CBI Prodrug Analogs of CC-1065 and the Duocarmycins

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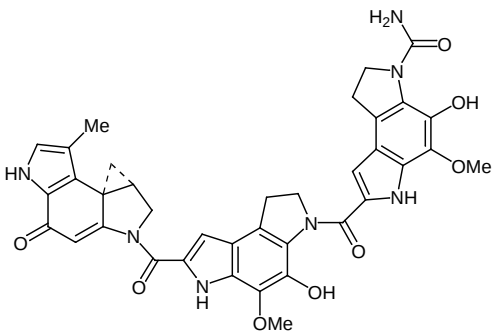
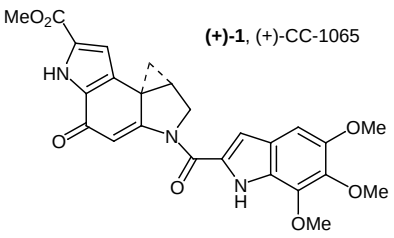
Abstract: The preparation of a small series of CBI-indole$_2$ prodrugs is detailed.

Key words: prodrugs, cytoxicity, CBI-indole$_2$, duocarmycins

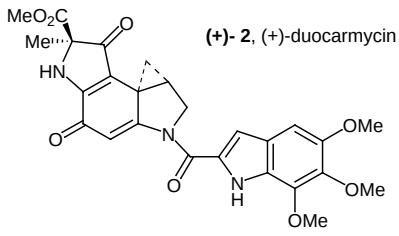
CC-1065 and the duocarmycins (**1–3**) are the parent members of a class of potent naturally occurring antitumor antibiotics which derive their biological properties through the sequence selective alkylation of duplex DNA.[1,2] In the course of our efforts on the evaluation of analogs bearing deep-seated structural modifications in the alkylation subunit, we described the first preparation and examination of agents containing the 1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one (CBI) alkylation subunit.[3] In these studies, the CBI-based analogs are chemically more stable (4×), biologically more potent (4×), and considerably more synthetically accessible than the corresponding agents incorporating the natural CPI alkylation subunit of CC-1065.[3] (+)-CBI-indole$_2$ (**4**), a simplified agent within our early series of analogs, not only exhibited cytotoxic potency comparable to that of the (+)-CC-1065 and greater (4×) than that of (+)-CPI-indole$_2$ (U71,184),[4] but it also exhibited potent and efficacious in vivo antitumor activity.[5] It is known that duocarmycin and CPI prodrugs formed by acylation of the C4 phenol of appropriate seco precursors such as **5** may show improved pharmacokinetics, synthesis scaleup safety, storage stability and safety, as well as improved efficacy[6] and two such compounds, KW-2189[7] and carzelesin (U-80,244),[8] are currently undergoing clinical development. Herein, we describe the synthesis and evaluation of six prodrug derivatives of (+)-CBI-indole$_2$ which have become especially attractive for their therapeutic properties.



(+)-1, (+)-CC-1065



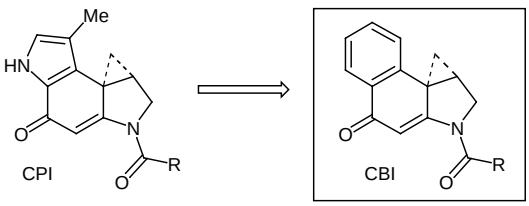
(+)- 2, (+)-duocarmycin SA

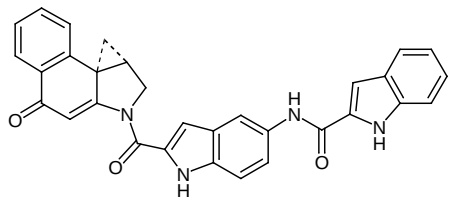


(+)-3, (+)-duocarmycin A

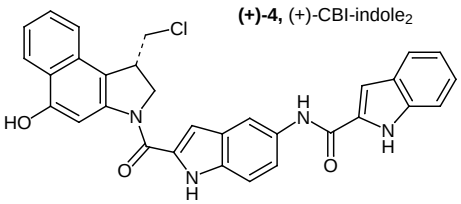
Synthesis

The *N*,*N*-dialkylcarbamoyl derivatives of *seco*-CBI-indole$_2$ (**5**)[5] were prepared in good yields by treatment of **5** with 4-nitrophenyl chloroformate (Et_3N, THF) to afford

