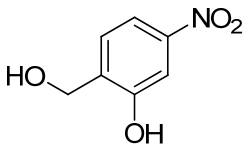
Total Synthesis and Evaluation of

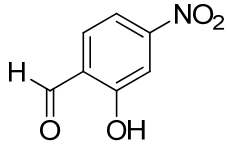
***iso*-Duocarmycin SA and *iso*-Yatakemycin**

Karen S. MacMillan, Trihn Nguyen, Inkyu Hwang, and Dale L. Boger*

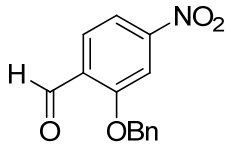
Department of Chemistry and the Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California, 92037



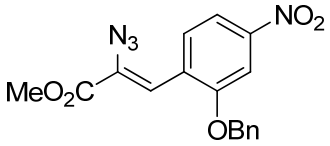
2-(Hydroxymethyl)-5-nitrophenol (11). 2-Hydroxy-4-nitrobenzoic acid (3.66 g, 20 mmol) was dissolved in anhydrous THF (33 mL) and cooled to 0 °C. A 1 M solution of borane–THF (100 mL, 100 mmol) was cannulated into the solution over 30 min. After complete addition, the reaction mixture was warmed slowly to room temperature and stirred overnight. Upon completion (TLC), the reaction mixture was cooled to 0 °C and MeOH was added carefully to quench the residual borane. This mixture was partitioned between water and EtOAc (3×) and the combined organic phases were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by chromatography (SiO_2, 0–80% EtOAc–hexanes gradient) to provide **11** (3.32 g, 98%) as a white solid: mp 128–130 °C; ^{1}H NMR (acetone-d_6, 600 MHz) δ 9.31 (d, 1H, J = 0.6 Hz), 7.75 (dd, 1H, J = 1.8, 8.4 Hz), 7.65 (d, 1H, J = 2.4 Hz), 7.62 (d, 1H, J = 8.4 Hz), 4.81 (d, 2H, J = 5.4 Hz), 4.65 (t, 1H, J = 5.4 Hz); ^{13}C NMR (acetone-d_6, 150 MHz) δ 156.7, 149.5, 138.2, 129.4, 116.2, 111.0, 61.4; ESITOF–HRMS m/z 168.0299 ($[M - H]^-$, $C_7H_6NO_4$ requires 168.0302).



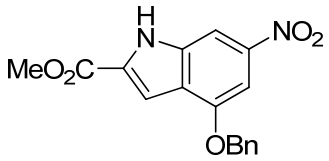
2-Hydroxy-4-nitrobenzaldehyde (12). 2-(Hydroxymethyl)-5-nitrophenol (**11**, 6.12 g, 36.2 mmol) and MnO_2 (24.8 g, 254 mmol) were slurried in EtOAc (225 mL) and the mixture was warmed at reflux for 2.5 h. After consumption of the starting material (TLC), the reaction mixture was filtered hot through Celite, and the residual black solid was washed copiously with hot EtOAc and THF. The filtrate was concentrated in vacuo to give **12** as a red solid (5.35 g, 88%): mp 129 °C; ^{1}H NMR (acetone-d_6, 500 MHz) 10.27 (s, 1H), 8.10 (d, 1H, J = 8.5 Hz), 7.87 (dd, 1H, J = 1.5, 8.5 Hz), 7.76 (d, 1H, J = 2.0 Hz); ^{13}C NMR (acetone-d_6, 125 MHz) δ 197.6, 163.1, 154.3, 136.3, 126.8, 116.1, 114.2; ESITOF–HRMS m/z 166.0144 ($[M - H]^-$, $C_7H_4NO_4$ requires 166.0146).



2-Benzyloxy-4-nitrobenzaldehyde (13). 2-Hydroxy-4-nitrobenzaldehyde (**12**, 1.90 g, 11.4 mmol) was dissolved in anhydrous DMF and cooled to 0 °C. K_2CO_3 (2.36 g, 17.1 mmol) was added portionwise to form a deep red mixture. After 20 min of vigorous stirring, benzyl bromide (2.7 mL, 22.8 mmol) was added and the reaction mixture was allowed to warm to room temperature overnight. The resulting opaque orange mixture was poured over acidified ice water (10% aqueous HCl) to precipitate aldehyde **13** which was collected by filtration, dried, and triturated with warm hexanes to yield **13** (2.64 g, 90%) as a pale yellow solid: mp 100 °C; ^{1}H NMR (acetone-d_6, 400 MHz) δ 10.58 (d, 1H, J = 0.8 Hz), 8.13 (d, 1H, J = 2.0 Hz), 8.00 (d, 1H, J = 8.4 Hz), 7.93 (ddd, 1H, J = 8.4, 2.0, 0.8 Hz), 7.63 (m, 1H), 7.61 (m, 1H), 7.46 (m, 2H), 7.40 (m, 1H), 5.52 (s, 2H); ^{13}C NMR (acetone-d_6, 125 MHz) δ 189.78, 189.76, 163.0, 154.1, 137.7, 131.0, 130.6, 130.2, 129.7, 117.5, 111.0, 73.1; GC/MS *m/z* 257 ($[M]^+$, $C_{14}H_{11}NO_4$ requires 257).

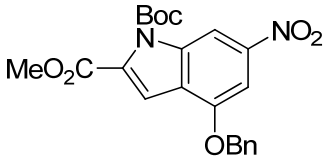


Methyl (*Z*)-2-Azido-3-(2-benzyloxy-4-nitrophenyl)acrylate (14). Sodium metal (143 mg, 6.23 mmol) was carefully added to distilled MeOH (5.7 mL), and the mixture was cooled to –15 °C under Ar. Aldehyde **13** (400 mg, 1.56 mmol) was added portionwise to the solution to form a red mixture, followed by methyl azidoacetate (953 mg, 6.23 mmol) dissolved in freshly distilled MeOH (0.8 mL). After 3 h at –15 °C, the reaction mixture was warmed to 0 °C and stirred for 24 h. Upon completion (TLC), saturated aqueous NH_4Cl was added and the mixture was partitioned between EtOAc and H_2O, washed with saturated aqueous NaCl, dried over Na_2SO_4, filtered, and concentrated to afford **14** as an orange solid (520 mg, 95%): mp 117–118 °C; ^{1}H NMR (acetone-d_6, 600 MHz) δ 8.48 (d, 1H, J = 8.4 Hz), 7.93 (d, 1H, J = 1.8 Hz), 7.90 (dd, 1H, J = 2.4, 8.4 Hz), 7.56 (m, 2H), 7.45 (m, 2H), 7.38 (m, 2H), 5.42 (s, 2H), 3.91 (s, 3H); ^{13}C NMR (acetone-d_6, 150 MHz) δ 165.1, 158.8, 150.3, 138.1, 132.8, 130.79, 130.75, 130.5, 130.1, 129.4, 117.7, 117.4, 109.1, 72.8, 54.6.

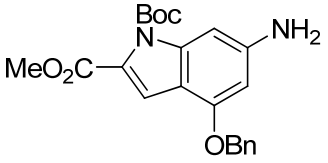


Methyl 4-Benzyloxy-6-nitroindole-2-carboxylate (15). Azide **14** (460 mg, 1.30 mmol) was suspended in xylenes (42 mL) and the mixture was warmed at reflux for 3 h under Ar. The yellow solution was cooled to room temperature, and then to 0 °C. The resulting precipitate was collected by filtration and washed with hexanes to yield the majority of the desired product. The supernatant was concentrated and purified by

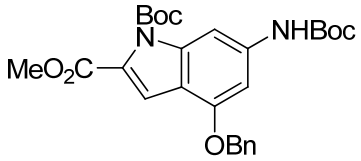
chromatography (SiO_2, 17% EtOAc–hexanes) to afford the remaining product **15** as an orange solid (313 mg, 74%): mp decomposes above 210 °C; ^{1}H NMR (acetone-d_6, 400 MHz) δ 11.68 (br s, 1H), 8.16 (dd, 1H, *J* = 0.8, 1.6 Hz), 7.63 (m, 2H), 7.54 (d, 1H, *J* = 1.6 Hz), 7.45 (m, 2H), 7.38 (m, 1H), 7.34 (d, 1H, *J* = 0.8 Hz), 5.45 (s, 2H), 3.94 (s, 3H); ^{13}C NMR (acetone-d_6, 150 MHz) δ 162.8, 155.1, 148.3, 138.6, 138.3, 133.0, 130.4, 129.9, 129.5, 125.0, 107.2, 104.9, 97.8, 72.2, 53.5; ESITOF–HRMS *m/z* 327.0969 ([M + H]$^+$, $C_{17}H_{15}N_2O_5$ requires 327.0975).



