# Unsymmetrical DNA Cross-Linking Agents: Combination of the CBI and PBD Pharmacophores

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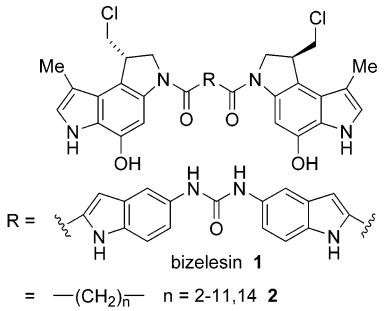
*Received November 20, 2002*

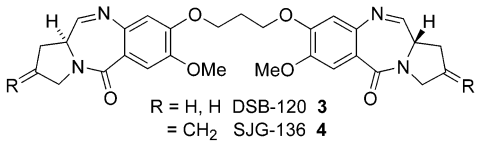
A set of 10 compounds, each combining the *seco*-1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one (*seco*-CBI) and pyrrolo[2,1-*c*][1,4]benzodiazepine (PBD) pharmacophores, was designed and prepared. These compounds were anticipated to cross-link between N3 of adenine and N2 of guanine in the minor groove of DNA. The compounds, which differ in the chain length separating the two alkylation subunits, and the configuration of the CBI portion, showed great variation in cellular toxicity (over 4 orders of magnitude in a cell line panel) with the most potent example exhibiting $IC_{50}$s in the pM range. Cytotoxicity correlated with the ability of the compounds to cross-link naked DNA. Cross-linking was also observed in living cells, at much lower concentrations than for a related symmetrical PBD dimer. A thermal cleavage assay was used to assess sequence selectivity, demonstrating that the CBI portion controlled the alkylation sites, while the PBD substituent increased the overall efficiency of alkylation. Several compounds were tested for in vivo activity using a tumor growth delay assay against WiDr human colon carcinoma xenografts, with one compound (the most cytotoxic and most efficient cross-linker) showing a statistically significant increase in survival time following a single iv dose.

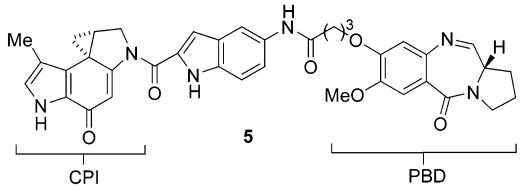
## Introduction

DNA cross-linking agents constitute an important class of antitumor drugs.[1] However, several of those used in the clinic (e.g., nitrogen mustards, cisplatin, mitomycin C) are compromised by serious side effects associated with their poor selectivity for tumor cells. Also, indiscriminate alkylation of multiple DNA sites, or other cellular targets, makes it difficult to determine their precise mechanism of action. Several attempts have been made in recent years to construct new cross-linking compounds by the dimerization of known and more discriminating monoalkylating agents. Unusual biological activities can be anticipated for those compounds which cross-link at new sites (e.g., major or minor groove, adenine or guanine bases), with different selectivity or efficiency, or with various degrees of distortion of the DNA structure. A further focus of recent studies has been to increase the size of the dimer recognition site. The hope is that enhanced sequence selectivity might increase selectivity for tumor cells,[2,3] with the ultimate goal of targeting predefined DNA sites[4] and thereby specifically inhibiting the expression of those proteins critical for tumor cell proliferation.[5]

Two classes of minor groove monoalkylating agents that have been used in these studies are the cyclopropaindolones[6] and pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs),[7] each of which possess significant sequence selectivity as monoalkylating agents. Cyclopropaindolone dimers[8] are exemplified by bizelesin **1**. In this compound two *seco*-CPI[9] alkylating units derived from the natural product CC-1065 are linked by a rigid bis(indolyl)urea.[10] Bizelesin very efficiently forms an interstrand cross-link between the N3 position of two adenines 6 or 7 base pairs (bps) apart in the minor groove, favoring AT-rich sequences such as 5′-TAATAA*-3′.[11,12] Bizelesin is both more sequence selective and cytotoxic than the monomeric agents from which it is derived. Excellent preclinical activity was observed in mice,[13] and bizelesin advanced to clinical trial,[14] although significant myelotoxicity has been reported[15] (in

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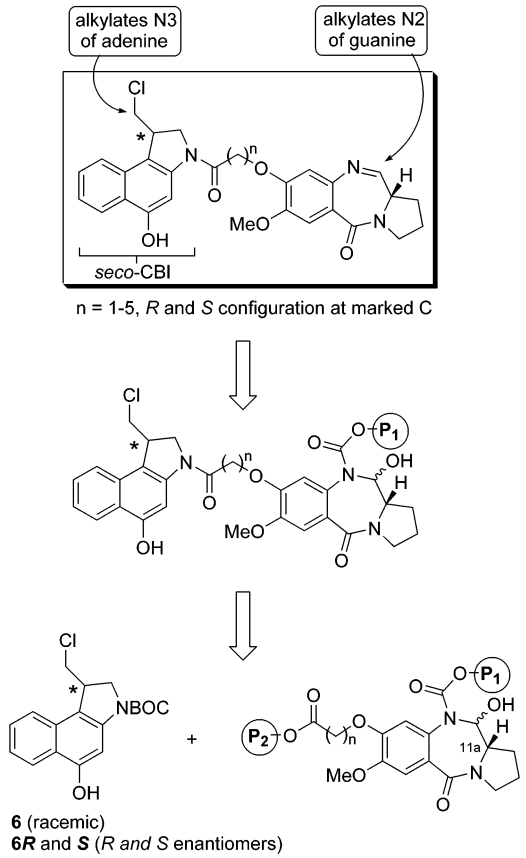
10.1021/jm020526p CCC: $25.00 
Published on Web 04/25/2003

**Scheme 1.** Target Compounds and Retrosynthesis Analysis




common with several cyclopropaindolone monomers[16]). Bizelesin itself was a development of earlier flexibly linked *seco*-CPI dimers **2** in which cross-linking ability and cytotoxicity were found to be related to the number of carbons in the linking polymethylene chain.[17] Other variants of cyclopropaindolone dimers have since been reported,[18] including extension to the *seco*-CBI (*seco*-1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one, see Scheme 1) alkylation subunit,[19] which has the advantages of being more synthetically accessible, more stable, and more cytotoxic than it's CPI analogue.[20]

An example of a recently developed PBD dimer is DSB-120 **3**,[21] formed by linking two molecules of the PBD monomer DC-81 (see Scheme 5). DSB-120 was one example selected from several dimers which differ in the length of the connecting chain.[22] Dimerization increases the ability of these compounds to raise the melting temperature of DNA, significantly enhances their cytotoxicity, and extends the sequence selectivity beyond the 5′-PuG*Pu-3′ motif favored by the monomers. Molecular modeling and NMR studies of DSB-120 demonstrated minor groove interstrand cross-linking between the exocyclic amino groups of two guanines 4 bps apart, with the molecule spanning a 6 bp sequence.[23] Although DSB-120 showed minimal antitumor activity in a murine model,[24] an analogue with C2 *exo*-methylene substitution (SJG-136, **4**)[25] has very recently been selected for clinical development on the basis of activity in the National Cancer Institute's In Vitro and Hollow Fiber Assays.[26]

Given the success of this dimerization strategy to provide interesting and biologically active molecules, we have combined the CBI and PBD units to construct a new class of unsymmetrical minor groove cross-linking agents. These were anticipated to alkylate between N3 of adenine and N2 of guanine, a previously unknown DNA lesion. During the course of this work a report appeared describing the synthesis of a single CPI−PBD dimer **5**, designed and demonstrated to cross-link the 6 bp sequence 5′-CAATTA*-3′.[27] Herein we provide full details of our own work in this area.

## Synthesis

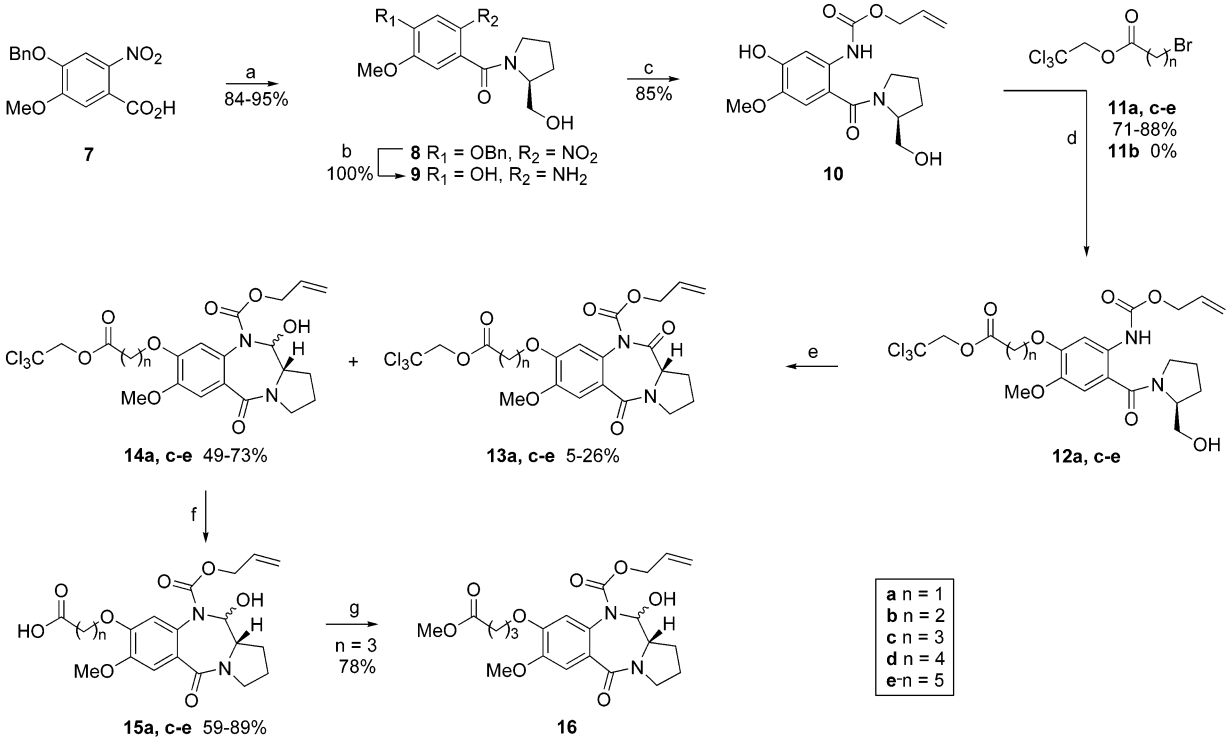
The target compounds for synthesis in the current study are represented by the general structure highlighted in Scheme 1, i.e., minimal CBI and PBD pharmacophores joined by the simplest possible linker, a polymethylene chain. A set of 10 compounds was envisaged in which the linking chain contained between one and five carbon atoms ($n = 1-5$, to span a range which should include matches to the bp register) and, for each chain length, both enantiomers of the CBI subunit. The latter was considered important since it is known that the cyclopropaindolone enantiomeric form affects sequence selectivity (and toxicity)[28,29] and, depending on the binding preferences of the PBD component of the dimers, could affect the balance of inter- versus intrastrand cross-linking.

The most sensitive functional group in the target molecules was anticipated to be the PBD imine, which is very reactive to nucleophilic addition. The formation of the imine was therefore planned as the final step, from a suitably protected carbamate ($\mathbf{P_1}$ in Scheme 1) of the corresponding hemiaminal. This $\mathbf{P_1}$ group must be removed under conditions which are not strongly basic, reductive, or nucleophilic—and the Aloc protecting group, used by others in PBD syntheses[25,30] was considered a suitable candidate. Retrosynthetic disconnection at the amide bond gives two fragments: the pair of resolved Boc-protected CBI enantiomers **6*R*** and **6*S***, and a set of variable chain length PBD esters. The former was prepared by the known route, in six steps from 1,3-dihydroxynaphthalene[20,31] and resolved by semipreparative chiral HPLC, also as previously described.[29] For the latter, an ester protecting group $\mathbf{P_2}$ was needed that was orthogonal to $\mathbf{P_1}$ and also did not require basic conditions for cleavage, as these would be expected to racemize the crucial C11a position.[32]

The synthesis of the requisite PBD acids is illustrated in Scheme 2. Vanillin was converted in three steps as described to the nitrobenzoic acid **7**,[33] which was coupled with commercially available (*S*)-2-pyrrolidinemethanol to give the known amide **8**.[34] Hydrogenation over Pd/C removed the benzyl protecting group and reduced the nitro substituent to the aniline. The latter was selectively reacted with allyl chloroformate at low temperature to introduce the Aloc group in **10**, while leaving the phenol unaffected (reaction at room temperature gave ca. 10% of the bis-Aloc product).

The phenol **10** was then alkylated with a variety of 4-bromobutanoic esters (i.e., $n = 3$ chain length) in test reactions to find an ester protecting group suitable for the following steps. For example, reaction with the TCE (trichloroethyl) ester **11c** gave **12c**, which was oxidized with DMP (the Dess−Martin periodinane). The intermediate aldehyde spontaneously cyclized to generate the

**Scheme 2**[a]




[a] Conditions: (a) $(COCl)_2$, DMF, then (*S*)-2-pyrrolidinemethanol, $K_2CO_3$; (b) $H_2$, Pd/C; (c) allyl chloroformate, py, −78 °C; (d) $K_2CO_3$, DMF, 20 °C; (e) DMP; (f) Zn powder, $HCO_2H$; (g) $CH_2N_2$.

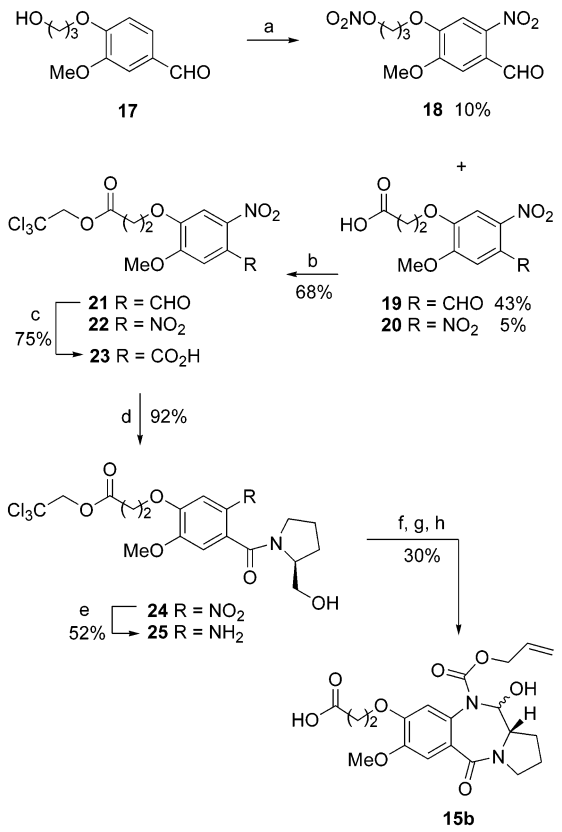
diazepine ring in **14c**. Although this reaction was accompanied by some overoxidation to the dione **13c**, requiring careful chromatographic separation, DMP oxidation was found to be considerably higher yielding than either Swern oxidation or the use of TPAP (tetrapropylammonium perruthenate). Cleavage of the TCE ester was accomplished using $Zn/HCO_2H$,[35] giving the desired acid **15c** in 89% yield. Not only was this higher yielding than some alternatives (Zn/aq $KH_2PO_4$[36] or Zn/aq $NH_4OAc$[37]), it also gave the product with the highest optical rotation, a value that was unchanged on repeated exposure to the reaction conditions. A portion of **15c** was also converted to the methyl ester **16** and examined by chiral HPLC: in comparison with a sample obtained via base cleavage of the ester **14c**, which caused complete epimerization, **16** was clearly uncontaminated by racemization at C11a (see Supporting Information).

Other possible ester protecting groups were less successful: although both the TMSE (trimethylsilylethyl) ester and t-Bu ester analogues of **11c** gave good yields in the alkylation and oxidation steps, deprotection of the ester resulted in decomposition (TMSE ester, HF/py), racemization (TMSE ester, TBAF or CsF), or considerably lower yields (t-Bu ester, HCl).

The TCE ester route was applied to the other chain lengths ($n$ = 1, 2, 4, 5) and gave similar yields in all steps, except for the propionate ester ($n$ = 2) for which the phenol alkylation failed completely, due to reverse Michael reaction. This necessitated an alternative synthesis for the $n$ = 2 analogue, as shown in Scheme 3. Vanillin was alkylated with 3-bromopropanol to give **17**, which underwent nitration using $HNO_3$ to incorporate the 5-$NO_2$ group and also oxidize the side-chain alcohol to the acid **19**. This reaction is well precedented for vanillic acid analogues and has been used in PBD syntheses in the past.[38] In this case the reaction was not clean, and along with the desired product there was also a significant amount of the nitrate **18**, as well as ipso substitution of the aldehyde to give the dinitro acid **20**. The latter cocrystallized with **19**, but was easily separated following the later aldehyde oxidation. The acid was protected as the TCE ester **21**, the aldehyde oxidized to the benzoic acid **23** (using sodium chlorite[39]), and the latter coupled with (*S*)-2-pyrrolidinemethanol to give **24**. Reduction of the nitro group in the presence of the TCE ester presented some problems—eventually, brief hydrogenation over Pd/C was chosen ahead of some reportedly more selective reagents ($Ni_2B$,[40] $PtO_2/H_2$) and gave the desired product **25** in a workable 52% yield. Steps as described above completed the synthesis of **15b** and furnished the complete set of five PBD acids.

The CBI and PBD units were linked together as shown in Scheme 4. The Boc protecting group was removed from the CBI with acid treatment, and the resulting amines immediately reacted with the PBD acids using EDCI. The final step, removal of the Aloc protecting group using catalytic Pd(0), was not initially successful and prompted some model reactions using an equimolar mixture of the PBD ester **16** and the Boc-protected CBI **6**. It was found that weakly basic allyl cation trapping reagents (e.g., dimedone[41]) were not able to prevent significant allylation of the CBI phenol (nor was *p*-methoxyphenol, tested as a more nucleophilic phenol competitor), while stronger bases such as morpholine or pyrrolidine caused competing spirocyclization of the CBI. Eventually it was found that very brief exposure to $Pd(PPh_3)_4$ and an excess of pyrrolidine (10 equiv, 1−5 min, 20 °C), followed by direct transfer of the concentrated reaction mixture to the top of a

**Scheme 3**[a]




[a] Conditions: (a) c. $HNO_3$, 0-20 °C; (b) $(COCl)_2$, DMF, then $Cl_3CCH_2OH$: (c) $NaClO_2$, $H_2O_2$; (d) $(COCl)_2$, DMF, then (*S*)-2-pyrrolidinemethanol, $K_2CO_3$; (e) $H_2$, Pd/C; (f) allyl chloroformate, py, −78 °C; (g) DMP; (h) Zn powder, $HCO_2H$.

chromatography column and immediate elution, provided the desired products in good yield (59−81%). This method was successful too for the $n = 2$ analogues where again a reverse Michael reaction could have interfered. In one case (**27a*S***) the final product could not be adequately purified, but the nine remaining compounds were shown to be pure by $^1H$ NMR and HPLC analysis (>97%), and were carried forward to further testing.