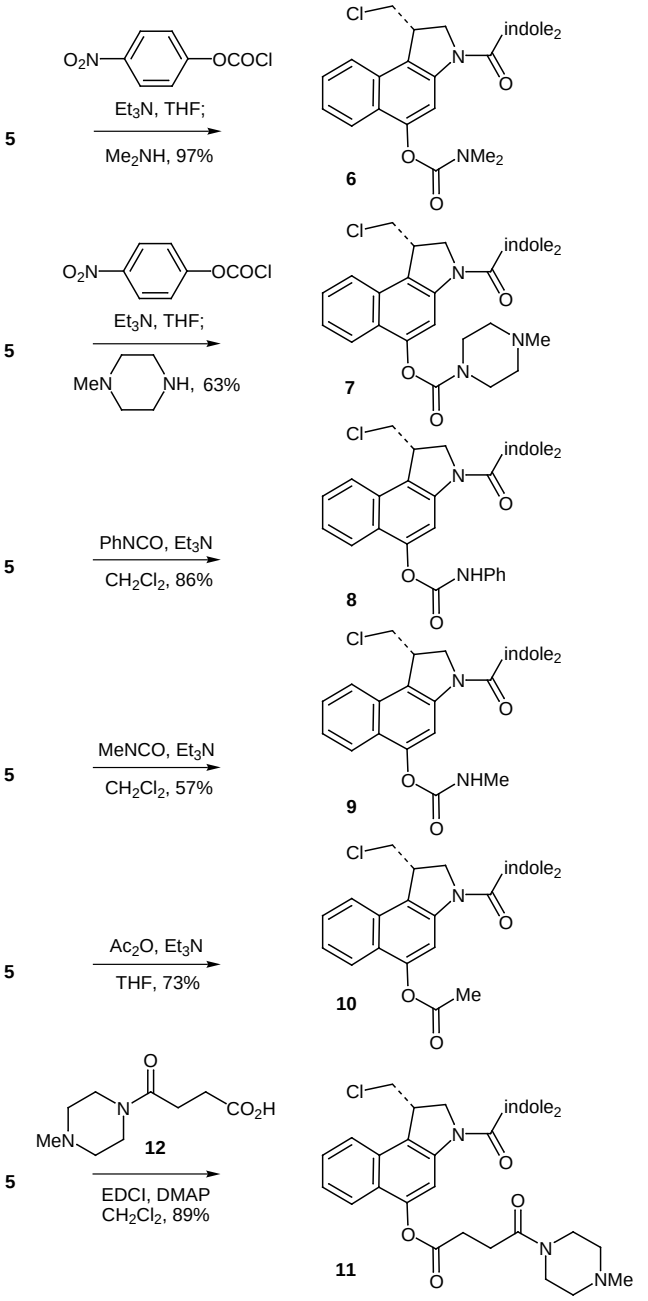




(+)-4, (+)-CBI-indole$_2$

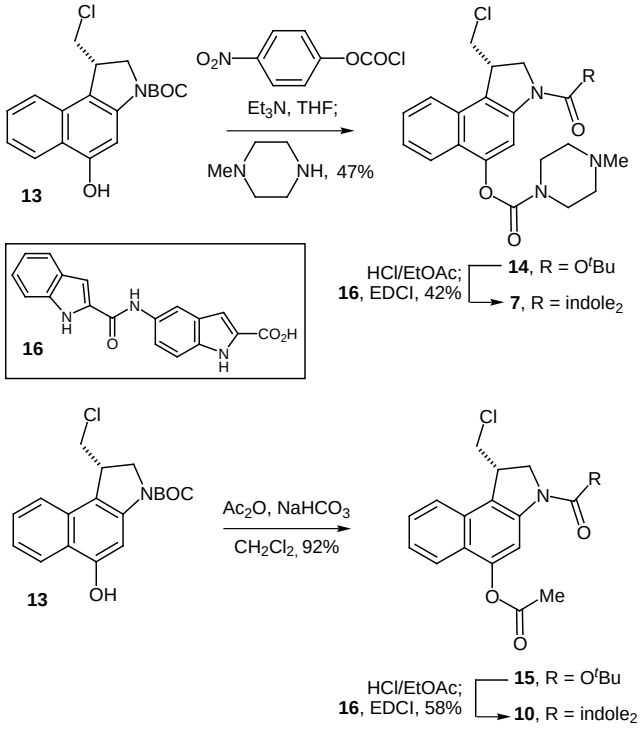


(+)-5, (+)-*seco*-CBI-indole$_2$



the carbonate as an intermediate, followed by the addition of dimethylamine (**6**, 97%) or 1-methylpiperazine (**7**, 63%). Reaction of **5** with phenyl or methyl isocyanate (Et_3N, CH_2Cl_2) gave the *N*-phenylcarbamoyl derivative **8** (86%) and *N*-methylcarbamoyl derivative **9** (57%). Treatment of **5** with acetic anhydride (Et_3N, THF, 73%) produced **10** while esterification of **5** with the acid **12**[6] (EDCI, DMAP, CH_2Cl_2, 89%) gave **11.**

An alternative synthesis of the prodrugs of *seco*-CBI-indole$_2$ was also explored. There are a number of advantages to an approach in which the prodrug substituent is added to the alkylation subunit, followed by its coupling to the DNA binding subunit including the safety issues surrounding large scale intermediate handling and the examination of alternative right-hand subunits. Treatment of **13**[3,5] with 4-nitrophenyl chloroformate (Et_3N, CH_2Cl_2) afforded the carbonate intermediate which was reacted immediately with 1-methylpiperazine (**14**, 47%). Similarly, treatment of **13** with acetic anhydride ($NaHCO_3$, CH_2Cl_2) gave the acetate in good yield (**15**, 92%). Deprotection of both **14** and **15** under acidic conditions (3.3 M HCl/EtOAc) followed by direct coupling with **16**[5] (EDCI, DMF) gave the two prodrugs in good yield (42% for **7,** 58% for **10**).



