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CC-1065/Duocarmycin and Bleomycin A_2 Hybrid Agents: Lack of Enhancement of DNA Alkylation by Attachment to Noncomplementary DNA Binding Subunits

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Abstract—Hybrid agents **5–11** containing the *C*-terminus DNA binding domain of bleomycin A_2 linked to the CBI analogue of the CC-1065 and duocarmycin DNA alkylation subunits were prepared and evaluated. The agents exhibited little or no enhancement of the DNA alkylation efficiency and in some cases the linkage resulted in diminished properties relative to the simple alkylation subunit itself. Moreover, the DNA alkylation selectivity (5′-AA>5′-TA) of the resulting agents proved identical to that of simple derivatives of the CBI alkylation subunit, e.g. *N*-BOC-CBI. Thus, the linkage to the DNA binding domain of bleomycin A_2 did not alter this inherent DNA alkylation selectivity to reflect a DNA binding or cleavage selectivity of bleomycin A_2, nor did it reflect the greater 5- or 3.5-base-pair AT-rich selectivities observed with CC-1065 or the duocarmycins, respectively. Consistent with these observations, the cytotoxic properties of **5–11** were diminished relative to those of even simple derivatives of the CC-1065/duocarmycin alkylation subunits, e.g. *N*-BOC-CBI.

Introduction

CC-1065 (**1**) and the duocarmycins (**2–3**) are characteristic members of a potent class of antitumor antibiotics.[1–3] Since their discovery, extensive studies have been conducted to identify their site(s) and mode of action (Fig. 1).[4–7] In these studies, it has been demonstrated that CC-1065 and the duocarmycins bind and alkylate 5- and 3.5-base-pair AT-rich DNA minor groove sites, respectively, through adenine N3 addition to the activated cyclopropane found in the agents left-hand subunits.[4–13] A series of studies with analogues containing deep-seated structural changes have defined functional and structural features of the natural products that contribute to their DNA alkylation efficiency and selectivity and the resulting biological properties.[4] Of these agents, those that contain the CBI modification in the alkylation subunit have proven especially interesting (Fig. 1).[14] They have been shown to be 4× more stable, 4× more cytotoxic and to exhibit identical DNA alkylation selectivities when compared with the corresponding CC-1065 or CPI-based analogues. This synthetically more accessible[15] class of agents alkylate DNA more efficiently and more rapidly than the CPI-based agents[16] and members within this class have exhibited efficacious in vivo antitumor activity.[17]

The bleomycins are a family of clinically effective glycopeptide antitumor antibiotics of which bleomycin A_2 (**4**) is the major constituent (Fig. 2).[18] It is generally accepted that they derive their therapeutic effects through the ability to mediate the oxidative cleavage of duplex DNA or RNA by a process that is metal ion and oxygen dependent. The *C*-terminus tripeptide S subunit of bleomycin A_2 including the sulfonium cation and the bithiazole provide the majority of the DNA binding affinity[18] ($K_{app}=0.26$ versus 1.0×10^5 M^{-1}),[19] while the amino terminus pyrimidoblamic acid subunit in conjunction with the adjacent *erythro*-β-hydroxy-L-histidine provides the metal chelation/oxygen activation and polynucleotide recognition. Recent detailed studies[19] of the DNA binding properties of *N*-BOC di-, tri-, tetra- and pentapeptide S and related structures ($K_{app}=0.1$, 0.26, 0.21 and 0.23×10^5 M^{-1}), including a determination of their apparent binding site sizes (2.2,

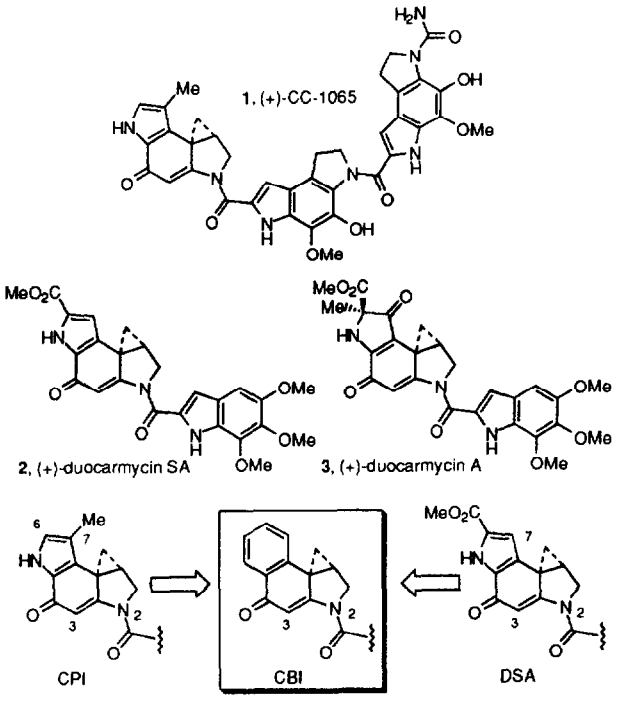


Figure 1.

3.6, 3.7, and 4.2 versus 3.8 base-pairs for **4**), suggested that the natural agent adopts a bent bound conformation with tripeptide S fully bound to DNA incorporating a reverse turn and swivel point in the peptide backbone at the tripeptide S-tetrapeptide S junction.[19] Recent NMR structural studies[20] of bleomycin A_2 bound to a cleavage site within an oligonucleotide were found to embody the basic tenants of this nonstructural model.[19,21]

Herein, we describe the preparation and evaluation of the series of hybrid agents **5–11** of CC-1065/duocarmycins and the bleomycins which incorporate the CBI analogue of the DNA alkylation subunits of the former natural products linked to the *C*-terminus di- and tripeptide S DNA binding domain of bleomycin A_2 (Fig. 3). Both a short rigid linker (**5–7**) and a longer flexible linker (**8–11**) were examined in the initial studies. The agents **5–11** incorporate the precursor to the CBI alkylation subunit which has been shown to function in an equivalent manner to the agents containing the preformed cyclopropane.[14–17] In addition to the potentially interesting biological properties of the resulting agents, the examination of **5–11** was expected to provide further insights into the polynucleotide recognition inherent in the CC-1065/duocarmycin alkylation subunits and/or the bleomycin A_2 *C*-terminus.

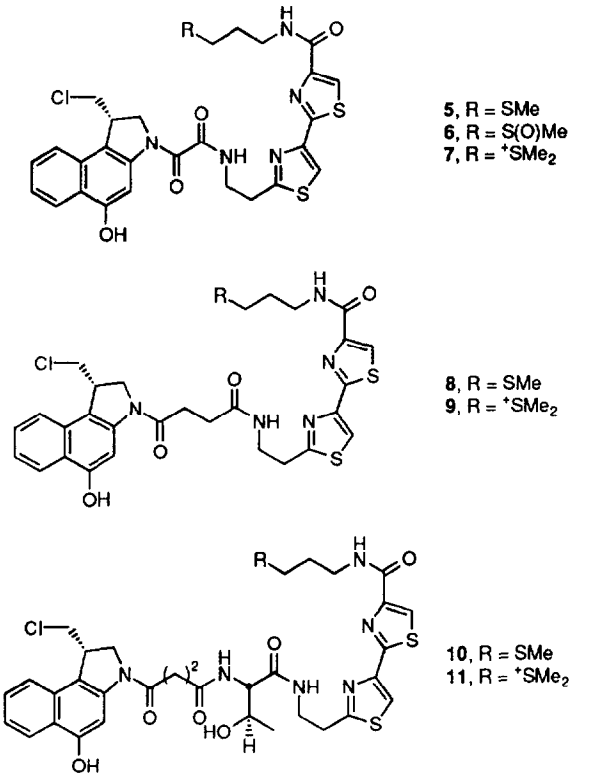


Figure 3.

