JOC The Journal of Organic Chemistry

Article

pubs.acs.org/joc

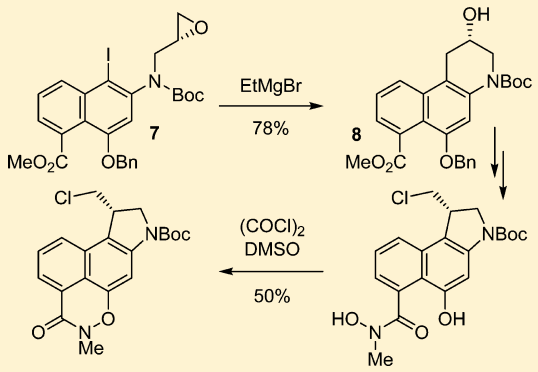
Asymmetric Synthesis of a CBI-Based Cyclic *N*-Acyl *O*-Amino Phenol Duocarmycin Prodrug

Mika Uematsu and Dale L. Boger*

Department of Chemistry and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 N. Torrey Pines Road, La Jolla, California 92037, United States

Supporting Information

ABSTRACT: A short, asymmetric synthesis of a cyclic *N*-acyl *O*-amino phenol duocarmycin prodrug subject to reductive activation based on the simplified 1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one (CBI) DNA alkylation subunit is described. A key element of the approach entailed treatment of iodo-epoxide **7**, prepared by N-alkylation of **6** with (*S*)-glycidal 3-nosylate, with EtMgBr at room temperature to directly provide the optically pure alcohol **8** in 78% yield (99% ee) derived from an effective metal−halogen exchange and subsequent regioselective intramolecular 6-*endo-tet* cyclization. Following O-debenzylation, introduction of a protected *N*-methylhydroxamic acid, direct trannannular spirocyclization, and subsequent stereoelectronically controlled acid-catalyzed cleavage of the resulting cyclopropane (HCl), further improvements in a unique intramolecular cyclization with N−O bond formation originally introduced for formation of the reductively labile prodrug functionality are detailed.



INTRODUCTION

Duocarmycin SA (**1**)[1] and CC-1065 (**2**)[2] are the two most widely recognized members of a class of exceptionally potent naturally occurring antitumor compounds that also include duocarmycin A[3] and yatakemycin[4] (Figure 1). Each of these natural products has been shown to derive its antitumor properties from its ability to alkylate DNA in a sequence-selective manner,[5] undergoing a stereoelectronically controlled adenine N3 alkylation within the minor groove at defined locations within 4−5 base pair A−T rich sites.[6] Extensive studies conducted with the natural products, their synthetic unnatural enantiomers,[7] and a systematic series of key analogues have defined a range of fundamental features that control their DNA alkylation selectivity, efficiency, and catalysis,[8] providing a detailed understanding of the relationships between structure, reactivity, and biological activity.

Recently, we reported the synthesis and examination of both acyclic[9] and cyclic[10] *N*-acyl *O*-amino phenol derivatives as members of a unique class of reductively cleaved prodrugs of the duocarmycin family of natural products.[11] These prodrugs were explored with analogues incorporating the synthetically more accessible 1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one (CBI) alkylation subunit[12,13] (Figure 1) and with the intention of attenuating the extraordinary potency of the compounds. The expectation was that they may be chemically tuned for cleavage selectively within hypoxic tumor environments that have intrinsically higher intracellular concentrations of reducing nucleophiles. The most recent of these, a class of cyclic *N*-acyl *O*-amino phenol prodrugs,[10] were designed to liberate the free drug without the release of an extraneous group. In vivo evaluation of the most stable of these latter prodrugs, **3**, showed that it exhibited extraordinary antitumor efficacy in a simple tumor model (T/C > 1500, L1210; 6/10 one year survivors) substantially exceeding that of the free drug, that its therapeutic window of activity was much larger than that of the free drug, and yet that it displayed a potency in vivo that approached the free drug.[10] These studies indicate that prodrug **3** may benefit from either its controlled slow release of the free drug or its preferential intracellular reductive cleavage.

In a continuation of these studies and prompted by the need for improved access to the materials, herein we report an asymmetric synthesis of **3**, avoiding the late-stage chiral phase resolution of our initial synthesis and improving key elements of the approach including a unique cyclization with N−O bond formation for introduction of the reductively labile prodrug functionality.

RESULTS AND DISCUSSION

Palladium(0)-catalyzed carbonylation of **4**,[14] in DMF−MeOH (2:1, 0.13 M, 0.1 equiv of $Pd(OAc)_2$, 0.2 equiv of xantphos, 1 equiv of K_2CO_3, CO atm, 100 °C, 17 h), provided methyl ester **5** in good conversion (60−65%) (Scheme 1). Regioselective iodination of **5** (2 equiv of *N*-iodosuccinimide (NIS), cat. HOAc, toluene, 25 °C, 17 h, 82%) followed by N-alkylation of **6** with (*S*)-glycidal 3-nosylate (99% ee) with clean S_N2 displacement of the nosylate (1.15 equiv, 1.5 equiv of NaH, DMF, 0−25 °C, 5 h, 91%) set the stage for a key cyclization. Treatment of iodo-epoxide **7** with EtMgBr at room temperature directly provided the optically pure alcohol **8** in 78% yield derived from selective metal−halogen