Several reference compounds were also synthesized. The PBD monomer DC-81 **31** was prepared as shown in Scheme 5. Reduction of **8** with $SnCl_2$ selectively reduced the nitro group as reported.[34] The resulting aniline **28** was subjected to the same steps as above: reaction with allyl chloroformate, DMP oxidation, and cleavage of the Aloc protecting group to give the intermediate **30**, previously prepared by an alternative route.[42] The benzyl ether was removed, employing the reported conditions[42] to give **31** in moderate yield, isolated for the first time as an analytically pure solid. Interestingly, the measured optical rotation of **31** ($[\alpha_D] = +1239°$) was considerably higher than the literature values for any of the previous synthetic samples ($[\alpha_D] = +310°$,[42] $+315°$,[43] $+371°$[44]).[45] We note that the solvent used in all cases was $CHCl_3$, which commonly contains 1−2% EtOH as a stabilizer. Our measurements were recorded using dried and fractionally distilled $CHCl_3$, and we found that addition of 2% EtOH to the $CHCl_3$ solution immediately reduced the measured $[\alpha_D]$ by more than half.[46]

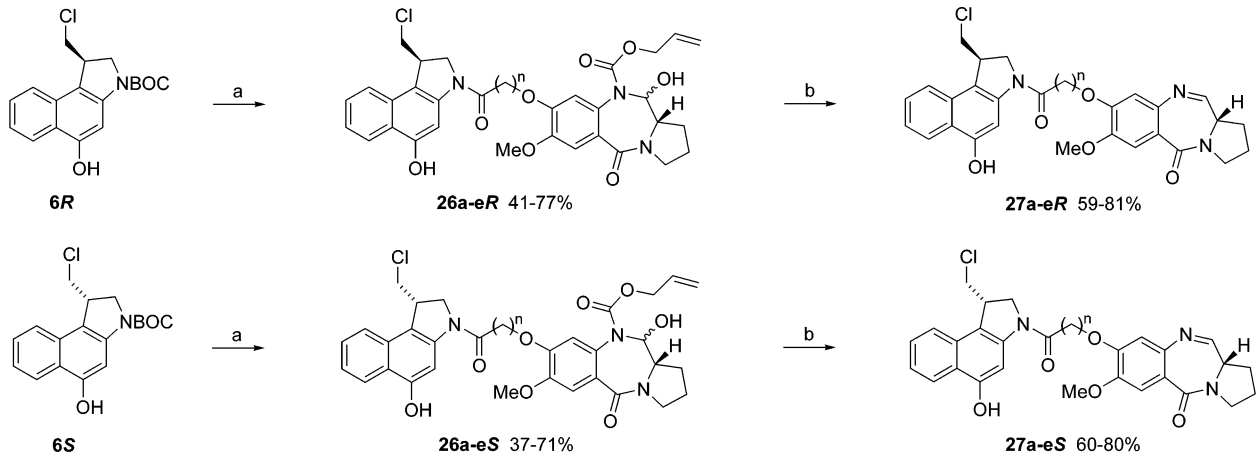
The symmetrical PBD dimer, DSB-120, was prepared using similar chemistry (Scheme 6): the intermediate **10** was alkylated with 0.5 equiv of 1,3-dibromopropane, the product was oxidized with DMP, and the Aloc protecting groups were removed. The measured optical rotation of the analytically pure solid ($[\alpha_D] = +1140°$) was again much higher than that previously reported for this compound as an oil ($[\alpha_D] = +330°$).[47]

The enantiomeric pair of CBIs in which the minor groove binding component is truncated to a simple acetyl substituent was also synthesized (**32*R*** and **32*S***, Scheme 7), along with the previously described CBI analogue **33**[29] bearing the trimethoxyindole substituent common to the duocarmycin natural products.

Finally, two compounds were prepared that closely mimic the structure of one of the cross-linking agents (**27e*S***, $n = 5$, *S*-CBI enantiomer), except for minor changes that render either end incapable of alkylating DNA. For the 'PBD inactive' compound, the diazepine-5,11-dione **35** was chosen. Retaining $sp^2$ hybridization at C11 is important to maintain the same overall shape of the PBD, and diazepine-5,11-diones such as this have been used in the past as nonalkylating structural mimics of the PBDs.[48] **35** was synthesized as shown in Scheme 8, starting from **13e**, the byproduct from the DMP oxidation step.[49]

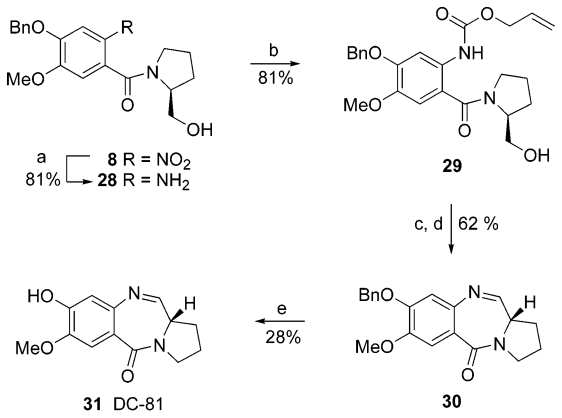
For the 'CBI-inactive' compound, the usual chloromethyl substituent was replaced with a simple methyl

**Scheme 4**[a]



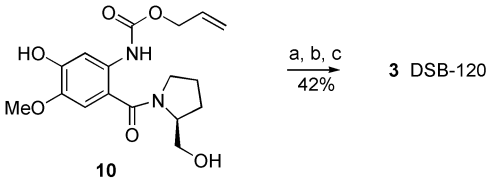

[a] Conditions: (a) HCl, dioxane, then **15a**−**e**, EDCI; (b) $Pd(PPh_3)_4$, pyrrolidine, $CH_2Cl_2$, 20 °C, ≤5 min.

**Scheme 5.** Synthesis of DC-81[a]



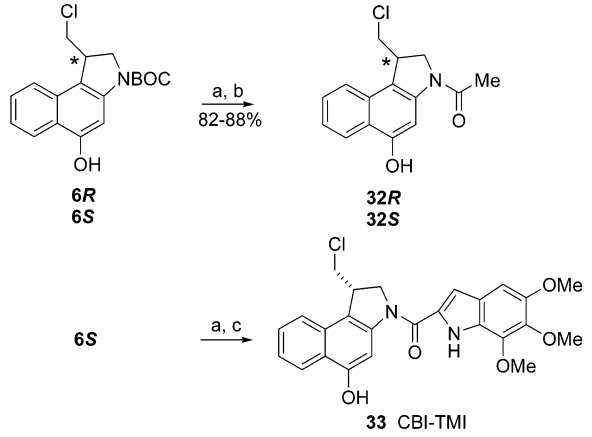

[a] Conditions: (a) $SnCl_2$; (b) allyl chloroformate, py, −0 °C; (c) DMP; (d) $Pd(PPh_3)_4$, pyrrolidine; (e) Pd/C, 1,4-cyclohexadiene.

**Scheme 6.** Synthesis of DSB-120[a]




[a] Conditions: (a) $Br(CH_2)_3Br$, $K_2CO_3$, DMF; (b) DMP; (c) $Pd(PPh_3)_4$, pyrrolidine.

**Scheme 7.** Synthesis of CBI Reference Compounds[a]




[a] Conditions: (a) HCl, dioxane; (b) AcCl, $NaHCO_3$; (c) TMI acid, EDCI.HCl.

group, as in structure **39**. The synthesis of this utilized an intermediate **36**[20] from the normal CBI synthesis (Scheme 8). Alkylation with allyl bromide provided **37**,[50] which underwent the expected radical ring closure, benzyl group deprotection, coupling with the PBD acid **15e**, and deprotection, to give the desired product **39**.

**In Vitro Cytotoxicity.** Cellular toxicities were determined as $IC_{50}$ values after 4 h of drug exposure in four cell lines: the Chinese hamster lines AA8 and UV4, the murine mammary carcinoma EMT6, and the human ovarian carcinoma Skov3.[51] The results for the CBI−PBD compounds, and for several reference compounds, are collected in Table 1.

Immediately apparent is the enormous variation in toxicity among the CBI−PBD compounds, with the ratio between the least potent **27b*R*** and most potent **27e*S*** being on the order of 35 000. There is also a clear pattern in the toxicity data, with the chain lengths $n =$ 3 and 5 being more potent than $n = 2$ and 4. Presumably this is due to a better match between the separation of the alkylating functional groups and the base pair register. A similar alternation in toxicity with polymethylene chain length has been observed with symmetrical dimers in both CPI[17] and PBD[22] classes, although the magnitude of the change was not so great. For each chain length the *S*-CBI enantiomer is the more potent, with the differential larger for the more toxic chain lengths. These trends are more clearly seen in Figure 1, where cytotoxicity in the AA8 cell line is plotted on a log scale against chain length of the CBI−PBD compounds.

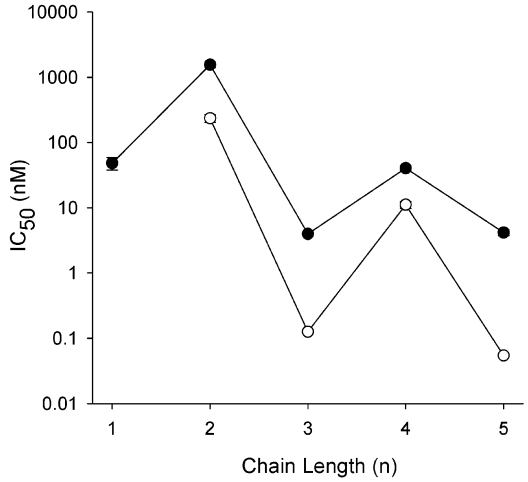


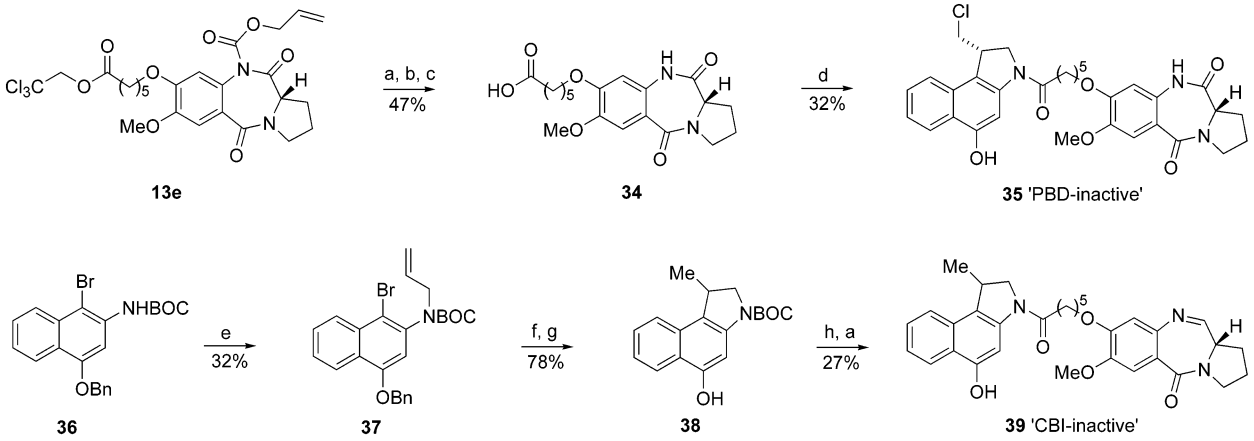

**Figure 1.** Cytotoxicity of the CBI−PBD compounds **27a**−**e*R*** and **27b**−**e*S*** in the AA8 cell line as a function of the linker chain length (*n*). Filled symbols, *R*-CBI enantiomer; open symbols, *S*-CBI enantiomer.

There are no particularly large variations in sensitivity to a given compound across the cell lines in Table 1. This is also true for **27c*S***, **27e*R***, **27e*S*** (and **3**) which were tested in a more extensive panel of human tumor cell lines: of colon (Colo205, HT29, WiDr), ovarian (Skov3), or cervical (SiHa) origin (Table 2). Since the Colo205 line is not adherent under culture conditions an alternative assay employing continuous 5 day drug exposure was used for this comparison.[52] Under these conditions of prolonged drug exposure Skov3 cells were 10−20 times more sensitive than in Table 1, but the relative toxicity of the four compounds is similar in both assays.

The similar sensitivity of the AA8 and UV4 cell lines (Table 1) is worthy of note. UV4 is a sub-line of AA8 that is defective in nucleotide excision repair as a result of a mutation in the ERCC1 gene[53] and is hypersensitive to many DNA alkylating agents. A hypersensitivity factor [HF = $IC_{50}$(AA8)/$IC_{50}$(UV4)] in the range 10−70 has been reported as typical of DNA cross-linking agents,[54] but for the CBI−PBD compounds the HF is clearly much lower than this and mostly falls in the range 1.5−3. It is possible that these compounds alkylate with little distortion of the DNA structure, so that the adducts are poorly recognized by the nucleotide excision repair pathway.

It is also interesting to compare cytotoxicities with those of the reference compounds in Table 1. DC-81 **31**, the PBD monomer, has $IC_{50}$s in the 1−2 $\mu$M range, whereas the symmetrical dimer based on DC-81, DSB-

**Scheme 8.** Synthesis of Monoalkylating Analogues[a]




[a] Conditions: (a) $Pd(PPh_3)_4$, pyrrolidine; (b) EDCI, THF; (c) Zn powder, $HCO_2H$; (d) **6*S***, HCl, doxane, then **34**, EDCI, DMA; (e) allyl bromide, NaH; (f) $Bu_3SnH$, AIBN, PhH; (g) Pd/C, $NH_4HCl_2$; (h) HCl, dioxane, then **15e**, EDCI, DMA.

**Table 1.** In Vitro Cytotoxicity (4 h exposure), NCI Results, and Cross-Linking Ability of CBI−PBDs and Reference Compounds

| compound | | | $IC_{50}$ (nM)[a] | | | | NCI screen[b] | | |
|---|---|---|---|---|---|---|---|---|---|
| | $n$[d] | enant[e] | AA8 | UV4 | EMT6 | Skov3 | $GI_{50}$ (nM)[f] | range[g] | $C_{50}$[c] ($\mu$M) |
| **27a*R*** | 1 | *R* | 48 | 6.1 | 5.0 | 16 | 91 | 200 | 1.21 |
| **27b*R*** | 2 | *R* | 1600 | 860 | 300 | 960 | 3200 | 35 | 7.90 |
| **27b*S*** | 2 | *S* | 240 | 160 | 51 | 200 | 500 | 50 | 5.00 |
| **27c*R*** | 3 | *R* | 4.0 | 0.83 | 0.30 | 1.9 | 13 | 3200 | 0.025 |
| **27c*S*** | 3 | *S* | 0.13 | 0.074 | 0.030 | 0.14 | <10 | >10000 | 0.017 |
| **27d*R*** | 4 | *R* | 40 | 14 | 12 | 35 | 130 | 190 | 1.05 |
| **27d*S*** | 4 | *S* | 11 | 5.9 | 3.1 | 11 | 79 | 160 | 0.56 |
| **27e*R*** | 5 | *R* | 4.2 | 1.5 | 0.56 | 3.0 | 18 | 2400 | 0.13 |
| **27e*S*** | 5 | *S* | 0.054 | 0.023 | 0.0078 | 0.032 | <10 | >10000 | 0.022 |
| **31** DC-81 | | | 2000 | 1500 | 1100 | 1900 | | | |
| **3** DSB-120 | | | 340 | 86 | 23 | 140 | | | 0.029 |
| **32*R*** CBI−Ac | | *R* | 14000 | 1800 | 12000 | 14000 | | | |
| **32*S*** CBI−Ac | | *S* | 210 | 59 | 280 | 360 | | | |
| **33** CBI-TMI | | *S* | 0.21 | 0.090 | 0.13 | 0.29 | | | |
| **35** 'PBD-inactive' | | *S* | 29 | 13 | 3.2 | 6.2 | | | |
| **39** 'CBI-inactive' | | | 4500 | 2500 | 840 | 2100 | | | |

[a] Drug concentration to reduce cell density to 50% of that of the controls. Values are the mean of 2−6 experiments. [b] Results from the National Cancer Institute's In Vitro Cell Line Screening Project. [c] Concentration required for 50% cross-linking of linearized pcDNA3 plasmid DNA following 18 h exposure at 37 °C. [d] Number of carbons in the linker chain. [e] Enantiomeric form at C1 of the *seco*-CBI. [f] Mean drug concentration required for 50% inhibition of growth compared to controls across the 60 cell line panel. [g] Ratio of $LC_{50}$ values between the least sensitive and most sensitive cell lines.

**Table 2.** In Vitro Cytotoxicity (5 day exposure) of Selected CBI−PBD Compounds and DSB-120 **3** against Human Tumor Cell Lines

| | $IC_{50}$ (nM)[a] | | | | |
|---|---|---|---|---|---|
| compound | Colo205 | HT29 | WiDr | Skov3 | SiHa |
| **27c*S*** | 0.037 | 0.021 | 0.014 | 0.021 | 0.012[b] |
| **27e*R*** | 0.18 | 0.25 | 0.28 | 0.20 | 0.20 |
| **27e*S*** | 0.0035 | 0.0027 | 0.0039 | 0.0033 | 0.0028 |
| **3** (DSB-120) | 7.0 | 8.7 | 10 | 6.3 | 7.3 |

[a] Drug concentration to reduce [$^3$H]thymidine uptake to 50% of that of the controls. Values are the mean of 2−3 experiments except where marked. [b] Single determination.

120 (**3**), is on average about 20 times as toxic. For DSB-120, the linker chain length was identified as being optimal for both toxicity and cross-linking ability.[22] However, if DC-81 carries an *S*-CBI substituent, even the poorest choice of linker ($n$ = 2) gives a compound **27b*S*** equitoxic with DSB-120, while the optimal linker ($n$ = 5), **27e*S***, increases toxicity by a further 3 orders of magnitude. This is clearly due to alkylation of DNA, rather than some favorable noncovalent interaction, since **39**, the 'CBI inactive' variant of **27e*S***, is no more toxic than DC-81 itself.

A similar comparison can be made considering the PBD as a substituent on a CBI parent. For example, the toxicity of the *S*-CBI acetamide **32*S*** is increased 13 000 times with an $n$ = 5 linked PBD substituent, **27e*S***, but only by 40-fold with a similarly spaced nonalkylating diazepinedione analogue, **35**. Compound **27e*S***, which has $IC_{50}$s in the pM range, is in fact more cytotoxic than **33**, although it lacks the indole substituent of the latter which is thought to be responsible for activation of CBI-type compounds in general on binding in the minor groove of DNA.[55]

Overall, the marked dependence of potency on linker chain length, and on the presence of two alkylation-competent functional groups, strongly suggests that DNA cross-linking is a major contributor to the observed toxicities of the CBI−PBD compounds.