Results

Preparation of 14–16: The nonlinked CBI-based agents

For a direct comparison with the di- and tripeptide S linked agents, the CBI agents **14–16** were prepared and incorporate the full acyl group used to join the CBI precursor to the bleomycin A_2 DNA binding domains. Acid-catalyzed deprotection of **12**[15] (3.6 N HCl–EtOAc, 25 °C, 30 min) followed direct coupling of **13** with ethyl oxalyl chloride (2 equiv, 3 equiv $NaHCO_3$, THF, 25 °C, 2 h, 95–100%) cleanly provided **14**[22] (Scheme 1). Similarly, direct coupling of freshly generated **13** with ethyl succinyl chloride (1 equiv, 2.5 equiv $NaHCO_3$, THF, 25 °C, 1 h, 95–100%) followed by treatment of **15** with 5% aq $NaHCO_3$: THF[14–17] (1:1, 25 °C, 9 h, 79%) provided **16**. Although this is illustrated in Scheme 1 with only the natural enantiomer series, both enantiomers of the agents **14–16** were prepared for comparative examination.

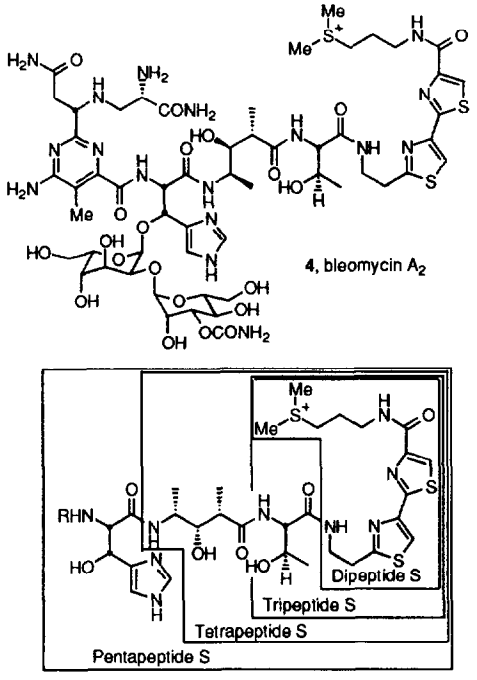
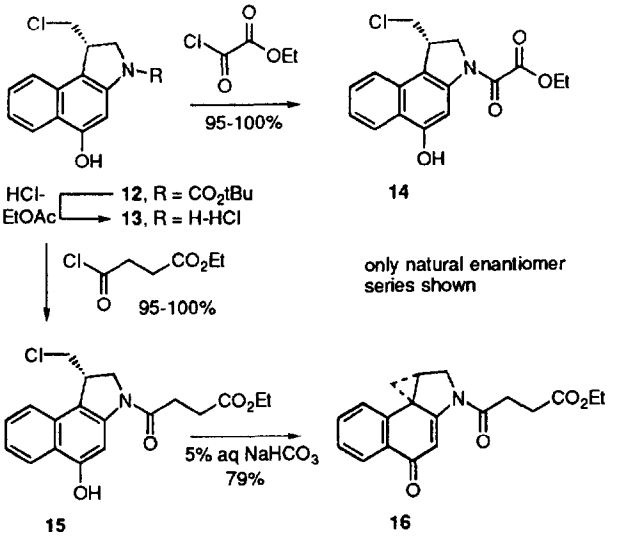


Figure 2.



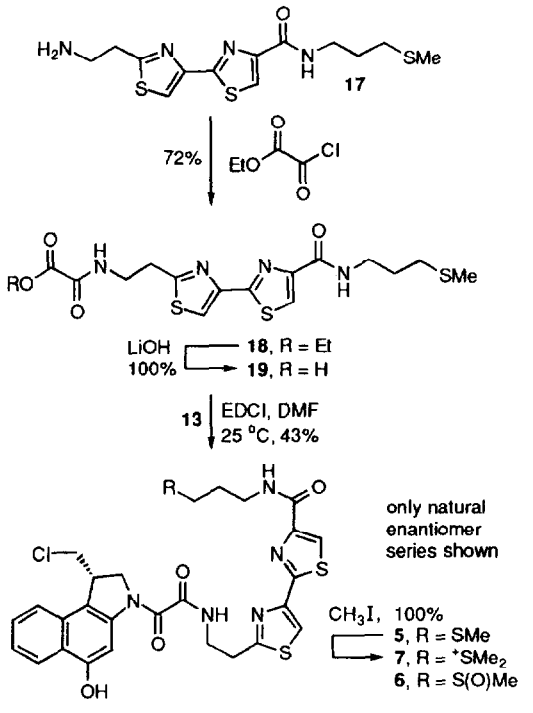
Scheme 1.

Preparation of 5–7: CBI joined with dipeptide S employing a rigid dicarbonyl linker

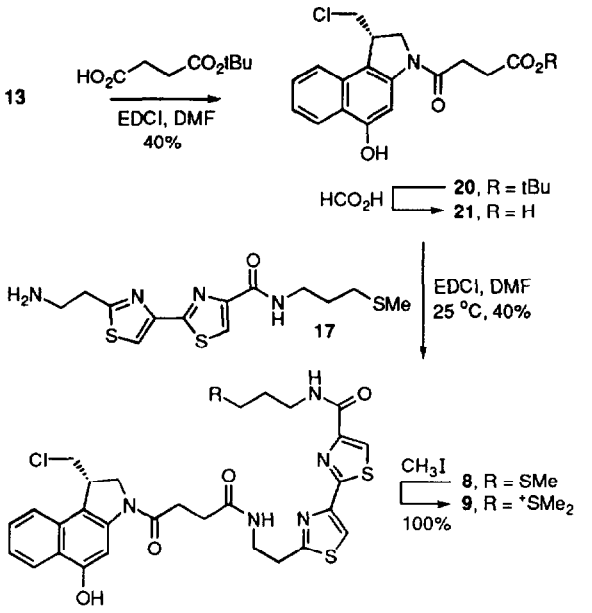
The first series of agents prepared include **5–7** in which the precursor to the CBI alkylation subunit was joined with dipeptide S through a rigid dicarbonyl linker (Scheme 2). Acylation of **17**[19] with ethyl oxalyl chloride (2 equiv, DMF, 25 °C, 20 h, 72%) followed by ethyl ester hydrolysis of **18** (5 equiv LiOH, 3:1:1 THF:CH_3OH:H_2O, 25 °C, 2 h, 100%) and direct coupling of **19** with freshly generated **13** (1.5 equiv ECDI, DMF, 25 °C, 17 h) deliberately conducted in the absence of added base[23] cleanly provided **5**. On occasions of prolonged exposure of **5** to air in the course of its purification, significant amounts of the corresponding sulfoxide **6** were isolated (20–25%) and independently characterized. This interesting variant of **5** and **7** embodies the DNA binding domain of bleomycin A_1, a minor constituent of the naturally occurring bleomycins. *S*-Methylation of **5** (100 equiv CH_3I, DMF, 25 °C, 67 h, 100%) cleanly provided the sulfonium salt **7**.

Preparation of 8 and 9: CBI joined with dipeptide S employing a flexible four-carbon linker

In efforts to ensure that the rigid and potentially labile dicarbonyl linker of **5–7** might not be uniquely influencing the properties of the agents, **8** and **9** were prepared in which the precursor to the CBI alkylation subunit was joined with dipeptide S through a flexible four-carbon linker (Scheme 3). Without optimization, coupling of freshly generated **13** with *t*-butyl hemisuccinate[24] (3 equiv EDCI, DMF, 25 °C, 21 h) deliberately conducted in the absence of added base[23] provided **20**. Acid-catalyzed deprotection of **20** (HCO_2H, 25 °C, 3 h) followed by coupling of crude carboxylic acid **21** with **17**[19] (3 equiv EDCI, DMF, 25 °C, 17 h, 40%) again conducted in the deliberate absence of added base[23] provided **8**. Subsequent *S*-methylation of **8** (100 equiv CH_3I, DMF, 25 °C, 144 h, 100%) cleanly provided **9**.



Scheme 2.



Scheme 3.