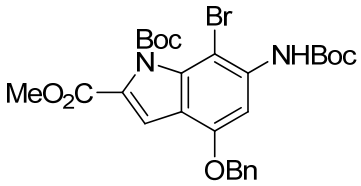
Methyl 4-Benzyloxy-1-(*tert*-butyloxycarbonyl)-6-nitroindole-2-carboxylate (16). 4-Dimethylaminopyridine (6 mg, 0.046 mmol) and di-*tert*-butyl-dicarbonate (150 mg, 0.69 mmol) were added sequentially to a solution of **15** (150 mg, 0.46 mmol) in THF (5 mL) and the solution was stirred at room temperature for 2 h. The reaction mixture was concentrated under a stream of nitrogen and purified by chromatography (SiO_2, 0–20% EtOAc–hexanes gradient) to yield **16** as a yellow powder (175 mg, 89%): mp 92 °C; ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.62 (dd, 1H, *J* = 0.8, 1.6 Hz), 7.76 (d, 1H, *J* = 1.6 Hz), 7.61 (m, 2H), 7.45 (m, 2H), 7.39 (m, 1H), 7.33 (d, 1H, *J* = 0.8 Hz), 5.48 (s, 2H), 3.95 (s, 3H), 1.66 (s, 9H); ^{13}C NMR (acetone-d_6, 150 MHz) δ 163.0, 154.7, 150.2, 149.5, 138.6, 138.2, 135.4, 130.5, 130.1, 129.5, 124.9, 111.9, 106.2, 101.8, 88.0, 72.5, 54.1, 28.8; ESITOF–HRMS *m/z* 449.1324 ([M + Na]$^+$, $C_{22}H_{22}N_2O_7Na$ requires 449.1319).



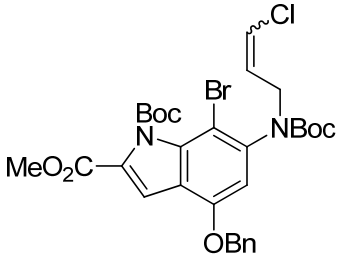
Methyl 6-Amino-4-benzyloxy-1-(*tert*-butyloxycarbonyl)-indole-2-carboxylate (17). Zinc nanopowder (306 mg, 4.7 mmol) and NH_4Cl (374 mg, 7.04 mmol) were added to a solution of **16** (200 mg, 0.47 mmol) in a 5:1 mixture of acetone/H_2O (9.4 mL). The reaction mixture was shaken for 5 min in a capped vial, filtered through a pad of Celite, and concentrated in vacuo. The residue was extracted with EtOAc and water (3×), washed with saturated aqueous NaCl, dried over Na_2SO_4, filtered, and concentrated to an orange oil. Chromatography (SiO_2, 0–50% EtOAc–hexanes gradient) provided **17** as a golden foam (175 mg, 95%): ^{1}H NMR (acetone-d_6, 500 MHz) δ 7.51 (d, 2H, *J* = 2.5 Hz), 7.40 (m, 2H), 7.34 (m, 1H), 7.15 (d, 1H, *J* = 0.5 Hz), 6.94 (d, 1H, *J* = 1.0 Hz), 6.35 (d, 1H, *J* = 1.5 Hz), 5.17 (s, 2H), 5.08 (br s, 2H), 3.83 (s, 3H), 1.58 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.0, 153.7, 149.8, 147.8, 141.3, 136.7, 128.5, 128.0, 127.2, 126.3, 114.5, 111.2, 95.2, 92.9, 84.1, 69.9, 62.6, 51.9, 27.7; ESITOF–HRMS *m/z* 397.1757 ([M + H]$^+$, $C_{22}H_{25}N_2O_5$ requires 397.1758).



Methyl 4-Benzyloxy-1-(*tert*-butyloxycarbonyl)-6-((*tert*-butyloxycarbonyl)amino)-indole-2-carboxylate (18). A solution of **17** (50 mg, 0.126 mmol) in 1 mL of THF was treated with Et_3N (21 μL, 0.152 mmol) and di-*tert*-butyldicarbonate (41 mg, 0.189 mmol). The resulting mixture was stirred at room temperature overnight, concentrated, and purified by chromatography (SiO_2, 0–50% EtOAc–hexanes gradient) to yield **18** (56 mg, 90%): mp 155–157 °C; ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.62 (br s, 1H), 8.05 (s, 1H), 7.55 (m, 2H), 7.42 (m, 2H), 7.35 (m, 1H), 7.20 (d, 1H, J = 0.8 Hz), 7.19 (d, 1H, J = 1.6 Hz), 5.24 (s, 2H), 3.87 (s, 3H), 1.62 (s, 9H), 1.51 (s, 9H); ^{13}C NMR (acetone-d_6, 100 MHz) δ 162.3, 153.8, 153.6, 152.3, 141.5, 140.6, 137.9, 129.3, 129.0, 128.8, 128.4, 114.2, 113.1, 98.3, 97.3, 85.1, 80.2, 70.6, 52.4, 28.5, 27.8; ESITOF–HRMS *m/z* 497.2279 ($[M + H]^+$, $C_{27}H_{33}N_2O_7$ requires 497.2282).

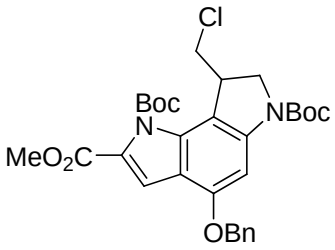


Methyl 4-Benzyloxy-7-bromo-1-(*tert*-butyloxycarbonyl)-6-((*tert*-butyloxycarbonyl)amino)-indole-2-carboxylate (19). NBS (75 mg, 0.423 mmol) was added to a solution of **18** (175 mg, 0.353 mmol) in anhydrous THF (12 mL). The reaction mixture was stirred for 4 h at room temperature in the absence of light. The reaction mixture was partitioned between EtOAc and saturated aqueous NaCl, dried over Na_2SO_4, filtered, and concentrated. Chromatography (SiO_2, 0–30% EtOAc–hexanes gradient) afforded **19** as a pale yellow solid (178 mg, 88%): mp 90–92 °C; ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.69 (br s, 1H), 7.66 (s, 1H), 7.59 (m, 2H), 7.43 (m, 2H), 7.37 (m, 1H), 7.34 (s, 1H), 5.28 (s, 2H), 3.89 (s, 3H), 1.68 (s, 9H), 1.53 (s, 9H); ^{13}C NMR (acetone-d_6, 100 MHz) δ 162.2, 154.4, 151.0, 138.6, 138.5, 136.8, 130.3, 129.8, 129.57, 128.55, 117.9, 110.5, 100.93, 100.90, 89.2, 87.9, 82.2, 72.0, 53.5, 29.4, 28.7; ESITOF–HRMS *m/z* 575.1388 ($[M + H]^+$, $C_{27}H_{31}BrN_2O_7$ requires 575.1387).