Received: August 8, 2014
Published: September 23, 2014

   dx.doi.org/10.1021/jo501839x | *J. Org. Chem.* 2014, 79, 9699−9703

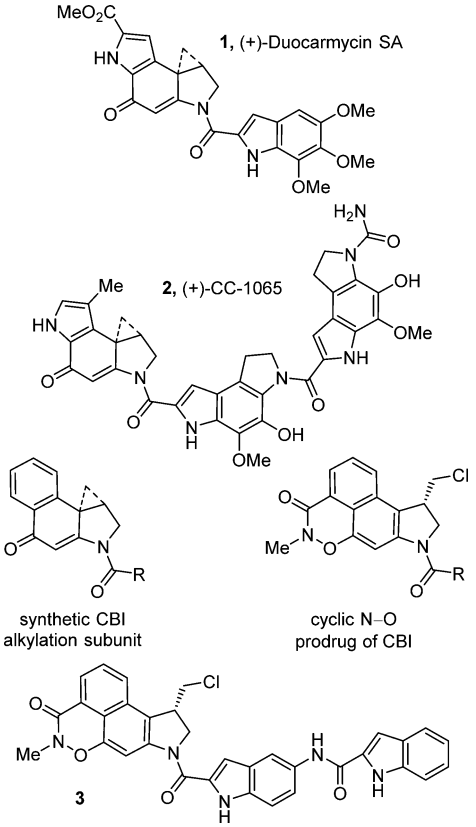
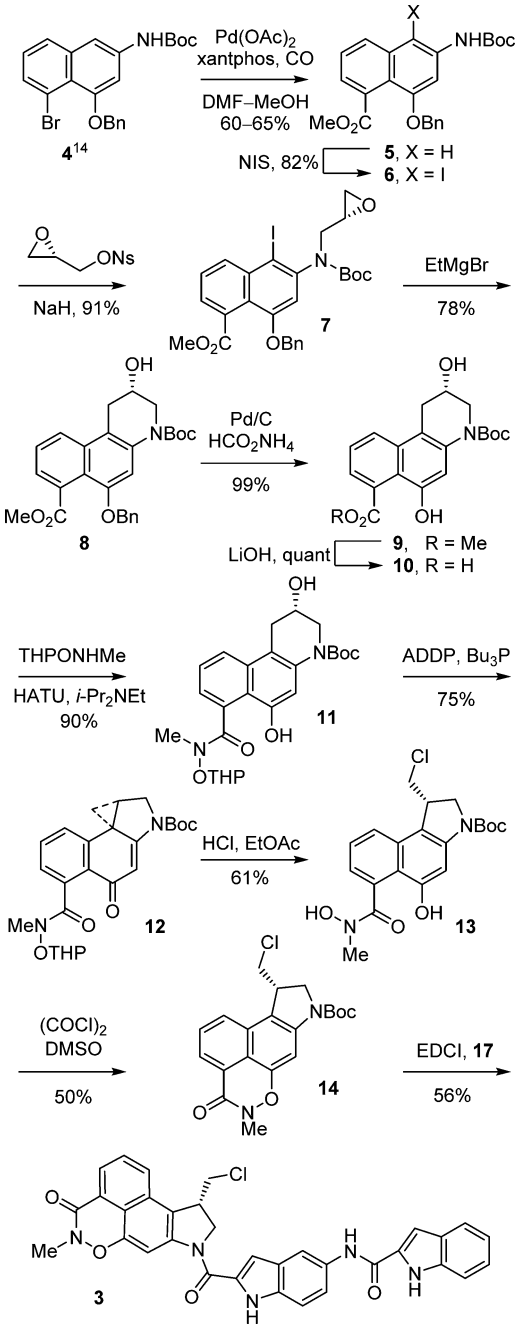


Figure 1. Structure of (+)-duocarmycin SA, (+)-CC-1065, CBI, the cyclic N−O prodrug of the CBI alkylation subunit, and prodrug **3**.

exchange and subsequent intramolecular 6-*endo*-*tet* cyclization. Analogous to observations made in an asymmetric synthesis of CBI itself,[13c] formation of the aryl Grignard reagent by metal−halogen exchange (2.0 equiv of EtMgBr, 23 °C) is followed by the rapid intramolecular epoxide ring opening to give near exclusively **8**, the result of intramolecular 6-*endo* versus 5-*exo* addition to the epoxide with only detection of trace amounts of the isomeric product (>13:1, <5%). A similar but technically more demanding protocol, entailing metal−halogen exchange (*i*-PrMgCl, THF, −40 °C, 20 min) followed by transmetalation with CuI−PBu_3 (−78 °C, 1 h),[15] also provided **8** (−40 °C, 30 min, 68%) in comparable conversions. O-Debenzylation of **8**, accomplished by transfer hydrogenolysis (cat. 10% Pd/C, 10 equiv of HCO_2NH_4, THF−MeOH, 25 °C, 1 h, 99%), followed by methyl ester hydrolysis (5 equiv of LiOH, THF−MeOH−H_2O 3:3:1, 70 °C, 4 h, quant.), provided carboxylic acid **10**. Coupling of **10** (1.5 equiv of (1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]-pyridinium 3-oxide hexafluorophosphate (HATU), 1.5 equiv of *i*-Pr_2NEt, 25 °C, 18 h, 90%) with *N*-methylhydroxylamine protected as its tetrahydropyranyl (THP) derivative (**20**, prepared as detailed in the Experimental Section) provided intermediate **11** functionalized for N−O bond formation yet permitted subsequent spirocyclization and HCl addition to the activated cyclopropane and could be readily deprotected without competitive *N*-Boc deprotection or N−O bond cleavage. Lactone **15** was observed in the reaction mixture, could be isolated and characterized, and converts to product **11** when subjected to the reaction conditions, indicating that **15** can serve as an intermediate in the generation of **11** (Figure 2). Direct transannular Ar-3′ spirocyclization upon Mitsunobu activation of the secondary alcohol **11** (5 equiv of 1,1′-(azodicarbonyl)-

Scheme 1. Asymmetric Synthesis of 14 and 3



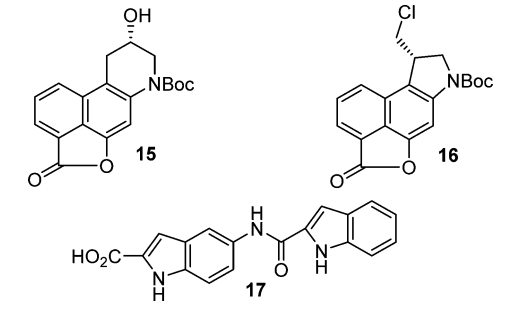


Figure 2. Structures of **15**−**17**.

dipiperidine (ADDP), 5 equiv of Bu_3P, THF, 23 °C, 1 h, 75%) provided **12**. Subsequent treatment of **12** with 4 N HCl in EtOAc

dx.doi.org/10.1021/jo501839x | J. Org. Chem. 2014, 79, 9699−9703

(−78 °C, 2 h, 61−78%) afforded **13** derived from a stereoelectronically controlled regioselective cyclopropane cleavage and acid-catalyzed THP deprotection without competitive *N*-Boc deprotection provided that the reaction was carried out and quenched at low temperature. The key oxazinone was closed with N−O bond formation upon exposure of **13** to Swern oxidation conditions (3 equiv of $(COCl)_2$, 6 equiv of DMSO, CH_2Cl_2), yielding **14** (50%) in an improved yield relative to our original report.[10] More careful control of the reaction conditions, especially the reaction temperature (−78 °C, 30 min and −15 °C, 2 h vs −78 to −10 °C, 2 h) served to substantially improve the conversion of **13** to **14** (50 vs 20%) originally reported.[10] As disclosed in our original work,[10] this represents a new and unique method for intramolecular formation of an N−O bond and presumably entails activation of the hydroxamic acid alcohol as its dimethyl sulfoxonium cation for subsequent intramolecular phenol displacement. Additionally and in the conversion of **12** to **13**, the lactone byproduct **16** (19%) was also isolated, and it was occasionally detected in the conversion of **13** to **14** as a minor byproduct but not quantitated.