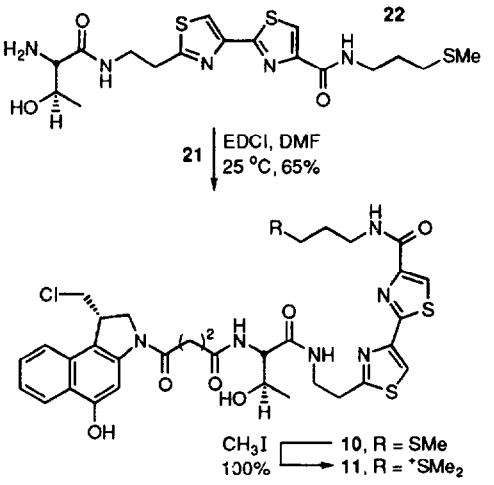
The alternative approach of first coupling **17** with succinic anhydride (2.5 equiv, cat $CoCl_2$, 2 equiv iPr_2NEt, CH_3CN, 25 °C, 58 h, 70%) followed by coupling of the resulting carboxylic acid[25] with freshly generated **13** necessarily conducted in the absence of added base[23] failed to provide **8** due to competitive internal iminolactone formation.

Preparation of 10 and 11: CBI joined with tripeptide S employing a flexible four carbon linker

The final series of agents prepared for examination include **10** and **11** in which the precursor to the CBI alkylation subunit was joined with tripeptide S through a flexible four-carbon linker. Following an approach analogous to that detailed for **8** and **9**, the acid-catalysed deprotection of **20** (HCO_2H, 25 °C, 1.5 h) and immediate coupling of **21** with **22**[19] (2.5 equiv EDCI, 1.1 equiv HOBt, 25 °C, 47 h, 65%) cleanly provided **10** (Scheme 4). Subsequent *S*-methylation of **10** (100 equiv CH_3I, DMF, 25 °C, 88 h, 100%) cleanly provided **11**.

In vitro cytotoxic activity

Summarized in Table 1 is the L1210 cytotoxic activity of the agents **5–11**, the comparison samples **14–16** of CBI acylated with the linkers only, and a representative range of additional comparison agents including the natural products **1–3**. The comparison agents **14–16** exhibited properties consistent with past observations in which the simple *N*-acyl CBI derivatives exhibited cytotoxic activity in the 5–200 nM range approximately



Scheme 4.

10^3–$10^4\times$ less potent than the natural products or the more advanced CBI-based analogues and the natural enantiomers were found to be 2–50× more potent than the corresponding unnatural enantiomers. Analogous to prior observations, no distinctions were observed between the ring-opened precursor **15** and the corresponding agent **16** containing the cyclopropane. Interestingly, the natural enantiomers of **15** and **16** exhibited low nM cytotoxic activity (5–6 nM IC_{50}, L1210) and are among the most potent simple derivatives disclosed to date, cf. **23**–**27** (Fig. 4).

In sharp contrast, the agents **5**–**11** incorporating the di- or tripeptide S DNA binding domain of bleomycin A_2 linked to the agents **14**–**16** exhibited much lower cytotoxic activity typically being 10^2–$10^3\times$ less potent than **14**–**16** themselves and 10^5–$10^6\times$ less potent than the natural products. Only the two enantiomers of **5** approached the cytotoxic potency of **14**, its corresponding CBI building block, and its structure represents that of the series which incorporates the least essential components of the bleomycin A_2 DNA binding domain. The remainder exhibited substantially diminished properties.

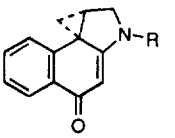
DNA alkylation properties

The agents **14**–**16** exhibited DNA alkylation properties analogous to those of *N*-BOC-CBI (Fig. 5). Within w794 DNA,[26] the agents alkylated DNA at concentrations of 10^{-1}–10^{-3} M which is 10^3–$10^4\times$ less efficient than **1**–**3** and did so with alkylation of the same sites (5′-AA>5′-TA) independent of the absolute configuration. Analogous to their relative cytotoxic potencies, the natural enantiomers were approximately 10× more effective than the corresponding unnatural enantiomers. Further consistent with its cytotoxic properties, the natural enantiomer of **16** was 10–100× more effective at alkylating DNA than *N*-BOC-CBI (**23**) and **14** was also found to be more effective. Thus, the attached linkers did not diminish, and in fact enhanced, the DNA alkylation efficiencies.

In contrast, the first set of hybrid agents examined including **5**–**7** failed to provide evidence of detectable, thermally sensitive alkylation[27] of DNA even under vigorous reaction conditions (37 °C, 72 h) at agent concentrations as high as 10^{-2} M. Analogous to the relative cytotoxic activity of the agents, the attachment of the CBI alkylation subunit to the bleomycin bithiazole *C*-terminus using the dicarbonyl linker resulted in diminished adenine N3 alkylation characteristic of **1**–**3**. Similarly, the methyl sulfides **8** and **10** incorporating the flexible four carbon linker and the unmethylated di- and tripeptide S *C*-terminus, respectively, failed to alkylate DNA at concentrations of 10^{-3} M or lower and failed to produce thermally labile adducts. This is illustrated nicely in Figure 6 with **8** where both enantiomers of *N*-BOC-CBI alkylate DNA at 10^{-2} M but no reaction is observed for **8**. Only the agents **9** and **11** incorporating the flexible four-carbon linker

Table 1. In vitro cytotoxic activity

Agent	Configuration	IC_{50} (L1210, nM)
(+)-**1**	Natural	0.02
ent-(−)-**1**	Unnatural	0.02
(+)-**2**	Natural	0.01
ent-(−)-**2**	Unnatural	0.10
(+)-**3**	Natural	0.5
ent-(−)-**3**	Unnatural	≥22
(1*S*)-**5**	Natural	500
ent-(1*R*)-**5**	Unnatural	500
(1*S*)-**6**	Natural	>1500
ent-(1*R*)-**6**	Unnatural	>1500
(1*S*)-**7**	Natural	>1300
ent-(1*R*)-**7**	Unnatural	>1300
(1*S*)-**8**	Natural	1000
(1*S*)-**9**	Natural	2100
(1*S*)-**10**	Natural	3100
(1*S*)-**11**	Natural	7700
(1*S*)-**14**	Natural	250
ent-(1*R*)-**14**	Unnatural	600
(1*S*)-**15**	Natural	6
ent-(1*R*)-**15**	Unnatural	200
(+)-**16**	Natural	5
ent-(−)-**16**	Unnatural	200
(+)-**23**	Natural	80
ent-(−)-**23**	Unnatural	1000
(+)-**24**	Natural	200
(+)-**25**	Natural	140
(+)-**26**	Natural	110
(+)-**27**	Natural	25



23, R = CO_2tBu
24, R = CONHMe
25, R = CO_2Me
26, R = COEt
27, R = SO_2Et

Figure 4.

and the fully functionalized di- and tripeptide S C-terminus provided a thermally labile DNA alkylation reaction, but did so in a manner only slightly more effective than **16**. Moreover, detectable alkylation required vigorous reaction conditions (37 °C, 48–72 h), prolonged reaction times, and proceeded with a selectivity (5′-AA, 5′-TA) that was analogous to that observed with *N*-BOC-CBI and **16**. Thus, while exhibiting properties better than **5–7** or **8** and **10**, the agents were only comparable to **15** and **16**. Thus, their DNA alkylation efficiency was not significantly enhanced by their attachment to the C-terminus of bleomycin A_2 and their inherent DNA alkylation selectivity was not altered.

Incubation of the agents with calf thymus DNA under comparable conditions (37 °C, 48–72 h, 1:51 agent: base-pair ratio) followed by recovery of unreacted agent by extraction (**5**, **6**, **8**, and **10**) or DNA precipitation (**9** and **11**) confirmed that the observations are the result of a diminished DNA alkylation capability and not attributable to alternative DNA alkylation reactions that fail to provide thermally labile adducts (Table 2). Both enantiomers of **5** and **6** and the natural enantiomers of **8** and **10** were recovered nearly quantitatively from the DNA reaction mixtures even under prolonged vigorous reaction conditions (37 °C, 72 h) conducted in the presence of excess DNA. Only **9** and **11** exhibited perceptible covalent attachment to the calf thymus DNA consistent with their modest DNA alkylation capabilities observed in the sequencing studies.