**In Vitro Activity in the NCI Screen.** All of the CBI−PBD compounds were submitted for testing in the National Cancer Institute's In Vitro Cell Line Screening Project.[56] Each compound was tested against a panel of about 60 different human cancer cell lines derived from nine different tissue types, and the results were expressed as the concentration required for 50% inhibi-

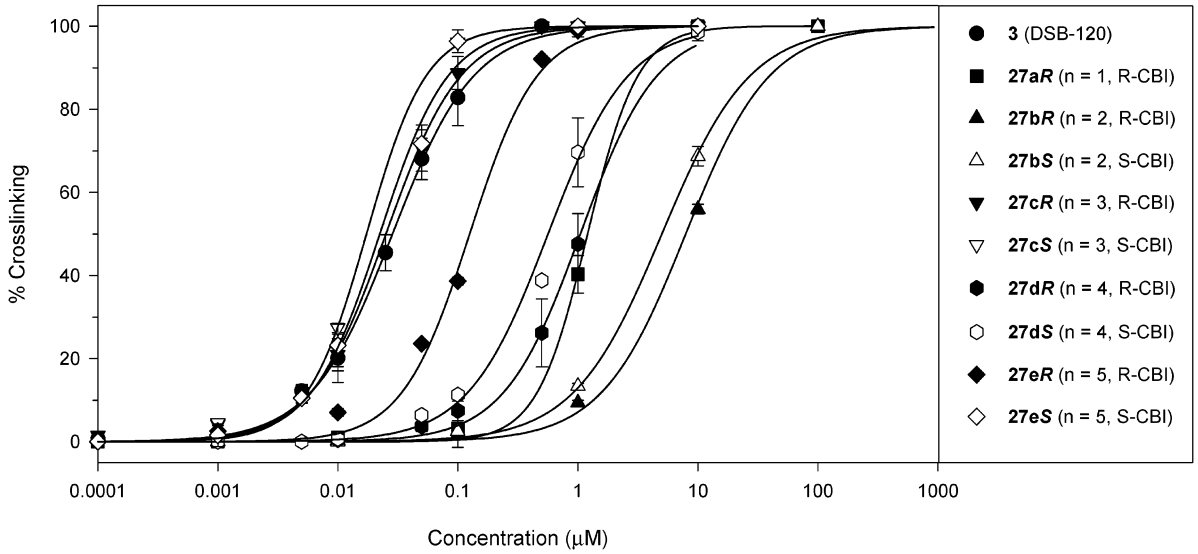



**Figure 2.** Extent of cross-linking of linearized pcDNA3 plasmid DNA by CBI−PBD compounds **27a**−**e*R*** and **27b**−**e*S*** and DSB-120 **3** following 18 h exposure at 37 °C. Fitted curves are Logistic 3-parameter sigmoidal curves with maximum *y* value constrained to 100%. Concentrations required for 50% cross-linking ($C_{50}$) are presented in Table 1.

tion of growth compared to controls ($GI_{50}$), no growth compared to controls (TGI), or 50% reduction in cells ($LC_{50}$). The mean $GI_{50}$ values (analogous to the $IC_{50}$ values reported above) for each of the CBI−PBDs across the cell line panel are reported in Table 1, and again these cover a large range of concentrations, with generally the same ranking of compound toxicity as reported above. With this more extensive range of cell lines some differential sensitivity was observed and was considerably greater for the more potent compounds (see range of $LC_{50}$ values in Table 1). Although the individual patterns varied, some cell lines repeatedly showed up as significantly more sensitive than the average, for example (based on $LC_{50}$ data) the NSCLC NCI−H522, the colon cancer Colo205, and several melanomas such as UACC-62 and SK-MEL-2. In contrast the CNS cancers and leukemia panel were generally more resistant than average. The latter is surprising given the myelotoxicity encountered in clinical trials of cyclopropaindolone compounds.[16]

A COMPARE analysis[57] was also performed for seven of the CBI−PBDs (all except the examples where $n$ = 2, **27b*R*** and **27b*S***) against the compounds in the NCI's Standard Agent Database. The COMPARE algorithm ranks compounds for the similarity of their in vitro growth patterns against the 60 cell line panel. The results are calculated as Pearson correlation coefficients (PCCs) where the 'seed compound' has a PCC = 1.00. High correlation values have been shown to indicate a common mechanism of action between various compounds, which may not be structurally related. For the CBI−PBDs using $LC_{50}$ data the highest PCCs were observed with intercalating agents (or aromatic chromophores) bearing minor groove binding side chains. In particular the RNA synthesis inhibitors actinomycin D, chromomycin A3, and mithramycin A, the quinoxaline bisintercalator echinomycin, and the topoisomerase II inhibitor adriamycin were ranked in the top seven matches for all of the CBI−PBDs with average PCCs greater than 0.73. (Full details are provided in the Supporting Information.) Surprisingly, alkylating agents hardly feature in the COMPARE matches, and those that do, for example cyanomorpholino-adriamycin, combine alkylating ability with intercalation and minor groove binding capability.

At the $GI_{50}$ level of comparison the data set was not complete since both **27c*S*** and **27e*S*** were too toxic to achieve 50% cell growth at the concentration ranges tested. The remaining compounds could be divided into two groups: **27c*R*** and **27e*R*** which showed some correlation (PCC 0.50−0.60) to a group of topoisomerase II inhibitors (e.g., adriamycin, daunomycin), and **27a*R***, **27d*R***, and **27d*S*** which showed stronger correlation (PCC > 0.74) to a set of DNA cross-linking agents (e.g., chlorambucil, uracil nitrogen mustard).

**Cross-Linking of Naked DNA.** The ability of the CBI−PBD compounds to cross-link naked DNA was determined using a slight modification of a previously described assay.[58] pcDNA3 plasmid DNA (5400 bp) was linearized and 3′-end labeled using $^{32}$P-dATP. The DNA was incubated with the compounds for 18 h at 37 °C and then denatured by alkali treatment. The basis of the assay is that if the DNA is cross-linked, the denatured strands are held in close proximity and readily renature, so that on agarose gel electrophoresis they run as double-stranded DNA. Following phosphorimaging the amount of cross-linking can be calculated.

This assay has been used in the past for symmetrical dimers of cyclopropaindolones[59] and PBDs.[22,25] In both cases denaturation was induced by heating (90 °C for 2 min), but because of the known susceptibility of cyclopropaindolone−adenine adducts to thermal depurination,[60] we chose to use alkali-induced denaturation.[58] It is also known that alkali can reverse cyclopropaindolone−adenine adduct formation,[61] but control experiments showed that prolonging the alkali exposure before electrophoresis did not alter the levels of cross-linking observed for the CBI−PBD compounds.

Each of the compounds was tested at five or more concentrations, and the assays repeated 2−4 times. Each gel also included **3** as an internal standard, which gave good reproducibility on repeat assay.[62] Figure 2 shows how the amount of cross-linked DNA varied with compound concentration. The data points were fitted to

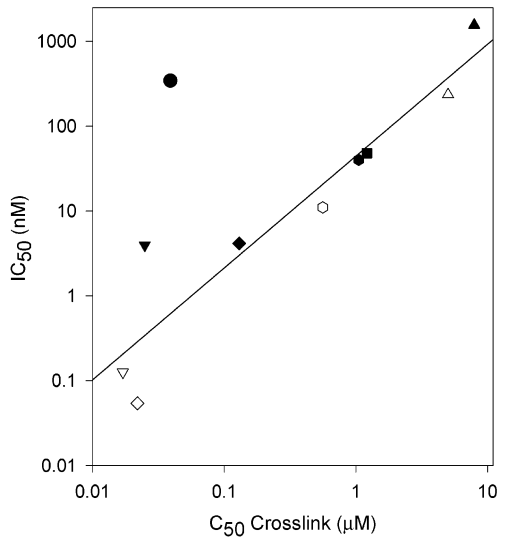



**Figure 3.** Relationship between cross-linking ability and cytotoxicity in the AA8 cell line for the CBI−PBD compounds **27a**−**e*R*** and **27b**−**e*S*** and DSB-120 **3**. Symbols as in Figure 2. Regression line calculated for all compounds except **3** ($r^2$ = 0.88, slope = 1.32).

a sigmoidal curve (constrained to a maximum *y* value of 100%) which in each case gave good convergence to the data, and allowed calculation of $C_{50}$ (the concentration required for 50% cross-linking). These values are collected in Table 1.

Inspection of Figure 2 reveals some interesting patterns. Cross-linking ability is dependent on chain length in the order $n = 2 < 1 < 4 < 5 \leq 3$, and for each chain length the *S*-CBI enantiomer (open symbols in Figure 2) is the more efficient cross-linker. Cross-linking ability spans a wide range ($C_{50}$ = 7.90−0.017 $\mu$M), with three of the CBI−PBDs (**27c*R***, **27c*S***, and **27e*S***) and DSB-120 **3**[63] ranking as extremely efficient cross-linkers. For these compounds cross-linking was detectable at concentrations as low as 1 nM, corresponding to less than one drug molecule for each segment of linear DNA in solution under the assay conditions. Even at the $C_{50}$ concentrations there are less than 20 molecules of these compounds per DNA segment in the assay.

Figure 3 plots cross-linking ability against cytotoxicity in AA8 cells and shows a good correlation between these properties. Excluding the PBD dimer **3** the log−log regression gave $r^2$ = 0.88 with a slope of 1.32, again supportive of cross-linking as the major mechanism of cytotoxicity. (Results for the other cell lines were similar with $r^2$ values from 0.89 to 0.92 and slopes of 1.31−1.39, see Supporting Information.) Compound **3** is clearly an outlier from this data set and is about 400-fold less toxic than would be predicted from its cross-linking ability. One possible explanation is that **3** may cross-link more slowly than the CBI−PBDs, so that comparable $C_{50}$ values measured after 18 h drug exposure do not reflect the level of cross-links generated during the cytotoxicity assay (4 h exposure). However, when the cross-linking assay was repeated for **3** and **27c*R*** (the CBI−PBD of most similar cross-linking ability) at 2 h drug exposure, again near identical $C_{50}$s (0.13 and 0.11 $\mu$M, respectively) and practically superimposable cross-link curves were observed (Supporting Information).

**DNA Cross-Linking in Cells.** To further investigate this difference between the CBI−PBDs and DSB-120 and to assess DNA cross-linking under more physiologically relevant cellular conditions, comet assays[64] were performed for the most potent CBI−PBD **27e*S*** and for DSB-120 **3**. A single cell suspension of Skov3 cells was exposed to the compounds for 1 h at various concentrations and then irradiated with a dose of 10 Gy in order to induce single strand breaks. The cells were immobilized in an agarose gel on a microscope slide and lysed with alkali. Electrophoresis draws any DNA fragments out of the nuclei to form cometlike tails. These are stained with propidium, and the tail moments (mean migration distance × percent DNA in the tail) were determined using an image analysis system.

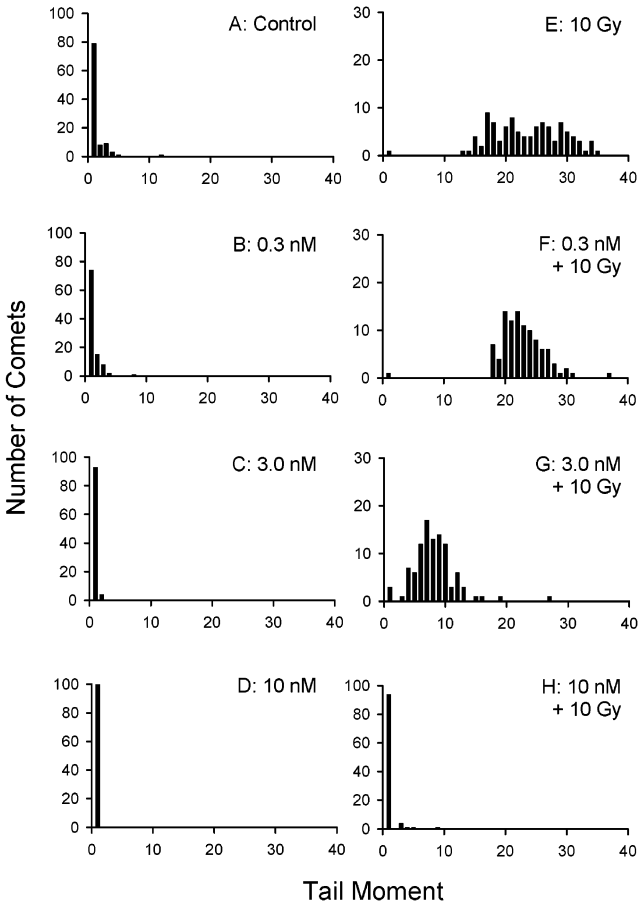



**Figure 4.** Comet assay of Skov3 cell suspensions following exposure to **27e*S*** at various concentrations for 1 h. Cells were washed, and then aliquots were irradiated on ice with a dose of 10 Gy. Cells were analyzed for DNA breakage or cross-linking, with the histograms showing the distribution of tail moments from 100 comets per treatment condition. Calculated cross-linking indices: (panel E, 0%); panel F, 6%; panel G, 70%; panel H, 101%.

Typical results for **27e*S*** are shown in Figure 4, from which it is apparent that treatment with **27e*S*** alone (panels B−D) does not significantly alter the control tail moment distribution (panel A). Irradiation causes significant DNA strand breakage as expected (panel E), and this fragmentation can effectively be reversed by pretreatment with increasing concentrations of **27e*S*** (panels F−H). A significant shift was apparent even at the lowest drug concentration tested (0.3 nM), a concentration at which clonogenic survival of the cells was reduced to 10% of that of controls (data not shown). Calculation of the cross-linking index (effectively, how much a given drug treatment shifts the tail moment

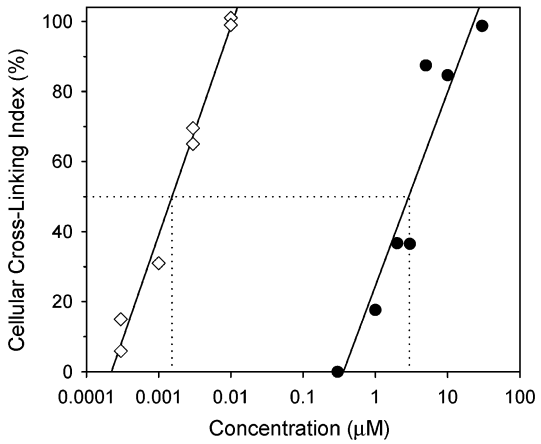



**Figure 5.** Extent of cross-linking of cellular DNA (as measured by the cross-linking index, CI, derived from the comet assay in Skov3 cells) by the CBI−PBD **27e*S*** (open diamond) and DSB-120 **3** (filled circle) following 1 h exposure at 37 °C.

distribution from the radiation-only condition back to the control condition) shows that for **27e*S*** a $CI_{50}$ (cross-linking index of 50%) is achieved at a concentration of approximately 1.5 nM (Figure 5).

A similar set of experiments was performed for DSB-120 **3** and generated a $CI_{50} = 3.0$ $\mu$M (Figure 5). Thus, although **3** and **27e*S*** have essentially equal ability to cross-link naked DNA ($C_{50}$ ratio = 1.3, Table 1), the latter is at least 1000 times more efficient at cross-linking cellular DNA. This ratio correlates much more closely with the observed cytotoxicity differentials for **3** versus **27e*S*** against Skov3 cells (4300 in Tables 1 and 1900 in Table 2).

**Sequence Selectivity.** To gain some information on the sequence selectivity of the alkylation events a thermal cleavage assay was performed using a 512 bp fragment of the *lac UV5* promoter as the target DNA. The 5′-$^{32}$P-end labeled DNA was incubated with the compounds of interest and then heated at 100 °C for 30 min. This assay is widely used with CBI-type compounds since it induces depurination and strand cleavage at all CBI alkylation sites, and the fragments can be readily identified following gel electrophoresis.[60,65]

Figure 6 shows results for a 90 bp section of the target DNA for three pairs of compounds: the CBI-acetamides **32*R*** and **32*S***, and the CBI−PBDs where $n = 2$ (**27b*R*** and ***S***) and 5 (**27e*R*** and ***S***). For each of these compounds on all occasions (a total sequence of 240 bps was analyzed by varying the electrophoresis conditions) the only alkylation site observed was adenine, almost exclusively with one or two AT bps on the 5′ side of the alkylation site. This sequence matches the reported binding preference of truncated CBI analogues[65] (such as **32*R*** and **32*S***), but it was surprising to observe exactly the same sites with approximately the same relative affinities for the CBI−PBDs. It appears that the CBI portion drives the overall sequence selectivity of the dimers, and the presence of the flexibly linked PBD neither limits the sites which are alkylated nor directs the CBI to sites that were not previously targeted. This was particularly unexpected for the pairs of compounds which differ only in CBI enantiomeric form (**27b*R*** and **27b*S***, **27e*R*** and **27e*S***), since for these to alkylate a given adenine on the labeled strand the bulky PBD substituent must necessarily point in the opposite direction along the minor groove and to therefore lie in a quite different sequence environment. The PBD does however make the alkylation more efficient, as similar intensity bands were observed for the CBI−PBDs at much lower concentrations than for the CBI-acetamides.

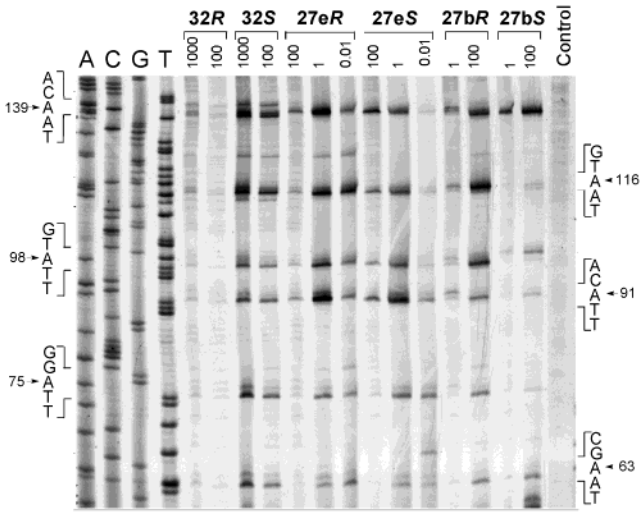



**Figure 6.** Thermal cleavage assay of DNA alkylated with CBI-acetamides **32*R*** and **32*S*** and CBI−PBDs where $n = 5$ (**27e*R*** and **27e*S***) and $n = 2$ (**27b*R*** and **27b*S***), resolved using denaturing polyacrylamide gel electrophoresis. The DNA was incubated with the compounds for 6 h at 37 °C at the concentrations indicated above the lanes ($\mu$M). The control lane contained no alkylating agent. Dideoxy sequencing lanes are labeled A, C, G, and T. The sequence is numbered relative to the labeled strand 5′ end of the 512 bp PCR product which was designated bp 1. Major alkylation sites are indicated by the numbered arrows.

**Table 3.** Maximum Tolerated Doses and Antitumor Activity of Selected CBI−PBD Compounds and DSB-120 3

| compound | MTD[a] ($\mu$mol/kg) ip | MTD ratio (iv/ip) | time to end point[b] [a]± SEM (days) |
|---|---|---|---|
| | iv | | |
| vehicle control | – | – | 20.1 ± 2.9 |
| **27c*S*** | 0.00316 | 0.316 | 100 | 26.0 ± 4.5 |
| **27e*R*** | 0.75 | 13.3 | 17.7 | 24.4 ± 2.1 |
| **27e*S*** | 0.0178 | 0.178 | 10 | 40.8 ± 7.9 |
| **3** (DSB-120) | 1.0 | 4.21 | 4.21 | 22.8 ± 2.5 |

[a] Maximum tolerated dose for single injection to non-tumor-bearing mice. [b] Average time for WiDr tumor xenograft to reach a diameter of 17 mm following single iv injection at day 0 (tumor diameter 8 mm).