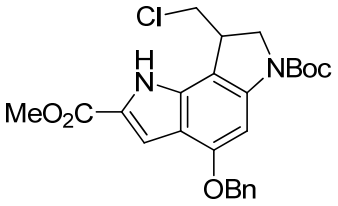


Methyl 4-Benzyloxy-7-bromo-1-(*tert*-butyloxycarbonyl)-6-(*tert*-butyloxycarbonyl(3-chloroallyl)amino)-indole-2-carboxylate (20). P_4-*t*-Bu-phosphazene base (200 μL, 1 M

in hexanes) and 1,3-dichloropropene (23 μL, 1.5 equiv) were added sequentially to a solution of **19** (96 mg, 0.167 mmol) in benzene (2.8 mL). After 3 h, the reaction mixture was directly subjected to chromatography (SiO_2, 0–30% EtOAc–hexanes gradient) to afford **20** (100 mg, 92%) as a mixture of *E* and *Z* isomers: ^{1}H NMR (acetone-d_6, 600 MHz) δ 7.55 (m, 4H), 7.44–7.41 (m, 6H), 7.37–7.34 (m, 2H), 6.90 (s, 1H), 6.86 (s, 1H), 6.23–6.19 (m, 2H), 6.12–6.05 (m, 2H), 5.34 (s, 4H), 4.54 (dd, 1H, *J* = 6.0, 16.2 Hz), 4.37–4.28 (m, 2H), 4.06–4.02 (m, 1H), 3.92 (s, 6H), 1.696 (s, 18H), 1.31 (s, 18H); ^{13}C NMR (acetone-d_6, 150 MHz) δ 162.2, 155.4, 155.2, 154.20, 154.18, 150.7, 142.3, 142.1, 138.6, 136.9, 131.1, 130.4, 129.9, 129.8, 129.3, 123.0, 121.9, 120.7, 110.1, 107.8, 107.6, 99.6, 99.5, 88.2, 88.1, 81.72, 81.70, 72.13, 72.08, 53.7, 50.9, 47.8, 29.3, 28.7; ESITOF–HRMS *m/z* 649.1318 ($[M + H]^+$, $C_{30}H_{35}BrClN_2O_7$ requires 649.1311).

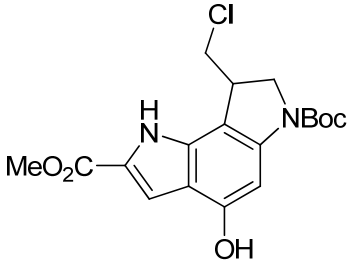


Methyl 5-Benzyloxy-3,8-bis(*tert*-butyloxycarbonyl)-1-chloromethyl-1,2-dihydropyrrolo[2,3-*g*]indole-7-carboxylate (21). A sample of **20** (125 mg, 0.192 mmol) and AIBN (9 mg, 0.058 mmol) were dissolved in anhydrous benzene (2.1 mL) and the mixture was degassed using the freeze-pump-thaw method three times. Bu_3SnH (62 μL, 0.231 mmol) was added, and the reaction mixture was capped and warmed at reflux for 3.5 h. Direct chromatography (SiO_2, 0–8% EtOAc–hexanes gradient) provided **21** (80 mg, 73%) as a white powder: ^{1}H NMR (acetone-d_6, 600 MHz) δ 7.75 (br s, 1H), 7.58 (d, 2H, *J* = 7.2 Hz), 7.43 (m, 2H), 7.36 (m, 1H), 7.27 (s, 1H), 5.27 (s, 2H), 4.19 (m, 2H), 4.06 (m, 1H), 3.90 (s, 3H), 3.75 (dd, 1H, *J* = 3.0, 10.8 Hz), 3.45 (dd, 1H, *J* = 9.0, 10.8 Hz), 1.61 (s, 9H), 1.58 (s, 9H); ^{13}C NMR (acetone-d_6, 150 MHz) δ 163.1, 155.7, 153.9, 151.7, 147.4, 138.8, 136.7, 130.3, 129.8, 129.6, 129.5, 116.2, 114.2, 108.5, 96.5, 87.1, 82.6, 71.8, 54.2, 53.5, 49.2, 29.5, 28.6; ESITOF–HRMS *m/z* 571.2214 ($[M + H]^+$, $C_{30}H_{36}BrClN_2O_7$ requires 571.2205).

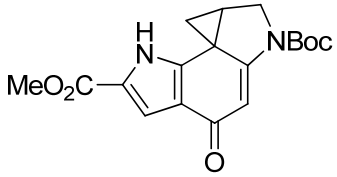


Methyl 5-Benzyloxy-3-(*tert*-butyloxycarbonyl)-1-chloromethyl-1,2-dihydropyrrolo[2,3-*g*]indole-7-carboxylate (22). A sample of **21** (15 mg, 0.0263 mmol) was dissolved in 4 N HCl/EtOAc (2 mL) and the solution was stirred at room temperature for 6 h. After complete removal of both Boc groups as established by LCMS, the reaction mixture was concentrated under a stream of nitrogen, dried under vacuum, and redissolved in THF (2 mL). Et_3N (7 μL, 0.053 mmol) and di-*tert*-butyldicarbonate (9 mg,

0.040 mmol) were added and the reaction mixture was stirred at room temperature overnight. Upon completion (TLC), the mixture was concentrated and the residue was purified by chromatography (SiO_2, 0–30% EtOAc–hexanes gradient) to afford **22** as a white solid (8 mg, 65%). The enantiomers were separated using chiral phase HPLC (ChiralCel OD, 2 × 25 cm, 2% *i*-PrOH–hexanes, 7 mL/min, α =1.23): ^{1}H NMR (acetone-d_6, 600 MHz) δ 7.59 (m, 2H), 7.43 (t, 2H, *J* = 7.8 Hz), 7.36 (t, 1H, *J* = 7.8 Hz), 7.24 (d, 1H, *J* = 1.8 Hz), 5.26 (s, 2H), 4.15 (d, 2H, *J* = 6.0 Hz), 4.08 (dd, 1H, *J* = 3.6, 10.8 Hz), 4.01 (m, 1H), 3.85 (s, 3H), 3.68 (dd, 1H, *J* = 9.0, 10.2 Hz), 1.58 (s, 9H); ^{13}C NMR (acetone-d_6, 150 MHz) δ 163.4, 155.9, 153.8, 139.2, 136.6, 130.3, 129.7, 129.4, 127.9, 117.5, 108.7, 106.4, 94.12, 94.09, 82.0, 71.5, 54.8, 52.9, 48.5, 42.4, 29.6; ESITOF–HRMS *m/z* 469.1534 ($[M - H]^-$, $C_{25}H_{26}ClN_2O_5$ requires 469.1536).
(1*S*)-**22**: $[\alpha]^{23}_D$ –54 (*c* 0.1, THF), natural enantiomer.
(1*R*)-**22**: $[\alpha]^{23}_D$ +56 (*c* 0.1, THF), unnatural enantiomer.

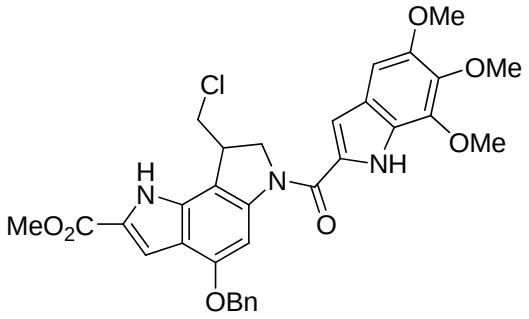


Methyl 3-(*tert*-Butyloxycarbonyl)-5-hydroxy-1-chloromethyl-1,2-dihydropyrrolo[2,3-*g*]indole-7-carboxylate (23). A solution of **22** (2 mg, 0.0043 mmol) in THF (0.5 mL) was treated with approximately 0.5 mg of 10% Pd/C. H_2 was bubbled through the solution for 1 h, after which it was filtered through a small plug of Celite and concentrated to a white residue. Chromatography (SiO_2, 0–100% EtOAc–hexanes gradient) afforded **23** (1.5 mg, 93%) as a white film: ^{1}H NMR (acetone-d_6, 600 MHz) δ 10.84 (br s, 1H), 8.80 (br s, 1H), 7.35 (s, 1H), 7.27 (d, 1H, *J* = 1.8 Hz), 4.11 (m, 2H), 4.07 (dd, 1H, *J* = 3.6, 10.8 Hz), 3.96 (m, 1H), 3.85 (s, 3H), 3.62 (dd, 1H, *J* = 9.6, 10.2 Hz), 1.56 (s, 9H); ^{13}C NMR (acetone-d_6, 150 MHz) δ 163.4, 154.4, 153.8, 137.1, 130.1, 127.6, 116.7, 108.8, 105.0, 96.7, 81.8, 54.7, 52.8, 48.5, 42.4, 29.6; ESITOF–HRMS *m/z* 379.1063 ($[M - H]^-$, $C_{18}H_{20}ClN_2O_5$ requires 379.1066).
(1*S*)-**23**: $[\alpha]^{23}_D$ –28 (*c* 0.1, THF), natural enantiomer.
(1*R*)-**23**: $[\alpha]^{23}_D$ +28 (*c* 0.1, THF), unnatural enantiomer.

