Supporting Information

A unique class of duocarmycin and CC-1065 analogues subject to reductive activation

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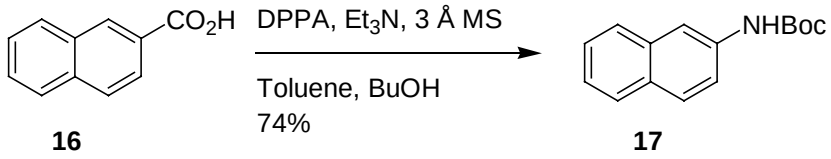
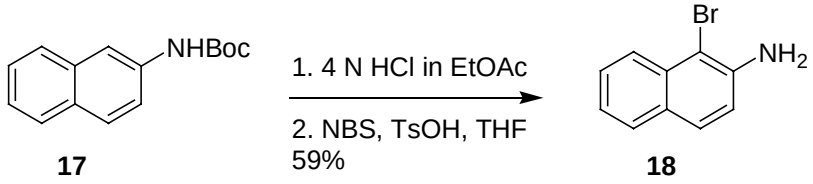
Experimental

DNA alkylation selectivity and efficiency. The DNA alkylation reactions were performed by treatment of 4.5 μL of singly ^{32}P 5'-end-labeled double-stranded w794 DNA[39] in TE buffer (10 mM Tris, 1 mM EDTA, pH 7.6) with 0.5 μL of agent in EtOH (at the specified concentration). The samples were incubated for 18 h at 4 ºC. Unbound agent was removed by EtOH precipitation of DNA (0.5 μL of 3.0 M sodium acetate and 12.5 μL of cold absolute EtOH) and the solutions were stored at –78 °C for 1 h or longer. The DNA was pelleted by centrifugation at 4 °C (13000 rpm, 25 min), dried in a Savant Speed Vac concentrator, and resuspended in 5 μL of TE buffer (pH 7.6). Thermal depurination (3 × 10 min at 100 °C) was performed and then 2.5 μL of formamide dye solution was added to the cooled samples. Thermally denatured samples were assayed by gel electrophoresis [8% denaturing gel with 8 M urea in TBE buffer (89 mM Tris-borate, 2 mM EDTA)] followed by autoradiography of the dried gel using Kodak BIOMAX XAR film and a Picker Spectra^{TM} intensifying screen.



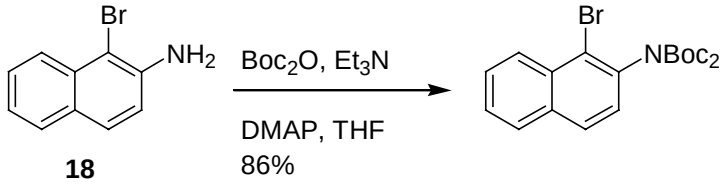
16 **17**

A solution of 2-naphthoic acid (**16**, 1.5 g, 8.7 mmol) in t-BuOH (50 mL) and toluene (50 mL) was treated with Et_3N (1.44 mL, 10 mmol), 3 Å molecular sieves (10 g) and diphenyl phosphorylazide (2.1 mL, 10 mmol). The reaction mixture was warmed at reflux for 24 h and then cooled to 23 °C. The solid was filtered off through Celite and the solvent was removed in vacuo. The residue was dissolved in EtOAc (75 mL), and the organic phase was washed with 1 N aqueous HCl (50 mL × 2), saturated aqueous $NaHCO_3$ (50 mL × 2), dried over anhydrous sodium sulfate, and concentrated. Chromatography (SiO_2, 10% EtOAc/hexane) afforded **17** as a pale yellow solid (1.56 g, 74%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (s, 1H), 7.72–7.75 (m, 3H), 7.42 (t, *J* = 7.5 Hz, 1H), 7.31–7.37 (m, 2H), 6.67 (s, 1H), 1.55 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.8, 135.7, 134.0, 130.0, 128.7, 127.5, 127.3, 126.4, 124.4, 119.1, 114.5, 80.7, 20.3; ESI-TOF HRMS *m/z* 266.1150 ($M+Na^+$, $C_{15}H_{17}NO_2$ requires 266.1151).



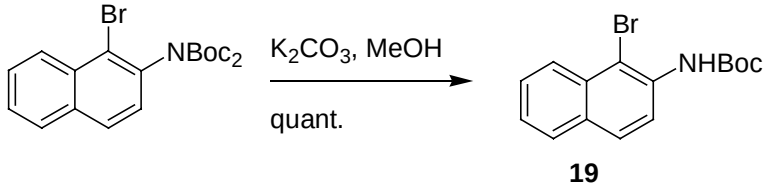
17 **18**

Compound **17** (1.5 g, 6.2 mmol) was treated with 4 N HCl–EtOAc (50 mL) for 1 h before the solvent was removed to yield a white powder. The crude HCl salt (790 mg, 5.5 mmol), and TsOH (170 mg, 1.1 mmol) in THF (50 mL) cooled to 0 °C was treated with NBS (982 mg, 5.5 mmol) in THF (30 mL), and the solution was allowed to warm to 23 °C. After stirring for 5 h, the reaction mixture was washed with saturated aqueous $NaHCO_3$ (30 mL × 2). The organic layer was dried over anhydrous sodium sulfate and was concentrated. Chromatography (SiO_2, 10% EtOAc/hexane) afforded **18** (863 mg, 59% for two steps): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, *J* = 8.6 Hz, 1H), 7.68 (d, *J* = 8.1 Hz, 1H), 7.60 (dd, *J* = 1.5, 8.7 Hz, 1H), 7.50 (t, *J* = 7.1 Hz, 1H), 7.28 (t, *J* = 7.4 Hz, 1H) 6.98 (dd, *J* = 2.1, 8.7 Hz, 1H), 4.36 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.0, 133.0, 128.5, 128.1, 127.7, 124.9, 124.8, 122.9, 117.6 104.0; ESI-TOF HRMS *m/z* 221.9910 ($M+H^+$, $C_{10}H_8BrN$ requires 221.9913).



