Supporting Information

A Novel, Unusually Efficacious Duocarmycin Carbamate Prodrug That Releases No Residual Byproduct

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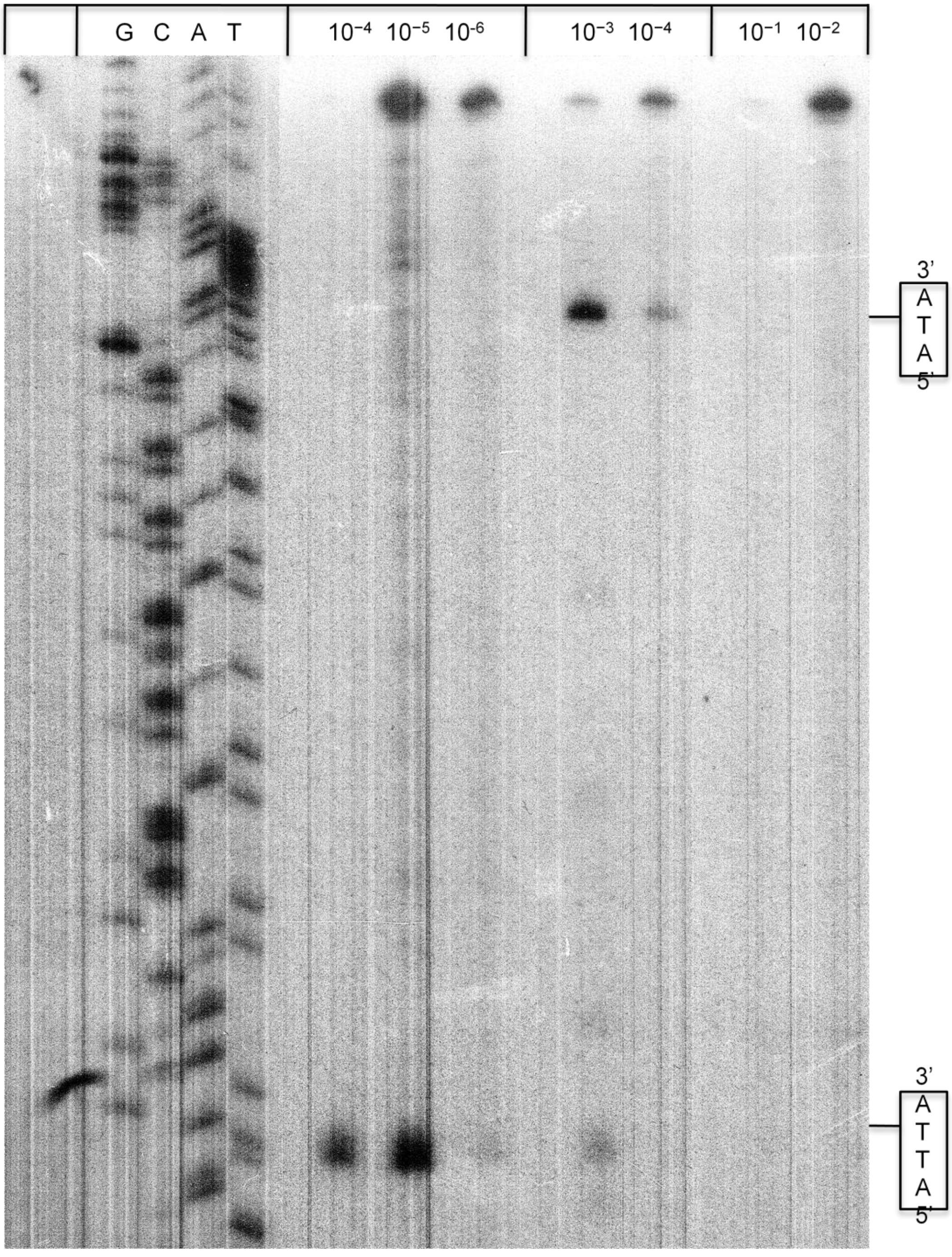
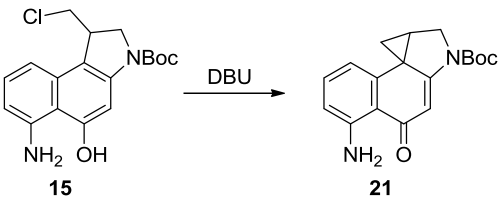