**In Vivo Activity.** Three CBI−PBDs were chosen for in vivo study (**27c*S***, **27e*R***, and **27e*S***), a subset that includes the most efficient cross-linking agents and representatives of two chain lengths ($n = 3, 5$) and both CBI configurations. DSB-120 **3** was also included as a comparison, although in vivo activity was not anticipated for this compound.[24] Maximum tolerated doses (MTDs) were determined for single intraperitoneal (ip) or intravenous (iv) injections in nontumor-bearing C3H/HeN mice (Table 3). Comparing routes of administration, iv injection was better tolerated for all compounds (up to 100 times for **27cS**) and caused acute toxicity at doses above the MTD (time to death ≤17 days, see Supporting Information), while higher doses by ip injection were associated with later deaths (≥33 days) which appeared to result from local abdominal toxicity (chemical peritonitis and subsequent organ adhesions). The CBI−PBDs appeared intermediate in toxicity be-

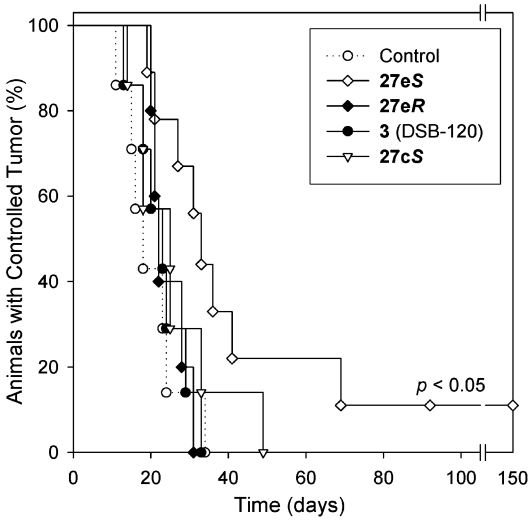



**Figure 7.** Survival curves for nude mice bearing WiDr xenografts treated with single iv doses of CBI−PBD compounds or DSB-120 **3**. Averages for groups of 5−9 mice. Controlled tumor implies mean tumor diameter less than 17 mm.

tween bizelesin **1** and DSB-120 **3**, which have reported MTDs for single iv injections of 0.018 and 9.4 $\mu$mol/kg, respectively.[13,24] However, given the large $IC_{50}$ differences compared to **3**, the mice tolerated considerably higher levels of the CBI−PBDs than anticipated, particularly by the iv route.

Antitumor activity was assessed using the human WiDr colon carcinoma grown as a xenograft in CD-1 nude mice. In cytotoxicity experiments using cell cultures (Table 2) this cell line displayed similar responses to the cross-linking agents as Colo205, which was highlighted in the NCI screening results as a particularly sensitive line. Single ip or iv doses were administered at the MTD to mice bearing established tumors with an average diameter of 8 mm. This treatment was well tolerated in the tumor-bearing mice as judged by minimal early body weight changes (for the first 5 days −0.3 to +0.8% compared to +0.9 to 1.3% for controls). No significant antitumor effects were observed for any of the compounds following ip injection, nor for **3**, **27c*S*** or **27e*R*** via the iv route. However **27eS** administered as a single iv dose gave a statistically significant ($p$ < 0.05) growth delay of 21 days: the average time to end point (mean tumor diameter of 17 mm) was 41 days after **27eS** treatment compared to 20 days for controls (Figure 7 and Table 3). The group of 9 treated mice also included one cure (no palpable tumor 150 days post-treatment), which was not included in the determination of growth delay. The individual tumor growth curves are presented in the Supporting Information section.

## Conclusions

In this study we describe the preparation and investigation of a set of CBI−PBD compounds designed as unsymmetrical DNA cross-linking agents. The convergent and efficient synthesis, which introduces the variable chain length linker at as late a stage as possible, provides the target compounds in high purity and reasonable overall yield and in no more than 12 linear steps from commercially available starting materials. Although this route would be adaptable to more complex structures (e.g., CBIs bearing a minor groove binding indole substituent, or PBDs with *exo*-unsaturation) the emphasis in this study was to use the minimal alkylating agents and simplest possible linker to define the structural requirements for efficient cross-linking.

Both linker chain length and CBI enantiomeric form appear critical, with an enormous variation in cytotoxicity, more than 35 000-fold in several cell lines, observed among the set of compounds prepared. A good correlation between toxicity and the ability to cross-link naked DNA (Figure 3) supports the proposal that DNA cross-linking is the major mechanism of cytotoxicity of these compounds. This is emphasized by the relative inactivity of the monoalkylating agents **35** and **39**, where even the latter is more than 2 orders of magnitude less toxic than its close structural analogue **27e*S***. The most efficient CBI−PBD cross-linkers are exceptionally cytotoxic with $IC_{50}$s in the pM range.

Interestingly, the correlation between cross-linking ability and cytotoxicity does not extend to the symmetrical PBD dimer **3**, which appeared much less toxic than anticipated (Figure 3). Compound **3** has previously been characterized as a highly efficient cross-linker of cellular DNA (using the technique of alkaline elution in K562 cells),[66] so it was remarkable to observe a CBI−PBD compound cross-linking cellular DNA at 1000-fold lower concentrations (Figure 5). The reasons for this different behavior are not clear—large differences in cellular uptake between the similar structures **3** and **27e*S*** would not be expected, and for a 1 h drug exposure time differential repair of DNA adducts also seems unlikely, especially as DSB-120 adducts are known to be poorly repaired.[66] One possible explanation lies in the different reactivity of the CBI and PBD alkylating agents—whereas PBDs react (reversibly) with a range of biological nucleophiles such as glutathione, cyclopropaindolones are highly selective for reaction with DNA, so it is possible that the CBI portion of the mixed dimers directs these compounds more productively to DNA as the cellular target.

The CBI portion also appears to drive the sequence selectivity of the dimers, overriding the sequence preference of the PBD and leading to alkylation at the same sites as those targeted by truncated CBIs (**32*R*** and **32*S***) (Figure 6). This is observed irrespective of the CBI configuration. However, the thermal cleavage assay used to generate Figure 6 only gives information on CBI alkylation sites. It is possible that the vast majority of these sites represent monoalkylation events, and only a very small subset, those sites where the PBD finds itself in a suitable environment for cross-linking, generate the lesions responsible for the observed toxicity. It would be interesting to conduct experiments with short oligonucleotides (as reported for **5**)[27] to confirm the expected adenine-N3 to guanine-N2 cross-link and to define the binding site size and sequence preference of the CBI−PBDs. Another open question concerns the reversibility of adduct formation, and in particular whether a CBI−PBD monoalkylated via the CBI can translocate to a site suitable for cross-linking, at which it may then become trapped.

Results from the NCI In Vitro Cell Line Assay and COMPARE analysis were also interesting. Although

correlation of the CBI−PBD differential toxicity patterns to those of intercalating agents seems initially surprising, cross-correlation between alkylating agents and topoisomerase poisons are not unknown.[67] When bizelesin itself is used as a seed compound for COMPARE analysis, it also generates a list from the Standard Agent Database that includes, for example, actinomycin D, chromomycin A3, mithramycin A, adriamycin, and daunomycin among the most closely correlated hits.[68]

The marked differential toxicities for the most potent CBI−PBDs in the 60 cell line panel (Table 1), coupled with the apparent resistance of leukemia cell lines, prompted an investigation of the in vivo antitumor activity of the CBI−PBDs using a growth delay assay. All three CBI−PBDs tested were better tolerated than expected, given their much greater in vitro toxicity compared to DSB-120 **3**, and one compound, **27e*S***, provided a statistically significant increase in lifespan for mice bearing WiDr human colon xenografts. Given the demanding nature of this assay, this positive result suggests that further testing is warranted against other tumor models and using alternative dosing schedules.

## Experimental Section

**Chemistry.** Analyses were carried out in the Microchemical Laboratory, University of Otago, NZ. Melting points were determined using an Electrothermal Model 9200 digital melting point apparatus and are as read. NMR spectra were obtained on a Bruker AM-400 spectrometer at 400 MHz ($^1$H) or 100 MHz ($^{13}$C). Mass spectra were obtained on a Varian VG 7070 mass spectrometer at nominal 5000 mass resolution. $CHCl_3$ used for optical rotation measurements was washed with $H_2O$ (×3), dried ($CaCl_2$), fractionally distilled from $P_2O_5$, and stored in the dark.

**5-Amino-4-{[(2*S*)-2-(hydroxymethyl)pyrrolidinyl]carbonyl}-2-methoxyphenol (9).** A solution of {(2*S*)-1-[4-(benzyloxy)-5-methoxy-2-nitrobenzoyl]pyrrolidinyl}methanol (**8**)[34] (3.43 g, 8.87 mmol) in MeOH (100 mL) was hydrogenated over Pd/C (5%, 0.37 g) at 50 psi for 55 min. The catalyst was filtered off through Celite, the filtrate was evaporated, and the residue was dissolved in $CH_2Cl_2$ and evaporated to give **9** as a tan foam (2.36 g, 100%); $^1$H NMR (DMSO-$d_6$) $\delta$ 9.09 (br s, 1 H), 6.69 (s, 1 H), 6.17 (s, 1H), 4.98 (s, 2 H), 4.75 (s, 1 H), 4.12−4.03 (m, 1 H), 3.64 (s, 3 H), 3.48−3.28 (m, 4 H), 1.98−1.78 (m, 3 H), 1.71−1.61 (m, 1 H); $^{13}$C NMR $\delta$ 169.1, 149.1, 142.0, 138.4, 113.5, 110.5, 103.2, 61.5, 58.4, 56.6, 49.1, 27.1, 24.0, which was used directly in the next step.

**Allyl 5-Hydroxy-2-{[(2*S*)-2-(hydroxymethyl)pyrrolidinyl]carbonyl}-4-methoxyphenylcarbamate (10).** The aniline **9** prepared in the preceding reaction (2.36 g, 8.87 mmol) was dissolved in THF (100 mL), pyridine (0.93 mL, 11.3 mmol) was added, and the solution was cooled to −78 °C. Allyl chloroformate (0.94 mL, 8.87 mmol) was added dropwise, and the mixture was stirred for 10 min at −78 °C. $H_2O$ and aq HCl (2 N, 5 mL) were added and the reaction was allowed to warm to room temperature. The THF was evaporated and the aq residue was extracted with $CH_2Cl_2$ (×3). The extracts were dried ($Na_2SO_4$) and evaporated, and the residue purified by column chromatography (100:1 then 95:5 EtOAc:MeOH) to give **10** as a white foam (2.65 g, 85%); $[\alpha]_D$ −133° (*c* 0.314, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 8.54 (br s, 1 H), 7.67 (s, 1 H), 6.79 (s, 1 H), 6.27 (br s, 1 H), 6.00−5.90 (m, 1 H), 5.34 (dq, $J$ = 17.3, 1.4 Hz, 1 H), 5.23 (dq, $J$ = 10.5, 1.3 Hz, 1 H), 4.68−4.57 (m, 2 H), 4.45−4.35 (m, 1 H), 4.33 (br s, 1 H), 3.90−3.83 (m, 1 H), 3.85 (s, 3 H), 3.73−3.66 (m, 1 H), 3.61−3.54 (m, 1 H), 3.52−3.44 (m, 1 H), 2.20−2.12 (m, 1 H), 1.95−1.84 (m, 1 H), 1.81−1.63 (m, 2 H); $^{13}$C NMR $\delta$ 171.0, 153.6, 148.1, 141.9, 132.5, 131.8, 118.1, 116.1, 110.3, 108.3, 66.4, 65.8, 61.0, 56.4, 51.6, 28.3, 25.1; HRMS (EI) calcd for $C_{17}H_{22}N_2O_6$ 350.1478, found 350.1476.

**General Method for the Preparation of TCE Esters of *ω*-Bromoalkanoic Acids. Preparation of 2,2,2-Trichloroethyl 4-Bromobutyrate (11c).** $(COCl)_2$ (5.1 mL, 59 mmol) and DMF (3 drops) were added to a solution of 4-bromobutyric acid (7.47 g, 45 mmol) in $CH_2Cl_2$ (70 mL), and the solution was stirred at 20 °C for 16 h. The solvent was evaporated, the residue was redissolved in $CH_2Cl_2$ (60 mL), and 2,2,2-trichloroethanol (4.7 mL, 50 mmol) was added. The solution was stirred at 20 °C for 4 h and then washed with aq $NaHCO_3$. The organic layer was dried ($Na_2SO_4$) and evaporated and the residue purified by column chromatography (20:1 petroleum ether:EtOAc) to give **11c** as a colorless liquid (8.56 g, 64%); $^1$H NMR ($CDCl_3$) $\delta$ 4.76 (s, 2 H), 3.50 (t, $J$ = 6.4 Hz, 2 H), 2.69 (t, $J$ = 7.2 Hz, 2 H), 2.25 (quint, $J$ = 6.8 Hz, 2 H), which was used directly in the next step.

The following compounds were prepared by the same general method.

**2,2,2-Trichloroethyl bromoacetate (11a)**[69] as a colorless liquid (93%); $^1$H NMR ($CDCl_3$) $\delta$ 4.82 (s, 2 H), 3.98 (s, 2 H).

**2,2,2-Trichloroethyl 3-bromopropionate (11b)** as a colorless liquid (45%); $^1$H NMR ($CDCl_3$) $\delta$ 4.80 (s, 2 H), 3.63 (t, $J$ = 6.7 Hz, 2 H), 3.09 (t, $J$ = 6.7 Hz, 2 H).

**2,2,2-Trichloroethyl 5-bromopentanoate (11d)**[70] as a colorless liquid (82%); $^1$H NMR ($CDCl_3$) $\delta$ 4.76 (s, 2 H), 3.43 (t, $J$ = 6.4 Hz, 2 H), 2.52 (t, $J$ = 7.2 Hz, 2 H), 1.99−1.83 (m, 4 H).

**2,2,2-Trichloroethyl 6-bromohexanoate (11e)**[71] as a colorless liquid (79%); $^1$H NMR ($CDCl_3$) $\delta$ 4.75 (s, 2 H), 3.41 (t, $J$ = 6.7 Hz, 2 H), 2.49 (t, $J$ = 7.4 Hz, 2 H), 1.94−1.86 (m, 2 H), 1.77−1.69 (m, 2 H), 1.57−1.49 (m, 2 H).

**General Method for Phenol Alkylation. Preparation of 2,2,2-Trichloroethyl 4-(5-{[(Allyloxy)carbonyl]amino}-4-{[(2*S*)-2-(hydroxymethyl)pyrrolidinyl]carbonyl}-2-methoxyphenoxy)butanoate (12c).** $K_2CO_3$ (0.58 g, 4.2 mmol) and then **11c** (TCE ester) (0.93 g, 3.1 mmol) were added to a solution of **10** (phenol) (0.73 g, 2.08 mmol) in DMF (6 mL), and the mixture was stirred at 20 °C for 8 h. $H_2O$ and aq HCl (2 N, 6 mL) were added, and the mixture was extracted with EtOAc (×3). The extracts were washed with aq NaCl (×3) and then dried ($Na_2SO_4$) and evaporated. The residue was purified by column chromatography (EtOAc) to give **12c** as a white foam (0.84 g, 71%); $[\alpha]_D$ −69° (*c* 0.346, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 8.74 (br s, 1 H), 7.78 (s, 1H), 7.27 (s, 1 H), 6.02−5.91 (m, 1 H), 5.36 (dq, $J$ = 17.3, 1.4 Hz, 1 H), 5.25 (br d, $J$ = 11.5 Hz, 1 H), 4.77 (s, 2 H), 4.65−4.62 (m, 2 H), 4.47−4.37 (m, 1 H), 4.25 (br s, 1 H), 4.16 (t, $J$ = 6.1 Hz, 2 H), 3.87−3.81 (m, 1 H), 3.83 (s, 3 H), 3.74−3.68 (m, 1 H), 3.63−3.56 (m, 1 H), 3.54−3.46 (m, 1 H), 2.71 (t, $J$ = 7.3 Hz, 2 H), 2.25 (quint, $J$ = 6.7 Hz, 2 H), 2.21−2.14 (m, 1 H), 1.95−1.86 (m, 1 H), 1.81−1.62 (m, 2 H); $^{13}$C NMR $\delta$ 171.4, 170.9, 153.6, 150.5, 144.0, 132.5, 132.0, 118.1, 115.5, 111.7, 105.7, 94.9, 74.0, 67.6, 66.7, 65.8, 61.2, 56.6, 51.6, 30.6, 28.4, 25.1, 24.3; HRMS (EI, $^{35}$Cl) calcd for $C_{23}H_{29}Cl_3N_2O_8$ 566.0990, found 566.0978.

The following compounds were prepared by the same general method.

**2,2,2-Trichloroethyl (5-{[(allyloxy)carbonyl]amino}-4-{[(2*S*)-2-(hydroxymethyl)pyrrolidinyl]carbonyl}-2-methoxyphenoxy)acetate (12a)** as a white foam (80% after a reaction time of 2 h, leaving the reaction for longer caused decomposition and much lower yields); $[\alpha]_D$ −71° (*c* 0.228, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 8.67 (br s, 1 H), 7.77 (s, 1H), 6.87 (s, 1 H), 6.00−5.90 (m, 1 H), 5.35 (dq, $J$ = 17.2, 1.5 Hz, 1 H), 5.25 (dq, $J$ = 10.4, 1.3 Hz, 1 H), 4.91 (s, 2 H), 4.86 (d, $J$ = 0.8 Hz, 2 H), 4.67−4.57 (m, 2 H), 4.45−4.37 (m, 1 H), 3.87 (s, 3 H), 3.86−3.82 (m, 1 H), 3.74−3.67 (m, 1 H), 3.61−3.46 (m, 2 H), 2.21−2.13 (m, 1 H), 1.96−1.88 (m, 1 H), 1.81−1.63 (m, 2 H); $^{13}$C NMR $\delta$ 170.5, 166.8, 153.5, 148.9, 144.1, 132.4, 131.7, 118.2, 117.1, 111.9, 106.3, 94.3, 74.1, 66.4, 65.8, 65.2, 61.1, 56.7, 51.5, 28.3, 25.1; HRMS (FAB, $^{35}$Cl) calcd for $C_{21}H_{26}Cl_3N_2O_8$ 539.0755, found 539.0747.

**2,2,2-Trichloroethyl 5-(5-{[(allyloxy)carbonyl]amino}-4-{[(2*S*)-2-(hydroxymethyl)pyrrolidinyl]carbonyl}-2-methoxyphenoxy)pentanoate (12d)** as a colorless oil (88%); $[\alpha]_D$

−68° (*c* 0.326, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 8.77 (br s, 1 H), 7.79 (s, 1H), 6.83 (s, 1 H), 6.02−5.92 (m, 1 H), 5.36 (dq, *J* = 17.3, 1.5 Hz, 1 H), 5.25 (dq, *J* = 10.5, 1.2 Hz, 1 H), 4.75 (s, 2 H), 4.69−4.60 (m, 2 H), 4.47−4.38 (m, 1 H), 4.15−4.07 (m, 2 H), 3.88−3.81 (m, 1 H), 3.83 (s, 3 H), 3.75−3.68 (m, 1 H), 3.64−3.57 (m, 1 H), 3.54−3.46 (m, 1 H), 2.58 (t, *J* = 7.1 Hz, 2 H), 2.22−2.14 (m, 1 H), 1.98−1.85 (m, 5 H), 1.81−1.61 (m, 2 H); $^{13}$C NMR $\delta$ 171.7, 171.0, 153.6, 150.7, 144.0, 132.5, 132.1, 118.1, 115.2, 111.7, 105.5, 95.0, 73.9, 68.2, 66.8, 65.8, 61.2, 56.7, 51.7, 33.6, 28.4, 28.3, 25.1, 21.4; HRMS (EI, $^{35}$Cl) calcd for $C_{24}H_{31}Cl_3N_2O_8$ 580.1146, found 580.1130.

