 0.23 μmol), and anhydrous CuI (0.04 mg, 0.2 μmol). The vial was flushed with N_2 and capped. The reaction mixture was stirred vigorously for 3.5 h at which time, the reaction was judged to be complete by TLC. Saturated aqueous NH_4Cl and ethyl acetate (2 mL) were added, the layers were separated and the aqueous layer was extracted with ethyl acetate (5×4 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated by rotary evaporation. The residue was purified by preparative TLC (ethyl acetate→ethyl acetate/ethanol, 3:1, v/v) to provide **1** (1.2 mg, 0.9 μmol, 38%) as a white film: TLC (silica gel, ethyl acetate/ethanol, 2:1, v/v)

$R_f = 0.2$; $[\alpha]_D^{23}$ +28.0 (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 7.58 (s, 1H), 7.43 (s, 1H), 6.69 (ddd, $J = 16.0$, 10.5, 6.0 Hz, 1H), 6.27 (m, 2H), 6.08 (dt, $J = 5.5$, 16.0 Hz, 1H), 5.92 (m, 2H), 5.69 (d, $J = 16.0$ Hz, 1H), 5.60 (dd, $J = 7.5$, 15.5 Hz, 1H), 5.48 (m, 1H), 5.38 (d, $J = 9.0$ Hz, 1H), 4.99 (m, 2H), 4.91 (s, 1H), 4.74 (dd, $J = 3.0$, 10.5 Hz, 1H), 4.62 (s, 1H), 4.50 (m, 2H), 4.41 (m, 2H), 4.32 (m, 2H), 4.22 (m, 2H), 4.17 (m, 1H), 4.09–3.99 (m, 4H), 3.85–3.73 (m, 3H), 3.70 (t, $J = 5.0$ Hz, 3H), 3.64 (m, 9H), 3.58 (m, 3H) 3.35 (s, 3H), 3.31 (s, 3H), 3.20–3.10 (m, 2H), 2.93 (dd, $J = 5.5$, 13.0 Hz, 1H), 2.72 (d, $J = 12.5$ Hz, 1H), 2.73 (d, $J = 13.0$ Hz, 1H), 2.62 (d, $J = 4.5$ Hz, 1H), 2.56–2.51 (m, 3H), 2.42–2.31 (m, 12H), 2.06 (d, $J = 13$ Hz, 1H), 1.99 (s, 3H), 1.96–1.81 (m, 4H), 1.80 (s, 3H), 1.77–1.63 (m, 3H), 1.58–1.53 (m, 6H), 0.99 (d, $J = 7.0$ Hz, 3H), 0.76 (d, $J = 6.5$ Hz, 3H); MS (MALDI-TOF) *m*/*z* cacld for $C_{72}H_{100}O_{19}N_4SNa$ $[(M + Na)^+]$: 1380.6573, found 1379.6; *m*/*z* cacld for $C_{72}H_{100}O_{19}N_4SNa_2$ $[(M + 2Na)^{2+}]$: 701.3245, found 701.3257.