Figure S1. Thermally induced strand cleavage of w794 DNA; DNA−agent incubation at 23 °C for 48 h, removal of unbound agent by EtOH precipitation, and 30 min of thermolysis (100 °C) followed by 8% denaturing PAGE and autoradiography. Lane 1, control DNA; lanes 2−5, Sanger G, C, A, and T sequencing reactions; lanes 6−8, (+)-**1** (1×10^{-4} to 1×10^{-6}); lanes 9−10, (−)-**1** (1×10^{-3} to 1×10^{-4}); lanes 11-12, (+)-**6** (1×10^{-1} to 1×10^{-2}).



***N-tert*-Butyloxycarbonyl-5-amino-1,2,9,9a-tetrahydrocyclopropa[*c*]benzo[*e*]indole-4-one (*N*-Boc-ACBI, 21)**. Compound **15** (4 mg, 11.4 μmol) in 0.2 mL of acetonitrile was treated with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 6 μL, 0.043 mmol). The reaction mixture was allowed to stir a room temperature for 90 min. After 90 min, the solvent was evaporated under reduced pressure and the residue was purified by PTLC (SiO_2, 50% EtOAc/hexanes) to provide **21** (3.5 mg, 100% yield) as an orange oil. ^1H NMR (acetone-d_6, 600 MHz) δ 7.25 (br, 1H), 7.12 (t, J = 7.8 Hz, 1H), 6.59 (d, J = 8.4 Hz, 1H), 6.12 (d, J = 7.2 Hz, 1H), 4.00−3.94 (m, 2H), 2.85 (m, 1H), 1.53 (s, 9H), 1.45−1.40 (m, 2H). ^{13}C NMR (acetone-d_6, 150 MHz) δ 191.0, 159.6, 153.1, 152.8, 144.3, 134.0, 116.3, 115.2, 109.9, 109.5, 85.5, 54.2, 35.0, 31.9, 29.0, 25.5. ESI-TOF HRMS m/z 313.1553 ($M+H^+$, $C_{18}H_{20}N_2O_3$ requires 313.1547).

Solvolysis of 21. Compound **21** was dissolved in CH_3OH (1.5 mL). The CH_3OH solution was mixed with aqueous buffer (pH 2, 1.5 mL). The buffer contained 4:1:20 (v:v:v) 1.0 M citric acid, 0.2 M Na_2HPO_4, and H_2O, respectively. After mixing, the solvolysis solutions were stoppered and kept at 25 °C in the dark. The UV spectrum of the solutions was measured 3−4 times in the first two days and once a day for 2−4 weeks. The UV monitoring was continued until no further change was detectable. The long-wavelength absorption at 380 nm and short-wavelength absorption at 255 nm were monitored. The solvolysis rate constant and half-life were calculated from the data recorded at the short wavelength (255 nm) from the least square treatment of the slopes of plots of time versus ln $[(A_{Final}-A_{Initial})/(A_{Final}-A)]$.

pH 1 buffer: 10 M citric acid: 0.2 M Na_2HPO_4: H_2O (4:1:20)

$t_{1/2} = 8.52$ h, $k = 1.5 \times 10^{-5}$ s^{-1}

pH 2 buffer: 1.0 M citric acid: 0.2 M Na_2HPO_4: H_2O (4:1:20)