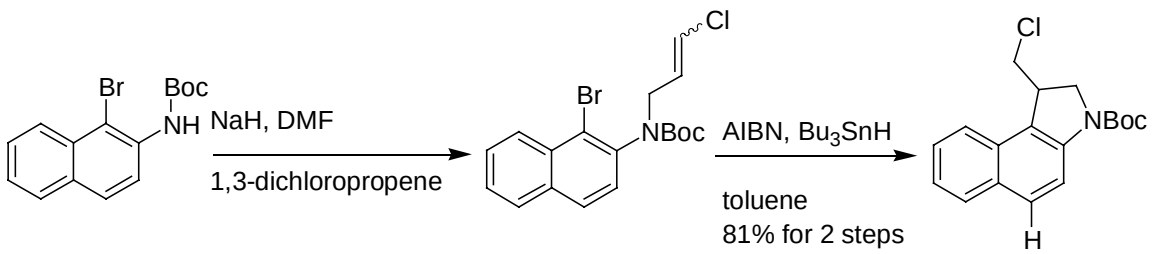
18

A solution of **18** (800 mg, 3.6 mmol) in CH_2Cl_2 was treated with Et_3N (496 mL, 3.6 mmol), DMAP (36 mg, 0.36 mmol), and Boc_2O (830 mg, 3.8 mmol) and the reaction mixture was stirred at 55 °C for 36 h. The reaction mixture was cooled to 23 °C and washed with aqueous 1 N HCl (30 mL × 2), and saturated aqueous $NaHCO_3$ (30 mL × 2). The organic layer was dried over anhydrous sodium sulfate, and concentrated. Chromatography (SiO_2, 10% EtOAc/hexanes) provided the product as a white solid (1.25 g, 83%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (d, *J* = 8.3 Hz, 1H), 7.84 (d, *J* = 7.9 Hz, 1H), 7.81 (d, *J* = 8.6 Hz, 1H), 7.60 (td, *J* = 1.3, 8.4 Hz, 1H), 7.53 (td, *J* = 1.3, 8.4 Hz, 1H), 7.53 (td, *J* = 1.3, 7.4 Hz, 1H), 1.38 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.4, 136.4, 133.2, 132.2, 128.0, 127.5, 127.4, 126.8, 126.7, 123.1, 82.7, 27.7; ESI-TOF HRMS *m/z* 444.0780 ($M+Na^+$, $C_{20}H_{24}BrNO_4$ requires 444.0781).



19

A solution of the product above (516 mg, 1.18 mmol) in MeOH (20 mL) was treated with K_2CO_3 (490 mg, 3.6 mmol), and the resulting mixture was warmed at reflux for 1.5 h. The reaction mixture was allowed to cool to 23 °C and filtered through Celite to remove solid residue. The solvent was removed to yield **19** as a white solid (448 mg, quant.), which was sufficiently pure to use for next step without further purification: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (d, *J* = 8.7 Hz, 1H), 8.12 (d, *J* = 7.6 Hz, 1H), 7.77 (d, *J* = 8.5 Hz, 1H), 7.76 (d, *J* = 8.5 Hz, 1H), 7.54 (t, *J* = 7.0 Hz, 1H), 7.40 (t, *J* = 6.9 Hz, 1H), 7.33 (s, 1H), 1.38 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.5, 134.7, 132.0, 130.8, 128.2, 128.0, 127.6, 126.3, 124.9, 119.5, 109.9, 81.2, 28.3; ESI-TOF HRMS *m/z* 344.0250 ($M+Na^+$, $C_{15}H_{16}BrNO_2$ requires 344.0257).



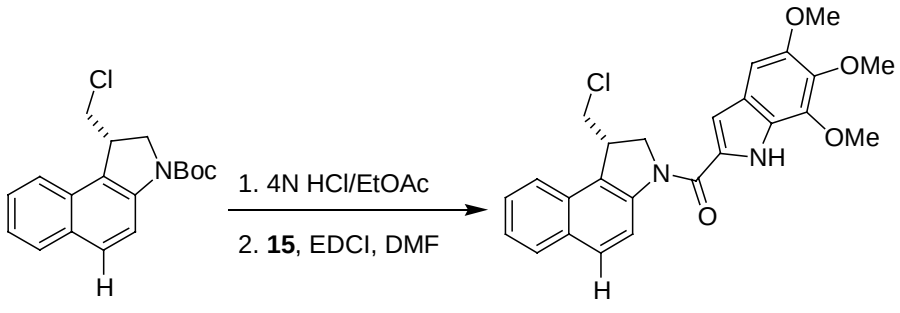
19 **20** **21**

A solution of **19** (980 mg, 3 mmol) in DMF (20 mL) was treated with NaH (60%, 304 mg, 7.5 mmol) and Bu_4NI (11 mg, 0.3 mmol) at 0 °C. After stirring for 15 min, 1,3-dichloropropene (0.8 mL, 9 mmol) was added, and the resulting mixture was warmed to 23 °C. and stirred for another 4 h. The reaction mixture was diluted with EtOAc (50 mL) and washed with saturated aqueous NH_4Cl (30 mL × 2). The organic layer was dried over anhydrous sodium sulfate and contentrated. The crude product **20** was used for the next step without further purification.