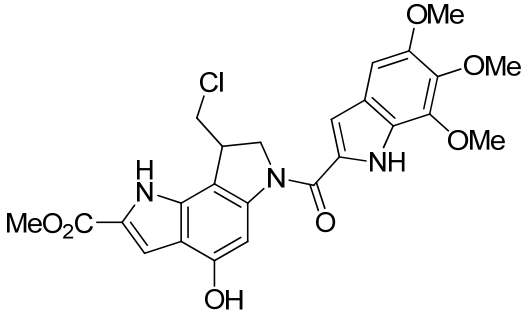


***N*-Boc-*iso*-Duocarmycin SA (7).** A sample of **23** (2.4 mg, 0.0063 mmol) was dissolved in DMF (1 mL), cooled to 0 °C, and saturated aqueous $NaHCO_3$ (0.5 mL) was added. The reaction mixture was stirred for 1 h at 0 °C until no yellow color persisted. An equal volume of THF was added to precipitate the inorganic salts and the solution was filtered through a short pad of silica gel. The eluent was concentrated and chromatography (SiO_2, 0–100% EtOAc–hexanes gradient) afforded *N*-Boc-*iso*-DSA (**7**, 1.9 mg, 88%) as a tan powder: ^{1}H NMR (acetone-d_6, 600 MHz) δ 11.14 (br s, 1H), 7.09 (s, 1H), 6.60 (br s, 1H), 4.00 (m, 2H), 3.80 (s, 3H), 3.16 (m, 1H), 1.92 (dd, 1H, *J* = 4.2, 8.4 Hz),1.52 (s, 9H),

1.48 (t, 1H, J = 4.2 Hz); ^{13}C NMR (acetone-d_6, 150 MHz) δ 184.0, 162.8, 159.0, 153.4, 143.7, 125.2, 124.5, 112.9, 110.7, 83.7, 54.9, 52.8, 32.1, 29.3, 27.5, 25.8; ESITOF–HRMS *m/z* 345.1439 ([M + H]$^+$, $C_{18}H_{21}N_2O_5$ requires 345.1445).
(+)-**7**: $[\alpha]^{23}_D$ +90 (*c* 0.1, THF), natural enantiomer.
(–)-**7**: $[\alpha]^{23}_D$ –90 (*c* 0.1, THF), unnatural enantiomer.



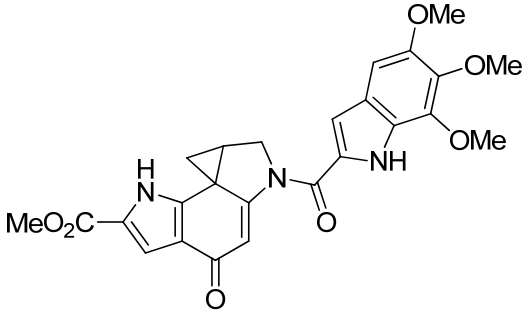
Methyl 5-Benzyloxy-3-[(5,6,7-trimethoxyindol-2-yl)carbonyl]-1-chloromethyl-1,2-dihydropyrrolo[2,3-*g*]indole-7-carboxylate (25). A sample of **22** (18.6 mg, 0.04 mmol) was dissolved 4 N HCl/EtOAc (2 mL) and the mixture was stirred at room temperature for 45 min, after which the blue solution was concentrated under a stream of nitrogen and dried under vacuum for 45 min. The residue was dissolved in 400 μL DMF and $NaHCO_3$ (3 mg, 0.04 mmol) was added, followed by EDCI (16.8 mg, 0.12 mmol) and 5,6,7-trimethoxyindole-2-carboxylic acid (**24**, 11 mg, 0.044 mmol). The mixture was stirred at room temperature for 30 min, after which an equal volume of THF was added to precipitate undesired salts, and the mixture was filtered through a plug of silica gel. The eluent was concentrated and chromatography (SiO_2, 0–40% EtOAc–hexanes gradient) afforded **25** as a pale yellow powder (19.4 mg, 82%): ^{1}H NMR (acetone-d_6, 600 MHz) δ 10.30 (s, 1H), 7.91 (br s, 1H), 7.58 (d, 2H, J = 7.2 Hz), 7.43 (t, 2H, J = 7.2 Hz), 7.35 (m, 1H), 7.29 (d, 1H, J = 2.4 Hz), 7.09 (d, 1H, J = 2.4 Hz), 6.98 (s, 1H), 5.26 (s, 2H), 4.76 (dd, 1H, J = 9.0, 10.2 Hz), 4.68 (dd, 1H, J = 3.0, 10.8 Hz), 4.18 (m, 1H), 4.13 (dd, 1H, J = 3.0, 10.8 Hz), 4.04 (s, 3H), 3.87 (s, 6H), 3.87 (s, 3H), 3.78 (dd, 1H, J = 9.0, 10.8 Hz); ^{13}C NMR (acetone-d_6, 150 MHz) δ 163.4, 161.8, 155.5, 152.1, 145.7, 142.5, 141.0, 139.2, 136.1, 132.9, 130.3, 129.7, 129.4, 128.5, 127.5, 125.8, 118.6, 108.7, 108.1, 108.0, 100.0, 96.2, 71.5, 62.5, 62.4, 57.5, 57.0, 53.0, 48.2, 43.6; UV (CH_3CN) λ_{max} 349 nm (ε 23,360), 320 nm (ε 20,740); ESITOF–HRMS *m/z* 604.1843 ([M + H]$^+$, $C_{32}H_{31}ClN_3O_7$ requires 604.1845).
(1*S*)-**25**: $[\alpha]^{23}_D$ –32 (*c* 0.1, THF), natural enantiomer.
(1*R*)-**25**: $[\alpha]^{23}_D$ +26 (*c* 0.1, THF), unnatural enantiomer.



***seco-iso*-Duocarmycin SA (26).** A sample of **25** (8.7 mg, 0.014 mmol) was dissolved in THF/MeOH (1 mL, 9:1). A spatula tip of 10% Pd/C was added, and H_2 was bubbled through the mixture for 3 h. Upon completion of the reaction as judged by TLC, the mixture was filtered through Celite, concentrated, and **26** was isolated as a pure yellow powder (6.5 mg, 88%): ^{1}H NMR (acetone-d_6, 600 MHz) δ 10.28 (s, 1H), 8.95 (s, 1H), 7.97 (s, 1H), 7.72 (s, 1H), 7.32 (d, 1H, *J* = 2.4 Hz), 7.09 (d, 1H, *J* = 2.4 Hz), 6.98 (s, 1H), 4.74 (dd, 1H, *J* = 9.0, 10.2 Hz), 4.66 (dd, 1H, *J* = 3.0, 10.8 Hz), 4.15 (m, 1H), 4.12 (m, 1H), 4.03 (s, 3H), 3.88 (s, 3H), 3.87 (s, 3H), 3.86 (s, 3H), 3.74 (dd, 1H, *J* = 9.0, 10.8 Hz); ^{13}C NMR (acetone-d_6, 150 MHz) δ 163.4, 161.7, 154.1, 152.0, 145.6, 142.4, 141.0, 136.6, 133.0, 128.2, 127.4, 125.7, 117.9, 108.8, 107.9, 106.6, 100.0, 98.7, 62.5, 62.4, 57.5, 57.0, 52.9, 48.3, 43.6; ESITOF–HRMS *m/z* 514.1376 ($[M + H]^+$, $C_{25}H_{25}ClN_3O_7$ requires 514.1375).

(1*S*)-**26**: $[\alpha]^{23}_D$ –50 (*c* 0.1, THF), natural enantiomer.

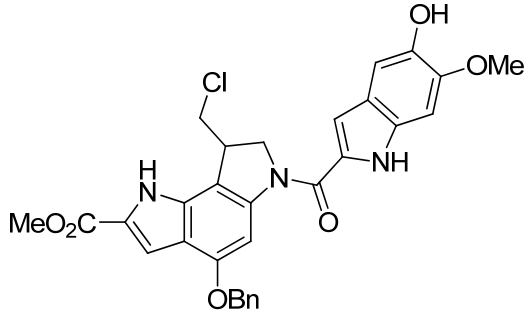
(1*R*)-**26**: $[\alpha]^{23}_D$ +46 (*c* 0.1, THF), unnatural enantiomer.