Discussion

Thus, the attachment of the CBI alkylation subunit characteristic of CC-1065 and the duocarmycins to the *C*-terminus DNA binding domain of bleomycin A_2 did not led to enhancement of the DNA alkylation or cytotoxic properties of the resulting hybrid agents and, in some instances, lead to diminished properties. This is in sharp contrast to the impact of the conventional DNA binding domains of CC-1065 and related analogues which leads to a 10^3–$10^4\times$ enhancement in DNA alkylation efficiencies and cytotoxic potencies. In addition to illustrating the important complementary nature of these two functions of DNA binding and subsequent DNA alkylation in the natural products and their closely related analogues, the results have significant implications on the behavior of both bleomycin A_2 and CC-1065/duocarmycin. The most obvious is that the *C*-terminus of bleomycin A_2 does not appear to

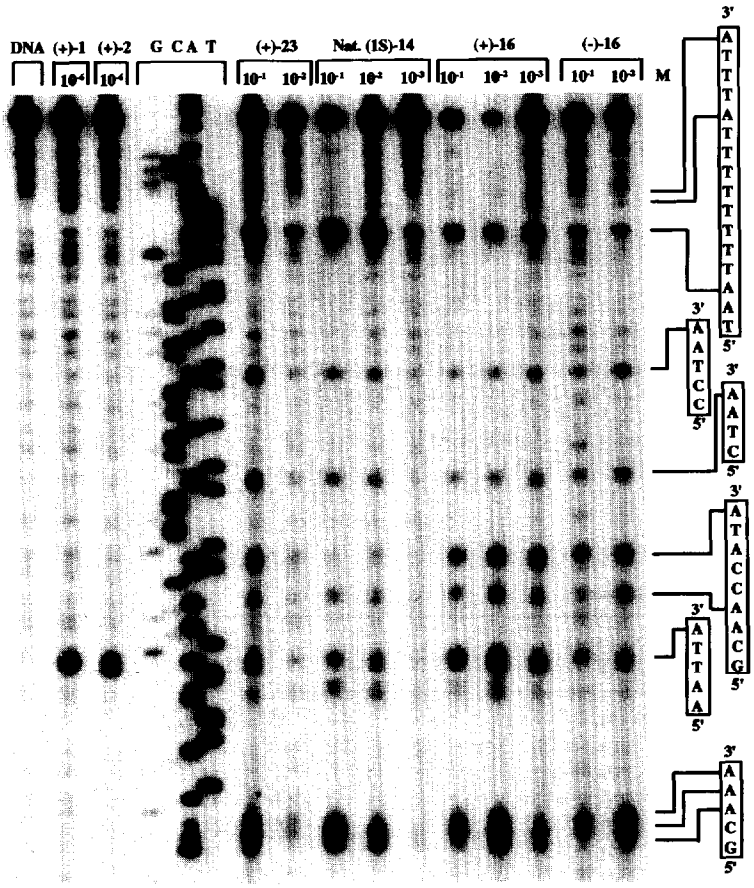


Figure 5. Thermally-induced strand cleavage of double-stranded DNA (SV40 DNA fragment, 144 bp, nucleotide no. 5238–138, clone w794) after 72 h incubation of agent with DNA at 37 °C followed by removal of unbound agent, and 30 min incubation at 100 °C, 8% denaturing PAGE, and autoradiography: lane 1, control DNA; lane 2, (+)-CC-1065 (**1**, 1×10^{-6} M); lane 3, (+)-duocarmycin SA (**2**, 1×10^{-6} M); lanes 4–7, Sanger G, C, A, and T sequencing reactions; lanes 8 and 9, (+)-*N*-BOC-CBI [(+)-**23**, 1×10^{-1} and 1×10^{-2} M]; lanes 10–12, (1*S*)-**14** (1×10^{-1} to 1×10^{-3} M); lanes 13–15, (+)-**16** (1×10^{-1} to 1×10^{-3} M); lanes 16 and 17, (−)-**16** (1×10^{-1} and 1×10^{-2} M).

behave as an AT-rich minor groove binding domain analogous to the right-hand subunits of CC-1065 and the duocarmycins. Although minor groove binding has been suggested to be a productive DNA binding mode for bleomycin A_2 and even suggested to be responsible for the sequence selective polynucleotide recognition, the results are more consistent with expectations resulting from bithiazole intercalative binding. This mode of binding would not be expected to selectively deliver the alkylation subunit to the DNA minor groove and might, in fact, inhibit such delivery. In addition, the alkylation selectivity of **9** and **11** was identical to that of *N*-BOC-CBI (**23**) and **16** which lack the bleomycin A_2 DNA binding domain and all were much less selective than **1–3**. Thus, the attachment of the DNA binding domain of bleomycin A_2 did not alter

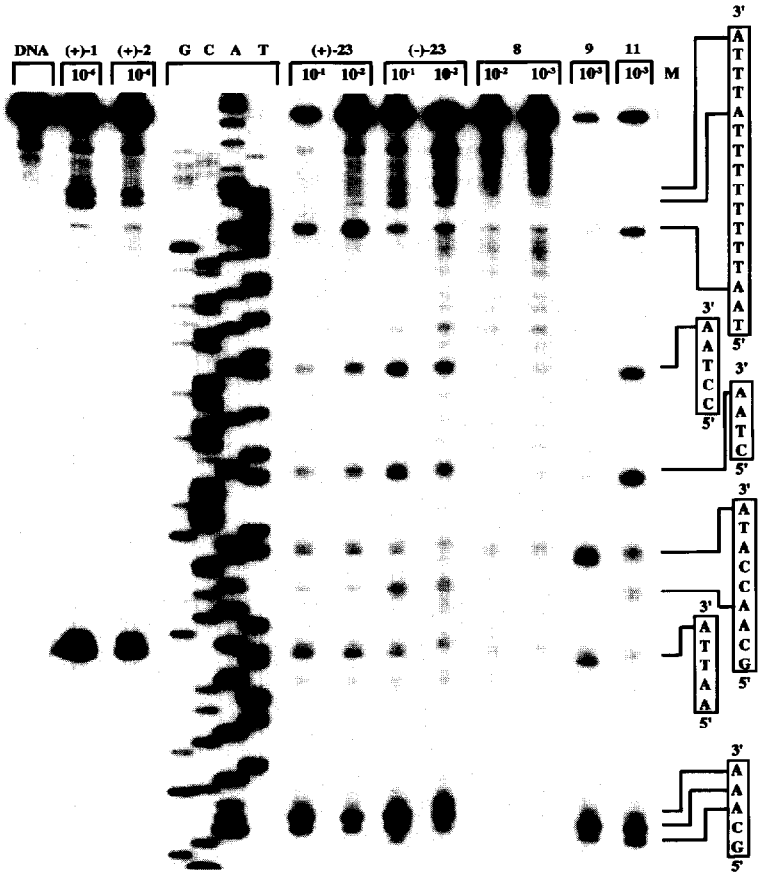


Figure 6. Thermally-induced strand cleavage of double-stranded DNA (SV40 DNA fragment, 144 bp, nucleotide no. 5238–138, clone w794) after 48 h incubation of agent with DNA at 37 °C followed by removal of unbound agent, and 30 min incubation at 100 °C, 8% denaturing PAGE, and autoradiography: lane 1, control DNA; lane 2, (+)-CC-1065 (**1**, 1×10^{-6} M); lane 3, (+)-duocarmycin SA (**2**, 1×10^{-6} M); lanes 4–7, Sanger G, C, A, and T sequencing reactions; lanes 8 and 9, (+)-*N*-BOC-CBI [(+)-**23**, 1×10^{-1} and 1×10^{-2} M]; lanes 10 and 11, (−)-*N*-BOC-CBI [(−)-**23**, 1×10^{-1} and 1×10^{-2} M]; lanes 12 and 13, **8** (1×10^{-2} and 1×10^{-3} M); lane 14, **9** (1×10^{-3} M); lane 15, **11** (1×10^{-3} M).