A solution of crude **20** (1.0 g, 2.52 mmol) and AIBN (41 mg, 0.25 mmol) in degassed toluene (40 mL) was treated with Bu_3SnH (0.75 mL, 2.77 mmol). The resulting solution was purged with N_2 gas for 10 min and then warmed at reflux overnight. The solvent was removed and the crude product was purified by chromatography (SiO_2, 10% EtOAc/hexanes) to yield racemic **21** as a white solid (780 mg, 97%). The two enantiomers were separated by chromatography (semiprep 2 × 25 cm Chiral OD column, 10% iPrOH/hexanes, flow rate = 0.5 mL/min, t_R = 35.5 min (natural), 25.0 min (unnatural), α = 1.42): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.07 (br s, 1H), 7.85–7.95 (m, 3H), 7.53 (t, J = 8.3 Hz, 1H), 7.39 (t, J = 8.1 Hz, 1H), 4.22 (d, J = 6.4 Hz, 1H), 4.15 (d, J = 8.2 Hz, 1H), 4.02–4.09 (m, 2H), 3.89 (dd, J = 7.0, 10.0 Hz, 1H), 1.54 (s, 9H); ESI-TOF HRMS *m/z* 340.1076 ($M+H^+$, $C_{18}H_{20}ClNO_2$ requires 340.1075).

1*S*-**21**: $[\alpha]^{23}_D$ –0.38 (*c* 0.18, CH_3OH), natural enantiomer.

1*R*-**21**: $[\alpha]^{23}_D$ +0.46 (*c* 0.13, CH_3OH), unnatural enantiomer.

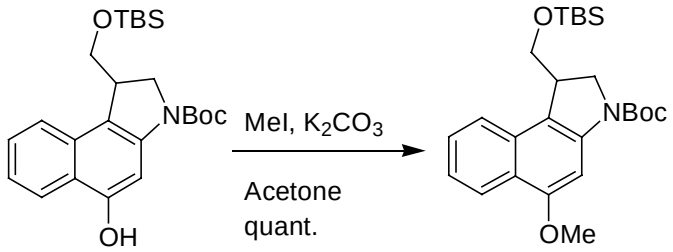


21 **9**

A sample of **21** (13 mg, 41 μmol) was treated with 4 N HCl–EtOAc (3 mL) for 30 min before the solvent was removed by a stream of N_2. The resulting crude HCl salt, 5,6,7-trimethoxyindol-2-carboxylic acid (**15**, 10.3 mg, 41 μmol) and EDCI (24 mg, 0.12 mmol) were dissolved in DMF (3 mL), and the resulting solution was stirred at 23 °C for 3 h. The reaction mixture was diluted with EtOAc (15 mL) and washed with aqueous 1 N HCl (5 mL × 2), and saturated aqueous $NaHCO_3$ (5 mL × 2). The organic layer was dried over anhydrous sodium sulfate, and concentrated. PTLC (SiO_2, 50% EtOAc/hexanes) gave **9** as a white solid (13.6 mg, 74%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.53 (s, 1H), 8.31 (br s, 1H), 7.90–7.97 (m, 3H), 7.53 (t, *J* = 7.1 Hz, 1H), 7.46 (t, *J* = 7.0 Hz, 1H), 7.08 (d, *J* = 2.1 Hz, 1H), 6.97 (s, 1H), 4.79 (dd, *J* = 9.6, 11.0 Hz, 1H), 4.53 (dd, *J* = 2.8, 11.0 Hz, 1H), 4.35 (td, *J* = 2.9, 7.9 Hz, 1H), 4.08 (dd, *J* = 3.2, 11.1 Hz, 1H), 3.94 (dd, *J* = 7.2, 10.8 Hz, 1H), 3.93 (s, 3H), 3.82 (s, 3H), 3.80 (s, 3H); ESI-TOF HRMS *m/z* 451.1420 ($M+H^+$, $C_{25}H_{23}ClN_2O_4$ requires 451.1419).

1*S*-**9**: $[\alpha]^{23}_D$ –0.26 (*c* 0.46, THF), natural enantiomer.