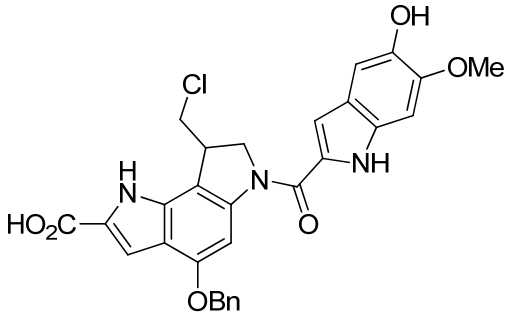
***iso*-Duocarmycin SA (5).** A sample of **26** (7.7 mg, 0.015 mmol) was dissolved in DMF and cooled to 0 °C. Saturated aqueous $NaHCO_3$ (1.5 mL) was added and the reaction mixture was stirred for 2 h at 0 °C. THF was added to precipitate the inorganic salts, and the mixture was passed through a plug of silica gel. The eluent was concentrated and chromatography (SiO_2, 0–10% MeOH–CH_2Cl_2 gradient) afforded *iso*-duocarmycin SA (**5**) as a beige powder (5.6 mg, 80%): ^{1}H NMR (CD_3CN, 600 MHz) δ 10.16 (br s, 1H), 9.74 (br s, 1H), 7.10 (s, 1H), 7.00 (d, 1H, *J* = 2.4 Hz), 6.91 (s, 1H), 6.60 (s, 1H), 4.41 (dd, 1H, *J* = 5.4, 10.2 Hz), 4.35 (d, 1H, *J* = 10.2 Hz), 3.99 (s, 3H), 3.854 (s, 3H), 3.847 (s, 3H), 3.83 (s, 3H), 3.09 (ddd, 1H, *J* = 3.0, 5.4, 4.2, 8.8 Hz), 1.89 (dd, 1H, *J* = 4.2, 7.8 Hz) 1.65 (t, 1H, *J* = 4.8 Hz); ^{13}C NMR (acetone-d_6, 150 MHz) δ 182.9, 162.0, 161.8, 158.6, 151.2, 142.8, 141.9, 140.1, 130.6, 127.1, 126.0, 124.4, 123.6, 112.7, 111.9, 108.3, 98.9, 61.5, 61.4, 56.4, 55.2, 51.8; UV (CH_3CN) λ_{max} 342 nm (ε 17,800), 267 nm (ε 15,600); ESITOF–HRMS *m/z* 478.1612 ($[M + H]^+$, $C_{25}H_{24}N_3O_7$ requires 478.1609).

(+)-**5**: $[\alpha]^{23}_D$ +160 (*c* 0.1, THF), natural enantiomer.

(–)-**5**: $[\alpha]^{23}_D$ –180 (*c* 0.1, THF), unnatural enantiomer.

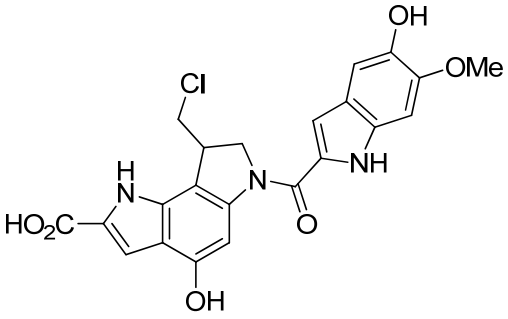


Methyl 3-[(5-Hydroxy-6-methoxyindol-2-yl)carbonyl]-1-chloromethyl-5-benzyloxy-1,2-dihydropyrrolo[2,3-*g*]indole-7-carboxylate (28). Compound **22** (17 mg, 0.036 mmol) was stirred in 4 N HCl/EtOAc (1.7 mL) for 1 h. The greenish-blue solution was concentrated and dried under vacuum for 2 h. The residue was suspended in anhydrous DMF (0.15 mL) to which $NaHCO_3$ (3.4 mg, 0.040 mmol) and EDCI (20 mg, 0.1447 mmol) were added. After 5 min, carboxylic acid **27** (11.2 mg, 0.054 mmol) was added, and the reaction mixture stirred for 2.5 h, after which the salts were precipitated by the addition of THF. The resulting mixture was passed through a silica gel plug and the eluent was concentrated and chromatography (SiO_2, 0–60% EtOAc–hexanes gradient) afforded **28** as a yellow powder (16.5 mg, 83%): mp decomposes above 155 °C; ^{1}H NMR (acetone-d_6, 600 MHz) δ 10.54 (s, 1H), 7.99 (s, 1H), 7.58 (d, 2H, J = 7.2 Hz), 7.42 (m, 2H), 7.34 (m, 1H), 7.28 (d, 1H, J = 2.4 Hz), 7.15 (s, 1H), 7.08 (s, 2H), 7.03 (d, 1H, J = 2.4 Hz), 5.28 (d, 2H, J = 1.8 Hz), 4.77 (dd, 1H, J = 9.0, 10.8 Hz), 4.70 (dd, 1H, J = 3.0, 10.8 Hz), 4.20 (m, 1H), 4.13 (dd, 1H, J = 3.0, 10.8 Hz), 3.871 (s, 3H), 3.868 (s, 3H), 3.80 (dd, 1H, J = 9.0, 10.8 Hz); ^{13}C NMR (acetone-d_6, 150 MHz) δ 163.4, 162.0, 155.4, 150.1, 146.0, 144.7, 139.2, 136.1, 133.2, 131.4, 130.3, 129.7, 129.4, 128.4, 123.5, 118.5, 108.7, 107.9, 107.5, 106.7, 96.4, 95.5, 71.6, 57.2, 57.0, 52.9, 48.3, 43.6; ESITOF–HRMS *m/z* 560.1589 ($[M + H]^+$, $C_{30}H_{27}ClN_3O_6$ requires 560.1583).
(1*S*)-**28**: $[\alpha]^{23}_D$ –32 (*c* 0.1, THF), natural enantiomer.
(1*R*)-**28**: $[\alpha]^{23}_D$ +32 (*c* 0.1, THF), unnatural enantiomer.

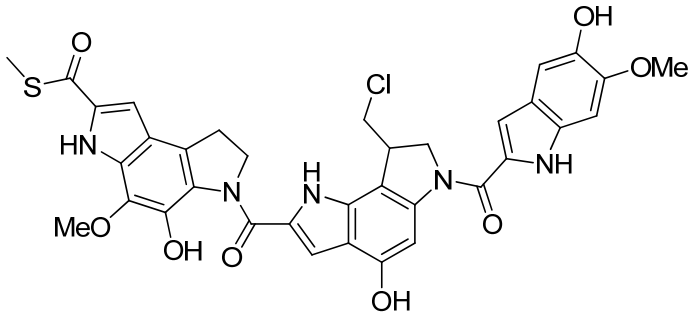


3-[5-Hydroxy-6-methoxyindol-2-yl)carbonyl]-1-chloromethyl-5-benzyloxy-1,2-dihydropyrrolo[2,3-*g*]indole-7-carboxylic acid (29). A sample of **28** (10.0 mg, 0.018 mmol) was dissolved in 3:2 THF–MeOH (0.22 mL), and thoroughly degassed by bubbling Ar through the solution for 15 min. Sodium dithionite (9.3 mg, 0.054 mmol) was added, followed by a solution of LiOH–H_2O (7.5 mg, 0.18 mmol) in degassed water (50 μL). The reaction mixture was stirred for 6.5 h at 23 °C, after which the mixture was concentrated, redissolved in H_2O (1 mL), and acidified with the addition of aqueous 1 N HCl. The resulting white mixture was extracted three times with EtOAc, and the organic layer was washed with saturated aqueous NaCl, dried over Na_2SO_4, filtered, and concentrated to give **29** as a pale yellow powder (9.3 mg, 95%): mp decomposes above 170 °C; ^{1}H NMR (acetone-d_6, 600 MHz) δ 10.49 (s, 1H), 7.91 (s, 1H), 7.60 (d, 2H, J = 7.8 Hz), 7.43 (m, 2H), 7.35 (m, 1H), 7.31 (d, 1H, J = 1.8 Hz), 7.15 (s, 1H), 7.11 (s, 1H), 7.09 (s, 1H), 7.05 (d, 1H, J = 1.8 Hz), 5.74 (s, 1H), 5.29 (d, 2H, J = 3.0 Hz), 4.79 (dd, 1H, J = 9.0, 10.8 Hz), 4.71 (dd, 1H, J = 3.0, 10.8 Hz), 4.23 (m, 1H), 4.16 (dd, 1H, J = 3.6, 10.8 Hz), 3.92 (s, 3H), 4.83 (dd, 1H, J = 9.0, 10.8 Hz); ^{13}C NMR (acetone-d_6, 150 MHz) δ 163.6, 161.9, 155.4, 150.1, 145.9, 144.7, 139.3, 136.2, 133.2, 131.5, 130.3, 129.7, 129.4, 128.8, 123.6, 118.6, 108.8, 107.9, 107.4, 106.8, 96.3, 95.6, 71.6, 57.3, 57.0, 48.4, 43.6; ESITOF–HRMS *m/z* 546.1444 ($[M + H]^+$, $C_{29}H_{25}ClN_3O_6$ requires 546.1426).