$t_{1/2} = 40.3$ h, $k = 5 \times 10^{-6}$ s^{-1}

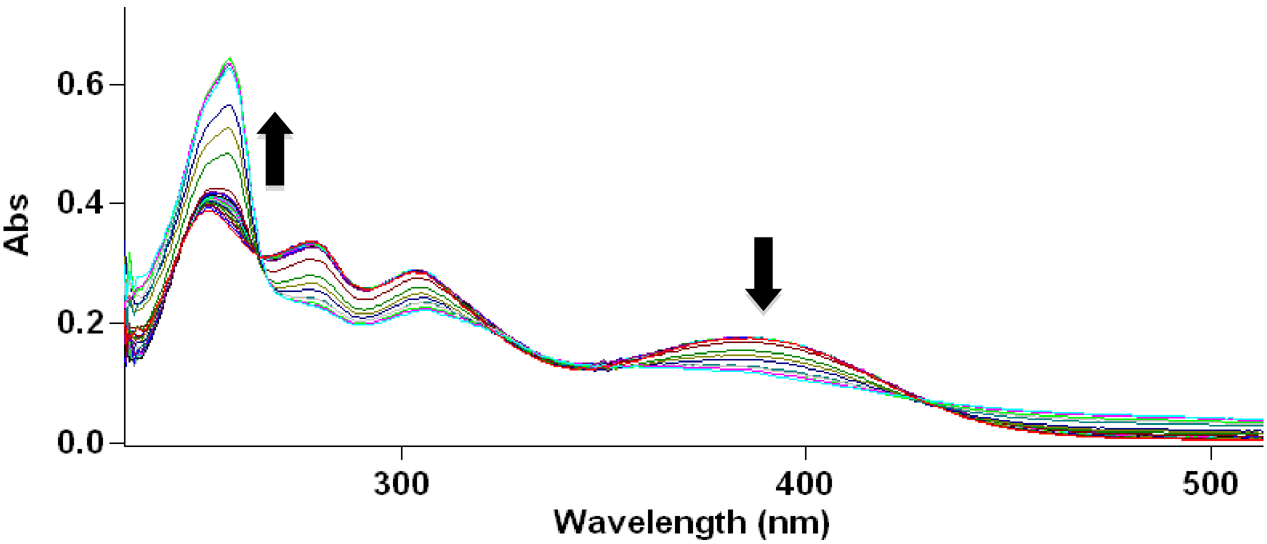
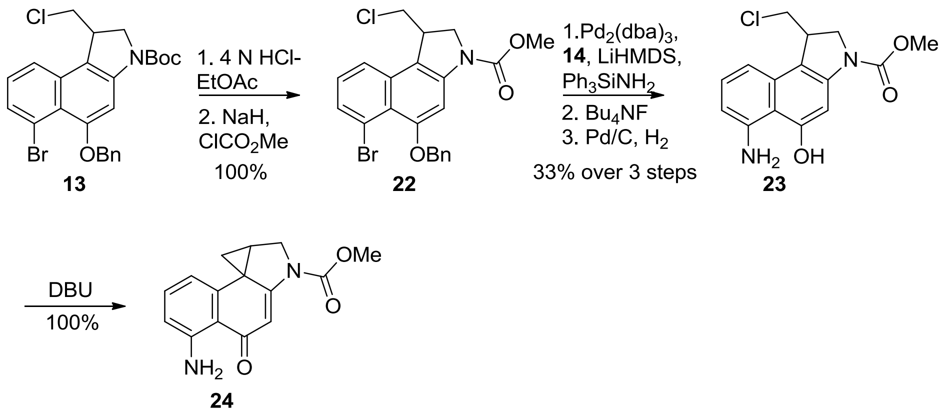


Figure S2. UV-visible spectra of **21** in 50% CH_3OH-aqueous buffer (pH 2) recorded at various time intervals.

Scheme S1



Methyl 5-(Benzyloxy)-6-bromo-1-(chloromethyl)-1*H*-benzo[*e*]indole-3(2*H*)-carboxylate (22). Compound **13** (50 mg, 0.099 mmol) was dissolved in 4 N HCl in EtOAc (2.0 mL) and the mixture was allowed to stir at room temperature for 15 min. The solvent was removed under a stream of nitrogen and the residue was redissolved in anhydrous DMF (0.9 mL) and the solution was cooled to 0 °C. Once cooled, 60% NaH in mineral oil (9.5 mg, 0.23 mmol) was added and the reaction mixture was allowed to stir at 0 °C for 30 min. Methyl chloroformate (36 µL, 0.47 mmol) was added and the solution was warmed to room temperature. After 4 h, the reaction mixture was quenched with the addition of saturated aqueous NH_4Cl and diluted with ethyl acetate. The organic layer was washed with H_2O, saturated aqueous NaCl, then dried over Na_2SO_4, and concentrated under reduced pressure. Flash chromatography (SiO_2, 2 × 8 cm, 15% EtOAc/hexanes elution) provided compound **22** (45 mg, 100%)