**2,2,2-Trichloroethyl 6-(5-{[(allyloxy)carbonyl]amino}-4-{[(2*S*)-2-(hydroxymethyl)pyrrolidinyl]carbonyl}-2-methoxyphenoxy)hexanoate** (**12e**) as a colorless oil (83%); $[\alpha]_D$ −66° (*c* 0.515, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 8.77 (br s, 1 H), 7.78 (s, 1H), 6.82 (s, 1 H), 6.02−5.91 (m, 1 H), 5.36 (dq, *J* = 17.2, 1.5 Hz, 1 H), 5.25 (dq, *J* = 10.4, 1.3 Hz, 1 H), 4.75 (s, 2 H), 4.69−4.59 (m, 2 H), 4.46−4.38 (m, 1 H), 4.12−4.04 (m, 2 H), 3.88−3.82 (m, 1 H), 3.83 (s, 3 H), 3.74−3.68 (m, 1 H), 3.64−3.57 (m, 1 H), 3.54−3.46 (m, 1 H), 2.51 (t, *J* = 7.4 Hz, 2 H), 2.22−2.14 (m, 1 H), 1.94−1.84 (m, 3 H), 1.83−1.62 (m, 4 H), 1.60−1.52 (m, 2 H); $^{13}$C NMR $\delta$ 171.8, 171.1, 153.6, 150.8, 143.9, 132.5, 132.1, 118.1, 115.1, 111.6, 105.5, 95.0, 73.9, 68.5, 66.7, 65.8, 61.2, 56.7, 51.7, 33.8, 28.6, 28.4, 25.5, 25.1, 24.5; HRMS (EI, $^{35}$Cl) calcd for $C_{25}H_{33}Cl_3N_2O_8$ 594.1303, found 594.1297.

**General Method for DMP Oxidation. Preparation of Allyl (11a*S*)-11-Hydroxy-7-methoxy-5-oxo-8-[4-oxo-4-(2,2,2-trichloroethoxy)butoxy]-2,3,11,11a-tetrahydro-1*H*-pyrrolo-[2,1-*c*][1,4]benzodiazepine-10(5*H*)-carboxylate (14c).** DMP (1.31 g, 3.1 mmol) was added to a solution of **12c** (1.17 g, 2.06 mmol) in $CH_2Cl_2$ (30 mL), causing slight warming, and the solution was stirred at room temperature for 30 min. Aqueous $Na_2SO_3$ (10%, 35 mL) was added, followed by aq $NaHCO_3$, and the mixture was stirred for a further 15 min. The organic layer was separated, the aq layer was extracted with $CH_2Cl_2$ (×2), and the combined extracts were dried ($Na_2SO_4$) and evaporated. The residue was purified by column chromatography (EtOAc, repeating with the mixed fractions to separate the slightly less polar over-oxidation product) to give **14c** as a white foam (770 mg, 66%); mp 124−126 °C (PhH-petroleum ether); $[\alpha]_D$ +110° (*c* 0.257, $CHCl_3$); $^1$H NMR ($CDCl_3$) (shows the presence of ca. 10% PhH) $\delta$ 7.24 (s, 1H), 6.68 (s, 1 H), 5.86−5.73 (m, 1 H), 5.67−5.59 (m, 1 H), 5.19−5.09 (m, 2 H), 4.76 (s, 2 H), 4.67 (dd, *J* = 13.3, 5.4 Hz, 1 H), 4.50−4.42 (m, 1 H), 4.12−4.02 (m, 2 H), 3.91 (s, 3 H), 3.77 (br s, 1 H), 3.73−3.66 (m, 1 H), 3.60−3.52 (m, 1 H), 3.50−3.44 (m, 1 H), 2.71 (t, *J* = 7.2 Hz, 2 H), 2.22 (quint, *J* = 6.7 Hz, 2 H), 2.16−2.09 (m, 2 H), 2.04−1.96 (m, 2 H); $^{13}$C NMR (one aromatic quaternary carbon possibly coincident with PhH signal at $\delta$ 128.3) $\delta$ 171.4, 166.9, 156.0, 149.9, 148.7, 131.8, 126.1, 118.0, 114.0, 110.8, 94.9, 86.0, 74.0, 67.7, 66.7, 59.8, 56.1, 46.3, 30.4, 28.7, 24.2, 23.0. Anal. ($C_{23}H_{27}Cl_3N_2O_8 \cdot {}^1/_8$PhH) C, H, N.

Also isolated from the same column was **allyl (11a*S*)-7-methoxy-5,11-dioxo-8-[4-oxo-4-(2,2,2-trichloroethoxy)butoxy]-2,3,11,11a-tetrahydro-1*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-10(5*H*)-carboxylate** (**13c**) as a colorless oil (208 mg, 18%); $[\alpha]_D$ +38° (*c* 0.170, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 7.33 (s, 1H), 6.73 (s, 1 H), 5.93−5.82 (m, 1 H), 5.30 (dq, *J* = 17.4, 1.5 Hz, 1 H), 5.25 (dq, *J* = 10.4, 1.1 Hz, 1 H), 4.76 (s, 2 H), 4.75−4.71 (m, 1 H), 4.67−4.62 (m, 1 H), 4.13−4.04 (m, 3 H), 3.93 (s, 3 H), 3.82−3.74 (m, 1 H), 3.60−3.51 (m, 1 H), 2.71 (t, *J* = 7.2 Hz, 2 H), 2.72−2.67 (m, 1 H), 2.23 (quint, *J* = 6.6 Hz, 2 H), 2.12−1.95 (m, 3 H); $^{13}$C NMR $\delta$ 171.3, 170.2, 165.0, 152.2, 150.1, 148.8, 130.8, 128.3, 124.5, 119.5, 111.6, 110.9, 94.9, 74.0, 68.1, 67.9, 59.6, 56.1, 46.6, 30.3, 26.4, 24.1, 23.6; HRMS (EI, $^{35}$Cl) calcd for $C_{23}H_{25}Cl_3N_2O_8$ 562.0677, found 562.0654.

The following compounds were prepared by the same general method.

**Allyl (11a*S*)-11-hydroxy-7-methoxy-5-oxo-8-[2-oxo-2-(2,2,2-trichloroethoxy)ethoxy]-2,3,11,11a-tetrahydro-1*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-10(5*H*)-carboxylate** (**14a**) as a white foam (73%); $[\alpha]_D$ +106° (*c* 0.279, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 7.29 (s, 1H), 6.71 (s, 1 H), 5.88−5.74 (m, 1 H), 5.64−5.54 (m, 1 H), 5.21−5.10 (m, 2 H), 4.87−4.82 (m, 2 H), 4.84 (s, 2 H), 4.65 (dd, *J* = 13.3, 5.3 Hz, 1 H), 4.51−4.43 (m, 1 H), 3.94 (s, 3 H), 3.76 (s, 1 H), 3.73−3.67 (m, 1 H), 3.60−3.52 (m, 1 H), 3.49−3.44 (m, 1 H), 2.18−2.08 (m, 2 H), 2.06−1.96 (m, 2 H); $^{13}$C NMR $\delta$ 166.9, 166.6, 156.0, 149.0, 148.5, 131.8, 128.1, 127.8, 118.3, 115.9, 111.5, 94.3, 86.0, 74.1, 66.9, 66.0, 59.7, 56.1, 46.4, 28.7, 23.0; HRMS (FAB, $^{35}$Cl) calcd for $C_{21}H_{24}Cl_3N_2O_8$ 537.0598, found 537.0593; and **allyl (11a*S*)-7-methoxy-5,11-dioxo-8-[2-oxo-2-(2,2,2-trichloroethoxy)ethoxy]-2,3,11,11a-tetrahydro-1*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-10(5*H*)-carboxylate** (**13a**) as a colorless oil (5%); $[\alpha]_D$ +35° (*c* 0.277, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 7.38 (s, 1H), 6.76 (s, 1 H), 5.94−5.81 (m, 1 H), 5.32 (dq, *J* = 17.0, 1.3 Hz, 1 H), 5.27 (br d, *J* = 10.4 Hz, 1 H), 4.93−4.77 (m, 4 H), 4.75−4.63 (m, 2 H), 4.13−4.09 (m, 1 H), 3.96 (s, 3 H), 3.82−3.76 (m, 1 H), 3.59−3.51 (m, 1 H), 2.73−2.67 (m, 1 H), 2.13−1.95 (m, 3 H); HRMS (FAB, $^{35}$Cl) calcd for $C_{21}H_{22}Cl_3N_2O_8$ 535.0442, found 535.0426.

**Allyl (11a*S*)-11-hydroxy-7-methoxy-5-oxo-8-{[5-oxo-5-(2,2,2-trichloroethoxy)pentyl]oxy}-2,3,11,11a-tetrahydro-1*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-10(5*H*)-carboxylate** (**14d**) as a white foam (60%); $[\alpha]_D$ +95° (*c* 0.378, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 7.24 (s, 1H), 6.66 (s, 1 H), 5.86−5.74 (m, 1 H), 5.66−5.58 (m, 1 H), 5.20−5.10 (m, 2 H), 4.75 (s, 2 H), 4.72−4.64 (m, 2 H), 4.49−4.41 (m, 1 H), 4.07−3.97 (m, 2 H), 3.91 (s, 3 H), 3.73−3.65 (m, 1 H), 3.60−3.45 (m, 2 H), 2.57 (t, *J* = 7.0 Hz, 2 H), 2.16−2.09 (m, 2 H), 2.05−1.97 (m, 2 H), 1.95−1.85 (m, 4 H); $^{13}$C NMR $\delta$ 171.7, 166.9, 156.1, 150.1, 148.7, 131.8, 128.3, 125.9, 118.0, 113.8, 110.8, 95.0, 86.0, 73.9, 68.5, 66.7, 59.8, 56.1, 46.4, 33.5, 28.7, 28.3, 23.0, 21.4; HRMS (EI, $^{35}$Cl) calcd for $C_{24}H_{29}Cl_3N_2O_8$ 578.0990, found 578.0983; and **allyl (11a*S*)-7-methoxy-5,11-dioxo-8-{[5-oxo-5-(2,2,2-trichloroethoxy)pentyl]oxy}-2,3,11,11a-tetrahydro-1*H*-pyrrolo-[2,1-*c*][1,4]benzodiazepine-10(5*H*)-carboxylate** (**13d**) as a colorless oil (7%); $[\alpha]_D$ +34° (*c* 0.134, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 7.32 (s, 1H), 6.71 (s, 1 H), 5.93−5.81 (m, 1 H), 5.30 (dq, *J* = 17.1, 1.5 Hz, 1 H), 5.25 (dq, *J* = 10.4, 1.1 Hz, 1 H), 4.82−4.71 (m, 1 H), 4.80 (s, 2 H), 4.67−4.61 (m, 1 H), 4.14−4.11 (m, 1 H), 4.05−3.98 (m, 2 H), 3.93 (s, 3 H), 3.82−3.76 (m, 1 H), 3.59−3.52 (m, 1 H), 2.74−2.68 (m, 1 H), 2.58 (t, *J* = 7.0 Hz, 2 H), 2.14−1.84 (m, 7 H); $^{13}$C NMR ($CCl_3$ carbon not seen) $\delta$ 171.6, 170.3, 165.1, 152.2, 150.3, 148.9, 130.8, 128.3, 124.3, 119.5, 111.4, 110.9, 73.9, 68.7, 68.1, 59.7, 56.2, 46.6, 33.4, 28.2, 26.4, 23.6, 21.4; HRMS (EI, $^{35}$Cl) calcd for $C_{24}H_{27}Cl_3N_2O_8$ 576.0833, found 576.0825.

**Allyl (11a*S*)-11-hydroxy-7-methoxy-5-oxo-8-{[6-oxo-6-(2,2,2-trichloroethoxy)hexyl]oxy}-2,3,11,11a-tetrahydro-1*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-10(5*H*)-carboxylate** (**14e**) as a white foam (49%); $[\alpha]_D$ +99° (*c* 0.348, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 7.24 (s, 1H), 6.65 (s, 1 H), 5.86−5.74 (m, 1 H), 5.66−5.57 (m, 1 H), 5.20−5.10 (m, 2 H), 4.75 (s, 2 H), 4.68 (dd, *J* = 13.4, 5.4 Hz, 1 H), 4.48−4.42 (m, 1 H), 4.04−3.96 (m, 2 H), 3.91 (s, 3 H), 3.73−3.63 (m, 2 H), 3.60−3.44 (m, 2 H), 2.51 (t, *J* = 7.4 Hz, 2 H), 2.15−2.09 (m, 2 H), 2.04−1.97 (m, 2 H), 1.92−1.84 (m, 2 H), 1.82−1.74 (m, 2 H), 1.59−1.51 (m, 2 H); $^{13}$C NMR $\delta$ 171.8, 167.0, 156.1, 150.1, 148.6, 131.8, 128.3, 125.7, 118.0, 113.7, 110.7, 95.0, 86.0, 73.9, 68.7, 66.7, 59.8, 56.1, 46.3, 33.8, 28.7, 28.6, 25.4, 24.4, 23.0; HRMS (EI, $^{35}$Cl) calcd for $C_{25}H_{31}Cl_3N_2O_8$ 592.1146, found 592.1139; and **allyl (11a*S*)-7-methoxy-5,11-dioxo-8-{[6-oxo-6-(2,2,2-trichloroethoxy)hexyl]oxy}-2,3,11,11a-tetrahydro-1*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-10(5*H*)-carboxylate** (**13e**) as a colorless oil (26%); $[\alpha]_D$ +31° (*c* 0.440, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 7.32 (s, 1H), 6.71 (s, 1 H), 5.93−5.82 (m, 1 H), 5.29 (dq, *J* = 17.2, 1.3 Hz, 1 H), 5.24 (dq, *J* = 10.5, 1.1 Hz, 1 H), 4.78−4.72 (m, 1 H), 4.75 (s, 2 H), 4.67−4.61 (m, 1 H), 4.16−4.11 (m, 1 H), 4.03−3.96 (m, 2 H), 3.93 (s, 3 H), 3.83−3.74 (m, 1 H), 3.60−3.51 (m, 1 H), 2.74−2.67 (m, 1 H), 2.51 (t, *J* = 7.4 Hz, 2 H), 2.12−1.96 (m, 3 H), 1.93−1.85 (m, 2 H), 1.82−1.74 (m, 2 H), 1.60−1.52 (m, 2 H); $^{13}$C NMR $\delta$ 171.7, 170.3, 165.1, 152.2, 150.4, 148.8, 130.8, 128.3, 124.1, 119.4, 111.2, 110.8, 95.0, 73.8, 68.9, 68.0, 59.7, 56.2, 46.6, 33.7, 28.5, 26.4, 25.4, 24.4, 23.6; HRMS (EI, $^{35}$Cl) calcd for $C_{25}H_{29}Cl_3N_2O_8$ 590.0990, found 590.0977.

**General Method for TCE Ester Cleavage.**[35] **Preparation of 4-({(11a*S*)-10-[(Allyloxy)carbonyl]-11-hydroxy-7-methoxy-5-oxo-2,3,5,10,11,11a-hexahydro-1*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-8-yl}oxy)butanoic Acid (15c).** Zn powder (0.68 g, 10.4 mmol) was added to a solution of (**14c**) (ester) (1.18 g, 2.09 mmol) in $HCO_2H$ (88%, 30 mL), causing slight warming, and the mixture was stirred at room temperature for 1 h. The mixture was diluted with EtOAc, filtered through Celite, and evaporated. The residue was dissolved in EtOAc and extracted with aq $NaHCO_3$ (×3). The aq extracts were acidified with c.HCl and then extracted with EtOAc (×4). The organic extracts were dried ($Na_2SO_4$) and evaporated, and the resulting oil was crystallized from EtOAc−PhH to give **15c** as a white solid (0.81 g, 89%); mp 163−165 °C; $[\alpha]_D$ +123° (*c* 0.237, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ ca. 7.5 (v br s, 1 H), 7.25 (s, 1H), 6.77 (s, 1 H), 5.82−5.72 (m, 1 H), 5.62 (d, $J$ = 9.8 Hz, 1 H), 5.17−5.07 (m, 2 H), 4.64 (dd, $J$ = 13.3, 5.4 Hz, 1 H), 4.47−4.39 (m, 1 H), 4.13−4.01 (m, 2 H), 3.90 (s, 3 H), 3.73−3.66 (m, 1 H), 3.59−3.44 (m, 2 H), 2.57 (t, $J$ = 6.8 Hz, 2 H), 2.19−2.07 (m, 4 H), 2.03−1.96 (m, 2 H); $^{13}$C NMR $\delta$ 177.3, 167.1, 156.2, 150.0, 148.7, 131.7, 125.8, 118.1, 114.1, 110.8, 86.0, 67.8, 66.9, 60.1, 56.1, 46.4, 30.0, 28.7, 23.8, 23.0. Anal. ($C_{21}H_{26}N_2O_8$·PhH) C, H, N.

The following compounds were prepared by the same general method.

**({(11a*S*)-10-[(Allyloxy)carbonyl]-11-hydroxy-7-methoxy-5-oxo-2,3,5,10,11,11a-hexahydro-1*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-8-yl}oxy)acetic acid** (**15a**) as a white solid (59%); $[\alpha]_D$ +136° (*c* 0.196, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 7.28 (s, 1H), 6.66 (s, 1 H), 5.84−5.72 (m, 1 H), 5.60 (d, $J$ = 9.8 Hz, 1 H), 5.19−5.07 (m, 2 H), 4.77−4.64 (m, 2 H), 4.60 (dd, $J$ = 13.3, 5.4 Hz, 1 H), 4.49−4.42 (m, 1 H), 3.92 (s, 3 H), 3.74−3.66 (m, 1 H), 3.58−3.41 (m, 2 H), 2.10−1.95 (m, 4 H); $^{13}$C NMR (one aromatic quaternary C not seen) $\delta$ 170.9, 166.9, 156.2, 148.8, 131.6, 128.0, 127.2, 118.3, 115.3, 111.2, 85.9, 67.1, 66.0, 60.1, 56.2, 46.5, 28.5, 23.0; HRMS (FAB) calcd for $C_{19}H_{23}N_2O_8$ 407.1454, found 407.1445.

**5-({(11a*S*)-10-[(Allyloxy)carbonyl]-11-hydroxy-7-methoxy-5-oxo-2,3,5,10,11,11a-hexahydro-1*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-8-yl}oxy)pentanoic acid** (**15d**) as a cream solid (67%); mp 96−98 °C (EtOAc-$Et_2O$); $[\alpha]_D$ +123° (*c* 0.349, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 7.25 (s, 1H), 6.70 (s, 1 H), 5.86−5.74 (m, 1 H), 5.63 (d, $J$ = 9.8 Hz, 1 H), 5.18−5.07 (m, 2 H), 4.67 (dd, $J$ = 13.4, 5.5 Hz, 1 H), 4.50−4.43 (m, 1 H), 4.09−3.97 (m, 2 H), 3.91 (s, 3 H), 3.73−3.67 (m, 1 H), 3.60−3.47 (m, 2 H), 2.44 (t, $J$ = 7.1 Hz, 2 H), 2.15−2.07 (m, 2 H), 2.03−1.96 (m, 2 H), 1.94−1.77 (m, 4 H); $^{13}$C NMR $\delta$ 177.5, 167.1, 156.2, 150.1, 148.7, 131.8, 128.3, 125.7, 118.1, 114.0, 110.8, 86.0, 68.6, 66.8, 60.0, 56.1, 46.4, 33.4, 28.7, 23.0, 21.3. Anal. ($C_{22}H_{28}N_2O_8$·$^1/_2H_2O$) C, H, N.