(1*S*)-**29**: $[\alpha]^{23}_D$ –50 (*c* 0.1, THF), natural enantiomer.
(1*R*)-**29**: $[\alpha]^{23}_D$ +50 (*c* 0.1, THF), unnatural enantiomer.



3-[5-Hydroxy-6-methoxyindol-2-yl)carbonyl]-1-chloromethyl-5-hydroxy-1,2-dihydropyrrolo[2,3-*g*]indole-7-carboxylic Acid (30). Carboxylic acid **29** (6 mg, 0.011 mmol) was dissolved in THF (0.4 mL). A spatula tip of 10% Pd/C was added, and H_2 was bubbled through solution for 6 h**.** Upon completion of the reaction as judged by TLC, the mixture was filtered through Celite and concentrated. The residue was triturated with Et_2O and hexanes to remove nonpolar impurities. The product **30** was isolated as a pale yellow powder (5 mg, 57%): ^{1}H NMR (DMF-d_7, 600 MHz) δ 10.08 (br s, 1H), 8.58 (br s, 1H), 7.73 (s, 1H), 7.35 (d, 1H, *J* = 2.4 Hz), 7.13 (s, 1H), 7.10 (s, 1H), 7.04 (d, 1H, *J* = 1.8 Hz), 4.78 (dd, 1H, *J* = 9.0, 10.8 Hz), 4.65 (dd, 1H, *J* = 2.4, 10.8 Hz), 4.26 (dd, 1H, *J* = 3.0, 10.8 Hz), 4.20 (m, 1H), 3.89 (s, 3H), 3.87 (dd, 1H, *J* = 9.6, 11.4 Hz); ^{13}C NMR (DMF-d_7, 150 MHz) δ 164.0, 161.2, 153.6, 149.8, 144.6, 144.3, 135.9, 132.6, 131.0, 122.6, 117.1, 107.5, 106.2, 105.5, 100.5, 97.6, 95.3, 56.5, 56.2, 48.1, 42.7; ESITOF–HRMS *m/z* 456.0956 ($[M + H]^+$, $C_{22}H_{19}ClN_3O_6$ requires 456.0957).
(1*S*)-**30**: $[\alpha]^{23}_D$ –48 (*c* 0.1, DMF), natural enantiomer.
(1*R*)-**30**: $[\alpha]^{23}_D$ +44 (*c* 0.1, DMF), unnatural enantiomer.

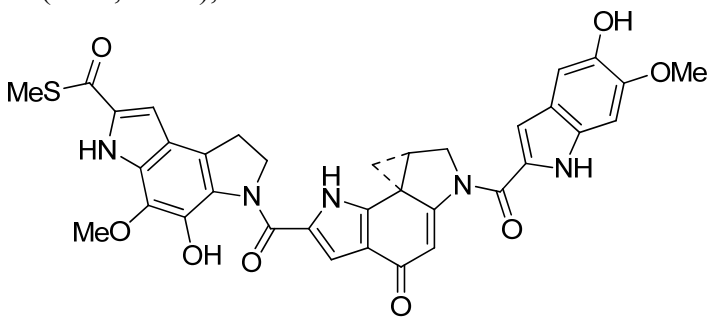


***seco-iso*-Yatakemycin (33).** Thioester **31** (2.6 mg, 0.007 mmol) was dissolved in 4 N HCl/EtOAc (0.26 mL) and the mixture was stirred at room temperature for 45 min. The resulting mixture was concentrated under a stream of nitrogen and dried under vacuum for 1 h. Carboxylic acid **30** (2.1 mg, 0.005 mmol) was dissolved in anhydrous DMF (70 μL) and added to the sample of thioester. EDCI (2.6 mg, 0.018 mmol) was added and the reaction mixture was stirred for 45 min. Upon completion, an equal volume of THF was added to precipitate the undesired salts, and the mixture was passed through a silica gel plug. Chromatography (SiO_2, 7–14% DMF–toluene gradient) yielded **33** as a yellow powder (1.6 mg, 49%): ^{1}H NMR (DMF-d_7, 600 MHz) δ 10.25 (s, 1H), 8.55 (s, 1H), 7.78 (s, 1H), 7.50 (d, 1H, *J* = 2.4 Hz), 7.35 (d, 1H, *J* = 1.8 Hz), 7.12 (s, 1H), 7.11 (s, 1H), 7.06 (d, 1H, *J* = 1.8 Hz), 4.85–4.88 (dt, 2H, *J* = 2.4, 8.4 Hz), 4.81 (m, 1H), 4.68 (dd, 1H, *J* =

1.8, 10.8 Hz), 4.26–4.28 (m, 2H), 3.97 (s, 3H), 3.92–3.94 (m, 1H), 3.90 (s, 3H), 2.49 (s, 3H); ^{13}C NMR (DMF-d_7, 150 MHz) δ 183.6, 162.0, 161.3, 153.8, 149.8, 145.2, 144.3, 141.0, 136.0, 135.2, 134.7, 133.5, 132.6, 130.9, 129.53, 129.48, 123.1, 122.6, 119.0, 117.4, 108.0, 107.9, 106.4, 106.2, 105.3, 97.8, 95.3, 68.4, 61.1, 56.5, 56.3, 54.6, 48.2, 42.7, 11.3; ESITOF–HRMS *m/z* 716.1549 ([M + H]$^+$, $C_{35}H_{31}ClN_5O_8S$ requires 716.1576).

(1*S*)-**33**: $[\alpha]^{23}_D$ –40 (*c* 0.1, DMF), natural enantiomer.

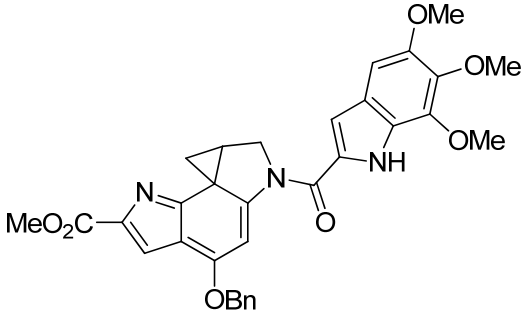
(1*R*)-**33**: $[\alpha]^{23}_D$ +26 (*c* 0.1, DMF), unnatural enantiomer.



***iso*-Yatakemycin (6).** A sample of **33** (1.5 mg, 0.002 mmol) was dissolved in DMF (0.5 mL) and saturated aqueous $NaHCO_3$ (0.25 mL) was added. The reaction mixture was stirred at ambient temperature for 1 h. Upon completion, THF was added to precipitate the inorganic salts, and the resulting mixture was passed through a plug of silica gel. Chromatography (SiO_2, 14–25% DMF–toluene gradient) afforded *iso*-yatakemycin (**6**, 0.89 mg, 62%) as a yellow powder: ^{1}H NMR (DMF-d_7, 600 MHz) δ 8.68 (br s, 1H), 7.34 (s, 1H), 7.33 (s, 1H), 7.10 (s, 1H), 7.09 (s, 2H), 6.86 (s, 1H), 4.79 (t, 2H, *J* = 7.8 Hz), 4.65 (dd, 1H, *J* = 4.8, 9.6 Hz), 4.53 (d, 1H, *J* = 10.2 Hz), 3.95 (s, 3H), 3.91 (s, 3H), 3.44–3.49 (m, 2H), 2.49 (s, 3H), 2.16 (dd, 1H, J = 3.6, 7.2 Hz), 1.78 (dd, 1H, J = 3.6, 4.8 Hz); ^{13}C NMR (DMF-d_7, 600 MHz) δ 183.7, 162.3, 161.0, 159.8, 150.3, 144.3, 140.5, 135.7, 133.3, 133.1, 132.5, 129.8, 129.1, 123.5, 122.9, 122.1, 118.8, 112.2, 111.7, 107.8, 106.0, 100.3, 94.9, 68.6, 61.0, 56.4, 55.6, 54.1, 39.6, 28.7, 25.8, 24.6, 23.7, 11.5, 11.2; ESITOF–HRMS *m/z* 680.1801 ([M + H]$^+$, $C_{35}H_{30}N_5O_8S$ requires 680.1810).