In Vitro Cytotoxicity Activity

Consistent with past observations, each of the prodrugs exhibited an in vitro cytotoxic activity in the L1210 assay that was essentially equivalent to that of **4** and **5**. Since the *O*-acylated or *O*-alkylated derivatives of **5** cannot as effectively alkylate DNA and would be expected to be >100x less potent, the cytotoxicity of the derivatives **6–11** indicate that each prodrug is effectively hydrolyzed to release **5** which serves as the penultimate precursor to the active constituent **4**. The agents were further examined in a human colon carcinoma cell line (HCT116) and two resistant variants. HCT116/VM46 overexpresses the cell surface protein gp 170 and embodies the MDR phenotype while HCT116/VP35 is resistant to topoisomerase II inhibitors by virtue of its lower expression. In these cell lines, the carbamate prodrugs incorporating a tertiary amine were inactive presumably because of the lack of

hydrolysis, but the remainder exhibited potent cytoxic activity essentially equipotent or more potent, against both the wild type and resistant cell lines. We had reported previously this behavior for **2**,[5] and the results suggest that the agents may prove especially effective against resistant variants of tumor cell lines and particularly useful in combination therapy or in relapse chemotherapy. The results of more detailed comparative in vitro and in vivo testing of **4–11** will be disclosed in due course.