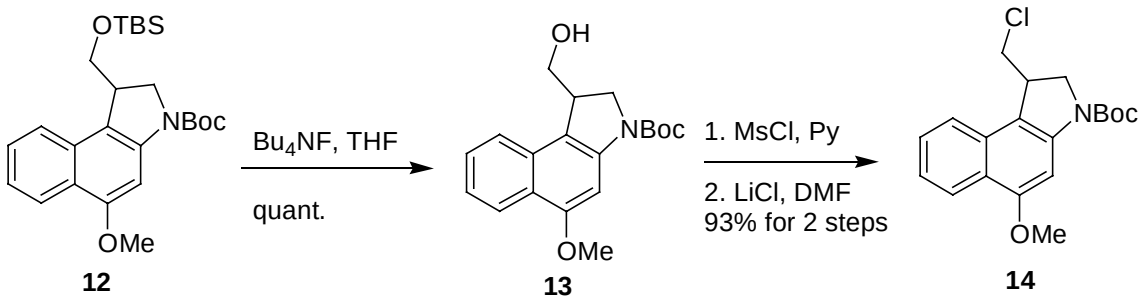
1*R*-**9**: $[\alpha]^{23}_D$ +0.27 (*c* 0.73, THF), unnatural enantiomer.



11 **12**

A solution of **11**[1] (50 mg, 0.116 mmol), and methyl iodide (14.5 μL, 0.233 mmol) in acetone (12 mL) was treated with K_2CO_3 (48 mg, 0.349 mmol) at 23 °C, and the resulting mixture was stirred at 23 °C for 3 h. The reaction was diluted with water (10 mL) and extracted with EtOAc (15 mL × 2). The combined organic layers were washed with water (15 mL × 2), saturated aqueous NaCl (15 mL) and dried over anhydrous sodium sulfate. The solvent was removed and the crude product **12** was sufficiently pure for use without further purification (55 mg, quant.): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (d, *J* = 8.8 Hz, 1H), 7.73 (br s, 1H), 7.68 (d, *J* = 8.6 Hz, 1H), 7.46 (t, *J* = 7.8 Hz, 1H), 7.30 (t, *J* = 7.4 Hz, 1H), 4.20–4.26 (m, 1H), 4.02 (s, 3H), 3.93–3.98 (m,

2H), 3.71–3.77 (m, 1H), 3.43–3.50 (m, 1H), 1.25 (s, 9H), 0.88 (s, 9H), 0.02 (s, 3H), –0.04 (s, 3H).

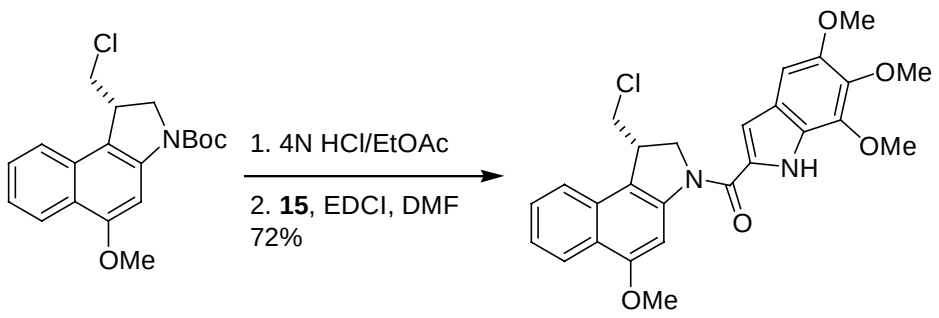


A solution of **12** (51 mg, 0.115 mmol) in THF (5 mL) was treated with Bu_4NF (1 M in THF, 575 μL, 0.575 mmol) at 23 °C. After stirring at 23 °C for 1 h, the reaction mixture was diluted with EtOAc (20 mL) and washed with water (10 mL), and saturated aqueous NaCl (10 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated to afford pure **13** (40 mg, quant.).

The above crude compound **13** (40 mg, 0.121 mmol) was dissolved in pyridine (2 mL). Methanesulfonyl chloride (59 μL, 0.607 mmol) was added at 0 °C. After stirring at 23 °C for 6 h, the reaction mixture was diluted with EtOAc (20 mL), and washed with water (10 mL × 2), and saturated aqueous NaCl (10 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated. The crude residue was dissolved in DMF (2 mL) and was treated with LiCl (26 mg, 0.607 mmol). After stirring at 23 °C for 3 days, the reaction mixture was diluted with EtOAc (20 mL) and washed with water (10 mL), saturated aqueous NaCl (10 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated. Chromatography (SiO_2, 20% EtOAc/hexanes) afforded **14** (37.5 mg, 93% for two steps). The two enantiomers were separated by chromatography (semiprep 2 × 25 cm Chiral OD column, 10% iPrOH/hexanes, flow rate = 0.5 mL/min, t_R = 14.4 min (natural), 12.1 min (unnatural), α = 1.19): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.21 (d, *J* = 8.4 Hz, 1H), 7.72 (br s, 1H), 7.64 (d, *J* = 8.1 Hz, 1H), 7.50 (t, *J* = 8.4 Hz, 1H), 7.33 (t, *J* = 7.5 Hz, 1H), 4.20–4.30 (m, 1H), 4.12 (t, *J* = 9.0 Hz, 1H), 4.02 and 4.00 (two s, 3H), 3.86–3.94 (m, 2H), 3.44 (t, *J* = 10.8 Hz, 1H), 1.61 and 1.58 (two s, 9H).

1*S*-**14**: $[\alpha]^{23}_D$ –0.43 (*c* 0.28, THF), natural enantiomer.

1*R*-**14**: $[a]^{23}_D$ +0.45 (*c* 0.53, THF), unnatural enantiomer.