The optical purity of **14** was established by chiral phase HPLC (Chiralcel OD column, 0.46 × 25 cm, 5% *i*-PrOH/hexane) and established to be 99% ee, reflecting the optical purity of the starting (*S*)-glycidal 3-nosylate (99% ee) (see Figure S1, Supporting Information). As described previously,[10] acid-catalyzed *N*-Boc deprotection of **14** (4 N HCl, EtOAc, 25 °C, 15 min) and immediate coupling of the resulting HCl salt with **17** (3 equiv of 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide (EDCI), DMF, 25 °C, 20 h, 56%) afforded **3**.

■ CONCLUSIONS

An effective, improved, and asymmetric synthesis of a reductively activated cyclic *N*-acyl *O*-amino phenol duocarmycin prodrug based on the simplified 1,2,9,9a-tetrahydrocyclopropa[*c*]benz-[*e*]indol-4-one (CBI) DNA alkylation subunit is described. Its use in furthering the preclinical exploration of such analogues of the natural products is in progress and will be disclosed in due course.

■ EXPERIMENTAL SECTION

Methyl 8-(Benzyloxy)-6-((*tert*-butyloxycarbonyl)amino)-1-naphthoate (5). A solution of **4** (500 mg, 1.17 mmol) in a 2:1 mixture of DMF−CH_3OH (0.13 M) was treated with $Pd(OAc)_2$ (26.2 mg, 0.117 mmol), xantphos (135 mg, 0.234 mmol), and K_2CO_3 (162 mg, 1.17 mmol) under N_2. CO gas was bubbled through the solution, and the reaction vessel atmosphere was exchanged with CO. The reaction vessel was sealed, after which the mixture was heated to 100 °C and stirred for 17 h. The reaction mixture was cooled to room temperature and filtered. The filtrate was diluted with EtOAc, washed with H_2O and saturated aqueous NaCl, and dried over Na_2SO_4. The solvent was concentrated, and the residue was purified by flash chromatography (10−20% EtOAc/hexanes gradient elution) to give **5** (286 mg, 60%) as an orange solid: mp 178−179 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.73 (dd, J = 8.4, 1.2 Hz, 1H), 7.48 (d, J = 7.2 Hz, 2H), 7.4−7.39 (m, 3H), 7.37 (t, J = 7.2 Hz, 1H), 7.26 (dd, J = 7.2, 1.2 Hz, 1H), 7.10 (s, 1H), 6.62 (s, 1H), 5.14 (s, 2H), 3.31 (s, 3H), 1.55 (s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 171.6, 154.7, 152.7, 137.0, 135.9, 135.4, 129.9, 128.95, 128.92 (2C), 128.7 (2C), 128.6, 126.1, 123.5, 118.1, 107.4, 100.5, 81.0, 71.4, 51.9, 28.5 (3C); IR (film) ν_{max} 1717, 1543, 1240, 1155 cm^{-1}; ESI-TOF HRMS m/z 408.1808 (M + H$^+$, $C_{24}H_{25}NO_5$ requires 408.1805).

Methyl 8-(Benzyloxy)-6-((*tert*-butyloxycarbonyl)amino)-5-iodo-1-naphthoate (6). A suspension of **5** (2.12 g, 5.20 mmol) and NIS (2.23 g, 10.4 mmol) in toluene (124 mL) was treated with acetic acid (1.1 mL) under Ar in the dark. The reaction flask was wrapped with aluminum foil and stirred at 25 °C in the dark for 17 h. The reaction mixture was poured into H_2O and extracted with EtOAc. The organic layer was washed with saturated aqueous NaCl, dried over Na_2SO_4, and concentrated. The residue was purified by flash chromatography (10−25% EtOAc/hexanes gradient elution) to give **6** (2.28 g, 82%) as a pale tan solid: mp 172 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.16−8.14 (m, 2H), 7.51−7.48 (m, 3H), 7.42 (t, J = 8.0 Hz, 2H), 7.38−7.35 (m, 2H), 7.31 (dd, J = 7.0, 1.0 Hz, 1H), 5.19 (s, 2H), 3.24 (s, 3H), 1.58 (s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 207.1, 171.1, 153.4, 152.5, 135.5, 134.3, 131.6, 130.5, 129.4 (2C), 128.7 (2C), 127.1, 125.2, 124.0, 118.6, 100.2, 81.6, 71.7, 52.0, 31.1, 28.5 (3C); IR (film) ν_{max} 2928, 1732, 1620, 1497, 1364, 1279, 1229, 1155 cm^{-1}; ESI-TOF HRMS m/z 534.0769 (M + H$^+$, $C_{24}H_{24}INO_5$ requires 534.0772).