Table 2. Calf thymus DNA alkylation and recovery[a]

Agent	(1) % Recovery (λ_{max}, A)[b]	(2) % Recovery (λ_{max}, A)[b]	Control[c] (λ_{max}, A)[b]
5	95% (298 nm, 0.39)	88% (297 nm, 0.36)	(297 nm, 0.41)
ent-**5**	100% (296 nm, 0.60)	100% (296 nm, 0.61)	(297 nm, 0.55)
6	100% (298 nm, 0.42)	100% (298 nm, 0.39)	(299 nm, 0.33)
ent-**6**	100% (298 nm, 0.44)	100% (298 nm, 0.42)	(297 nm, 0.38)
8	100% (296 nm, 0.76)	100% (297 nm, 0.77)	(297 nm, 0.68)
9	63% (291 nm, 0.32)	nd	(294 nm, 0.51)
10	90% (297 nm, 0.47)	80% (298 nm, 0.41)	(298 nm, 0.52)
11	72% (290 nm, 0.33)	nd	(295 nm, 0.46)

[a]Incubation carried out at 37 °C, 48–72 h. Unreacted agent recovered by extraction or DNA precipitation, see text.
[b]UV λ_{max} and absorbance.
[c]Control recovery without DNA following identical incubation conditions.

the DNA alkylation selectivity of the CBI alkylation subunit (5′-AA>5′-TA) in a manner that would reflect any sequence selective binding by this component of bleomycin nor did it enhance the selectivity in manner that approaches the five base-pair AT-rich alkylation selectivity of **1**.

Experimental

1-Chloromethyl-3-(2-ethoxy-1,2-dioxoethyl)-5-hydroxy-1,2-dihydro-3*H*-benz[*e*]indole (14). A sample of **13** freshly generated from **12**[15] (3.6 mg. 0.01 mmol) by treatment with 3.6 N HCl–EtOAc (25 °C, 30 min) was treated with ethyl oxalyl chloride (3.0 mg, 0.022 mmol, 2.0 equiv) in THF (0.5 mL) in the presence of $NaHCO_3$ (2.7 mg, 0.03 mmol, 3.0 equiv) and the reaction mixture was stirred at 25 °C for 2 h. The solvent was removed under a stream of N_2. Chromatography (SiO_2, 0.8×5 cm, 50% EtOAc–hexane) afforded **14** (3.6 mg, 3.6 mg theoretical, 100%) as a solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.13 (br s, 1H), 8.37 (s, 1H), 8.31 (d, J=8.3 Hz, 1H), 8.68 (d, J=8.2 Hz, 1H), 7.54 (dd, J=7.2, 8.3 Hz, 1H), 7.44 (dd, J=7.2, 8.2 Hz, 1H), 4.40–4.60 (m, 4H), 4.07 (m, 1H), 3.94 (dd, J=3.1, 11.3 Hz, 1H), 3.43 (t, J=11.1 Hz, 1H), 1.56 (t, J=7.2 Hz, 3H); IR (neat) ν_{max} 3239, 1732, 1640, 1581, 1438; 1397, 1360, 1245, 1227, 1121, 854, 776, 753 cm^{-1}; FABHRMS (NBA–NaI) *m/z* 356.0678 (M+Na$^+$, $C_{17}H_{16}ClNO_4$ requires 356.0666). Natural (1*S*)-**14**: $[\alpha]_D^{25}$ −81 (*c* 0.2, $CHCl_3$). *Ent*-(1*R*)-**14**: $[\alpha]_D^{25}$ +90 (*c* 0.25, $CHCl_3$).

1-Chloromethyl-3-(4-ethoxy-1,4-dioxobutyl)-5-hydroxy-1,2-dihydro-3*H*-benz[*e*]indole (15). A sample of **13** freshly generated from **12**[15] (5.3 mg, 0.016 mmol) by treatment with 3.6 N HCl–EtOAc (25 °C, 30 min) was treated with ethyl succinyl chloride (2.6 mg, 0.016 mmol, 1.0 equiv) in THF (0.5 mL) in the presence of $NaHCO_3$ (3.3 mg, 0.04 mmol, 2.5 equiv) and the mixture was stirred at 25 °C for 1 h. The solvent was removed under a stream of N_2. PTLC (SiO_2, 0.25 mm×20×20 cm, 50% EtOAc–hexane) afforded **15** (5.8 mg, 5.8 mg theoretical, 100%) as a solid: ^{1}H NMR (acetone-d_6, 400 MHz) δ 9.20 (s, 1H), 8.20 (d, J=8.4 Hz, 1H), 8.05 (s, 1H), 7.81 (d, J=8.4 Hz, 1H), 7.50 (dd, J=2.9, 8.4 Hz, 1H), 7.34 (dd, J=7.9, 8.4 Hz, 1H), 4.36 (m, 1H), 4.18 (m, 1H), 4.10 (q, J=7.2 Hz, 2H), 4.02 (dd, J=3.1, 11.1 Hz, 1H), 3.72 (dd, J=9.1, 11.1 Hz, 1H), 2.83 (t, J=6.4 Hz, 2H), 2.66 (t, J=6.4 Hz, 2H), 1.22 (t, J=7.2 Hz, 3H); IR (neat) ν_{max} 3290, 1718, 1635, 1578, 1473, 1430, 1395, 1382, 1246, 1181, 1132, 861 770, 748 cm^{-1}; FABHRMS (NBA–NaI) *m/z* 362.1148 (M + H$^+$, $C_{19}H_{20}ClNO_4$ requires 362.1159). Natural (1*S*)-**15**: $[\alpha]_D^{25}$ −32 (*c* 0.3, THF). *Ent*-(1*R*)-**15**: $[\alpha]_D^{25}$ +39 (*c* 0.15, THF).

***N*^{2}-(4-Ethoxy-1,4-dioxobutyl)-1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]indole-4-one (16).** A sample of **15** (3.5 mg, 9.7 μmol) was placed in 5% aq $NaHCO_3$: THF (1:1, 500 μL) and the mixture was stirred at 25 °C for 9 h before the solvent was removed under a stream of N_2. PTLC (SiO_2, 0.25 mm×20×20 cm, 50% THF–hexane) afforded **16** (2.5 mg, 3.2 mg theoretical, 79%) as a white solid: ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.07 (d, J=7.8 Hz, 1H), 7.54 (dd, J=7.6, 7.8 Hz, 1H), 7.39 (dd, J=7.6, 7.8 Hz, 1H), 7.12 (d, J=7.8 Hz, 1H), 4.28 (m, 2H), 4.09 (q, J=7.1 Hz, 2H), 3.10 (m, 1H), 2.81 (t, J=6.8 Hz, 2H), 2.62 (t, J=6.8 Hz, 2H), 1.70 (dd, J=4.2, 4.8 Hz, 1H), 1.54 (apparent t, J=4.6 Hz, 1H), 1.21 (t, J=7.1 Hz, 3H); IR (neat) ν_{max} 2933, 1727, 1693, 1624, 1594, 1560, 1402, 1389, 1368, 1235, 1167, 1017, 859, 782 cm^{-1}; FABHRMS (NBA) *m/z* 326.1382 (M+H$^+$, $C_{19}H_{19}NO_4$ requires 326.1392). Natural (+)-**16**: $[\alpha]_D^{25}$ +133 (*c* 0.13, THF). *Ent*-(−)-**16**: $[\alpha]_D^{25}$ −150 (*c* 0.12, THF).