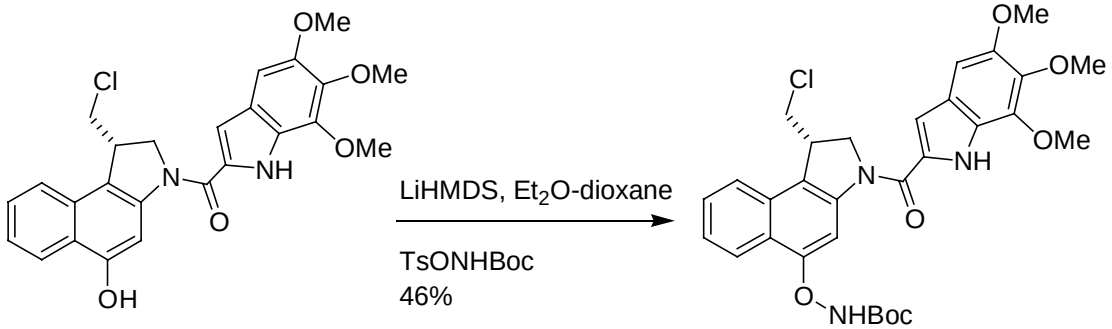


14 **10**

A sample of **14** (6.1 mg, 17 μmol) was treated with 4 N HCl–EtOAc (0.6 mL) for 30 min before the solvent was removed by a steam of N_2. The resulting crude HCl salt, 5,6,7-trimethoxyindol-2-carboxylic acid (**15**, 4.8 mg, 19 μmol) and EDCI (10.1 mg, 0.05 mmol) were dissolved in DMF (0.15 mL) and the resulting solution was stirred at 23 °C for 3 h. EtOAc (10 mL) was added to the reaction mixture and the resulting solution was washed with aqueous 1 N HCl (5 mL × 2), saturated aqueous $NaHCO_3$ (5 mL × 2), dried over anhydrous sodium sulfate and concentrated. PTLC (SiO_2, 50% EtOAc/hexanes) gave **10** as a white solid (5.5 mg, 65%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.41 (br s, 1H), 8.28 (d, *J* = 8.4 Hz, 1H), 8.08 (s, 1H), 7.72 (d, *J* = 8.4 Hz, 1H), 7.56 (t, *J* = 7.4 Hz, 1H), 7.42 (t, *J* = 7.6 Hz, 1H), 7.04 (d, *J* = 2.0 Hz, 1H), 6.89 (s, 1H), 4.81 (dd, *J* = 2.0, 10.8 Hz, 1H), 4.68 (t, *J* = 9.6 Hz, 1H), 4.11–4.19 (m, 1H), 4.09 (s, 3H), 4.07 (s, 3H), 3.99 (dd, *J* = 3.6, 10.8 Hz, 1H), 3.96 (s, 3H), 3.93 (s, 3H), 3.47 (t, *J* = 11.0 Hz, 1H); ESI-TOF HRMS *m/z* 481.1521 ($M+H^+$, $C_{26}H_{25}ClN_2O_5$ requires 481.1525).

1*S*-**10**: $[\alpha]^{23}_D$ –0.50 (*c* 0.31, THF), natural enantiomer.

1*R*-**10**: $[\alpha]^{23}_D$ +0.86 (*c* 0.14, THF), unnatural enantiomer.



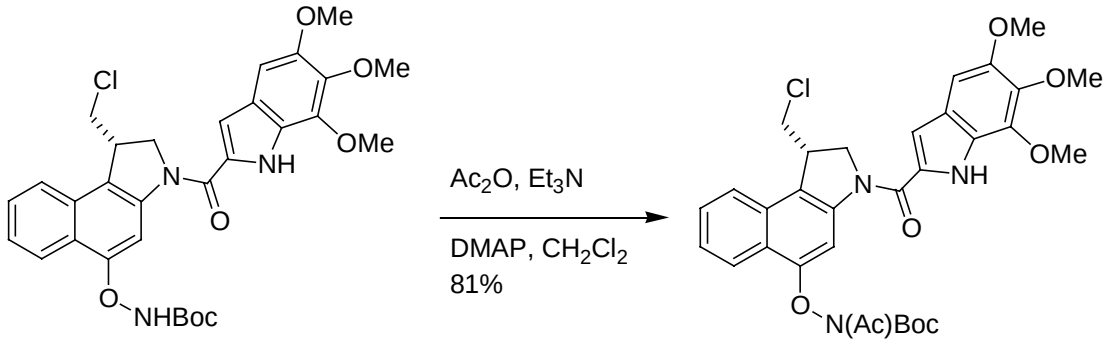
2 **4**

A solution of seco-CBI-TMI[2] (**2**, 30 mg, 0.064 mmol) in ether–dioxane (1:1, 3 mL) was treated with LiHMDS (1 M in THF, 193 μL, 0.193 mmol) at 0 °C, and the resulting mixture was stirred at 0 °C for 30 min. The resulting solution was treated with *t*-butyl-N-tosyloxycarbamate (55 mg,