**6-({(11a*S*)-10-[(Allyloxy)carbonyl]-11-hydroxy-7-methoxy-5-oxo-2,3,5,10,11,11a-hexahydro-1*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-8-yl}oxy)hexanoic acid** (**15e**) as a white foam (78%); $[\alpha]_D$ +116° (*c* 0.203, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 7.24 (s, 1 H), 6.68 (s, 1 H), 5.83−5.71 (m, 1 H), 5.62 (d, $J$ = 9.8 Hz, 1 H), 5.16−5.06 (m, 2 H), 4.65 (dd, $J$ = 13.4, 5.2 Hz, 1 H), 4.49−4.40 (m, 1 H), 4.02−3.95 (m, 2 H), 3.90 (s, 3 H), 3.72−3.65 (m, 1 H), 3.58−3.45 (m, 2 H), 2.33 (t, $J$ = 7.3 Hz, 2 H), 2.15−2.07 (m, 2 H), 2.03−1.95 (m, 2 H), 1.87−1.78 (m, 2 H), 1.72−1.63 (m, 2 H), 1.52−1.42 (m, 2 H); $^{13}$C NMR $\delta$ 179.0, 167.1, 156.3, 150.1, 148.7, 131.7, 128.3, 125.7, 118.0, 114.0, 110.7, 86.0, 68.8, 66.8, 60.2, 56.1, 46.4, 34.4, 28.7, 28.2, 25.2, 24.5, 23.0; HRMS (EI) calcd for $C_{23}H_{30}N_2O_8$ 462.2002, found 462.1998.

**4-(3-Hydroxypropoxy)-3-methoxybenzaldehyde (17).** A mixture of vanillin (5.08 g, 33.4 mmol), 3-bromopropanol (3.6 mL, 40.1 mmol), and $K_2CO_3$ (6.00 g, 43.4 mmol) in DMF (40 mL) was stirred at 90 °C for 30 min and then cooled to room temperature. $H_2O$ was added, and the mixture was extracted with EtOAc. The organic extracts were evaporated (to remove extracted DMF), and the residue partitioned between EtOAc and $H_2O$. The organic layer was dried ($Na_2SO_4$) and evaporated and the residue purified by column chromatography (1:1 EtOAc:petroleum ether) to give **17** as a colorless oil (5.07 g, 72%). A sample crystallized from PhH−petroleum ether, mp 79−80 °C; $^1$H NMR ($CDCl_3$) $\delta$ 9.85 (s, 1 H), 7.44 (dd, $J$ = 8.1, 1.9 Hz, 1 H), 7.41 (d, $J$ = 1.8 Hz, 1 H), 6.99 (d, $J$ = 8.1 Hz, 1 H), 4.28 (t, $J$ = 6.0 Hz, 2 H), 3.92 (s, 3 H), 3.89 (t, $J$ = 5.6 Hz, 2 H), 2.40 (br s, 1 H), 2.17−2.10 (m, 2 H); $^{13}$C NMR $\delta$ 190.9, 153.7, 149.8, 130.2, 126.7, 111.4, 109.1, 67.8, 60.8, 56.0, 31.6. Anal. ($C_{11}H_{14}O_4$) C, H.

**3-(4-Formyl-2-methoxy-5-nitrophenoxy)propionic Acid (19).** The alcohol **17** (3.57 g, 17.0 mmol) was added in portions over several minutes with stirring to c.$HNO_3$ (50 mL) at 0 °C. The internal temperature rose over 15 min to 18 °C, then fell again after 10 min to 5 °C. The ice bath was removed and the mixture allowed to warm to room temperature, with no further exotherm. After 20 min the mixture was poured into ice-cold $H_2O$ (400 mL) and the solid was filtered off. The solid was dissolved in EtOAc, and the solution was washed with $H_2O$ until the washings were only slightly acidic and then extracted with aq $NaHCO_3$ (×3). The aq extracts were acidified (c.HCl) and then extracted with EtOAc (×3). The EtOAc extracts were dried ($Na_2SO_4$) and evaporated to give **19** as a pale yellow solid (2.19 g, 48%); $^1$H NMR (DMSO-$d_6$) $\delta$ 12.47 (br s, 1 H), 10.20 (s, 1 H), 7.73 (s, 1 H), 7.37 (s, 1 H), 4.38 (t, $J$ = 5.9 Hz, 1 H), 3.95 (s, 3 H), 2.78 (t, $J$ = 5.9 Hz, 1 H); $^{13}$C NMR $\delta$ 188.5, 171.7, 152.5, 150.9, 143.5, 124.7, 110.1, 108.2, 65.3, 56.4, 33.6; HRMS (EI) calcd for $C_{11}H_{11}NO_7$ 269.0536, found 269.0534. $^1$H NMR analysis also showed the presence of an impurity (ca. 10%) which was not removed by attempted crystallization. This impurity is tentatively identified as 3-(2-methoxy-4,5-dinitrophenoxy)propionic acid **20**.

The EtOAc layer from the $NaHCO_3$ extraction was also dried ($Na_2SO_4$) and evaporated and the residue purified by column chromatography (1:2 EtOAc:petroleum ether) to give **3-(4-formyl-2-methoxy-5-nitrophenoxy)propyl nitrate** (**18**) as a yellow solid (0.49 g, 10%); $^1$H NMR ($CDCl_3$) $\delta$ 10.46 (s, 1 H), 7.61 (s, 1 H), 7.42 (s, 1 H), 4.71 (t, $J$ = 6.1 Hz, 1 H), 4.26 (t, $J$ = 6.0 Hz, 2 H), 4.00 (s, 3 H), 2.37−2.31 (m, 2 H); $^{13}$C NMR $\delta$ 187.7, 153.6, 151.2, 143.6, 126.0, 110.1, 108.3, 69.3, 65.5, 56.6, 26.7; HRMS (EI) calcd for $C_{11}H_{12}N_2O_8$ 300.0594, found 300.0589.

**2,2,2-Trichloroethyl 3-(4-Formyl-2-methoxy-5-nitrophenoxy)propionate (21).** $(COCl)_2$ (2.7 mL, 30.7 mmol) and DMF (2 drops) were added to a suspension of **19** (6.89 g, 25.6 mmol) in $CH_2Cl_2$ (120 mL), and the suspension was stirred at 20 °C for 18 h. The solution was evaporated, the residue was redissolved in $CH_2Cl_2$ (80 mL), and the solution was cooled to 0 °C. 2,2,2-Trichloroethanol (2.7 mL, 28 mmol) and pyridine (2.7 mL, 33 mmol) were added, the ice bath was removed, and the mixture was stirred for 90 min. Aqueous HCl (2 N, 40 mL) was added, and the mixture was extracted with $CH_2Cl_2$ (×3). The extracts were dried ($Na_2SO_4$) and evaporated, and the residue was purified by column chromatography (1:4 then 1:2 EtOAc:petroleum ether) to give **21** as a yellow oil (6.94 g, 68%); $^1$H NMR ($CDCl_3$) $\delta$ 10.46 (s, 1 H), 7.66 (s, 1 H), 7.41 (s, 1 H), 4.82 (s, 2 H), 4.49 (t, $J$ = 6.1 Hz, 2 H), 3.98 (s, 3 H), 3.09 (t, $J$ = 6.1 Hz, 2 H); $^{13}$C NMR $\delta$ 187.7, 168.8, 153.5, 151.2, 143.5, 126.0, 110.1, 108.5, 94.6, 74.2, 64.7, 56.6, 33.9; HRMS (EI, $^{35}$Cl) calcd for $C_{13}H_{12}Cl_3NO_7$ 398.9679, found 398.9680. $^1$H NMR analysis also showed the presence of an impurity (ca. 10%), identified as 2,2,2-trichloroethyl 3-(2-methoxy-4,5-dinitrophenoxy)propionate (**22**) (see below).

**5-Methoxy-2-nitro-4-[3-oxo-3-(2,2,2-trichloroethoxy)-propoxy]benzoic Acid (23). 21** (6.43 g, 16.0 mmol) was dissolved in $CH_3CN$ (80 mL), and a solution of $NaH_2PO_4$·$2H_2O$ (0.67 g, 4.3 mmol) in $H_2O$ (12 mL) was added, followed by aq $H_2O_2$ (35%, 1.64 mL, 16.8 mmol). A solution of $NaClO_2$ (80%, 2.54 g, 22.4 mmol) in $H_2O$ (25 mL) was then added dropwise over 20 min, causing slight warming.[39] The mixture was stirred at room temperature for 90 min, then $Na_2SO_3$ (0.42 g, 3.2 mmol) was added and the mixture stirred for a further 5 min to decompose excess $H_2O_2$. The mixture was diluted with aq NaCl and extracted with EtOAc (×3), and the extracts were washed with aq NaCl (×2) and extracted with aq $NaHCO_3$ (×3). The alkaline extracts were acidified (c.HCl) and extracted with EtOAc (×2), and the organic extracts were dried ($Na_2SO_4$) and evaporated to give **23** as a yellow foam (5.04 g, 75%);

$^1$H NMR ($CDCl_3$) $\delta$ 7.45 (s, 1 H), 7.22 (s, 1 H), 4.82 (s, 2 H), 4.44 (t, $J$ = 6.2 Hz, 2 H), 3.96 (s, 3 H), 3.07 (t, $J$ = 6.1 Hz, 2 H); $^{13}$C NMR $\delta$ 169.5, 168.9, 152.5, 149.9, 141.9, 120.2, 111.7, 108.7, 94.6, 74.2, 64.7, 56.6, 33.9; HRMS (EI, $^{35}$Cl) calcd for $C_{13}H_{12}Cl_3NO_8$ 414.9629, found 414.9628.

The EtOAc layer from the $NaHCO_3$ extraction was also dried ($Na_2SO_4$) and evaporated, and the residue triturated with MeOH to give **2,2,2-trichloroethyl 3-(2-methoxy-4,5-dinitrophenoxy)propionate** (**22**) as a pale yellow solid (0.85 g, 13%); $^1$H NMR ($CDCl_3$) $\delta$ 7.40 (s, 1 H), 7.31 (s, 1 H), 4.82 (s, 2 H), 4.46 (t, $J$ = 6.0 Hz, 2 H), 3.97 (s, 3 H), 3.08 (t, $J$ = 6.1 Hz, 2 H); $^{13}$C NMR $\delta$ 168.6, 152.3, 150.6, 137.3, 136.3, 108.5, 107.3, 94.6, 74.2, 65.1, 56.9, 33.8; HRMS (EI, $^{35}$Cl) calcd for $C_{12}H_{11}Cl_3N_2O_8$ 415.9581, found 415.9585.

**2,2,2-Trichloroethyl 3-(4-{[(2*S*)-2-(Hydroxymethyl)pyrrolidinyl]carbonyl}-2-methoxy-5-nitrophenoxy)propionate (24).** $(COCl)_2$ (0.25 mL, 2.9 mmol) and DMF (1 drop) were added to a solution of **23** (1.00 g, 2.40 mmol) in $CH_3CN$ (20 mL,) and the was solution stirred at 20 °C for 16 h. This solution was then added dropwise over 15 min to a mixture of (*S*)-(+)-2-pyrrolidinemethanol (242 mg, 2.4 mmol) and $K_2CO_3$ (0.80 g, 5.8 mmol) in $CH_3CN$ (15 mL) with stirring at −40 °C. The mixture was allowed to warm to 0 °C over 40 min, then $H_2O$ and aq HCl (2 N, 7 mL) were added. The $CH_3CN$ was evaporated, and the aq residue was extracted with EtOAc (×3). The extracts were dried ($Na_2SO_4$) and evaporated, and the residue was purified by column chromatography (1:50 MeOH:EtOAc) to give **24** as a pale yellow foam (1.10 g, 92%); $[\alpha]_D$ −67° (*c* 0.323, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 7.76 (s, 1 H), 6.80 (s, 1 H), 4.82 (s, 2 H), 4.44 (t, $J$ = 6.1 Hz, 2 H), 4.42−4.37 (m, 1 H), 3.95 (s, 3 H), 3.94−3.87 (m, 1 H), 3.82−3.77 (m, 1 H), 3.17 (t, $J$ = 6.7 Hz, 2 H), 3.06 (t, $J$ = 6.1 Hz, 2 H), 2.23−2.14 (m, 1 H), 1.95−1.65 (m, 3 H); HRMS (FAB, $^{35}$Cl) calcd for $C_{18}H_{22}Cl_3N_2O_8$ 499.0442, found 499.0440.

**2,2,2-Trichloroethyl 3-(5-{[(Allyloxy)carbonyl]amino}-4-{[(2*S*)-2-(hydroxymethyl)pyrrolidinyl]carbonyl}-2-methoxyphenoxy)propionate. 24** (4.77 g, 9.55 mmol) was dissolved in MeOH (70 mL) and hydrogenated over Pd/C (5%, 0.19 g) at 50 psi for 30 min. TLC analysis (1:20 MeOH:EtOAc) showed mostly starting material, so more Pd/C (0.19 g) was added and hydrogenation continued at 50 psi for a further 30 min. This process was repeated until TLC analysis showed mostly one product, slightly more polar than the starting material. The product was resolved from small amounts of over-reduction products that were slightly more polar again. Reduction to this point required a total of 160 min hydrogenation and 0.53 g of Pd/C. The catalyst was filtered off through Celite, the filtrate was evaporated, and the residue was purified by column chromatography (1:20 then 1:10 MeOH:EtOAc) to give crude 2,2,2-trichloroethyl 3-(5-amino-4-{[(2*S*)-2-(hydroxymethyl)pyrrolidinyl]carbonyl}-2-methoxyphenoxy)-propionate **25** (2.31 g, 52%) that was used directly in the next step.

Allyl chloroformate (0.52 mL, 4.9 mmol) was added dropwise to a solution of **25** (2.31 g, 4.92 mmol) and pyridine (0.52 mL, 6.4 mmol) in THF (120 mL) at −78 °C. After 10 min $H_2O$ and aq HCl (2 N, 10 mL) were added, and the mixture was allowed to warm to room temperature. The THF was evaporated, and the aq residue was extracted with $CH_2Cl_2$ (×3). The extracts were dried ($Na_2SO_4$) and evaporated, and the residue was purified by column chromatography (EtOAc) to give the title compound as a white foam (1.53 g, 56%); $[\alpha]_D$ −73° (*c* 0.295, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 8.71 (br s, 1 H), 7.83 (s, 1 H), 6.83 (s, 1 H), 6.02−5.91 (m, 1 H), 5.36 (dq, $J$ = 17.2, 1.4 Hz, 1 H), 5.25 (dq, $J$ = 10.4, 1.1 Hz, 1 H), 4.80 (s, 2 H), 4.69−4.60 (m, 2 H), 4.46−4.37 (m, 3 H), 3.89−3.81 (m, 1 H), 3.80 (s, 3 H), 3.75−3.68 (m, 1 H), 3.62−3.55 (m, 1 H), 3.53−3.45 (m, 1 H), 3.02 (t, $J$ = 6.2 Hz, 2 H), 2.22−2.14 (m, 1 H), 1.95−1.86 (m, 1 H), 1.81−1.60 (m, 2 H); $^{13}$C NMR $\delta$ 170.8, 169.2, 153.6, 150.1, 144.1, 132.5, 132.0, 118.2, 116.1, 112.0, 106.2, 94.7, 74.1, 66.7, 65.8, 64.0, 61.2, 56.7, 51.6, 34.1, 28.4; HRMS (FAB, $^{35}$Cl) calcd for $C_{22}H_{28}Cl_3N_2O_8$ 553.0911, found 553.0903.

**Allyl (11a*S*)-11-Hydroxy-7-methoxy-5-oxo-8-[3-oxo-3-(2,2,2-trichloroethoxy)propoxy]-2,3,11,11a-tetrahydro-1*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-10(5*H*)-carboxylate.** The allyl carbamate was oxidized with DMP using the general method described above to give the titile compound as a white foam (78%); $[\alpha]_D$ +110° (*c* 0.265, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 7.24 (s, 1 H), 6.73 (s, 1 H), 5.87−5.74 (m, 1 H), 5.66−5.58 (m, 1 H), 5.20−5.11 (m, 2 H), 4.80 (s, 2 H), 4.67 (dd, $J$ = 13.3, 5.5 Hz, 1 H), 4.50−4.42 (m, 1 H), 4.37−4.28 (m, 2 H), 3.89 (s, 3 H), 3.78 (br s, 1 H), 3.73−3.66 (m, 1 H), 3.60−3.44 (m, 2 H), 3.02 (t, $J$ = 6.5 Hz, 2 H), 2.17−2.09 (m, 2 H), 2.04−1.96 (m, 2 H); $^{13}$C NMR $\delta$ 169.2, 166.8, 156.0, 149.5, 148.9, 131.8, 128.2, 126.6, 118.2, 114.7, 111.1, 94.7, 86.0, 74.1, 66.8, 64.4, 59.8, 56.1, 46.4, 34.0, 28.7, 23.0; HRMS (FAB, $^{35}$Cl) calcd for $C_{22}H_{26}Cl_3N_2O_8$ 551.0755, found 551.0754.

**3-({(11a*S*)-10-[(Allyloxy)carbonyl]-11-hydroxy-7-methoxy-5-oxo-2,3,5,10,11,11a-hexahydro-1*H*-pyrrolo[2,1-*c*][1,4]-benzodiazepin-8-yl}oxy)propionic Acid (15b).** The TCE ester was cleaved using $Zn/HCO_2H$ according to the general method described above to give **15b** as a white solid (69%); mp 168−170 °C (EtOAc); $[\alpha]_D$ +144° (*c* 0.295, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 7.26 (s, 1 H), 6.75 (s, 1 H), 5.84−5.73 (m, 1 H), 5.63 (d, $J$ = 9.7 Hz, 1 H), 5.18−5.10 (m, 2 H), 4.65 (dd, $J$ = 13.3, 5.3 Hz, 1 H), 4.49−4.41 (m, 1 H), 4.34−4.25 (m, 2 H), 3.89 (s, 3 H), 3.73−3.66 (m, 1 H), 3.59−3.45 (m, 2 H), 2.89 (t, $J$ = 6.6 Hz, 2 H), 2.15−2.07 (m, 2 H), 2.04−1.96 (m, 2 H); $^{13}$C NMR $\delta$ 174.5, 167.0, 156.2, 149.5, 149.0, 131.6, 128.2, 126.5, 118.3, 115.0, 111.0, 86.0, 66.9, 64.6, 60.0, 56.1, 46.4, 33.9, 28.7, 23.0. Anal. ($C_{20}H_{24}N_2O_8$) C, H, N.