Methyl (*R*)-8-(Benzyloxy)-6-((*tert*-butyloxycarbonyl)(oxiran-2-ylmethyl)amino)-5-iodo-1-naphthoate (7). A solution of **6** (2.28 g, 4.27 mmol) and (*S*)-glycidal 3-nosylate (99% ee, 1.44 g, 5.55 mmol) in DMF (40 mL) was cooled to 0 °C and treated with NaH (60% dispersion in mineral oil, 256 mg, 6.41 mmol). The reaction mixture was stirred at 0 °C for 2 h, after which it was warmed to room temperature and stirred for 3 h. The solution was poured into ice-cold H_2O and extracted with EtOAc. The organic layer was washed with H_2O and saturated aqueous NaCl, dried over Na_2SO_4, and concentrated. The residue was purified by flash chromatography (5−20% EtOAc/hexanes gradient elution) to provide **7** (2.3 g, 91%) as a pale orange solid and as a mixture of rotamers (1:1): mp 105−106 °C; ^{1}H NMR (acetone-d_6, 600 MHz) δ 8.36 (t, J = 7.5 Hz, 1H), 7.71−7.67 (m, 1H), 7.54−7.53 (m, 3H), 7.44−7.42 (m, 2H), 7.39 (d, J = 7.5 Hz, 1H), 7.30 (s, 0.5H), 7.20 (s, 0.5H), 5.32 (s, 2H), 4.05 (ddd, J = 13.8, 13.8, 3.6 Hz, 1H), 3.46 (s, 1.5H), 3.42 (s, 1.5H), 3.36−3.27 (m, 2H), 2.67 (dd, J = 4.5, 4.5 Hz, 0.5H), 2.62 (dd, J = 4.5, 4.5 Hz, 0.5H), 2.41−2.37 (m, 1H), 1.30 (s, 4.5H), 1.29 (s, 4.5H); ^{13}C NMR (acetone-d_6, 150 MHz) δ 170.89, 170.85, 155.8, 155.7, 154.3, 154.2, 145.8, 145.4, 136.98, 136.93, 136.40, 136.34, 135.19, 135.16, 132.1, 129.76, 129.66, 129.50, 129.48, 129.35, 129.31, 129.28, 128.69, 128.67, 122.1, 110.5, 110.4, 96.9, 96.1, 80.93, 80.88, 72.3, 72.2, 53.4, 52.30, 52.25, 52.18, 52.13, 50.4, 50.0, 46.6, 46.2, 28.5 (3C); IR (film) ν_{max} 2927, 1713, 1701, 1365, 1278, 1152 cm^{-1}; $[\alpha]^{27}_D$ +9 (c 0.1, acetone); ESI-TOF HRMS m/z 590.1035 (M + H$^+$, $C_{27}H_{28}INO_6$ requires 590.1034).

4-(*tert*-Butyl) 7-Methyl (*S*)-6-(Benzyloxy)-2-hydroxy-2,3-dihydrobenzo[*f*]quinoline-4,7(1*H*)-dicarboxylate (8). A solution of **7** (250 mg, 0.424 mmol) in anhydrous THF (2.4 mL) was treated with EtMgBr (0.94 mL, 0.848 mmol, 0.9 M in THF) at 0 °C. The reaction mixture was stirred at room temperature for 1 h, after which the reaction was quenched with the addition of saturated aqueous NH_4Cl, diluted with EtOAc, washed with H_2O and saturated aqueous NaCl, and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the residue was purified by PTLC (50% EtOAc/hexanes elution) to provide **8** (153 mg, 78%) as a white amorphous solid: mp 60−61 °C; ^{1}H NMR (acetone-d_6, 600 MHz) δ 7.98 (dd, J = 8.7, 1.2 Hz, 1H), 7.56−7.53 (m, 3H), 7.45−7.42 (m, 3H), 7.38 (t, J = 7.2 Hz, 1H), 7.34 (dd, J = 7.2, 1.2 Hz, 1H), 5.21 (dd, J = 13.2, 10.8 Hz, 2H), 4.30−4.28 (m, 2H), 3.96 (dd, J = 12.0, 3.0 Hz, 1H), 3.59 (dd, J = 12.6, 7.2 Hz, 1H), 3.40−3.37 (m, 1H), 3.37 (s, 3H), 2.94 (dd, J = 16.8, 6.0 Hz, 1H), 1.52 (s, 9H); ^{13}C NMR (acetone-d_6, 150 MHz) δ 171.6, 154.7, 152.5, 138.1, 137.6, 134.3, 131.9, 129.6 (2C), 129.4 (2C), 129.2, 126.9, 125.1, 124.6, 120.1, 115.2, 107.0, 81.5, 72.0, 65.1, 52.0, 51.0, 34.3, 28.6 (3C); IR (film) ν_{max} 3430, 2929, 1695, 1366, 1247, 1151, 1084 cm^{-1}; $[\alpha]^{30}_D$ +40 (c 0.1, acetone); ESI-TOF HRMS m/z 464.2065 (M + H$^+$, $C_{27}H_{29}NO_6$ requires 464.2068).

4-(*tert*-Butyl) 7-Methyl (*S*)-2,6-dihydroxy-2,3-dihydrobenzo-[*f*]quinoline-4,7(1*H*)-dicarboxylate (9). A solution of **8** (160 mg, 0.345 mmol) in 9:1 mixture of THF−CH_3OH (0.03 M) was treated with ammonium formate (210 mg, 3.45 mmol) under Ar, after which 10% Pd/C (73 mg, 0.069 mmol) was added. The reaction mixture was stirred at 25 °C for 1 h. The reaction mixture was filtered through Celite and concentrated under reduced pressure. The residue was purified by PTLC (50% EtOAc/hexanes elution) to give **9** as a pale yellow amorphous solid. The product was triturated with hexane, providing **9** (128 mg, 99%) as a pale yellow amorphous solid: mp 39−40 °C; ^{1}H NMR (acetone-d_6, 600 MHz) δ 9.22 (br, 1H), 7.96 (dd, J = 8.7, 0.9 Hz, 1H), 7.52 (dd, J = 8.7, 6.9 Hz, 1H), 7.37 (dd, J = 6.9, 0.9 Hz, 1H), 7.34 (s, 1H), 4.28−4.26 (m, 1H), 3.96 (dd, J = 12.6, 3.0 Hz, 1H), 3.85 (s, 3H), 3.56

dx.doi.org/10.1021/jo501839x | J. Org. Chem. 2014, 79, 9699−9703

(dd, J = 12.6, 7.8 Hz, 1H), 3.36 (dd, J = 10.8, 6.0 Hz, 1H), 2.91 (dd, J = 10.8, 6.0 Hz, 1H), 1.51 (s, 9H); ^{13}C NMR (acetone-d_6, 150 MHz) δ 172.1, 154.6, 151.0, 138.2, 134.5, 131.7, 126.4, 125.3, 124.2, 119.7, 113.8, 109.2, 81.3, 65.2, 52.5, 50.9, 34.3, 28.5 (3C); IR (film) ν_{max} 3305, 2929, 1693, 1389, 1252, 1153, 1083 cm^{-1}; $[\alpha]^{27}_D$ +35 (c 0.1, acetone); ESI-TOF HRMS m/z 374.1596 (M + H$^+$, $C_{20}H_{23}NO_6$ requires 374.1598).