(+)-**6**: $[\alpha]^{23}_D$ +40 (*c* 0.1, THF), natural enantiomer.

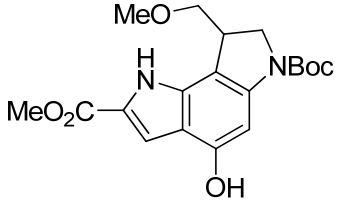
(–)-**6**: $[\alpha]^{23}_D$ –32 (*c* 0.1, THF), unnatural enantiomer.



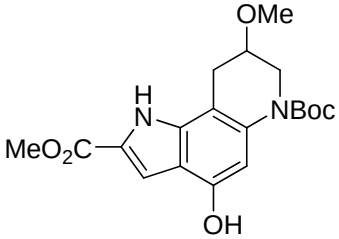
Methyl 2-[(5,6,7-Trimethoxyindol-2-yl)carbonyl]-4-benzyloxy-1,2,8,8a-tetrahydrocycloprop[*c*]pyrrolo[2,3-*g*]indole-6-carboxylate (34). A sample of **25** (8.5 mg, 0.014 mmol) was dissolved in anhydrous MeCN (282 μL) under Ar. A solution of phosphazene P_4-*t*Bu (1 M in hexanes, 47 μL, 0.047 mmol) was added and the reaction mixture was stirred at room temperature for 3 h. Upon complete consumption of the starting material by LC/MS, an equal volume of toluene was added to the reaction

mixture and the MeCN was removed under a stream of nitrogen. Direct chromatography (SiO_2, 0–30% acetone–hexane gradient) afforded **34** (5.2 mg, 65%) as a beige powder: ^{1}H NMR (acetone-d_6, 600 MHz) δ 10.88 (br s, 1H), 7.45 (m, 2H), 7.36 (m, 2H), 7.29 (m, 1H), 7.04 (d, 1H, *J* = 2.4 Hz), 6.94 (s, 1H), 6.71 (s, 1H), 4.99 (d, 1H, *J* = 14.4 Hz), 4.94 (d, 1H, *J* = 14.4 Hz), 4.62 (s, 1H), 4.00 (d, 1H, *J* = 14.4 Hz), 3.85 (s, 3H), 3.78 (s, 3H), 3.72 (s, 3H), 3.47 (dd, 1H, *J* = 4.2, 14.4 Hz), 3.37 (s, 3H), 2.37 (dd, 1H, *J* = 7.2, 7.8 Hz), 1.99 (ddd, 1H, *J* = 3.6, 6.0, 8.4 Hz), 0.79 (dd, 1H, *J* = 6.0, 6.6 Hz); ^{13}C NMR (acetone-d_6, 150 MHz) δ 164.5, 162.6, 157.2, 152.5, 142.1, 141.9, 139.0, 138.9, 135.8, 130.8, 130.2, 129.6, 129.2, 125.1, 122.7, 119.0, 112.1, 100.3, 99.4, 93.8, 92.2, 71.0, 62.1, 61.6, 57.4, 52.4, 45.2, 37.0, 29.6, 13.6; UV (CH_3CN) λ_{max} 304 nm (ε 21,800); ESITOF–HRMS *m/z* 568.2079 ($[M + H]^+$, $C_{32}H_{30}N_3O_7$ requires 568.2078).
(–)-**34**: $[\alpha]^{23}_D$ –136 (*c* 0.1, THF), natural enantiomer.
(+)-**34**: $[\alpha]^{23}_D$ +88 (*c* 0.1, THF), unnatural enantiomer.

Acid-Catalyzed Addition of CH_3OH to *N*-Boc-*iso*-DSA. A solution of **7** (1.4 mg, 4.1 μmol) was dissolved in 505 μL of CF_3SO_3H/CH_3OH (0.97 mM) at 0 °C and the mixture was stirred for 4 h. The reaction was quenched at 0 °C by addition of $NaHCO_3$ and the mixture was partitioned between water and EtOAc. The organic layer was dried over Na_2SO_4, filtered and concentrated. LC/MS analysis (30–98% H_2O/MeCN + 0.1% formic acid over 5 min, 98% H_2O/MeCN + 0.1% formic acid for 6 min, flow rate = 0.75 mL/min) of the crude reaction mixture indicated two distinct products (t_R = 6.21 and 5.41 min) produced in a 13:1 ratio. PTLC (SiO_2, 50% EtOAc–hexane) of the crude mixture afforded **35** (1.3 mg, 85%) and **36** as white films.



For **35**: ^{1}H NMR (acetone-d_6, 600 MHz) δ 10.15 (br s, 1H), 8.69 (br s, 1H), 7.37 (br s, 1H), 7.24 (d, 1H, *J* = 1.8 Hz), 4.14 (d, 1H, *J* = 10.2, 11.4 Hz), 3.87 (s, 3H), 3.83–3.80 (m, 1H), 3.73 (dd, 1H, *J* = 6.0, 11.4 Hz), 3.63 (t, 1H, *J* = 8.4 Hz), 3.56 (dd, 1H, J = 6.0, 9.0 Hz), 3.45 (s, 3H), 1.54 (s, 9H); ^{13}C NMR (acetone-d_6, 150 MHz) δ 163.5, 153.8, 153.7, 137.4, 127.1, 116.5, 108.4, 96.6, 77.1, 60.0, 53.3, 52.9, 40.3, 29.6; ESITOF–HRMS *m/z* 377.1702 ($[M + H]^+$, $C_{19}H_{25}N_2O_6$ requires 377.1707).



For **36**: ^{1}H NMR (acetone-d_6, 600 MHz) δ 10.46 (br s, 1H), 8.52 (s, 1H), 7.24 (d, 1H, *J* = 2.4 Hz), 6.90 (s, 1H), 3.87 (s, 3H), 3.81–3.79 (m, 1H), 3.74 (dd, 1H, *J* = 2.4, 12.6 Hz), 3.42 (s, 3H), 3.26 (dd, 1H, *J* = 6.0, 17.4 Hz), 2.87 (dd, 1H, *J* = 4.8, 17.4 Hz), 1.51 (s, 9H); ESITOF–HRMS *m/z* 399.1539 ($[M + Na]^+$, $C_{19}H_{24}N_2O_6Na$ requires 399.1526).

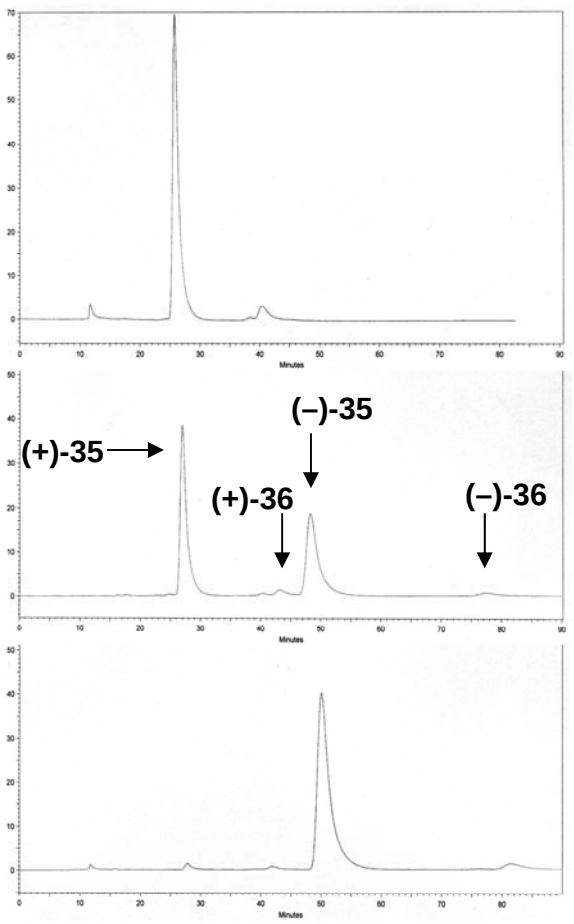


Figure S1. Acid-catalyzed CH_3OH addition to (+)-**7** (top), (±)-**7** (center), and (–)-**7** (bottom). ChiralCel AD HPLC (2.0 cm × 25 cm), 6 mL/min, 15% *i*-PrOH/hexane.