as a off white solid. ^{1}H NMR (acetone-d_6, 600 MHz) δ 7.95 (br, 1H), 7.80 (d, *J* = 8.4 Hz, 1H), 7.65 (d, *J* = 7.2 Hz, 3H), 7.41 (t, *J* = 7.8 Hz, 2H), 7.32 (m, 2H), 5.33 (s, 2H), 4.23 (dd, *J* = 2.4, 11.4 Hz, 1H), 4.18 (m, 1H), 4.11 (m, 1H), 3.96 (dd, *J* = 3, 10.8 Hz, 1H), 3.83 (s, 3H), 3.71 (dd, *J* = 10.8, 8.4 Hz, 1H). ^{13}C NMR (acetone-d_6, 150 MHz) δ 157.6, 154.8, 143.9, 138.4, 134.8, 132.6, 130.1, 129.9, 129.6, 129.3, 124.2, 121.6, 119.2, 117.2, 100.4, 72.5, 54.1, 53.9, 48.4, 43.2. IR (film) ν_{max} 2951, 2357, 1614, 1361, 1133 cm^{-1}.ESI-TOF HRMS *m/z* 460.0317 (M+H$^+$, $C_{22}H_{19}BrClNO_3$ requires 460.0310).

Methyl 6-Amino-1-(chloromethyl)-5-hydroxy-1*H*-benzo[*e*]indole-3(2*H*)-carboxylate (23). See compound **15** for procedure. Compound **23** was isolated as a tan solid (33% over 3 steps). ^{1}H NMR (THF-d_8, 600 MHz) δ 7.39 (br, 1H), 7.07 (t, *J* = 7.8 Hz, 1H), 6.75 (d, *J* = 7.8 Hz, 1H), 6.30 (d, *J* = 7.8 Hz, 1H), 4.19 (d, *J* = 11.4 Hz, 1H), 4.03 (t, *J* = 9Hz, 1H), 3.90 (dd, *J* = 2.4, 10.8 Hz, 1H), 3.83 (m, 1H), 3.76 (s, 3H), 3.39 (t, *J* = 10.2 Hz, 1H). ^{13}C NMR (THF-d_8, 150 MHz) δ 158.6, 154.1, 148.9, 142.0, 134.3, 129.3, 114.4, 112.0, 110.0, 107.3, 98.2, 53.4, 52.7, 47.2, 43.6. IR (film) ν_{max} 3197, 1672, 1584, 1483, 1411, 1336, 1150, 748 cm^{-1}. ESI-TOF HRMS *m/z* 307.0845 (M+H$^+$, $C_{15}H_{15}ClN_2O_3$ requires 307.0844).

Methyl 5-Amino-1,2,9,9a-tetrahydro-1*H*-benzo[*e*]cyclopropa[*c*]indol-4-one-2-carboxylate (24). See compound **21** for procedure. Compound **24** was isolated as a bright yellow solid (70%). ^{1}H NMR (acetone-d_6, 600 MHz) δ 7.22 (br, 1H), 7.13 (t, *J* = 7.8 Hz, 1H), 6.60 (d, *J* = 8.4 Hz, 1H), 6.13 (d, *J* = 6.6 Hz, 1H), 4.05−3.99 (m, 2H), 3.80 (s, 3H), 2.89−2.86 (m, 1H), 1.46 (d, *J* = 6Hz, 2H). ^{13}C NMR (acetone-d_6, 150 MHz) δ 190.2, 159.7, 153.8, 152.0, 143.5, 133.3, 115.5, 114.4, 109.3, 108.8, 53.4, 53.2, 34.1, 31.0, 24.9. IR (film) ν_{max} 3441, 1730, 1600, 1537, 1440, 1291, cm^{-1}. ESI-TOF HRMS *m/z* 271.1077 (M+H$^+$, $C_{15}H_{14}N_2O_3$ requires 271.1077).