(*S*)-4-(*tert*-Butoxycarbonyl)-2,6-dihydroxy-1,2,3,4-tetrahydrobenzo[*f*]quinoline-7-carboxylic Acid (10). Compound **9** (123 mg, 0.329 mmol) was dissolved in a 3:3:1 mixture of THF−CH_3OH−H_2O (0.03 M). LiOH−H_2O (69 mg, 1.65 mmol) was added, and the reaction mixture was stirred at 70 °C for 4 h. The reaction mixture was acidified to pH 1 with aqueous 1 N HCl, after which it was diluted with EtOAc. The organic layer was separated, washed with H_2O and saturated aqueous NaCl, and dried over Na_sSO_4. The organic extract was concentrated to give **10** (118 mg, 100%) as a pale tan solid: mp 102−104 °C; ^{1}H NMR (acetone-d_6, 600 MHz) δ 9.55 (br, 1H), 8.03 (dd, J = 8.4, 1.2 Hz, 1H), 7.64 (dd, J = 7.2, 1.2 Hz, 1H), 7.54 (dd, J = 8.4, 7.2 Hz, 1H), 7.36 (s, 1H), 4.27 (br, 1H), 3.96 (dd, J = 12.6, 2.4 Hz, 1H), 3.56 (dd, J = 12.6, 7.5 Hz, 1H), 3.38 (dd, J = 16.5, 5.7 Hz, 1H), 2.93 (dd, J = 16.5, 5.7 Hz, 1H), 1.52 (s, 9H); ^{13}C NMR (acetone-d_6, 150 MHz) δ 173.1, 154.7, 151.4, 138.4, 134.8, 131.3, 126.5, 126.3, 126.1, 120.0, 113.9, 110.1, 81.3, 65.2, 50.9, 34.5, 28.6 (3C); IR (film) ν_{max} 3299, 2929, 1681, 1392, 1256, 1156 cm^{-1}; $[\alpha]^{26}_D$ +34 (c 0.1, acetone); ESI-TOF HRMS m/z 360.1443 (M + H$^+$, $C_{19}H_{21}NO_6$ requires 360.1442).

***tert*-Butyl (2*S*)-2,6-Dihydroxy-7-(methyl((tetrahydro-2*H*-pyran-2-yl)oxy)carbamoyl)-2,3-dihydrobenzo[*f*]quinoline-4(1*H*)-carboxylate (11).** A suspension of **10** (113 mg, 0.314 mmol) and **20** (206 mg, 1.57 mmol) in CH_2Cl_2 (3.14 mL) was treated with HATU (179 mg, 0.471 mmol). i-Pr_2NEt (82 μL, 0.471 mmol) was added at room temperature under Ar, after which the reaction mixture was stirred for 18 h. The reaction mixture was poured into H_2O and extracted with EtOAc. The organic extract was washed with H_2O and saturated aqueous NaCl, dried over Na_2SO_4, and concentrated. The residue was purified by PTLC (88% EtOAc/hexanes elution) to provide **11** as a pale yellow oil. The compound was further triturated with hexanes to give **11** (134 mg, 90%) as a pale yellow amorphous solid and as a mixture of rotamers (1:3): mp 72−73 °C; ^{1}H NMR (acetone-d_6, 500 MHz) δ 9.09 (br, 1H), 7.91 (d, J = 9.0 Hz, 0.25H), 7.87 (d, J = 9.0 Hz, 0.75H), 7.55 (br, 0.25H), 7.50 (t, J = 7.8 Hz, 0.75H), 7.36 (br, 0.25H), 7.29 (d, J = 1.5 Hz, 0.75H), 7.25 (d, J = 7.0 Hz, 1H), 4.62 (br, 1H), 4.26 (m, 2H), 4.01−3.95 (m, 1H), 3.57−3.48 (m, 2H), 3.40 (s, 3H), 3.37−3.34 (m, 1H), 3.08 (br, 1H), 2.92−2.86 (m, 1H), 1.81 (m, 1H), 1.62−1.57 (m, 1H), 1.52 (s, 9H), 1.39−1.27 (m, 3H), 1.15 (m, 1H); ^{13}C NMR (acetone-d_6, 150 MHz) δ 154.66, 154.60, 151.4, 138.2, 137.8, 134.5, 134.0, 126.9, 126.4, 124.6, 124.0, 120.4, 113.7, 113.3, 108.8, 108.3, 103.9, 101.9, 81.1, 65.2, 63.0, 62.8, 50.94, 50.89, 39.9, 34.3, 34.2, 28.5, 26.3, 25.8, 20.8; IR (film) ν_{max} 2940, 1620, 1390, 1154 cm^{-1}; $[\alpha]^{25}_D$ +15 (c 0.1, acetone); ESI-TOF HRMS m/z 473.2280 (M + H$^+$, $C_{25}H_{32}N_2O_7$ requires 473.2282).

***tert*-Butyl (*S*)-9-Hydroxy-4-oxo-4,8,9,10-tetrahydro-7*H*-isobenzofuro[7,1-*fg*]quinoline-7-carboxylate (15).** In the conversion of **10** to **11**, the lactone **15** could be isolated and characterized if the reaction was prematurely quenched. For **15**: mp 190 °C; ^{1}H NMR (acetone-d_6, 500 MHz) δ 8.29 (d, J = 8.5 Hz, 1H), 8.11 (d, J = 7.5 Hz, 1H), 7.92 (dd, J = 8.5, 7.5 Hz, 1H), 7.52 (s, 1H), 4.36−4.34 (m, 2H), 3.93 (dd, J = 12.5, 2.5 Hz, 1H), 3.75 (dd, J = 12.5, 6.5 Hz, 1H), 3.48 (dd, J = 17.0, 5.5 Hz, 1H), 3.07 (dd, J = 17.0, 5.0 Hz, 1H), 1.54 (s, 9H); ^{13}C NMR (acetone-d_6, 150 MHz) δ 167.7, 154.9, 148.3, 140.0, 131.1, 130.2, 129.8, 127.9, 125.5, 121.9, 118.1, 106.8, 81.9, 64.7, 51.4, 33.1, 28.5 (3C); IR (film) ν_{max} 3404, 2926, 1776, 1699, 1648, 1455, 1392, 1366, 1242, 1154 cm^{-1}; ESI-TOF HRMS m/z 364.1161 (M + Na$^+$, $C_{19}H_{19}NO_5$ requires 364.1155).