0.193 mmol). The reaction mixture was allowed to warm to 23 °C and stirred for an additional 4 h. The solution was diluted with EtOAc (20 mL) and washed with water (10 mL), and saturated aqueous NaCl (10 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated. PTLC (SiO_2, 50% EtOAc/hexanes) afforded crude product (31.2 mg). To ensure the complete removal of any **2**, the product (12 mg) was dissolved in THF (6 mL) and saturated aqueous $NaHCO_3$ (6 mL) was added. After stirring at 23 °C for 2 h to promote spirocyclization of any residual **2** to the much more polar and easily separable CBI-TMI, the reaction mixture was diluted with EtOAc (20 mL), washed with water (10 mL) and saturated aqueous NaCl (10 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated. PTLC (SiO_2, 20% EtOAc/hexanes) afforded **4** (6.6 mg, 46%) as a pale yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.40 (br s, 1H), 8.42 (s, 1H), 7.94 (d, *J* = 8.4 Hz, 1H), 7.78 (d, *J* = 8.0 Hz, 1H), 7.57 (t, *J* = 8.4 Hz, 1H), 7.46 (t, *J* = 8.0 Hz, 1H), 7.46 (br s, 1H), 7.02 (d, *J* = 2.0 Hz, 1H), 6.88 (s, 1H), 4.83 (dd, *J* = 2.0, 10.8 Hz, 1H), 4.69 (t, *J* = 8.8 Hz, 1H), 4.16–4.21 (m, 1H), 4.09 (s, 3H), 3.99 (dd, *J* = 2.6, 11.4 Hz, 1H), 3.95 (s, 3H), 3.92 (s, 3H), 3.51 (t, *J* = 11.0 Hz, 1H), 1.65 (s, 9H); ESI-TOF HRMS *m/z* 582.2000 ($M+H^+$, $C_{30}H_{32}ClN_3O_7$ requires 582.2001).

1*S*-**4**: $[\alpha]^{23}_D$ –0.39 (*c* 0.31, THF), natural enantiomer.

1*R*-**4**: $[\alpha]^{23}_D$ +0.68 (*c* 0.44, THF), unnatural enantiomer.



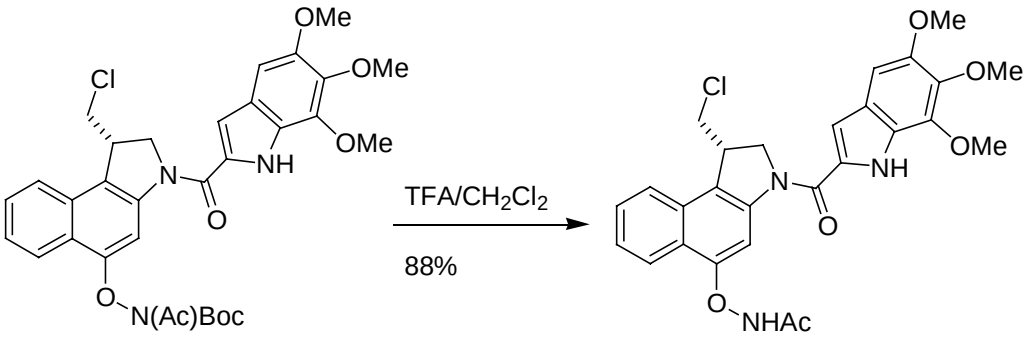
4 **6**

A solution of **4** (3.4 mg, 0.00584 mmol) in CH_2Cl_2 (0.34 mL) was treated with acetic anhydride (2.7 μL, 0.0292 mmol), Et_3N (4.1 μL, 0.0292 mmol) and DMAP (cat). After the resulting mixture was stirred at 23 °C for 12 h, the solvent was removed and the residue was purified by PTLC (SiO_2, 50% EtOAc/hexanes) to afford **6** (2.9 mg, 81%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.40 (br s, 1H), 8.53 (d, *J* = 12.4 Hz, 1H), 8.02 (d, *J* = 8.4 Hz, 1H), H), 7.81 (d, *J* = 8.4 Hz, 1H), 7.61 (td, *J* = 1.2, 5.4 Hz, 1H), 7.49 (t, *J* = 8.0 Hz, 1H), 7.03 (d, *J* = 2.0 Hz, 1H), 6.89 (s,

1H), 4.85 (dd, *J* = 1.2, 10.8 Hz, 1H), 4.72 (t, *J* = 9.6 Hz, 1H), 4.20–4.27 (m, 1H), 4.09 (s, 3H), 3.99–4.02 (m, 1H), 3.95 (s, 3H), 3.92 (s, 3H), 3.53 (t, *J* = 10.8 Hz, 1H), 2.56 and 2.58 (two s, 3H), 1.45 (s, 9H); ESI-TOF HRMS *m/z* 642.2102 ($M+H^+$, $C_{32}H_{34}ClN_3O_8$ requires 642.2107).

1*S*-**6**: $[\alpha]^{23}_D$ –0.43 (*c* 0.23, THF), natural enantiomer.

1*R*-**6**: $[\alpha]^{23}_D$ +0.54 (*c* 0.52, THF), unnatural enantiomer.