Solvolysis of **24**:
pH 1 buffer: 10 M citric acid: 0.2 M Na_2HPO_4: H_2O (4:1:20)

$t_{1/2}$ = 11.6 h, $k = 1.8 \times 10^{-5}$ sec^{-1}

pH 2 buffer: 1.0 M citric acid: 0.2 M Na_2HPO_4: H_2O (4:1:20)

$t_{1/2}$ = 57.6 h, $k = 3.3 \times 10^{-6}$ sec^{-1}

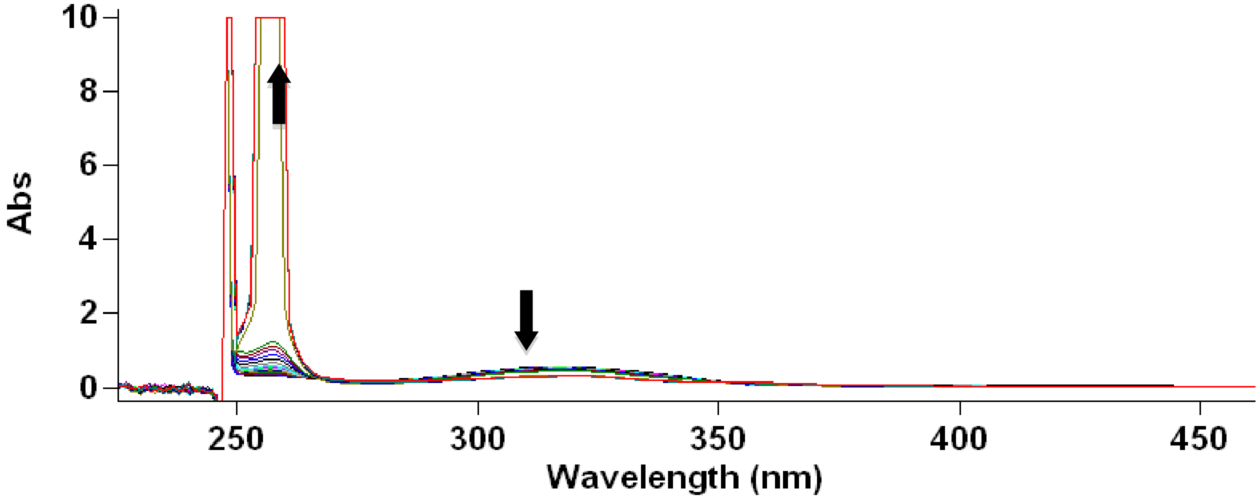


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Figure S3. UV-visible spectra of **24** in 50% CH_3OH-aqueous buffer (pH 1) recorded at various time intervals.

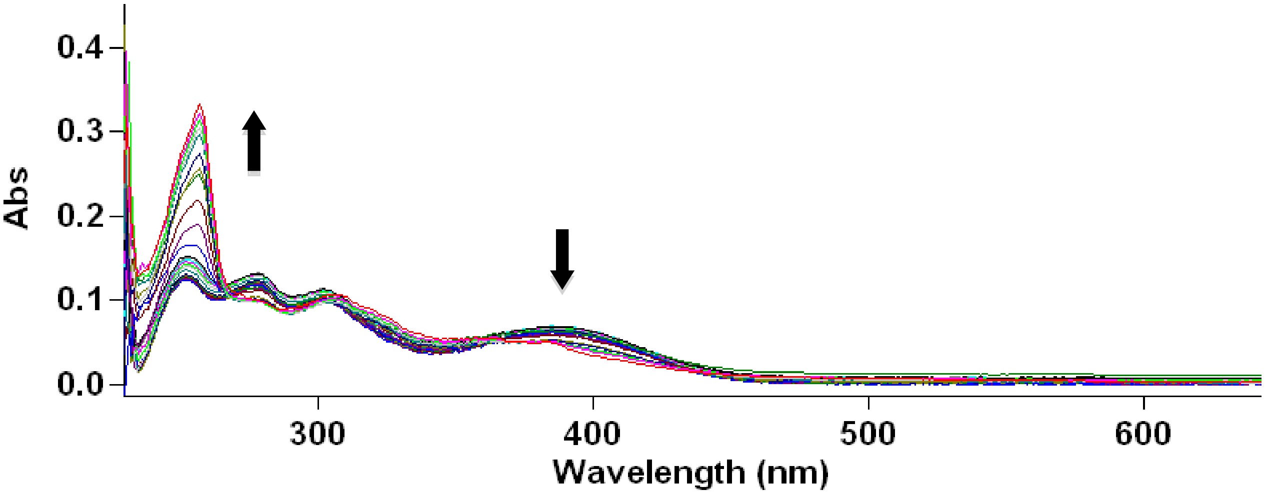

Figure S4. UV-visible spectra of **24** in 50% CH_3OH-aqueous buffer (pH 2) recorded at various time intervals.