***tert*-Butyl (9a*S*)-5-(Methyl((tetrahydro-2*H*-pyran-2-yl)oxy)-carbamoyl)-4-oxo-9,9a-dihydro-1*H*-benzo[*e*]cyclopropa[*c*]-indole-2(4*H*)-carboxylate (12).** A solution of **11** (66 mg, 0.14 mmol) in THF (14 mL) was treated with tributylphosphine (176 μL, 0.70 mmol), after which the reaction mixture was treated with ADDP (176 mg, 0.70 mmol). The reaction mixture was stirred for 1 h, quenched with the addition of H_2O, and extracted with EtOAc. The organic layer was washed with H_2O and saturated aqueous NaCl, dried over Na_2SO_4, and concentrated. The residue was purified by PTLC (88% EtOAc/hexanes elution) to provide **12** as a colorless oil. The compound was further triturated with hexanes to give **12** (48 mg, 75%) as a white amorphous solid and as a mixture of rotamers (1:1): mp 90−92 °C; ^{1}H NMR (acetone-d_6, 500 MHz) δ 7.56 (ddd, J = 7.5, 7.5, 3.5 Hz, 1H), 7.22 (t, J = 6.5 Hz, 1H), 7.14 (d, J = 7.5 Hz, 1H), 6.72 (d, J = 8.5 Hz, 1H), 4.53 (br, 0.5H), 4.49 (br, 0.5H), 4.09−4.02 (m, 2H), 3.89 (m, 1H), 3.51−3.47 (m, 1H), 3.40 (s, 1.5H), 3.39 (s, 1.5H), 3.11−3.08 (m, 1H), 1.72−1.59 (m, 3H), 1.55 (s, 9H), 1.42−1.41 (m, 2H), 1.33−1.21 (m, 3H); ^{13}C NMR (acetone-d_6, 150 MHz) δ 185.0, 184.7, 177.0, 176.5, 152.5, 142.0, 141.6, 138.0, 132.1, 125.4, 125.2, 123.22, 123.16, 108.6, 108.2, 105.2, 105.1, 102.0, 83.3, 63.14, 63.06, 53.98, 53.93, 38.58, 38.53, 32.5, 29.2, 28.40, 28.38, 25.96, 25.90, 23.4, 19.87, 19.84, 14.5; IR (film) ν_{max} 3728, 3627, 1730, 1965, 1730, 1625, 1377, 1274 cm^{-1}; $[\alpha]^{25}_D$ +89 (c 0.1, acetone); ESI-TOF HRMS m/z 455.2177 (M + H$^+$, $C_{25}H_{30}N_2O_6$ requires 455.2177).

***tert*-Butyl (*S*)-1-(Chloromethyl)-5-hydroxy-6-(hydroxy(methyl)carbamoyl)-1,2-dihydro-3*H*-benzo[*e*]indole-3-carboxylate (13).** A sample of **12** (45 mg, 0.099 mmol) at −78 °C was treated with 4 N HCl in EtOAc (4.5 mL), and the solution was stirred at −78 °C for 2 h. The reaction mixture was diluted with EtOAc (13.5 mL) at the same temperature, after which H_2O (9.0 mL) was added. The organic layer was separated, washed with H_2O and saturated aqueous NaCl, dried over Na_2SO_4, and concentrated. The residue was purified by PTLC (50% EtOAc/hexanes elution) to provide **13** and **16**. Compound **13** was further triturated with hexanes to give **13** (25 mg, 61%) as a pale yellow solid and as a mixture of rotamers (1:2): mp 115−116 °C; ^{1}H NMR (CD_3CN, 600 MHz) δ 8.23 (br, 0.33H), 8.07 (s, 0.67H), 7.76−7.67 (m, 2H), 7.44 (dd, J = 14.4, 7.2 Hz, 1H), 7.15 (d, J = 7.2 Hz, 1H), 7.16 (d, J = 7.0 Hz, 1H), 4.60−4.50 (br, 1H), 4.15−4.08 (m, 2H), 3.91−3.86 (m, 1H), 3.68−3.61 (m, 1H), 3.47 (br, 1H), 3.36 (s, 2H), 1.58 (s, 9H); ^{13}C NMR (CD_3CN, 150 MHz) δ 176.1, 154.7, 153.2, 142.9, 134.4, 131.4, 127.7, 127.3, 127.2, 124.4, 123.9, 105.0, 100.8, 81.6, 63.2, 53.6, 48.3, 48.2, 41.7, 37.9, 26.0, 25.6; IR (film) ν_{max} 2934, 1701, 1622, 1406, 1370, 1334, 1249, 1142 cm^{-1}; $[\alpha]^{25}_D$ +5 (c 0.08, acetone); ESI-TOF HRMS m/z 407.1367 (M + H$^+$, $C_{20}H_{23}ClN_2O_5$ requires 407.1368).

Byproduct **16** was further triturated with hexanes to give **16** (6.8 mg, 19%) as a yellow solid: mp 185−186 °C; ^{1}H NMR (acetone-d_6, 600 MHz) δ 8.34 (d, J = 8.4 Hz, 1H), 8.19−8.05 (br, 1H), 8.03 (d, J = 6.6 Hz, 1H), 7.91 (dd, J = 8.4, 6.6 Hz, 1H), 4.34−4.27 (m, 2H), 4.23 (dd, J = 11.4, 3.6 Hz, 1H), 3.95 (dd, J = 11.4, 7.2 Hz, 1H), 1.60 (s, 9H); ^{13}C NMR (acetone-d_6, 150 MHz) δ 167.6, 152.9, 151.7, 145.9, 131.7, 129.3, 127.6, 126.7, 124.6, 122.6, 118.3, 99.0, 82.1, 54.1, 48.4, 41.4, 28.6 (3C); IR (film) ν_{max} 1784, 1704, 1401, 1327, 1137 cm^{-1}; $[\alpha]^{28}_D$ −41 (c 0.1, acetone); ESI-TOF HRMS m/z 360.0997 (M + H$^+$, $C_{19}H_{18}ClNO_4$ requires 360.0997).