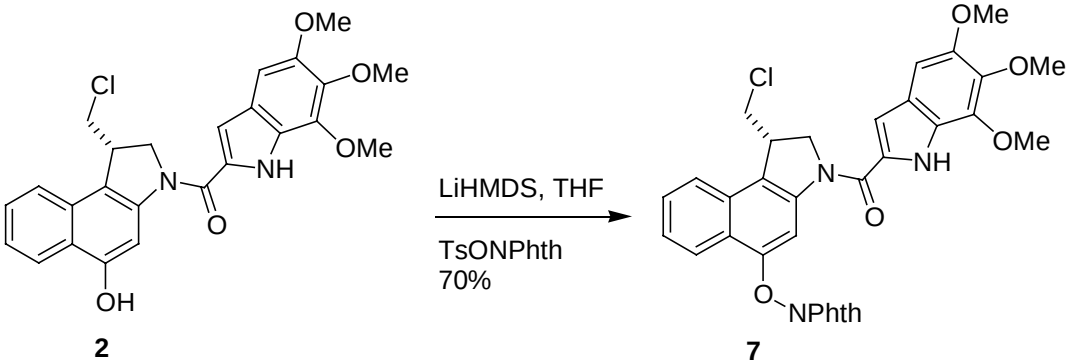


6 **5**

A solution of **6** (3.1 mg, 0.0053 mmol) in CH_2Cl_2 (1 mL) was treated with TFA (1 mL) at 23 °C for 3 h. The solvent and excess TFA were removed and the residue was purified by PTLC (SiO_2, 50% EtOAc/hexanes) to afford **5** (2.3 mg, 88%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.74 (br s, 1H), 8.83 (br s, 1H), 8.58 (s, 1H), 8.02 (d, *J* = 8.4 Hz, 1H), 7.81 (d, *J* = 8.0 Hz, 1H), 7.60 (t, *J* = 7.6 Hz, 1H), 7.49 (t, *J* = 8.0 Hz, 1H), 7.03 (d, *J* = 2.0 Hz, 1H), 6.88 (s, 1H), 4.85 (dd, *J* = 2.0, 10.8 Hz, 1H), 4.73 (t, *J* = 9.6 Hz, 1H), 4.22 (t, *J* = 8.2 Hz, 1H), 4.10 (s, 3H), 4.00 (dd, *J* = 3.2, 11.6 Hz, 1H), 3.96 (s, 3H), 3.92 (s, 3H), 3.54 (t, *J* = 11.0 Hz, 1H), 2,64 (s, 3H); ESI-TOF HRMS *m/z* 522.1431 ($M-H^-$, $C_{27}H_{26}ClN_3O_6$ requires 522.1437).

1*S*-**5**: $[\alpha]^{23}_D$ –1.2 (*c* 0.10, THF), natural enantiomer.

1*R*-**5**: $[\alpha]^{23}_D$ +0.76 (*c* 0.21, THF), unnatural enantiomer.

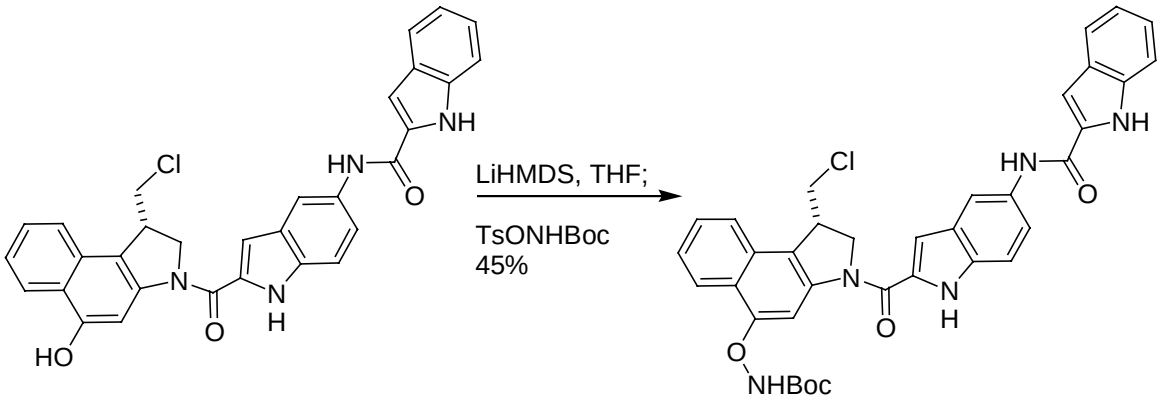


2 **7**

A solution of seco-CBI-TMI (**2**, 5.0 mg, 0.011 mmol) in THF (0.5 mL) was treated with LiHMDS (1 M in THF, 13 μL, 0.013 mmol) at −78 °C, and the resulting mixture was stirred at −78 °C for 30 min. The resulting solution was treated with N-p-tolylsulfonyloxyphthalimide (5.1 mg, 0.016 mmol). The reaction mixture was stirred at 23 °C for an additional 60 min. The solution was diluted with EtOAc (10 mL) and washed with water (5 mL), and saturated aqueous NaCl (5 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated. PTLC (SiO_2, 50% EtOAc/hexanes) afforded **7** (4.6 mg, 70%) as a pale yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.01 and 10.23 (two s, 1H), 9.37 (s, 1H), 8.45–8.66 (m, 2H), 7.92–7.99 (m, 1H), 7.72–7.87 (m, 1H), 7.59–7.77 (m, 1H), 7.39–7.56 (m, 1H), 7.14–7.22 (m, 1H), 7.00–7.05 (m, 1H), 6.88 and 6.89 (two s, 1H), 4.78–4.89 (m, 1H), 4.65–4.76 (m, 1H), 4.15–4.31 (m, 1H), 4.08 and 4.09 (two s, 3H), 3.98–4.05 (m, 1H), 3.94 and 3.95 (two s, 3H), 3.92 and 3.93 (two s, 3H), 3.46–3.60 (m, 1H).