3-[2′-(2-(2-Ethoxy-1,2-dioxoethyl)aminoethyl)-2,4′-bithiazole-4-carboxamido]propyl methyl sulfide (18). A solution of **17**[19] (13.7 mg, 0.04 mmol) in DMF (0.04 mL) was treated with ethyl oxalyl chloride (9.1 μL, 0.08 mmol, 2.0 equiv) and the mixture was stirred under Ar at 25 °C for 20 h before the solvent was removed under vacuum. Chromatography (SiO_2, 0.8×12 cm, 70% EtOAc–hexane) afforded **18** (12.7 mg, 17.7 mg theoretical, 72%) as an off white solid: R_f = 0.62 (SiO_2, 1×6.5 cm, 10% CH_3OH–CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.11 (s, 1H), 8.06 (t, J=6.7 Hz, 1H), 7.87 (s, 1H), 7.55 (t, J=6.1 Hz, 1H), 4.35 (q, J=7.2 Hz, 2H), 3.85 (dt, J=6.1, 6.3 Hz, 2H), 3.58 (dt, J=6.7, 6.7 Hz, 2H), 3.28 (t, J=6.3 Hz, 2H), 2.60 (t, J=7.2 Hz), 2.11 (s, 3H), 1.95 (tt, J=7.2, 6.7 Hz, 2H); IR (neat) ν_{max} 3325, 3113, 2920, 1733, 1693, 1658, 1545, 1480, 1436, 1371, 1297, 1205, 1114, 1053, 1018, 805, 766 cm^{-1}; FABHRMS (NBA) *m/z* 443.0889 (M+H$^+$, $C_{17}H_{22}N_4O_4S_3$ requires 443.0881).

3-[2′-(2-(2-Hydroxy-1,2-dioxoethyl)aminoethyl)-2,4′-bithiazole-4-carboxamido]propyl methyl sulfide (19). A solution of **18** (12.7 mg, 0.029 mmol) in THF:H_2O:CH_3OH (3:1:1, 0.45 mL) was treated with LiOH (6.0 mg, 0.14 mmol, 5.0 equiv) and the mixture was stirred at 25 °C for 2 h before the solvent was removed in vacuo. The crude product was dissolved in H_2O and was acidified to pH 0.5 with the addition of 10% aqueous HCl. The product was extracted with 30% isopropanol–$CHCl_3$ (7×1.2 mL), and the combined extracts were concentrated to afford **19** (11.9 mg, 11.9 mg theoretical, 100%) as an off-white solid which was sufficiently pure to use in the next reaction directly: ^{1}H NMR (CD_3OD, 400 MHz) δ 8.16 (s, 1H), 8.14 (s, 1H), 3.71 (t, J = 6.8 Hz, 2H), 3.51 (t, J = 7.0 Hz, 2H), 3.30 (t, 2H, overlapped with CH_3OH), 2.58 (t, J = 7.2 Hz, 2H), 2.10 (s, 3H), 1.92 (tt, J = 7.2, 7.0 Hz, 2H); IR (film) ν_{max} 3346, 3102, 2916, 1656, 1543, 1480, 1436, 1362, 1294, 1240, 1128, 1054 cm^{-1}; FABHRMS (NBA) *m/z* (M+H$^+$, $C_{15}H_{18}N_4O_4S_3$ requires 415.0568).

3-[2′-(2-(2-(1-Chloromethyl-5-hydroxy-1,2-dihydro-3*H*-benz[*e*]indol-3-yl)-1,2-dioxoethyl)aminoethyl)-2,4′-bithiazole-4-carboxamido]propyl methyl sulfide (5). A sample of **13** freshly generated from **12**[15] (5.3 mg, 0.016

mmol, 1.5 equiv) by treatment with 4 N HCl–EtOAc (25 °C, 30 min) was treated with **19** (4.4 mg, 0.011 mmol, 1.0 equiv) and EDCI (3.0 mg, 0.016 mmol, 1.5 equiv) in DMF (0.2 mL) under Ar and the mixture was stirred at 25 °C for 17 h. The DMF was removed and the crude product was placed in H_2O (0.2 mL). The aqueous phase was extracted with $CHCl_3$ (3 × 0.3 mL) and 50% hexane–EtOAc (2 × 0.3 mL). The combined organic extracts were concentrated in vacuo. PCTLC (SiO_2, 0.25 mm × 20 × 20 cm, 5% CH_3OH–CH_2Cl_2) afforded **5** (2.9 mg, 6.7 mg theoretical, 43%) as a tan solid: ^1H NMR ($CDCl_3$, 400 MHz) δ 8.34 (t, J = 6.1 Hz, 1H), 8.22 (d, J = 8.2 Hz, 1H), 8.14 (s, 1H), 7.95 (s, 1H), 7.71 (d, J = 8.3 Hz, 1H), 7.55 (m, 2H), 7.43 (dd, J = 8.1, 7.1 Hz, 1H), 3.89 (m, 3H), 3.53 (dt, J = 5.9, 6.1 Hz, 2H), 3.45 (t, J = 11.0 Hz, 1H), 3.38 (t, J = 6.4 Hz, 2H), 2.56 (t, J = 7.2 Hz, 2H), 2.09 (s, 3H), 1.90 (tt, J = 7.2, 6.1 Hz, 2H); IR (neat) ν_{max} 3322, 3115, 2917, 2842, 1645, 1574, 1541, 1518, 1410, 1391, 1358, 1254, 1123, 1019, 854, 806, 759 cm^{-1}; FABHRMS (NBA) *m/z* 630.1054 ($M+H^+$, $C_{28}H_{28}ClN_5O_4S_3$ requires 630.1070). Natural (1*S*)-**5**: $[\alpha]_D^{25}$ −22 (*c* 0.05, $CHCl_3$). *Ent*-(1*R*)-**5**: $[\alpha]_D^{25}$ +22 (*c* 0.05, $CHCl_3$).

3-[2′-(2-(2-(1-Chloromethyl-5-hydroxy-1,2-dihydro-3*H*-benz[*e*]indol-3-yl)-1,2-dioxoethyl)aminoethyl)-2,4′-bithiazole-4-carboxamido]propyl methyl sulfoxide (6). Samples of **6** (1.5 mg, 22%) were obtained as byproducts in the preparation of **5**. For **6**: ^1H NMR ($CDCl_3$, 400 MHz) δ 8.66 (m, 1H), 8.25 (d, J = 8.5 Hz, 1H), 8.07 (s, 1H), 7.91 (m, 1H), 7.76 (s, 1H), 7.68 (d, J = 8.3 Hz, 1H), 7.52 (dd, J = 8.2, 8.2 Hz, 1H), 7.40 (dd, J = 7.7, 7.5 Hz, 1H), 4.96 (dt, J = 12.7, 1.8 Hz, 1H), 4.63 (dt, J = 12.7, 2.4 Hz, 1H), 4.02 (m, 1H), 3.89 (m, 3H), 3.67 (m, 3H), 3.42 (dd, J = 10.0, 11.0 Hz, 1H), 3.35 (m, 3H), 2.64 (s, 3H), 2.19 (m, 2H); IR (neat) ν_{max} 3324, 3272, 3113, 3012, 2920, 2851, 1643, 1580, 1548, 1516, 1480, 1446, 1412, 1395, 1360, 1290, 1249, 1149, 1122, 1059, 1005, 948, 855 812, 755 cm^{-1}; FABHRMS (NBA) *m/z* 777.9980 ($M+Cs^+$, $C_{28}H_{28}ClN_5O_5S_3$ requires 777.9995). Natural (1*S*)-**6**: $[\alpha]_D^{25}$ −10 (*c* 0.07, $CHCl_3$). *Ent*-(1*R*)-**6**: $[\alpha]_D^{25}$ +10 (*c* 0.050, $CHCl_3$).