***tert*-Butyl (*S*)-10-(Chloromethyl)-5-methyl-4-oxo-4,5,9,10-tetrahydro-8*H*-pyrrolo[3′,2′:5,6]naphtho[1,8-*de*][1,2]oxazine-8-carboxylate (14).** A stirred solution of $(COCl)_2$ (6.2 μL, 0.074 mmol) in freshly distilled CH_2Cl_2 (1.0 mL) at −78 °C was treated with Me_2SO (10.5 μL, 0.148 mmol) in 0.25 mL of freshly distilled CH_2Cl_2 dropwise. After 30 min, compound **13** (10.0 mg, 0.0246 mmol) in 2.0 mL of freshly distilled CH_2Cl_2 was added dropwise and the reaction mixture was stirred at −78 °C for 30 min, after which the reaction mixture was warmed to −15 °C and stirred for 2 h. The reaction was quenched with the addition of saturated aqueous NH_4Cl, and the mixture was extracted with EtOAc. The organic layer was washed with saturated aqueous NaCl, dried over Na_2SO_4, and concentrated. The residue was purified by PTLC (50% EtOAc/hexanes elution) to provide **14** (4.75 mg, 50%) as a pale yellow solid: mp 147 °C; ^{1}H NMR (acetone-d_6, 600 MHz) δ 7.99 (d, J = 8.4 Hz, 1H), 7.82 (d, J = 7.2 Hz, 1H), 7.77 (br, 1H), 7.63 (t, J = 7.8 Hz, 1H), 4.21 (m, 2H), 4.14 (m, 1H), 4.00 (dd, J = 11.1, 3.6 Hz, 1H), 3.79 (dd, J = 8.4, 11.4 Hz, 1H), 3.56 (s, 3H), 1.58 (s, 9H); ^{13}C NMR (acetone-d_6, 150 MHz) δ 161.8, 153.7, 153.3, 144.8, 131.2, 130.0, 127.9, 123.9, 121.6, 118.5, 118.1, 99.2, 82.7, 54.7, 48.7, 45.5, 36.2, 29.5 (3C); IR (film) ν_{max} 2978, 1702, 1404, 1141 cm^{-1}; $[\alpha]^{23}_D$ −45 (c 1.0, THF); ESI-TOF HRMS m/z 389.1268 (M + H$^+$, $C_{20}H_{21}ClN_2O_4$ requires 389.1263).

Benzyl ((Tetrahydro-2*H*-pyran-2-yl)oxy)carbamate (18). A solution of *O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (2.0 g, 17.1 mmol) in CH_2Cl_2 (20 mL) was treated with i-Pr_2NEt (3.28 mL, 18.8

dx.doi.org/10.1021/jo501839x | J. Org. Chem. 2014, 79, 9699−9703

mmol) and benzyl chloroformate (2.56 mL, 18.0 mmol) at 0 °C and stirred at room temperature overnight. The reaction mixture was poured into ice-cold H_2O, extracted with CH_2Cl_2, washed with saturated aqueous NaCl, and dried over Na_2SO_4. The solvent was removed, and the residue was purified by flash chromatography (10−30% EtOAc/hexanes gradient elution) to give **18** (4.30 g, 100%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.64 (br, 1H), 7.37−7.31 (m, 5H), 7.79 (d, J = 8.5 Hz, 1H), 5.18 (dd, J = 16.2, 12.0 Hz, 2H), 4.93 (t, J = 3.0 Hz, 1H), 3.93 (ddd, J = 12.0, 9.6, 3.0 Hz, 1H), 3.61 (dddd, J = 11.4, 4.2, 4.2, 1.8 Hz, 1H), 1.80−1.76 (m, 3H), 1.64−1.54 (m, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 157.0, 135.7, 128.7, 128.5, 128.4, 102.6, 67.6, 62.6, 28.2, 25.1, 18.8; IR (film) ν_{max} 3265, 2945, 1723, 1454, 1242, 1205, 1108, 1036, 898, 874, 742 cm^{-1}; ESI-TOF HRMS m/z 252.1231 (M + H$^+$, $C_{13}H_{17}NO_4$ requires 252.1230).

Benzyl Methyl((tetrahydro-2*H*-pyran-2-yl)oxy)carbamate (19). A solution of **18** (4.30 g, 17.1 mmol) in DMF (50 mL) was treated with NaH (60% dispersion in mineral oil, 821 mg, 20.5 mmol) at 0 °C and stirred at the same temperature for 1 h, after which iodomethane (3.2 mL, 51.3 mmol) was added. The reaction mixture was stirred at room temperature overnight before being poured into ice-cold H_2O. The mixture was extracted with EtOAc, washed with H_2O and saturated aqueous NaCl, and dried over Na_2SO_4. The solvent was removed, and the residue was purified by flash chromatography (10−15% EtOAc/hexanes gradient elution) to give **19** (3.89 g, 86%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.37−7.31 (m, 5H), 5.19 (d, J = 12.0 Hz, 1H), 5.16 (d, J = 12.0 Hz, 1H), 5.02 (t, J = 3.0 Hz, 1H), 4.02 (ddd, J = 12.0, 9.0, 3.0 Hz, 1H), 3.60 (dddd, J = 11.4, 4.2, 4.2, 1.8 Hz, 1H), 3.28 (s, 3H), 1.79−1.72 (m, 3H), 1.64−1.54 (m, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 157.8, 136.2, 128.6, 128.3, 128.1, 103.0, 67.9, 62.8, 38.7, 28.6, 25.3, 19.0; IR (film) ν_{max} 2940, 2857, 1703, 1037 cm^{-1}; ESI-TOF HRMS m/z 266.1390 (M + H$^+$, $C_{14}H_{19}NO_4$ requires 266.1387).

***N*-Methyl-*O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (20).** A solution of **19** (1.0 g, 3.77 mmol) in anhydrous Et_2O (30 mL) was treated with 10% Pd/C (80 mg, 0.075 mmol), after which the atmosphere was exchanged with H_2. The reaction mixture was stirred under H_2 at room temperature for 2 h. The reaction mixture was diluted with Et_2O, filtered through Celite, and concentrated in an ice-cold bath under reduced pressure to give **20** (530 mg, 87%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 600 MHz) δ 5.68 (m, 1H), 4.80 (dd, J = 6.0, 3.0 Hz, 1H), 3.98−3.92 (m, 1H), 3.58−3.55 (m, 1H), 2.78 (s, 3H), 1.83−1.75 (m, 1H), 1.74−1.69 (m, 1H), 1.59−1.46 (m, 4H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 101.3, 63.3, 39.7, 29.4, 25.5, 20.4; IR (film) ν_{max} 2940, 2857, 1073, 1037 cm^{-1}; ESI-TOF HRMS m/z 132.1018 (M + H$^+$, $C_6H_{13}NO_2$ requires 132.1019).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Copies of the ^{1}H and ^{13}C NMR spectra and a figure of the HPLC chiral phase separation and establishment of the optical purity of **14**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: boger@scripps.edu.

Notes

The authors declare no competing financial interests.

■ ACKNOWLEDGMENTS

We gratefully acknowledge the financial support from the National Institutes of Health (CA042056, DLB).

■ REFERENCES

(1) Ichimura, M.; Ogawa, T.; Takahashi, K.; Kobayashi, E.; Kawamoto, I.; Yasuzawa, T.; Takahashi, I.; Nakano, H. *J. Antibiot.* **1990**, *43*, 1037−1038.