1*S*-**7**: $[\alpha]^{23}_D$ –0.42 (*c* 0.28, THF), natural enantiomer.

1*R*-**7**: $[\alpha]^{23}_D$ +0.53 (*c* 0.36, THF), unnatural enantiomer.



3 **8**

A solution of seco-CBI-indole$_2$[3] (**3**, 16.5 mg, 0.031 mmol) in THF (1.5 mL) was treated with LiHMDS (1 M in THF, 93 μL, 0.093 mmol) at 0 °C and the mixture was stirred at 0 °C for 30 min. The resulting solution was treated with *t*-butyl-N-tosyloxycarbamate (26.6 mg, 0.093 mmol), and the reaction mixture was allowed to warm to 23 °C and stirred for an additional 4 h. The solution was diluted with EtOAc (20 mL) and washed with water (10 mL), and saturated aqueous NaCl (10 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated. PTLC (SiO_2, 50% THF/hexanes) afforded **8** (12.0 mg). The product (12 mg) was

dissolved in THF (6 mL) and treated with saturated aqueous $NaHCO_3$ (6 mL) to promote the spirocyclization of any residual **3**. After stirring at 23 °C for 2 h, the reaction mixture was diluted with EtOAc (20 mL), washed with water (10 mL) and saturated aqueous NaCl (10 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated. PTLC (SiO_2, 20% THF/hexanes) afforded **8** (9.0 mg, 45%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.81 (d, *J* = 2.0 Hz, 1H), 11.74 (d, *J* = 1.6 Hz, 1H), 10.19 (br s, 1H), 8.26 (d, *J* = 8.4Hz, 1H), 8.07 (d, *J* = 8.4 Hz, 1H), 7.68 (d, *J* = 7.6 Hz, 1H), 7.65 (t, *J* = 7.8 Hz, 1H), 7.60 (dd, *J* = 2.0, 9.2 Hz, 1H), 7.54 (t, *J* = 8.0 Hz, 1H), 7.49 (t, *J* = 9.2 Hz, 1H), 7.44 (d, *J* = 1.6 Hz, 1H), 7.29 (d, *J* = 2.4 Hz, 1H), 7.22 (t, *J* = 7.4 Hz, 1H), 7.07 (t, *J* = 7.6 Hz, 1H), 4.93 (dd, *J* = 9.4, 11.0 Hz, 1H), 4.68 (dd, *J* = 2.4, 10.8 Hz, 1H), 4.41–4.46 (m, 1H), 4.11 (dd, *J* = 3.2, 11.2 Hz, 1H), 3.81 (t, *J* = 5.2 Hz, 1H). ESI-TOF HRMS *m/z* 650.2150 ($M+H^+$, $C_{36}H_{32}ClN_5O_5$ requires 650.2165).

1*S*-**8**: $[\alpha]^{23}_D$ +2.1 (*c* 0.50, THF), natural enantiomer.

1*R*-**8**: $[\alpha]^{23}_D$ –2.0 (*c* 0.89, THF), unnatural enantiomer.

(1) Kastrinsky, D. B.; Boger, D. L. *J. Org. Chem.* **2004**, *69*, 2284.

(2) Boger, D.L.; Yun, W. *J. Am. Chem. Soc.* **1994**, *116*, 7996.

(3) Boger, D. L.; Yun, W.; Han, N. *Bioorg. Med. Chem.* **1995**, *3*, 1429.

In Vivo Antitumor Activity. DBA/2J mice were purchased from Jackson Laboratory (Bar Harbor, ME) and housed in the animal facility at The Scripps Research Institute. L1210 tumor cells, originally isolated from DBA/2 mice, were cultured in DMEM medium containing 5% fetal calf serum. For tumor implantation, DBA/2J mice were i.p. injected with 1 x 10^5 L1210 cells at day 0. Compounds **3** and **8** were formulated with 30% DMSO plus 0.1% glucose solution. Treatment doses of drugs (0, 10, 30, 100 1g/kg wt. of animal) were i.p. injected consecutively on day 1, 5 and 9. The experiment was performed with six mice per group. Tumor growth in the peritoneal cavity was monitored daily or weekly for 52 weeks (1 year) and the death of animals was recorded (Figure 6). If necessary, weights of animals were measured once a week.

An analogous study with 10 mice per group was performed at the University of Kansas with the distinction that the compounds were administered in neat DMSO (0, 10, 30, 60, 100 1g/kg wt. of animal) and the study was terminated after 120 days (Figure 7). B6D2F1 (a cross strain of C57BL/6 and DBA/2) mice were purchased from Taconic (Hudson, NY) and injected

intraperitoneal (i.p.) with syngeneic L1210 cells (1 x 10^6) on day 0. Animals were monitored and weighed daily following injection of tumor cells. Ten mice were randomly assigned to treatment groups for compound **3**, **8** or vehicle at doses of 0, 10, 30, 60, and 100 1g/ kg/ inj. On days 1, 5, and 9 following tumor cell inoculation, mice received vehicle, **3** or **8** via an intraperitoneal injection (i.p.). Tumor burden in the peritoneal cavity and survivorship were determined for animals in each treatment group.