**General Method for Coupling CBI Amines and PBD Acids. Preparation of Allyl (11a*S*)-8-{2-[(1*R*)-1-(Chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-benzo[*e*]indol-3-yl]-2-oxoethoxy}-11-hydroxy-7-methoxy-5-oxo-2,3,11,11a-tetrahydro-1*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-10(5*H*)-carboxylate (26a*R*).** A solution of *tert*-butyl (1*R*)-1-(chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-benzo[*e*]indole-3-carboxylate (**6*R***)[29,31] (161 mg, 0.48 mmol) in dioxane (10 mL) was saturated with HCl gas and allowed to stand until TLC analysis indicated complete consumption of the starting material (ca. 45 min). The solvent was evaporated, and **15a** (PBD acid $n$ = 1) (196 mg, 0.48 mmol), EDCI·HCl [1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride] (184 mg, 0.96 mmol), and DMA (5 mL) were added. The mixture was stirred at 20 °C for 16 h, and then aq $NaHCO_3$ was added. The mixture was extracted with EtOAc (×3), and the extracts were washed with aq NaCl (×2), then dried ($Na_2SO_4$) and evaporated. The residue was purified by column chromatography (EtOAc) to give **26a*R*** as a pale green solid (230 mg, 77%); $[\alpha]_D$ +90° (*c* 0.162, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 9.28 (s, 1 H), 8.13 (d, $J$ = 8.4 Hz, 1 H), 8.10 (s, 1 H), 7.52 (d, $J$ = 8.1 Hz, 1 H), 7.47 (dt, $J$ = 7.5, 1.1 Hz, 1 H), 7.35 (dt, $J$ = 7.5, 1.2 Hz, 1 H), 7.28 (s, 1 H), 6.96 (s, 1 H), 5.68−5.55 (m, 1 H), 5.05 (d, $J$ = 14.7 Hz, 1 H), 5.00−4.90 (m, 2 H), 4.88 (d, $J$ = 14.7 Hz, 1 H), 4.75 (br s, 1 H), 4.44−4.32 (m, 3 H), 4.20 (t, $J$ = 9.8 Hz, 1 H), 3.95 (s, 3 H), 3.88−3.80 (m, 2 H), 3.66−3.60 (m, 1 H), 3.51−3.42 (m, 1 H), 3.39−3.30 (m, 2 H), 1.99−1.80 (m, 4 H); $^{13}$C NMR $\delta$ 167.0, 166.8, 155.8, 154.8, 149.1, 148.8, 140.7, 131.8, 129.7, 128.2, 127.8, 127.6, 123.7 (two coincident CBI tertiary carbons), 122.9, 122.1, 117.8, 115.9, 115.0, 111.2, 100.3, 85.9, 69.3, 66.8, 60.2, 56.2, 52.4, 46.4, 46.1, 42.5, 28.5, 23.0; HRMS (FAB, $^{35}$Cl) calcd for $C_{32}H_{32}ClN_3O_8$ 621.1878, found 621.1864.

The following compounds were prepared by the same general method.

**Allyl (11a*S*)-8-{3-[(1*R*)-1-(chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-benzo[*e*]indol-3-yl]-3-oxopropoxy}-11-hydroxy-7-methoxy-5-oxo-2,3,11,11a-tetrahydro-1*H*-pyrrolo-[2,1-*c*][1,4]benzodiazepine-10(5*H*)-carboxylate (26b*R*)** as a cream solid (60%); $[\alpha]_D$ +90° (*c* 0.187, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 8.84 (s, 1 H), 8.23 (d, $J$ = 8.3 Hz, 1 H), 8.02 (s, 1 H), 7.52−7.38 (m, 3 H), 7.23 (s, 1 H), 6.92 (s, 1 H), 5.78−5.64 (m, 2 H), 5.10−5.01 (m, 2 H), 4.81 (br s, 1 H), 4.62−4.55 (m, 2 H), 4.45−4.38 (m, 1 H), 4.36−4.30 (m, 1 H), 4.19−4.14 (m, 2 H), 3.85 (s, 3 H), 3.84−3.78 (m, 1 H), 3.73−3.65 (m, 1 H), 3.59−3.38 (m, 3 H), 3.31 (t, $J$ = 10.3 Hz, 1 H), 3.23−3.14 (m, 1 H), 3.04−2.95 (m, 1 H), 2.10−1.90 (m, 4 H); $^{13}$C NMR $\delta$ 169.2,

167.0, 155.9, 154.3, 149.6, 148.3, 140.8, 131.8, 129.8, 128.6, 127.5, 126.5, 123.7, 123.6, 122.4, 122.2, 117.8, 115.0, 114.3, 110.7, 100.4, 86.1, 66.7, 64.5, 60.4, 56.1, 53.4, 46.5, 46.4, 41.9, 35.6, 28.7, 23.1; HRMS (FAB, $^{35}$Cl) calcd for $C_{33}H_{34}ClN_3O_8$ 635.2034, found 635.2031.

**Allyl (11a*S*)-8-{4-[(1*R*)-1-(chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-benzo[*e*]indol-3-yl]-4-oxobutoxy}-11-hydroxy-7-methoxy-5-oxo-2,3,11,11a-tetrahydro-1*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-10(5*H*)-carboxylate (26c*R*)** as a white solid (46%); $[\alpha]_D$ +152° (*c* 0.155, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 9.61 (s, 1 H), 8.23 (d, *J* = 8.2 Hz, 1 H), 8.19 (s, 1 H), 7.59 (d, *J* = 8.3 Hz, 1 H), 7.49 (dt, *J* = 7.6, 1.0 Hz, 1 H), 7.35 (dt, *J* = 7.7, 0.8 Hz, 1 H), 7.24 (s, 1 H), 6.92 (s, 1 H), 5.70−5.58 (m, 2 H), 5.04−4.95 (m, 1 H), 4.88 (br s, 1 H), 4.54−4.46 (m, 1 H), 4.37−4.29 (m, 1 H), 4.19−4.06 (m, 3 H), 4.01−3.93 (m, 1 H), 3.91−3.83 (m, 2 H), 3.89 (s, 3 H), 3.72−3.65 (m, 1 H), 3.57−3.43 (m, 2 H), 3.29 (t, *J* = 11.2 Hz, 1 H), 2.74−2.65 (m, 1 H), 2.60−2.51 (m, 1 H), 2.39−2.23 (m, 2 H), 2.12−1.95 (m, 4 H); $^{13}$C NMR $\delta$ 171.7, 167.1, 156.3, 154.8, 149.9, 148.6, 141.1, 131.6, 129.9, 128.4, 127.6, 125.9, 123.8, 123.4, 122.6, 122.0, 117.9, 114.5, 114.2, 110.6, 100.6, 86.0, 67.7, 66.8, 60.2, 56.1, 53.3, 46.4 (two coincident carbons: $CH_2Cl$ and PBD C-3), 42.1, 31.7, 28.6, 23.7, 23.0; HRMS (FAB, $^{35}$Cl) calcd for $C_{34}H_{36}ClN_3O_8$ 649.2191, found 649.2193.

**Allyl (11a*S*)-8-({5-[(1*R*)-1-(chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-benzo[*e*]indol-3-yl]-5-oxopentyl}oxy)-11-hydroxy-7-methoxy-5-oxo-2,3,11,11a-tetrahydro-1*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-10(5*H*)-carboxylate (26d*R*)** as a gray solid (41%); $[\alpha]_D$ +138° (*c* 0.189, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 9.27 (s, 1 H), 8.25 (d, *J* = 8.3 Hz, 1 H), 8.19 (s, 1 H), 7.63 (d, *J* = 8.3 Hz, 1 H), 7.50 (dt, *J* = 7.5, 0.8 Hz, 1 H), 7.35 (dt, *J* = 7.4, 0.7 Hz, 1 H), 7.20 (s, 1 H), 6.86 (s, 1 H), 5.79−5.65 (m, 1 H), 5.64 (br d, *J* = 9.5 Hz, 1 H), 5.12−5.02 (m, 2 H), 4.64−4.57 (m, 1 H), 4.46−4.39 (m, 1 H), 4.28−4.06 (m, 5 H), 4.02−3.91 (m, 2 H), 3.87 (s, 3 H), 3.71−3.64 (m, 1 H), 3.57−3.41 (m, 2 H), 3.40 (t, *J* = 10.6 Hz, 1 H), 2.76−2.56 (m, 2 H), 2.13−2.06 (m, 2 H), 2.05−1.92 (m, 6 H); $^{13}$C NMR $\delta$ 172.2, 167.1, 156.3, 154.8, 150.0, 148.7, 141.3, 131.7, 130.0, 128.3, 127.7, 125.8, 123.8, 123.5, 122.6, 122.0, 118.0, 114.7, 114.2, 110.6, 100.3, 86.1, 69.0, 66.9, 60.0, 56.1, 53.3, 46.4, 46.3, 42.3, 35.4, 28.7, 27.9, 23.1, 20.8; HRMS (FAB, $^{35}$Cl) calcd for $C_{35}H_{39}ClN_3O_8$ 664.2426, found 664.2431.

**Allyl (11a*S*)-8-({6-[(1*R*)-1-(chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-benzo[*e*]indol-3-yl]-6-oxohexyl}oxy)-11-hydroxy-7-methoxy-5-oxo-2,3,11,11a-tetrahydro-1*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-10(5*H*)-carboxylate (26e*R*)** as a pale gray-green solid (49%): $[\alpha]_D$ +157° (*c* 0.161, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 9.43 (s, 1 H), 8.28 (d, *J* = 8.3 Hz, 1 H), 8.25 (s, 1 H), 7.63 (d, *J* = 8.3 Hz, 1 H), 7.50 (dt, *J* = 7.5, 1.2 Hz, 1 H), 7.36 (dt, *J* = 7.6, 1.0 Hz, 1 H), 7.24 (s, 1 H), 6.79 (s, 1 H), 5.78−5.65 (m, 2 H), 5.12−5.01 (m, 2 H), 4.63 (dd, *J* = 13.3, 5.2 Hz, 1 H), 4.47−4.41 (m, 1 H), 4.32 (br s, 1 H), 4.28 (dd, *J* = 10.9, 1.7 Hz, 1 H), 4.22−4.16 (m, 1 H), 4.12−3.98 (m, 3 H), 3.95 (dd, *J* = 11.2, 3.0 Hz, 1 H), 3.88 (s, 3 H), 3.73−3.66 (m, 1 H), 3.58−3.43 (m, 3 H), 3.41 (t, *J* = 10.7 Hz, 1 H), 2.69−2.51 (m, 2 H), 2.14−2.08 (m, 2 H), 2.03−1.95 (m, 2 H), 1.93−1.82 (m, 4 H), 1.66−1.56 (m, 2 H); $^{13}$C NMR $\delta$ 172.5, 167.2, 156.2, 155.0, 149.9, 149.0, 141.2, 131.6, 130.0, 128.3, 127.6, 126.0, 123.9, 123.4, 122.7, 122.0, 117.9, 114.9, 114.6, 110.7, 100.6, 86.0, 69.1, 66.8, 60.4, 56.1, 53.4, 46.4 (two coincident carbons: $CH_2Cl$ and PBD C-3), 42.2, 35.7, 28.7, 28.2, 25.3, 24.2, 23.1; HRMS (FAB, $^{35}$Cl) calcd for $C_{36}H_{41}ClN_3O_8$ 678.2582, found 678.2561.

The same general method, starting from *tert*-butyl (1*S*)-1-(chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-benzo[*e*]indole-3-carboxylate (**6S**)[29,31] was used to prepare compounds **26a**−**e*S***, which differ from those described above only in the configuration of the chiral center at C1 of the *seco*-CBI. These compounds were not fully characterized but were used directly in the next step. In general their $^1$H NMR were very similar to those of the corresponding analogues **26a**−**e*R***, except for the case where *n* = 1 (**26a*S***) where very broad signals, presumably a consequence of amide conformers, were observed.

**Allyl (11a*S*)-8-{2-[(1*S*)-1-(chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-benzo[*e*]indol-3-yl]-2-oxoethoxy}-11-hydroxy-7-methoxy-5-oxo-2,3,11,11a-tetrahydro-1*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-10(5*H*)-carboxylate (26a*S*)** as a pale green solid (71%); $[\alpha]_D$ −88° (*c* 0.122, $CHCl_3$).

**Allyl (11a*S*)-8-{3-[(1*S*)-1-(chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-benzo[*e*]indol-3-yl]-3-oxopropoxy}-11-hydroxy-7-methoxy-5-oxo-2,3,11,11a-tetrahydro-1*H*-pyrrolo-[2,1-*c*][1,4]benzodiazepine-10(5*H*)-carboxylate (26b*S*)** as a cream solid (42%): $[\alpha]_D$ +23° (*c* 0.105, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 8.73 (s, 1 H), 8.25−8.19 (m, 2 H), 7.65 (d, *J* = 8.2 Hz, 1 H), 7.52 (t, *J* = 7.4 Hz, 1 H), 7.40 (t, *J* = 7.7 Hz, 1 H), 7.23 (s, 1 H), 6.92 (s, 1 H), 5.77−5.64 (m, 2 H), 5.09−5.02 (m, 2 H), 4.60−4.53 (m, 2 H), 4.47−4.30 (m, 4 H), 4.09−4.02 (m, 1 H), 3.99−3.93 (m, 1 H), 3.88 (s, 3 H), 3.70−3.63 (m, 1 H), 3.51−3.32 (m, 3 H), 3.27−3.17 (m, 1 H), 3.14−3.04 (m, 1 H), 2.02−1.80 (m, 4 H).

**Allyl (11a*S*)-8-{4-[(1*S*)-1-(chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-benzo[*e*]indol-3-yl]-4-oxobutoxy}-11-hydroxy-7-methoxy-5-oxo-2,3,11,11a-tetrahydro-1*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-10(5*H*)-carboxylate (26c*S*)** as a white solid (45−55%); $[\alpha]_D$ +57° (*c* 0.176, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 9.44 (s, 1 H), 8.29−8.24 (m, 2 H), 7.64 (d, *J* = 8.3 Hz, 1 H), 7.51 (dt, *J* = 7.6, 0.9 Hz, 1 H), 7.37 (dt, *J* = 7.7, 0.7 Hz, 1 H), 7.23 (s, 1 H), 6.85 (s, 1 H), 5.67−5.57 (m, 2 H), 5.05−4.95 (m, 2 H), 4.54−4.47 (m, 1 H), 4.45 (br s, 1 H), 4.33−4.10 (m, 4 H), 4.05−3.98 (m, 1 H), 3.93 (dd, *J* = 11.3, 2.8 Hz, 1 H), 3.88 (s, 3 H), 3.72−3.65 (m, 1 H), 3.58−3.49 (m, 1 H), 3.47−3.41 (m, 1 H), 3.37 (t, *J* = 10.8 Hz, 1 H), 2.89−2.74 (m, 2 H), 2.46−2.29 (m, 2 H), 2.12−1.93 (m, 4 H).

**Allyl (11a*S*)-8-({5-[(1*S*)-1-(chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-benzo[*e*]indol-3-yl]-5-oxopentyl}oxy)-11-hydroxy-7-methoxy-5-oxo-2,3,11,11a-tetrahydro-1*H*-pyrrolo-[2,1-*c*][1,4]benzodiazepine-10(5*H*)-carboxylate (26d*S*)** as a gray solid (37%); $[\alpha]_D$ +84° (*c* 0.161, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 8.97 (s, 1 H), 8.27 (d, *J* = 8.3 Hz, 1 H), 8.18 (s, 1 H), 7.64 (d, *J* = 8.4 Hz, 1 H), 7.51 (t, *J* = 7.6 Hz, 1 H), 7.37 (t, *J* = 7.7 Hz, 1 H), 7.20 (s, 1 H), 6.98 (s, 1 H), 5.83−5.72 (m, 1 H), 5.65 (d, *J* = 9.6 Hz, 1 H), 5.16−5.05 (m, 2 H), 4.66 (dd, *J* = 13.3, 4.8 Hz, 1 H), 4.49−4.42 (m, 1 H), 4.30−4.12 (m, 4 H), 4.07−3.99 (m, 1 H), 3.95 (dd, *J* = 11.1, 2.6 Hz, 1 H), 3.86 (s, 3 H), 3.72−3.64 (m, 1 H), 3.57−3.49 (m, 1 H), 3.46−3.40 (m, 1 H), 3.38 (t, *J* = 10.8 Hz, 1 H), 2.74−2.55 (m, 2 H), 2.10−1.93 (m, 8 H).

**Allyl (11a*S*)-8-({6-[(1*S*)-1-(chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-benzo[*e*]indol-3-yl]-6-oxohexyl}oxy)-11-hydroxy-7-methoxy-5-oxo-2,3,11,11a-tetrahydro-1*H*-pyrrolo-[2,1-*c*][1,4]benzodiazepine-10(5*H*)-carboxylate (26e*S*)** as a pale gray-green solid (45%); $[\alpha]_D$ +92° (*c* 0.176, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 9.11 (s, 1 H), 8.29 (d, *J* = 8.4 Hz, 1 H), 8.18 (s, 1 H), 7.65 (d, *J* = 8.3 Hz, 1 H), 7.51 (dt, *J* = 7.5, 1.1 Hz, 1 H), 7.37 (dt, *J* = 7.7, 0.7 Hz, 1 H), 7.25 (s, 1 H), 6.81 (s, 1 H), 5.79−5.68 (m, 2 H), 5.14−5.02 (m, 2 H), 4.65 (dd, *J* = 13.5, 5.3 Hz, 1 H), 4.49−4.42 (m, 1 H), 4.41 (br s, 1 H), 4.32−4.14 (m, 3 H), 4.08−3.99 (m, 2 H), 3.96 (dd, *J* = 11.3, 3.0 Hz, 1 H), 3.90 (s, 3 H), 3.74−3.67 (m, 1 H), 3.59−3.44 (m, 2 H), 3.40 (t, *J* = 10.9 Hz, 1 H), 2.70−2.52 (m, 2 H), 2.14−2.08 (m, 2 H), 2.03−1.94 (m, 2 H), 1.92−1.82 (m, 4 H), 1.65−1.50 (m, 2 H).

**General Method for Removal of the Aloc Protecting Group. Preparation of (11a*S*)-8-{2-[(1*R*)-1-(Chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-benzo[*e*]indol-3-yl]-2-oxoethoxy}-7-methoxy-1,2,3,11a-tetrahydro-5*H*-pyrrolo-[2,1-*c*][1,4]benzodiazepin-5-one (27a*R*).** Pyrrolidine (0.30 mL, 3.6 mmol) then $Pd(PPh_3)_4$ (21 mg, 5%) was added to a solution of **26a*R*** (222 mg, 0.36 mmol) in $CH_2Cl_2$ (4 mL) and the solution stirred at 20 °C. TLC analysis (1:15 MeOH:EtOAc) indicated that the reaction was complete within 30 s. After 5 min the solution was concentrated to ca. 1 mL at 20 °C and directly purified by column chromatography (1:20 MeOH:EtOAc). The product-containing fractions were dissolved in and evaporated from $CH_2Cl_2$ (×3, to convert any hemiaminal to the imine form[44]), and the residue was triturated with EtOAc (4 mL) to give **27a*R*** as a cream solid (126 mg, 68%); mp 190−196 °C (dec); $[\alpha]_D$ +557° (*c* 0.176, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 9.62 (s, 1 H), 8.09 (d, *J* = 8.2 Hz, 1 H), 8.04 (s, 1 H), 7.60 (s, 1

H), 7.55 (d, $J$ = 4.3 Hz, 1 H), 7.44 (t, $J$ = 7.4 Hz, 1 H), 7.38−7.31 (m, 2 H), 6.84 (s, 1 H), 5.25 (d, $J$ = 15.0 Hz, 1 H), 4.99 (d, $J$ = 15.0 Hz, 1 H), 4.26−4.20 (m, 2 H), 4.03 (s, 3 H), 3.85−3.74 (m, 2 H), 3.60−3.47 (m, 3 H), 3.30 (t, $J$ = 10.5 Hz, 1 H), 2.19−2.13 (m, 2 H), 2.01−1.94 (m, 2 H); $^{13}$C NMR $\delta$ 166.5, 164.5, 162.8, 154.9, 149.8, 148.1, 140.8, 140.4, 129.6, 127.2, 123.7, 123.3, 122.7, 122.1, 121.5, 114.3, 112.0, 111.2, 100.3, 68.1, 56.2, 53.7, 52.0, 46.7, 46.3, 42.2, 29.4, 24.1. Anal. ($C_{28}H_{26}ClN_3O_5\cdot H_2O$) C, H; N: calcd, 7.81; found 7.38.