Table 1. In Vitro Cytotoxic Activity

	IC_{50} (pM)			
compound	L1210	HCT116 wild type	HCT116/VM46 (MDR)	HCT116/VP35 (reduced topo II)
(+)-**2**	10	30	10	2
(+)-**4**	10	100	60	10
(+)-**5**	20	80	100	30
(-)-**5**	6000			
(+)-**6**	35	>1600	>1600	>1600
(+)-**7**	30	>1500	>1500	>1500
(+)-**8**	5	150	400	300
(-)-**8**	15000			
(+)-**9**	10	50	100	50
(+)-**10**	30	100	350	150
(+)-**11**	35	150	450	250
vincristine		4000	200000	6000
doxorubicin		250000	2500000	600000

3-[5'-(((1*H*-Indol-2"-yl)carbonyl)amino)-1*H*-indol-2'-yl)carbonyl]-1-(chloromethyl)-5-[[(dimethylamino)carbonyl]oxy]-1,2-dihydro-3*H*-benz[*e*]indole (6)

A solution of **5** (2.8 mg, 5.2 μmol) in THF (0.3 mL) was treated with 4-nitrophenylchloroformate (2.8 mg, 14.0 μmol) and Et_3N (1.6 μL, 11.2 μmol) and stirred at 25 °C for 2.5 h under Ar. Me_2NH (8.4 μL, 2 M solution in THF, 16.8 μmol) was added and the solution was stirred for an additional 3 h at 25 °C. The mixture was concentrated and directly subjected to chromatography (PTLC, 20 × 20 cm, 50% THF/hexanes) to yield **6** (3.1 mg, 97%) as a light yellow film. (+)-(1*S*)-**6**: $[\alpha]^{25}_D$ +54 (*c* = 0.16, DMF).

^{1}H NMR (DMF-d_7, 400 MHz): δ = 11.69 (s, 1H), 11.56 (s, 1H), 10.18 (s, 1H), 8.23 (d, 1H, *J* = 1.8 Hz), 8.17 (s, 1H), 7.90 (d, 1H, *J* = 8.1 Hz), 7.78 (d, 1H, *J* = 8.8 Hz, partially obscured by DMF), 7.55 (dd, 1H, *J* = 1.8, 8.8 Hz), 7.46 (d, 1H, *J* = 7.4 Hz), 7.43 (m, 1H), 7.39 (s, 1H), 7.37 (m, 1H), 7.31 (m, 2H), 7.14 (d, 1H, *J* = 1.5 Hz), 7.05 (m, 1H), 6.88 (m, 1H), 4.79 (m, 1H), 4.62 (dd, 1H, *J* = 2.2, 10.6 Hz), 4.29 (m, 1H), 3.99 (dd, 1H, *J* = 3.3, 11.0 Hz), 3.91 (dd, 1H, *J* = 7.4, 11.0 Hz), 3.10 (s, 3H), 2.84 (s, 3H).

IR (film) ν_{max} = 3286, 2934, 1704, 1633, 1558, 1524, 1463, 1417, 1317, 1313, 1246, 1172 cm^{-1}.

FAB/HRMS (NBA/CsI) *m/z* = 738.0862 (M+Cs$^+$, $C_{34}H_{28}N_5O_4Cl$ requires 738.0864).

3-[5'-(((1*H*-Indol-2"-yl)carbonyl)amino)-1*H*-indol-2'-yl)carbonyl]-1-(chloromethyl)-5-[[(4-methyl-1-piperazinyl)carbonyl]oxy]-1,2-dihydro-3*H*-benz[*e*]indole (7)

A solution of **5** (2.0 mg, 3.7 μmol) in CH_2Cl_2 (0.1 mL) was treated with 4-nitrophenylchloroformate (7.5 mg, 37 μmol) and Et_3N (5.0 μL, 37 μmol) and stirred at 4 °C for 2.5 h under Ar. 1-Methylpiperazine (6.2 μL, 56 μmol) was added and the solution was allowed to warm to 25 °C. After 12 h, the mixture was concentrated and directly subjected to chromatography (PTLC, 20 × 10 cm, THF) to yield **7** (1.5 mg, 63%) as a beige film, (+)-(1*S*)-**7**: $[\alpha]^{25}_D$ +24 (*c* = 0.08, DMSO).