3-[2′-(2-(2-(1-Chloromethyl-5-hydroxy-1,2-dihydro-3*H*-benz[*e*]indol-3-yl)-1,2-dioxoethyl)aminoethyl)-2,4′-bithiazole-4-carboxamido]propyl dimethyl sulfonium iodide (7). A solution of **5** (2.9 mg, 0.0046 mmol) in DMF (0.2 mL) was treated with CH_3I (29 μL, 0.46 mmol, 100 equiv) and the mixture was stirred under Ar at 25 °C for 67 h. Evaporation of solvent and trituration with $CHCl_3$ (5 × 0.1 mL) afforded pure **8** (3.6 mg, 3.6 mg theoretical, 100%) as a yellow solid: ^1H NMR (CD_3OD, 400 MHz) δ 8.19 (m, 3H), 7.86 (s, 1H), 7.75 (d, J = 8.3 Hz, 1H), 7.52 (m, 1H), 7.37 (m, 1H), 4.59 (dd, J = 12.2, 2.0 Hz, 1H), 4.42 (dd, J = 12.1, 8.6 Hz, 1H), 4.07 (m, 1H), 3.90 (dd, J = 11.2, 3.2 Hz, 1H), 3.81 (t, J = 6.7 Hz, 2H), 3.57 (m, 3H), 3.39 (m, 4H), 2.94 (s, 6H), 2.15 (tt, J = 6.7, 6.6 Hz, 2H); IR (neat) ν_{max} 3426, 3015, 2769, 1646, 1467, 1431, 1410, 1390, 1251, 1112, 1051, 1015 cm^{-1}; FABHRMS (NBA) *m/z* 644.1238 (M^+, $C_{29}H_{31}ClN_5O_4S_3$ requires 644.1227). Natural (1*S*)-**7**: $[\alpha]_D^{25}$ −8.3 (*c* 0.08, CH_3OH). *Ent*-(1*R*)-**7**: $[\alpha]_D^{25}$ +8.5 (*c* 0.06, CH_3OH).

1-Chloromethyl-5-hydroxy-1,2-dihydro-3*H*-3-[*N*-(4-*tert*-butyloxy-1,4-dioxobutyl)]benz[*e*]indole (20). A freshly prepared sample of **13** generated by treatment of **12**[15] (10 mg, 0.03 mmol) with 4 N HCl–EtOAc (25 °C, 30 min) in DMF (0.75 mL) was treated with *tert*-butyl hemisuccinate[24] (7.8 mg, 0.045 mmol, 1.5 equiv) and EDCI (17.3 mg, 0.09 mmol, 3.0 equiv) and the mixture was stirred under Ar at 25 °C for 21 h. The solvent was removed under vacuum. Chromatography (SiO_2, 8 × 10 cm, 7% Et_2O–CH_2Cl_2) afforded **20** (4.7 mg, 11.7 mg theoretical, 40%): ^1H NMR ($CDCl_3$, 400 MHz) δ 9.25 (s, 1H), 8.30 (d, J = 8.3 Hz, 1H), 8.20 (s, 1H), 7.58 (d, J = 8.3 Hz, 1H), 7.49 (dd, J = 7.0, 8.3 Hz, 1H), 7.37 (dd, J = 7.0, 8.3 Hz, 1H), 4.28 (m, 2H), 3.89 (m, 2H), 3.36 (t, J = 10.6 Hz, 1H), 2.81 (m, 4H), 1.45 (s, 9H); IR (neat) ν_{max} 3133, 2971, 1726, 1649, 1582, 1476, 1451, 1429, 1415, 1388, 1375, 1334, 1249, 1145, 844, 754 cm^{-1}; FABHRMS (NBA–NaI) *m/z* 389.1399 (M^+, $C_{21}H_{24}ClNO_4$ requires 389.1394). Natural (1*S*)-**20**: $[\alpha]_D^{25}$ −58 (*c* 0.1, $CHCl_3$). *Ent*-(1*R*)-**20**: $[\alpha]_D^{25}$ +60 (*c* 0.4, $CHCl_3$).

3-[2′-(2-(2-(1-Chloromethyl-5-hydroxy-1,2-dihydro-3*H*-benz[*e*]indol-3-yl)-1,4-dioxobutyl)aminoethyl)-2,4′-bithiazole-4-carboxamido]propyl methyl sulfide (8). A sample of **20** (3.7 mg, 0.009 mmol) was treated with formic acid (2 mL) at 25 °C for 3 h. The formic acid was removed by evaporation under a stream of N_2. The crude acid **21** in DMF (0.35 mL) was treated with **17**[19] (3.9 mg, 0.011 mmol, 1.2 equiv), EDCI (5.5 mg, 0.029 mmol, 3.0 equiv) and the mixture was stirred under Ar at 25 °C for 38 h before the solvent was removed in vacuo. PCTLC (SiO_2, 0.25 mm × 20 cm × 20 cm, 3% CH_3OH–CH_2Cl_2) afforded **8** as a light yellow solid (2.3 mg, 6.2 mg theoretical, 37%): ^1H NMR (CD_3OD, 400 MHz) δ 8.15 (d, J = 8.3 Hz, 1H), 8.10 (s, 1H), 8.06 (s, 1H), 7.83 (s, 1H), 7.70 (d, J = 7.9 Hz, 1H), 7.48 (dd, J = 7.0, 7.9 Hz, 1H), 7.32 (dd, J = 7.0, 8.3 Hz, 1H), 4.27 (m, 2H), 4.05 (m, 1H), 3.94 (dd, J = 3.0, 11.0 Hz, 1H), 3.65 (t, J = 6.4 Hz, 2H), 3.58 (dd, J = 9.0, 11.0 Hz, 1H), 3.48 (t, J = 6.8 Hz, 2H), 3.26 (m, 2H, overlapped with solvent), 2.70–2.90 (m, 1H), 2.59 (m, 2H), 2.09 (s, 3H), 1.91 (tt, J = 6.8, 7.1 Hz, 2H); IR (neat) ν_{max} 3302, 3112, 2911, 1643, 1574, 1542, 1479, 1416, 1389, 1363, 1247, 1131, 756 cm^{-1}; FABHRMS (NBA) *m/z* 790.0330 ($M^+ + Cs$, $C_{30}H_{32}ClN_5O_4S_3$ requires 790.0359). Natural (1*S*)-**8**: $[\alpha]_D^{25}$ +10 (*c* 0.13, $CHCl_3$).

3-[2′-(2-(4-(1-Chloromethyl-5-hydroxy-1,2-dihydro-3*H*-benz[*e*]indol-3-yl)-1,4-dioxobutyl)aminoethyl)-2,4′-bithiazole-4-carboxamido]propyl dimethyl sulfonium iodide (9). A solution of **8** (1.9 mg, 0.003 mmol) in DMF (0.19 mL) was treated with CH_3I (41 mg, 0.29 mmol, 100 equiv) in DMF and the mixture was stirred under Ar at 25 °C for 120 h. Additional CH_3I (41 mg, 0.29 mmol, 100 equiv) was added and after an additional 22 h, DMF was removed in vacuo. The residue was purified by trituration with $CHCl_3$ (7 × 0.3 mL) to afford **9** (2.3 mg, 2.3 mg theoretical, 100%): ^1H

NMR (DMSO-d_6, 400 MHz) δ 10.36 (s, 1H), 8.68 (t, J = 6.1 Hz, 1H), 8.31 (s, 1H), 8.20 (t, J = 5.7 Hz, 1H), 8.16 (s, 1H), 8.09 (d, J = 8.4 Hz, 1H), 7.97 (s, 1H), 7.80 (d, J = 8.4 Hz, 1H), 7.51 (dd, J = 7.6, 8.4 Hz, 1H), 7.33 (dd, J = 7.6, 8.4 Hz, 1H), 4.35 (t, J = 10.7 Hz, 1H), 4.18 (m, 2H), 4.01 (dd, J = 1.9, 11.0 Hz, 1H), 3.80 (dd, J = 7.9, 10.8 Hz, 1H), 3.40–3.60 (m, 8H, overlapped with H_2O in DMSO-d_6), 3.33 (t, J = 7.6 Hz, 2H), 3.22 (t, J = 6.9 Hz, 2H), 2.09 (s, 6H), 2.01 (tt, J = 7.4, 7.6 Hz, 2H); IR (neat) ν_{max} 3422, 1651, 1646, 1635, 1557, 1539, 1521, 1506, 1473, 1457, 1418, 1056, 1028, 1008 cm^{-1}; FABHRMS (NBA–CsI) m/z 672.1566 (M^+, $C_{31}H_{35}ClN_5O_4S_3$ requires 672.1540). Natural (1*S*)-**9**: $[\alpha]_D^{25}$ −12 (*c* 0.18, DMSO).