Solvolysis of *N*-Boc-*iso*-DSA. *N*-Boc-*iso*-DSA (**7**, 50 μg, 0.15 mmol) was dissolved in a 50% MeOH–buffer solution (pH 3 buffer = 4:1:20 0.1 M citric acid: 0.2 M Na_2HPO_4: H_2O) and the reaction was monitored by UV–Vis spectrophotometry (Figure S2). Time points were taken every 1 h for 72 h, and then every 24 h for 15 d. The cuvette was sealed with parafilm and kept in the dark for the duration of timecourse. The reaction was monitored until no further change was detectable, and both the decrease in the long-wavelength absorption at 295 nm and the increase in the short-wavelength absorption at 266 nm were monitored. The solvolysis rate was calculated from the data recorded at 295 nm from the least-squares treatment (R^2 = 0.99) of the slope of a plot of time versus $\ln[(A_f–A_i)/(A_f–A)]$; $k = 2.2 \times 10^{-6}$ s^{-1}, $t_{1/2}$ = 89 h.

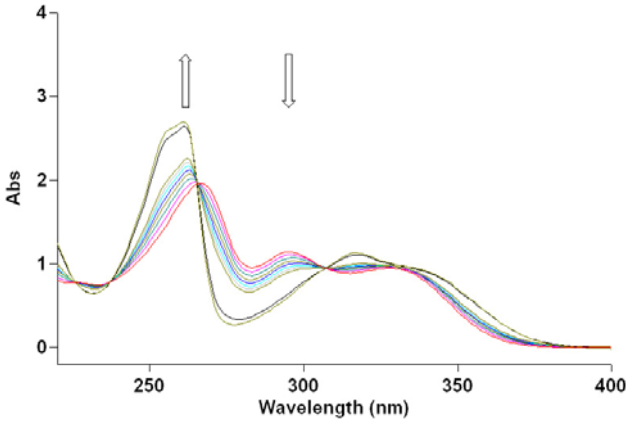


Figure S2. UV trace of acid-catalyzed solvolysis monitoring an increasing UV maximum at 266 nm and a decreasing UV maximum at 295 nm.

Solvolysis of *o*-spiro-*iso*-duocarmycin SA (34). MeOH (1.5 mL) was mixed with a universal aqueous buffer (pH 2–7; 1.5 mL, 0.2 M boric acid, 0.05 M citric acid, 0.1 M Na_3PO_4, and nanopure water). A sample of **34** (10 μg) was dissolved in MeCN (100 μL) and added to this solution immediately before data collection. The increase in absorbance at 240 nm was monitored by UV at regular intervals until no further change in absorbance was noted. The solvolysis rates were calculated from the least-squares treatment of the slope of plots of time versus ln $[(A_f - A_i)/(A_f - A)]$.

DNA Alkylation Studies. General procedures, the preparation of singly 5' end-labeled double-stranded DNA, gel electrophoresis, and autoradiography were conducted according to procedures described in full detail elsewhere.[15] Eppendorf tubes containing the 5' end-labeled DNA (4.5 μL) in TE buffer (10mM Tris, 1mM EDTA, pH 7.6) were treated with the agent in DMSO (0.5 μL at the specified concentration). The solution was vortexed and centrifuged prior to incubation at 25 °C or 37 °C for the specified period of time. Unbound agent was removed by EtOH precipitation. The covalently modified DNA was resuspended in TE buffer (5 μL) and heated at 100 °C (3 × 10 min) to effect thermal depurination at the alkylation sites. Samples were allowed to cool to 25 °C, centrifuged, and 2.5 μL of formamide dye (0.03% xylene cyanol FF, 0.03% bromophenol blue, 8.7% Na_2EDTA 250 mM) was added to each sample. Prior to electrophoresis, each sample was denatured by heating at 100 °C for 5 min, centrifuged and placed in an ice block. The sample was loaded directly onto the gel (4.5 μL) alongside Sanger dideoxynucleotide sequencing reactions run as standards and analyzed by polyacrylamide gel electrophoresis (PAGE, 8% sequencing gel) under denaturing conditions (8 M urea) in TBE buffer (100 mM Tris, 100 mM boric acid, 0.2 mM Na_2EDTA) followed by autoradiography.

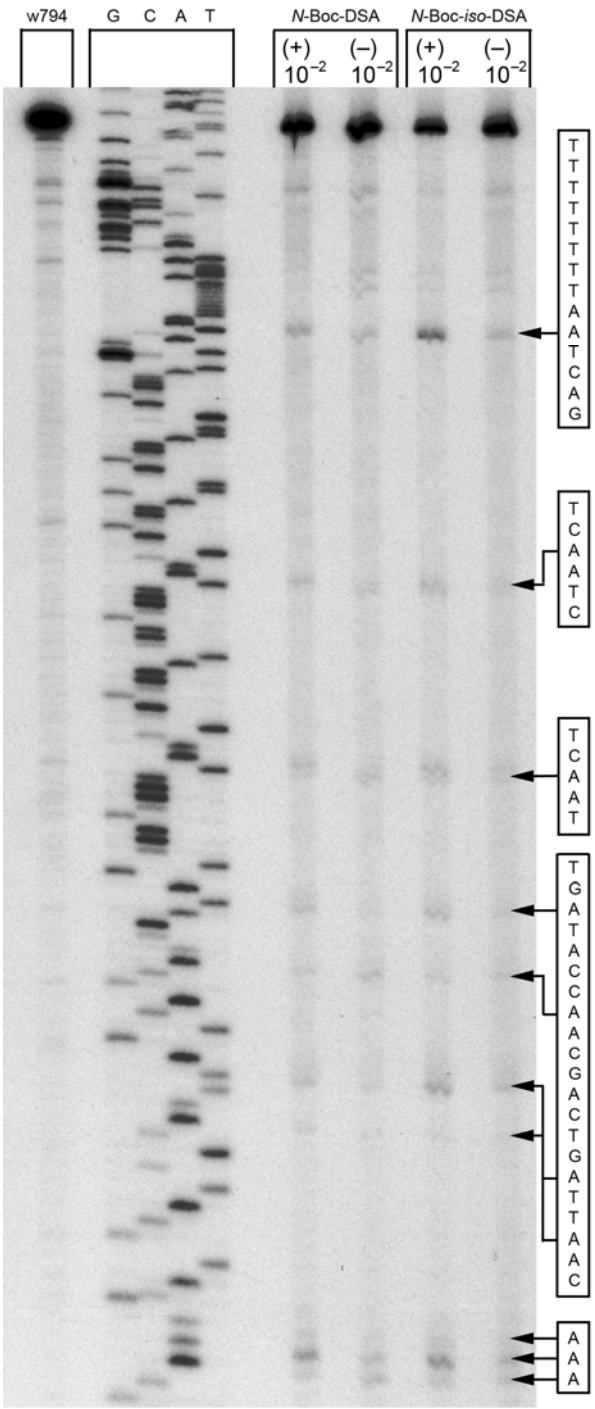


Figure S3. Thermally-induced strand cleavage of w794 DNA (144 bp, nucleotide no. 5238–138) after DNA–agent incubation with *N*-Boc-DSA and *N*-Boc-*iso*-DSA (24 h, 37 °C), removal of unbound agent by EtOH precipitation and 30 min thermolysis (100 °C), followed by denaturing 8% PAGE and autoradiography. Lane 1, control DNA; lanes 2–5, Sanger G, C, A, and T sequencing standards; lanes 6 and 7, (+)-*N*-Boc-DSA and *ent*-(–)-*N*-Boc-DSA (**8**, 1×10^{-2} M); lanes 8 and 9, (+)-*N*-Boc-*iso*-DSA and *ent*-(–)-*N*-Boc-*iso*-DSA (**7**, 1×10^{-2} M).