^{1}H NMR (DMS*O*-d_6, 400 MHz): δ = 11.70 (s, 1H), 11.65 (s, 1H), 10.19 (s, 1H), 8.32 (s, 1H), 8.24 (s, 1H), 8.07 (d, 1H, *J* = 9.5 Hz), 7.85 (d, 1H, *J* = 8.1 Hz), 7.69 (d, 1H, *J* = 8.1 Hz), 7.66 (m, 2H), 7.53 (m, 2H), 7.45 (d, 1H), 7.43 (s, 1H), 7.29 (s, 1H), 7.21 (dd, 1H, *J* = 1.9, 8.4 Hz), 7.10 (dd, 1H, *J* = 1.9, 8.5 Hz), 4.92 (m, 1H), 4.67 (d, 1H, *J* = 9.2 Hz), 4.44 (s, 1H), 4.10 (m, 2H), 4.05 (m, 2H), 3.82 (m, 1H), 3.51 (m, 1H), 3.45 (m, 2H, obscured by H_2O), 3.17 (d, 2H, *J* = 7.0 Hz), 2.30 (s, 3H).

IR (film) ν_{max} = 3310, 2925, 1734, 1653, 1559, 1478, 1405, 1368, 1335 cm^{-1}.

FAB/HRMS (NBA/CsI) *m/z* = 661.2347 (M+H$^+$, $C_{37}H_{33}N_6O_4Cl$ requires 661.2330).

3-[5'-(((1*H*-Indol-2"-yl)carbonyl)amino)-1*H*-indol-2'-yl)carbonyl]-1-(chloromethyl)-5-[[(phenylamino)carbonyl]oxy]-1,2-dihydro-3*H*-benz[*e*]indole (8)

A solution of **5** (1.9 mg, 3.6 μmol) in CH_2Cl_2 (0.3 mL) was treated with phenylisocyanate (1.7 μL, 16.0 μmol) and Et_3N (2.5 μL, 18.0 μmol) and stirred at 25 °C for 6 h under Ar. The mixture was concentrated and directly subjected to chromatography (PTLC, 20 × 20 cm, 50% THF/hexanes) to yield **8** (2.0 mg, 86%) as a light yellow film, (+)-(1*S*)-**8**: $[\alpha]^{25}_D$ +31 (*c* = 0.08, DMF); (–)-(1*R*)-**8**: $[\alpha]^{25}_D$ –35 (*c* = 0.02, DMF).

^{1}H NMR (DMF-d_7, 400 MHz): δ = 11.79 (s, 1H), 11.77 (s, 1H), 10.56 (br s, 1H), 10.31 (s, 1H), 8.51 (s, 1H), 8.43 (s, 1H), 8.15 (d, 1H, *J* = 8.4 Hz), 8.10 (d, 1H, *J* = 8.4 Hz), 7.71 (m, 4H), 7.65 (d, 1H, *J* = 8.4 Hz), 7.57 (m, 4H), 7.40 (t, 2H, *J* = 8.1 Hz), 7.37 (d, 1H, *J* = 1.5 Hz), 7.27 (t, 1H, *J* = 7.0 Hz), 7.11 (m, 2H), 5.03 (t, 1H, *J* = 10.7 Hz), 4.86 (dd, 1H, *J* = 1.8, 10.7 Hz), 4.53 (m, 1H), 4.23 (dd, 1H, *J* = 3.3, 11.0 Hz), 4.13 (dd, 1H, *J* = 7.0, 11.0 Hz).

IR (film): ν_{max} = 3318, 2916, 2838, 1723, 1614, 1557, 1515, 1465, 1418, 1335, 1240 cm^{-1}.

FAB/HRMS (NBA/CsI) *m/z* = 654.1932 (M+H$^+$, $C_{28}H_{28}N_5O_4Cl$ requires 654.1908).;

3-[5'-(((1*H*-Indol-2"-yl)carbonyl)amino)-1*H*-indol-2'-yl)carbonyl]-1-(chloromethyl)-5-[[(methylamino)carbonyl]oxy]-1,2-dihydro-3*H*-benz[*e*]indole (9)

A sample of **5** (3.1 mg, 5.8 μmol) was dissolved in CH_2Cl_2 (0.3 mL) and treated with methyl isocyanate (15 μL, 10% solution in CH_2Cl_2, 26.0 μmol) and Et_3N (4 μL, 29.0 μmol). The resulting solution was stirred at 25 °C for 12 h under Ar. The mixture was concentrated and directly subjected to flash chromatography (silica gel, 1.5 × 5 cm, 75% THF/hexanes) to yield **9** (2.4 mg, 57%) as a beige film, (+)-(1*S*)-**9**: $[\alpha]^{25}_D$ +56 (*c* = 0.11, THF).