3-[2′-(2-((4-(1-Chloromethyl-5-hydroxy-1,1-dihydro-3*H*-benz[*e*]indol-3-yl)-1,4-dioxobutyl)-L-threonyl)aminoethyl)-2,4′-bithiazole-4-carboxamido]propyl methyl sulfide (10). A sample of **20** (3.6 mg, 0.009 mmol) was treated with formic acid (1 mL) at 25 °C for 1.5 h before the solvent was removed by a stream of N_2. Crude **21** in DMF (0.3 mL) was treated with **22**[19] (4.0 mg, 0.009 mmol, 1.0 equiv), EDCI (4.4 mg, 0.023 mmol, 2.5 equiv) and HOBt (1.4 mg, 0.01 mmol, 1.1 equiv) and the mixture was stirred at 25 °C for 47 h. The solvent was removed in vacuo. PCTLC (SiO_2, 0.25 mm × 20 × 20 cm, 5% CH_3OH–CH_2Cl_2) afforded **10** (4.5 mg, 6.9 mg theoretical, 65%) as an orange solid: ^1H NMR (CD_3OD, 400 MHz) δ 8.06 (d, J = 8.4 Hz, 1H), 7.94 (s, 1H), 7.91 (s, 1H), 7.75 (s, 1H), 7.64 (d, J = 8.3 Hz, 1H), 7.44 (dd, J = 7.7, 8.3 Hz, 1H), 7.27 (dd, J = 7.7, 8.4 Hz, 1H), 4.40 (m, 1H), 4.27 (m, 3H), 4.05 (m, 1H), 3.96 (dd, J = 3.1, 11.2 Hz, 1H), 3.06 (m, 1H), 2.78 (m, 2H), 2.55 (t, J = 7.0 Hz, 3H), 2.09 (s, 3H), 1.89 (tt, J = 7.0, 7.2 Hz, 2H), 1.22 (d, J = 5.8 Hz, 3H); IR (neat) ν_{max} 3320, 2924, 1652, 1637, 1579, 1545, 1478, 1420, 1246, 749 cm^{-1}; FABHRMS (NBA–CsI) m/z 891.0827 (M^+ + Cs, $C_{34}H_{39}ClN_6O_6S_3$ requires 891.0836). Natural (1*S*)-**10**: $[\alpha]_D^{25}$ −218 (*c* 0.2, $CHCl_3$).

3-[2′-(2-((4-(1-Chloromethyl-5-hydroxy-1,2-dihydro-3*H*-benz[*e*]indol-3-yl)-1,4-dioxobutyl)-L-threonyl)aminoethyl)-2,4′-bithiazole-4-carboxamido]propyl dimethyl sulfonium iodide (11). A solution of **10** (2.2 mg, 0.003 mmol) in DMF (0.17 mL) was treated with CH_3I (41.2 mg, 0.29 mmol, 100 equiv) and the mixture was stirred under Ar at 25 °C for 88 h. The solvent was removed by evaporation. Pure **10** was obtained by trituration with $CHCl_3$ (8 × 0.5 mL) to afford **11** (2.6 mg, 2.6 mg theoretical, 100%) as a yellow solid: ^1H NMR (DMSO-d_6, 400 MHz) δ 10.33 (s, 1H), 8.63 (t, J = 5.9 Hz, 1H), 8.27 (s, 1H), 8.08 (s, 1H), 8.07 (d, J = 8.3 Hz, 1H), 8.00 (t, J = 5.7 Hz, 1H), 7.94 (s, 1H), 7.83 (d, J = 8.4 Hz, 1H), 7.77 (d, J = 8.3 Hz, 1H), 7.47 (dd, J = 7.5, 8.3 Hz, 1H), 7.30 (dd, J = 7.5, 8.4 Hz, 1H), 4.34 (t, J = 10.4 Hz, 1H), 3.56 (m, 1H), 3.40–3.50 (m, 8H, overlapped with H_2O in DMSO-d_6), 3.30 (t, J = 7.5 Hz, 2H), 3.15 (t, J = 6.8 Hz, 2H), 2.87 (s, 6H), 1.98 (tt, J = 6.6, 6.8 Hz, 2H), 1.03 (d, J = 6.4 Hz, 3H); IR (neat) ν_{max} 3317, 1648, 1633, 1555, 1535, 1516, 1502, 1473, 1453, 1414 cm^{-1}; FABHRMS (NBA) m/z 773.2050 (M^+, $C_{35}H_{42}ClN_6O_6S_3$ requires 773.2017). Natural (1*S*)-**11**: $[\alpha]_D^{25}$ −9.2 (*c* 0.1, DMSO).

DNA alkylation of w794 DNA. Eppendorf tubes containing the 5′-end labeled DNA (9 μL) in TE buffer (10 mM Tris, 1 mM EDTA, pH 7.2) were treated with the agent in DMSO (1 μL at the specified concentration). The solution was mixed by vortexing and brief centrifugation and subsequently incubated at 37 °C for 76 h (both enantiomers of **5**, **6**, **7**, **14**, **16** and **23**) and 48 h (**8**, **9**, **10** and **11**). The covalently modified DNA was separated from unbound agent by EtOH precipitation and resuspended in TE buffer (10 μL). The solution of DNA in an Eppendorf tube sealed with Teflon tape was warmed at 100 °C for 30 min to induce cleavage at the alkylation sites, allowed to cool to 25 °C and centrifuged. Formamide dye (0.03% xylene cyanol FF, 0.03% bromophenol blue, 8.7% Na_2EDTA 250 mM) was added (5 μL) to the DNA solution. Prior to electrophoresis, the sample was denatured by warming at 100 °C for 5 min, placed in an ice bath, and centrifuged, and the solution (4 μL) was loaded onto the gel. Sanger dideoxynucleotide sequencing reactions were run as standards adjacent to the reaction samples. Polyacrylamide gel electrophoresis (PAGE) was run on an 8% sequencing gel under denaturing conditions (8 M urea) in TBE buffer (10 mM Tris, 100 mM boric acid, 0.2 mM Na_2EDTA) followed by autoradiography.

DNA alkylation of calf thymus DNA. An aliquot of agent (5 μL, 0.01 M in DMSO) was added to a calf thymus DNA solution (0.45 mL, 3.79 mg mL^{-1}, 10 mM sodium phosphate, pH 7.0, base-pair:agent = 51:1). The DNA–agent mixtures were incubated at 37 °C for 72 h for both enantiomers of **5**, **6** and **7** or at 37 °C for 48 h for **8**, **9**, **10** and **11**. For both enantiomers of **5**, **6**, **8** and **10**, the unreacted materials were extracted with EtOAc (0.5 mL × 4). The combined extracts were dried and dissolved in EtOAc (0.9 mL) and the quantities of **5**, **6**, **8**, and **10** were determined by UV. Additional extraction with EtOAc (0.5 mL × 4) was carried out, and the amounts of combined material were determined by UV. No additional material was recovered. For the ionic agents **7**, **9** and **11**, the unreacted materials were recovered from the supernatants of EtOH precipitation of the DNA. The EtOH in the supernatant was removed by a stream of N_2. The supernatants were diluted to 0.9 mL with H_2O and the recovered quantities of **7**, **9**, and **11** were determined by UV.

These studies were conducted alongside control reactions conducted in the absence of DNA. An aliquot of agent (5 μL, 0.01 M in DMSO) was added to sodium phosphate buffer (0.45 mL, 10 mM, pH 7.0). The agent–buffer mixtures were incubated at 37 °C for 72 h for both enantiomers of **5**, **6** and **7** or at 37 °C for 48 h for **8**, **9**, **10** and **11**. For **5**, **6**, **8** and **10**, the materials were extracted with EtOAc (0.5 mL × 4). The combined extracts were dried and dissolved in EtOAc (0.9 mL) and the agent quantities were determined by UV. For the ionic agents **7**, **9** and **11**, the agent–buffer

mixtures were diluted to 0.9 mL with H_2O and their quantities were determined by UV.

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