(2) Martin, D. G.; Biles, C.; Gerpheide, S. A.; Hanka, L. J.; Krueger, W. C.; McGovren, J. P.; Mizsak, S. A.; Neil, G. L.; Stewart, J. C.; Visser, J. *J. Antibiot.* **1981**, *34*, 1119−1125.

(3) Takahashi, I.; Takahashi, K.; Ichimura, M.; Morimoto, M.; Asano, K.; Kawamoto, I.; Tomita, F.; Nakano, H. *J. Antibiot.* **1988**, *41*, 1915−1917.

(4) Igarashi, Y.; Futamata, K.; Fujita, T.; Sekine, A.; Senda, H.; Naoki, H.; Furumai, T. *J. Antibiot.* **2003**, *56*, 107−113.

(5) For duocarmycin SA, see: (a) Boger, D. L.; Johnson, D. S.; Yun, W. *J. Am. Chem. Soc.* **1994**, *116*, 1635−1656. For yatakemycin, see: (b) Parrish, J. P.; Kastrinsky, D. B.; Wolkenberg, S. E.; Igarashi, Y.; Boger, D. L. *J. Am. Chem. Soc.* **2003**, *125*, 10971−10976. For CC-1065, see (c) Hurley, L. H.; Lee, C.-S.; McGovren, J. P.; Warpehoski, M. A.; Mitchell, M. A.; Kelly, R. C.; Aristoff, P. A. *Biochemistry* **1988**, *27*, 3886−3892. (d) Boger, D. L.; Johnson, D. S.; Yun, W.; Tarby, C. M. *Bioorg. Med. Chem.* **1994**, *2*, 115−135. (e) Boger, D. L.; Zarrinmayeh, H.; Munk, S. A.; Kitos, P. A.; Suntornwat, O. *Proc. Natl. Acad. Sci. U. S. A.* **1991**, *88*, 1431−1435. For duocarmycin A, see: (f) Boger, D. L.; Ishizaki, T.; Zarrinmayeh, H.; Munk, S. A.; Kitos, P. A.; Suntornwat, O. *J. Am. Chem. Soc.* **1990**, *112*, 8961−8971. (g) Boger, D. L.; Yun, W.; Terashima, S.; Fukuda, Y.; Nakatani, K.; Kitos, P. A.; Jin, Q. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 759−765.

(6) Reviews: (a) Boger, D. L.; Johnson, D. S. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1438−1474. (b) Boger, D. L. *Acc. Chem. Res.* **1995**, *28*, 20−29. (c) Boger, D. L.; Johnson, D. S. *Proc. Natl. Acad. Sci. U. S. A.* **1995**, *92*, 3642−3649. (d) Boger, D. L.; Garbaccio, R. M. *Acc. Chem. Res.* **1999**, *32*, 1043−1052. (e) Searcey, M. *Curr. Pharm. Des.* **2002**, *8*, 1375−1389. (f) MacMillan, K. S.; Boger, D. L. *J. Med. Chem.* **2009**, *52*, 5771−5780.

(7) Review: Boger, D. L.; Boyce, C. W.; Garbaccio, R. M.; Goldberg, J. A. *Chem. Rev.* **1997**, *97*, 787−828.

(8) Review: Boger, D. L.; Garbaccio, R. M. *Bioorg. Med. Chem.* **1997**, *5*, 263−276.

(9) (a) Jin, W.; Trzupek, J. D.; Rayl, T. J.; Broward, M. A.; Vielhauer, G. A.; Weir, S. J.; Hwang, I.; Boger, D. L. *J. Am. Chem. Soc.* **2007**, *129*, 15391−15397. (b) Lajiness, J. P.; Robertson, W. M.; Dunwiddie, I.; Broward, M. A.; Vielhauer, G. A.; Weir, S. J.; Boger, D. L. *J. Med. Chem.* **2010**, *53*, 7731−7738.

(10) Wolfe, A. L.; Duncan, K. K.; Parelkar, N. K.; Brown, D.; Vielhauer, G. A.; Boger, D. L. *J. Med. Chem.* **2013**, *56*, 4104−4115.

(11) Reviews: (a) Chen, Y.; Hu, L. *Med. Res. Rev.* **2009**, *29*, 29−64. (b) Ghosh, N.; Sheldrake, H. M.; Searcey, M.; Pors, K. *Curr. Top. Med. Chem.* **2009**, *9*, 1494−1524. (c) Tietze, L. F.; Krewer, B. *Anti-Cancer Agents Med. Chem.* **2009**, *9*, 304−325. (d) Wolkenberg, S. E.; Boger, D. L. *Chem. Rev.* **2002**, *102*, 2477−2495.

(12) (a) Boger, D. L.; Ishizaki, T.; Kitos, P. A.; Suntornwat, O. *J. Org. Chem.* **1990**, *55*, 5823−5832. (b) Boger, D. L.; Ishizaki, T. *Tetrahedron Lett.* **1990**, *31*, 793−796. (c) Boger, D. L.; Wysocki, R. J.; Ishizaki, T. *J. Am. Chem. Soc.* **1990**, *112*, 5230−5240. (d) Boger, D. L.; Munk, S. A. *J. Am. Chem. Soc.* **1992**, *114*, 5487−5496.

(13) (a) Boger, D. L.; McKie, J. A.; Boyce, C. W. *Synlett* **1997**, 515−517. (b) Kastrinsky, D. B.; Boger, D. L. *J. Org. Chem.* **2004**, *69*, 2284−2289. (c) Lajiness, J. P.; Boger, D. L. *J. Org. Chem.* **2011**, *76*, 583−587. (d) Ling, L.; Xie, Y.; Lown, J. W. *Heterocycl. Commun.* **1997**, *3*, 405−408. (e) Tietze, L. F.; von Hof, J. M.; Krewer, B.; Muller, M.; Major, F.; Schuster, H. J.; Schuberth, I.; Alvers, F. *ChemMedChem.* **2008**, *3*, 1946−1955.

(14) Wolfe, A. L.; Duncan, K. K.; Parelkar, N. K.; Weir, S. J.; Vielhauer, G. A.; Boger, D. L. *J. Med. Chem.* **2012**, *55*, 5878−5886.

(15) Tichenor, M. S.; Trzupek, J. D.; Kastrinsky, D. B.; Shiga, F.; Hwang, I.; Boger, D. L. *J. Am. Chem. Soc.* **2006**, *128*, 15683−15696.