^{1}H NMR (DMF-d_7, 400 MHz): δ = 11.76 (s, 2H), 10.29 (s, 1H), 8.41 (s, 1H), 8.38 (s, 1H), 8.09 (d, 1H, *J* = 8.4 Hz), 7.98 (d, 1H, *J* = 8.4 Hz), 7.88 (m, 1H), 7.72 (dd, 1H, *J* = 1.8, 8.8 Hz), 7.69 (d, 1H, *J* = 9.5 Hz), 7.60 (m, 3H), 7.51 (m, 2H), 7.33 (s, 1H), 7.25 (m, 1H), 7.09 (m, 1H), 4.99 (m, 1H), 4.82 (dd, 1H, *J* = 1.8, 11.0 Hz), 4.48 (m, 1H), 4.18 (dd, 1H, *J* = 3.0, 11.0 Hz), 4.08 (dd, 1H, *J* = 7.3, 11.0 Hz), 2.86 (d, 3H, *J* = 4.4 Hz).

IR (film): ν_{max} = 3290, 2952, 1738, 1633, 1615, 1557, 1520, 1417, 1315, 1246, 1139 cm^{-1}.

FAB/HRMS (NBA/CsI): *m/z* 724.0751 (M+Cs$^+$, $C_{33}H_{26}N_5O_4Cl$ requires 724.0728).

3-[5'-(((1*H*-Indol-2"-yl)carbonyl)amino)-1*H*-indol-2'-yl)carbonyl]-5-(acetyloxy)-1-(chloromethyl)-1,2-dihydro-3*H*-benz[*e*]indole (10)

A sample of **5** (1.4 mg, 2.6 μmol) was dissolved in THF (0.1 mL) and treated with Ac_2O (1.2 μL, 13.1 μmol) and Et_3N (0.7 μL, 5.2 μmol). The resulting solution was stirred at 25 °C for 8 h under Ar. The mixture was concentrated and directly subjected to chromatography (PTLC, 20 × 10 cm, 50% THF/hexanes) to yield **10** (1.1 mg, 73%) as a beige film. (+)-(1*S*)-**10**: $[\alpha]^{25}_D$ +42 (*c* = 0.12, DMF).

^{1}H NMR (DMF-d_7, 400 MHz): δ = 11.79 (s, 1H), 11.77 (s, 1H), 10.32 (s, 1H), 8.43 (d, 1H, *J* = 1.9 Hz), 8.37 (s, 1H), 8.13 (d, 1H, *J* = 8.4 Hz), 8.02 (m, 1H, obscured by DMF), 7.74 (dd, 1H, *J* = 2.0, 8.9 Hz), 7.70 (d, 1H, *J* = 8.6 Hz), 7.66 (m, 1H), 7.61 (s, 1H), 7.59 (m, 1H), 7.53 (m, 2H), 7.36 (d, 1H, *J* = 1.7 Hz), 7.28 (m, 1H), 7.10 (m, 1H), 5.02 (t, 1H, *J* = 10.8 H), 4.85 (dd, 1H, *J* = 2.3, 11.0 Hz), 4.51 (m, 1H), 4.21 (dd, 1H, *J* = 3.4, 11.3 Hz), 4.12 (dd, 1H, *J* = 7.0, 11.1 Hz), 2.56 (s, 3H).

IR (film): ν_{max} = 3294, 2925, 1717, 1653, 1559, 1507, 1457, 1405, 1368, 1335 cm^{-1}.

FAB/HRMS (NBA/CsI): *m*/*z* = 709.0630 (M+Cs$^+$, $C_{33}H_{25}N_4O_4Cl$ requires 709.0619).

3-[5'-(((1*H*-Indol-2"-yl)carbonyl)amino)-1*H*-indol-2'-yl)carbonyl]-1-(chloromethyl)-5-[4-(4-methyl-1-piperazinyl)-1,4-dioxobutoxy]-1,2-dihydro-3*H*-benz[*e*]indole (11)

A sample of **5** (3.5 mg, 5.8 μmol) was dissolved in CH_2Cl_2 (0.3 mL) and treated with **12**[6] (1.5 mg, 7.5 μmol), EDCI (2.6 mg, 13.0 μmol) and catalytic dimethylaminopyridine (DMAP). The resulting solution was stirred at 25 °C for 2 h under Ar. The mixture was diluted with H_2O (5 mL), and extracted with EtOAc (3 × 5 mL). The organic layers were combined, washed with brine (5 mL), dried (Na_2SO_4), and concentrated under reduced pressure. Semi-preparative reverse-phase HPLC (Waters Bondapak C-18 column, 300 Å, 25 × 100 mm, 70% CH_3OH/30% (0.07% TFA/H_2O), 10 mL/min) afforded **11** (4.2 mg, 89%) as a beige film, (+)-(1*S*)-**12**: $[\alpha]^{25}_D$ +35 (*c* = 0.15, THF).

^{1}H NMR (DMF-d_7, 400 MHz): δ = 11.76 (s, 1H), 11.75 (s, 1H), 10.30 (s, 1H), 8.41 (s, 1H), 8.36 (s, 1H), 8.11 (d, 1H, *J* = 8.8 Hz), 7.98 (d, 1H, under DMF), 7.72 (dd, 1H, *J* = 1.8, 8.8 Hz), 7.69 (d, 1H, *J* = 9.5 Hz), 7.64 (m, 1H), 7.60 (m, 2H), 7.51 (m, 2H), 7.33 (s, 1H), 7.25 (m, 1H), 7.09 (m, 1H), 5.00 (m, 1H), 4.83 (dd, 1H, *J* = 1.7, 11.0 Hz), 4.51 (m, 1H), 4.20 (dd, 1H, *J* = 3.3, 11.4 Hz), 4.10 (dd, 1H, *J* = 7.0, 11.0 Hz), 3.5 (m, 3H, under H_2O), 3.12 (m, 3H), 3.00 (m, 6H), 2.75 (s, 3H).

IR (film): ν_{max} = 3282, 1757, 1653, 1521, 1465, 1418, 1314, 1202, 1138 cm^{-1}.

FAB/HRMS (NBA/CsI): *m*/*z* 849.1594 (M+Cs$^+$, $C_{40}H_{37}N_6O_5Cl$ requires 849.1568).

3-(*tert*-Butyloxycarbonyl)-1-(chloromethyl)-5-[[(4-methyl-1-piperazinyl)carbonyl]oxy]-1,2-dihydro-3*H*-benz[*e*]indole (14)

A solution of **13** (3.0 mg, 9.0 μmol) in CH_2Cl_2 (0.3 mL) was treated with 4-nitrophenyl chloroformate (3.6 mg, 18 μmol) and Et_3N (3 μL, 21 μmol) and stirred at 25 °C for 2 h. 1-Methylpiperazine (15 μL, 0.13 μmol) was added and the mixture was stirred for 16 h, diluted with CH_2Cl_2 (10 mL) and washed with 10% aq $NaHCO_3$ (5 x 10 mL). The organic layer was dried ($MgSO_4$), concentrated and subjected to chromatography (PTLC, 20 × 20 cm, 10% CH_3OH/EtOAc) to give **14** (2 mg, 47%) as a colorless film, (–)-(1*S*)-**14**: $[\alpha]^{25}_D$ –7.7 (*c* = 0.35, CH_2Cl_2).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.22 (s, 1H), 7.83 (d, 1H, *J* = 8.5 Hz), 7.67 (d, 1H, *J* = 8.3 Hz), 7.47 (dd, 1H, *J* = 1.8, 8.3 Hz), 7.35 (dd, 1H, *J* = 1.9, 8.5 Hz), 4.22 (m, 1H), 4.11 (dd, 1H, *J* = 3.3, 10.5 Hz), 3.93 (dd, 1H, *J* = 7.4, 11.0 Hz), 3.91 (d, 1H, *J* = 11.0 Hz), 3.82 (s, 2H), 3.61 (s, 2H), 3.46 (t, 1H, *J* = 11.1 Hz), 2.52 (s, 2H), 2.49 (s, 2H), 1.66 (s, 3H), 1.59 (s, 9H).

IR (film): ν_{max} = 2919, 1717, 1699, 1520, 1478, 1405, 1368, 1335 cm^{-1}.

FAB/HRMS (NBA/CsI): *m*/*z* = 460.2018 (M+H$^+$, $C_{24}H_{30}N_3O_4Cl$ requires 460.2003).

Alternative Synthesis of 7

Compound **14** (2.3 mg, 5.0 μmol) was treated with 3.3 M HCl/EtOAc (0.3 mL) and stirred at 25 °C for 30 min. The solvent was removed under a stream of N_2 and the resulting salt was dried under vacuum. This salt was dissolved in DMF (0.2 mL) and treated with EDCI (2.9 mg, 15 μmol) and **16** (2.9 mg, 8.9 μmol) and stirred at 25 °C. After 16 h, the mixture was concentrated and directly subjected to chromatography (PTLC, 20 × 20 cm, 10% $CH_3OH/CHCl_3$) to give **7** (1.4 mg, 42%).

5-(Acetyloxy)-3-(*tert*-butyloxycarbonyl)-1-(chloromethyl)-1,2-dihydro-3*H*-benz[*e*]indole (15)

A solution of **13** (3.1 mg, 9.1 μmol) in CH_2Cl_2 (0.3 mL) was treated with Ac_2O (13 μL, 93 μmol, 10 equiv) and $NaHCO_3$ (7.8 mg, 93 μmol, 10 equiv) and stirred at 25 °C for 19 h. The mixture was concentrated and directly subjected to chromatography (PTLC, 20 × 20 cm, 10% EtOAc/hexanes) to give **15** (3.2 mg, 92%) as a colorless film, (–)-(1*S*)-**15**: $[\alpha]^{25}_D$ –15 (*c* = 0.16, EtOAc).

^{1}H NMR ($CDCI_3$, 400 Mhz: δ = 8.01 (br s, 1H), 7.78 (d, 1H, *J* = 8.5 Hz), 7.68 (d, 1H, *J* = 8.3 Hz), 7.49 (dd, 1H, *J* = 1.8, 8.3 Hz), 7.35 (dd, 1H, *J* = 1.9, 8.5 Hz), 4.30 (m, 1H), 4.14 (dd, 1H, *J* = 3.3, 10.5 Hz), 4.00 (dd, 1H, *J* = 7.4, 11.0 Hz), 3.91 (d, 1H, *J* = 11.0 Hz), 3.45 (t, 1H, *J* = 10.6 Hz), 2.42 (s, 3H), 1.56 (s, 9H).

IR (film): ν_{max} = 2979, 1769, 1703, 1630, 1595, 1478, 1406, 1368, 1332 cm^{-1}.

FAB/HRMS (NBA/CsI): *m*/*z* = 375.1249 (M$^+$, $C_{20}H_{22}NO_4Cl$ requires 375.1237).

Alternative Synthesis of 10

Compound **15** (2.7 mg, 7.2 μmol) was treated with 3.3 M HCl/EtOAc (0.2 mL) and stirred at 25 °C for 30 min. The solvent was removed under a stream of N_2 and the resulting salt was dried under vacuum. This salt was dissolved in DMF (0.2 mL) and treated with EDCI (4.1 mg, 22 μmol) and **16** (2.8 mg, 8.6 μmol) and stirred at 25 °C. After 16 h, the mixture was concentrated and directly subjected to chromatography (PTLC, 20 × 20 cm, 50% EtOAc/hexanes) to give **10** (2.4 mg, 58%).

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