The following compounds were prepared by the same general method.

**(11a*S*)-8-{3-[(1*R*)-1-(Chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-benzo[*e*]indol-3-yl]-3-oxopropoxy}-7-methoxy-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-5-one (27b*R*)** as a cream solid (74%); mp 185−190 °C (dec); $[\alpha]_D$ +591° ($c$ 0.158, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 9.88 (s, 1 H), 8.37 (d, $J$ = 8.3 Hz, 1 H), 8.24 (s, 1 H), 7.70 (d, $J$ = 4.3 Hz, 1 H), 7.55 (d, $J$ = 8.2 Hz, 1 H), 7.50 (t, $J$ = 7.1 Hz, 1 H), 7.49 (s, 1 H), 7.36 (dt, $J$ = 7.5, 0.9 Hz, 1 H), 7.12 (s, 1 H), 4.61−4.46 (m, 1 H), 4.15−4.11 (m, 1 H), 3.94−3.73 (m, 4 H), 3.88 (s, 3 H), 3.60−3.48 (m, 2 H), 3.31 (t, $J$ = 10.8 Hz, 1 H), 3.08−3.01 (m, 2 H), 2.30−2.24 (m, 2 H), 2.09−1.98 (m, 2 H); $^{13}$C NMR $\delta$ 169.6, 164.6, 162.3, 154.9, 150.4, 147.4, 140.8, 129.8, 127.5, 124.3, 123.5, 122.6, 122.0, 120.4, 114.7, 111.6, 110.9, 100.6, 64.6, 56.1, 53.7, 53.5, 46.7, 46.5, 42.0, 35.8, 29.6, 24.2. Anal. ($C_{29}H_{28}ClN_3O_5\cdot H_2O$) C, H, N.

**(11a*S*)-8-{4-[(1*R*)-1-(Chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-benzo[*e*]indol-3-yl]-4-oxobutoxy}-7-methoxy-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-5-one (27c*R*)** as a cream solid (81%); mp 220−221 °C (dec); $[\alpha]_D$ +626° ($c$ 0.159, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 9.64 (s, 1 H), 8.30 (s, 1 H), 8.25 (d, $J$ = 8.4 Hz, 1 H), 7.62 (d, $J$ = 8.3 Hz, 1 H), 7.58 (d, $J$ = 4.3 Hz, 1 H), 7.50 (t, $J$ = 7.3 Hz, 1 H), 7.49 (s, 1 H), 7.35 (t, $J$ = 7.6 Hz, 1 H), 6.85 (s, 1 H), 4.33−4.17 (m, 4 H), 4.03−3.88 (m, 5 H), 3.86 (s, 3 H), 3.83−3.76 (m, 1 H), 3.59−3.53 (m, 2 H), 3.38 (t, $J$ = 10.7 Hz, 1 H), 2.90−2.75 (m, 2 H), 2.45−2.37 (m, 2 H), 2.24−2.17 (m, 2 H), 2.05−1.95 (m, 2H); $^{13}$C NMR $\delta$ 172.0, 164.6, 162.4, 155.0, 150.5, 147.8, 141.2, 140.6, 129.9, 127.5, 123.9, 123.4, 122.7, 122.0, 120.3, 114.7, 111.6, 110.8, 100.7, 67.5, 56.1, 53.6, 53.5, 46.7, 46.4, 42.2, 32.5, 29.6, 24.2, 24.1; HRMS (FAB, $^{35}$Cl) calcd for $C_{30}H_{31}ClN_3O_5$ 548.1952, found 548.1915. Anal. ($C_{30}H_{30}ClN_3O_5\cdot H_2O$) C, H, N.

**(11a*S*)-8-({5-[(1*R*)-1-(Chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-benzo[*e*]indol-3-yl]-5-oxopentyl}oxy)-7-methoxy-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-5-one (27d*R*)** as a cream solid (75%); mp 170−178 °C (dec); $[\alpha]_D$ +611° ($c$ 0.164, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 9.72 (s, 1 H), 8.29 (s, 1 H), 8.26 (d, $J$ = 8.3 Hz, 1 H), 7.65 (d, $J$ = 4.6 Hz, 1 H), 7.63 (d, $J$ ca. 9.1 Hz, 1 H), 7.50 (s, 1 H), 7.49 (t, $J$ ca. 7.1 Hz, 1 H), 7.35 (t, $J$ = 7.6 Hz, 1 H), 6.84 (s, 1 H), 4.30 (dd, $J$ = 10.8, 1.5 Hz, 1 H), 4.26−4.10 (m, 3 H), 4.05−3.98 (m, 1 H), 3.95 (dd, $J$ = 11.2, 2.7 Hz, 1 H), 3.89 (s, 3 H), 3.85−3.78 (m, 1 H), 3.70−3.65 (m, 1 H), 3.62−3.53 (m, 1 H), 3.41 (t, $J$ = 10.8 Hz, 1 H), 2.79−2.62 (m, 2 H), 2.33−2.26 (m, 2 H), 2.10−1.99 (m, 6 H); $^{13}$C NMR $\delta$ 172.4, 164.7, 162.4, 155.1, 150.8, 147.9, 141.3, 140.6, 129.9, 127.5, 123.9, 123.4, 122.7, 122.0, 120.2, 114.6, 111.6, 110.6, 100.6, 68.7, 60.4, 56.1, 53.7, 53.5, 46.7, 46.3, 42.3, 35.8, 29.6, 28.4, 24.2, 21.5. Anal. ($C_{31}H_{32}ClN_3O_5\cdot 1^1/_2H_2O$) H, N; C: calcd, 63.21; found 63.64.

**(11a*S*)-8-({6-[(1*R*)-1-(Chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-benzo[*e*]indol-3-yl]-6-oxohexyl}oxy)-7-methoxy-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-5-one (27e*R*)** as a cream solid (59%); mp 181−185 °C (dec); $[\alpha]_D$ +598° ($c$ 0.134, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 9.59 (s, 1 H), 8.30 (s, 1 H), 8.28 (d, $J$ = 8.7 Hz, 1 H), 7.65 (d, $J$ = 8.9 Hz, 1 H), 7.64 (d, $J$ = 5.0 Hz, 1 H), 7.50 (s, 1 H), 7.49 (t, $J$ ca. 8.1 Hz, 1 H), 7.35 (t, $J$ = 7.6 Hz, 1 H), 6.82 (s, 1 H), 4.31 (br d, $J$ = 10.7 Hz, 1 H), 4.26−4.20 (m, 1 H), 4.19−4.00 (m, 3 H), 3.95 (dd, $J$ = 11.3, 2.8 Hz, 1 H), 3.89 (s, 3 H), 3.86−3.78 (m, 1 H), 3.71−3.66 (m, 1 H), 3.62−3.54 (m, 1 H), 3.41 (t, $J$ = 10.8 Hz, 1 H), 2.72−2.54 (m, 2 H), 2.33−2.26 (m, 2 H), 2.09−1.88 (m, 6 H), 1.70−1.60 (m, 2 H). Anal. ($C_{32}H_{34}ClN_3O_5\cdot H_2O$) C, H, N.

**(11a*S*)-8-{2-[(1*S*)-1-(Chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-benzo[*e*]indol-3-yl]-2-oxoethoxy}-7-methoxy-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-5-one (27a*S*)** as a cream solid (61%); mp 225−230 °C (dec); $^1$H NMR (DMSO-$d_6$) $\delta$ 10.41 (s, 1 H), 8.10 (d, $J$ = 8.3 Hz, 1 H), 7.90 (s, 1 H), 7.80 (d, $J$ = 8.4 Hz, 1 H), 7.75 (d, $J$ = 4.5 Hz, 1 H), 7.52 (t, $J$ = 7.5 Hz, 1 H), 7.34 (t, $J$ = 7.6 Hz, 1 H), 6.91 (s, 1 H), 5.10 (q, $J$ = 15.8 Hz, 1 H), 4.45−4.37 (m, 1 H), 4.28−4.18 (m, 2 H), 4.00 (dd, $J$ = 10.9, 2.8 Hz, 1 H), 3.87 (s, 3 H), 3.84−3.57 (m, 3 H), 3.43−3.35 (m, 1 H), 2.32−2.17 (m, 2 H), 1.98−1.89 (m, 2 H); $^{13}$C NMR $\delta$ 165.3, 164.2, 163.2, 154.3, 149.6, 146.8, 141.5, 140.3, 129.8, 127.3, 123.1, 122.9, 122.6, 121.9, 120.3, 113.8, 111.5, 110.9, 99.4, 66.8, 55.6, 53.3, 51.1, 47.5, 46.3, 41.2, 28.7, 23.6; HRMS (FAB, $^{35}$Cl) calcd for $C_{28}H_{26}ClN_3O_5$ 519.1561, found 519.1559. $^1$H NMR analysis also showed the presence of an impurity (ca. 10%) which could not be removed by trituration or attempted crystallization from a number of solvents.

**(11a*S*)-8-{3-[(1*S*)-1-(Chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-benzo[*e*]indol-3-yl]-3-oxopropoxy}-7-methoxy-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-5-one (27b*S*)** as a cream solid (76%); mp 185−195 °C (dec); $[\alpha]_D$ +372° ($c$ 0.137, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 9.49 (s, 1 H), 8.30 (d, $J$ = 8.3 Hz, 1 H), 8.25 (s, 1 H), 7.68 (d, $J$ = 4.4 Hz, 1 H), 7.55 (d, $J$ = 8.2 Hz, 1 H), 7.51 (s, 1 H), 7.48 (dt, $J$ = 8.2, 1.0 Hz, 1 H), 7.35 (dt, $J$ = 7.6, 0.9 Hz, 1 H), 7.08 (s, 1 H), 4.56−4.40 (m, 2 H), 4.26 (br d, $J$ = 11.0 Hz, 1 H), 4.17−4.10 (m, 1 H), 3.90 (s, 3 H), 3.89−3.76 (m, 3 H), 3.61−3.48 (m, 2 H), 3.31 (t, $J$ = 11.3 Hz, 1 H), 3.16−3.08 (m, 1 H), 3.02−3.13 (m, 1 H), 2.29−2.18 (m, 2 H), 2.08−1.97 (m, 2 H). Anal. ($C_{29}H_{28}ClN_3O_5\cdot 1^1/_2H_2O$) C, H, N.

**(11a*S*)-8-{4-[(1*S*)-1-(Chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-benzo[*e*]indol-3-yl]-4-oxobutoxy}-7-methoxy-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-5-one (27c*S*)** as a cream solid (71%); mp 189−195 °C (dec); $[\alpha]_D$ +475° ($c$ 0.153, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 9.16 (s, 1 H), 8.26 (d, $J$ = 8.3 Hz, 1 H), 8.21 (s, 1 H), 7.64 (d, $J$ = 8.4 Hz, 1 H), 7.61 (d, $J$ = 4.4 Hz, 1 H), 7.50 (t, $J$ = 7.8 Hz, 1 H), 7.48 (s, 1 H), 7.37 (t, $J$ = 7.7 Hz, 1 H), 6.87 (s, 1 H), 4.35−4.21 (m, 2 H), 4.05−3.98 (m, 1 H), 3.95−3.89 (m, 1 H), 3.85 (s, 3 H), 3.84−3.77 (m, 1 H), 3.64−3.52 (m, 2 H), 3.38 (t, $J$ = 10.8 Hz, 1 H), 2.93−2.85 (m, 1 H), 2.82−2.73 (m, 1 H), 2.47−2.33 (m, 2 H), 2.28−2.21 (m, 2 H), 2.06−1.99 (m, 2H). Anal. ($C_{30}H_{30}ClN_3O_5\cdot 1^1/_2H_2O$) H, N; C: calcd, 64.69; found 64.24.

**(11a*S*)-8-({5-[(1*S*)-1-(Chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-benzo[*e*]indol-3-yl]-5-oxopentyl}oxy)-7-methoxy-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-5-one (27d*S*)** as a cream solid (80%); mp 178−186 °C (dec); $[\alpha]_D$ +463° ($c$ 0.133, $CHCl_3$); $^1$H NMR ($CDCl_3$): apart from phenol signal ($\delta$ 9.86 instead of 9.72) identical (± 0.03 ppm) to that reported for **27*R*d**, $^{13}$C NMR identical (± 0.1 ppm) to that reported for **27*R*d**. Anal. ($C_{31}H_{32}ClN_3O_5\cdot 1^1/_2H_2O$) C, H, N.

**(11a*S*)-8-({6-[(1*S*)-1-(Chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-benzo[*e*]indol-3-yl]-6-oxohexyl}oxy)-7-methoxy-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-5-one (27e*S*)** as a cream solid (64%); mp 118−125 °C (dec); $[\alpha]_D$ +505° ($c$ 0.111, $CHCl_3$); $^1$H NMR ($CDCl_3$): apart from phenol signal ($\delta$ 9.83 instead of 9.59) identical (± 0.03 ppm) to that reported for **27*R*e**, $^{13}$C NMR ($CDCl_3$) $\delta$ 172.6, 164.7, 162.3, 155.2, 150.9, 147.9, 141.3, 140.6, 130.0, 127.5, 123.9, 123.4, 122.8, 122.0, 120.1, 114.6, 111.6, 110.7, 100.7, 68.7, 56.1, 53.7, 53.5, 46.7, 46.3, 42.3, 36.1, 29.6, 28.7, 25.6, 24.4, 22.2. Anal. ($C_{32}H_{34}ClN_3O_5\cdot 1^1/_2H_2O$) C, H, N.

**2,2,2-Trichloroethyl 6-{[(11a*S*)-7-Methoxy-5,11-dioxo-2,3,5,10,11,11a-hexahydro-1*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-8-yl]oxy}hexanoate.** A sample of **13e** which had been left to stand at 20 °C for 16 months appeared to be mostly hydrolyzed to the corresponding acid (2S)-1-(2-{[(allyloxy)carbonyl]amino}-5-methoxy-4-{[6-oxo-6-(2,2,2-trichloroethoxy)hexyl]oxy}benzoyl)-2-pyrrolidinecarboxylic acid; $^1$H NMR ($CDCl_3$) $\delta$ 8.63 (br s, 1 H), 7.78 (br s, 1 H), 6.84 (s, 1 H), 5.99−5.89 (m, 1 H), 5.34 (dq, $J$ = 17.1, 1.4 Hz, 1 H), 5.22 (dq, $J$ = 10.3, 1.4 Hz, 1 H), 4.75 (s, 2 H), 4.64−4.60 (m, 2 H), 4.08 (t, $J$ = 6.7 Hz, 2 H), 3.82 (s, 3 H), 3.70−3.50 (m, 3 H), 2.51 (t, $J$ = 7.5 Hz, 2 H), 2.40−2.20 (m, 2 H), 2.07−1.98 (m, 2 H), 1.94−

1.83 (m, 3 H), 1.78 (quint, $J$ = 7.6 Hz, 2 H), 1.60−1.50 (m, 2 H); MS (APCI -ve, $^{35}Cl$), 607 (100%, M − $H^+$).

This acid (374 mg, 0.61 mmol) was dissolved in $CH_2Cl_2$ (10 mL) and pyrrolidine (0.26 mL, 3.1 mmol) and $Pd(PPh_3)_4$ (24 mg, 3%) were added. After stirring for 30 min at 20 °C, the solvent was evaporated and the residue dissolved in and evaporated from THF several times, until the residue was free of the excess pyrrolidine. The residue was redissolved in THF (10 mL), EDCI·HCl (0.24 g, 1.2 mmol) was added, and the mixture was stirred at 20 °C for 20 h. The THF was evaporated and the residue partitioned between $H_2O$ and EtOAc. The EtOAc layer and two further EtOAc extracts were dried ($Na_2SO_4$) and evaporated. The residue was purified by column chromatography (EtOAc) to give the title compound as a white solid (ca. 170 mg, ca. 55%), contaminated with a little coeluting $OPPh_3$; $^1H$ NMR ($CDCl_3$) $\delta$ 7.80 (s, 1 H), 7.44 (s, 1H), 6.43 (s, 1 H), 4.75 (s, 2 H), 4.08−4.06 (m, 1 H), 4.02 (t, $J$ = 6.5 Hz, 2 H), 3.90 (s, 3 H), 3.81−3.74 (m, 1 H), 3.65−3.57 (m, 1 H), 2.78−2.69 (m, 1 H), 2.51 (t, $J$ = 7.4 Hz, 2 H), 2.09−1.98 (m, 3 H), 1.90 (quint, $J$ = 7.1 Hz, 2 H), 1.78 (quint, $J$ = 7.6 Hz, 2 H), 1.60−1.52 (m, 2 H); $^{13}C$ NMR $\delta$ 171.8, 170.7, 165.2, 151.7, 146.7, 129.3, 119.4, 112.4, 104.9, 95.0, 73.9, 68.8, 56.8, 56.2, 47.3, 33.7, 28.5, 26.2, 25.4, 24.4, 23.5; HRMS (EI, $^{35}Cl$) calcd for $C_{21}H_{25}Cl_3N_2O_6$ 506.0778, found 506.0772.

**6-{[(11a*S*)-7-Methoxy-5,11-dioxo-2,3,5,10,11,11a-hexahydro-1*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-8-yl]oxy}-hexanoic Acid (34).** The TCE ester was cleaved using Zn/$HCO_2H$ according to the general method described above to give **34** as a colorless oil (85%); $[\alpha]_D$ +265° (*c* 0.145, $CHCl_3$); $^1H$ NMR ($CDCl_3$) $\delta$ 9.45 (s, 1 H), 7.44 (s, 1 H), 6.50 (s, 1 H), 4.08−3.99 (m, 3 H), 3.89 (s, 3 H), 3.81−3.74 (m, 1 H), 3.64−3.56 (m, 1 H), 2.77−2.68 (m, 1 H), 2.39 (t, $J$ = 7.3 Hz, 2 H), 2.07−1.96 (m, 3 H), 1.88 (quint, $J$ = 7.1 Hz, 2 H), 1.75−1.67 (m, 2 H), 1.57−1.49 (m, 2 H); $^{13}C$ NMR $\delta$ 178.1, 171.8, 165.4, 151.8, 146.7, 129.5, 119.0, 112.3, 105.0, 68.8, 56.8, 56.2, 47.4, 33.8, 28.4, 26.1, 25.2, 24.3, 23.5; HRMS (EI) calcd for $C_{19}H_{24}N_2O_6$ 376.1634, found 376.1628.

**(11a*S*)-8-({6-[(1*S*)-1-(Chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-benzo[*e*]indol-3-yl]-6-oxohexyl}oxy)-7-methoxy-2,3-dihydro-1*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11-(10*H*,11a*H*)-dione (35).** The acid **34** was coupled with **6*S*** using the general method described above, and the product triturated with EtOAc to give **35** as a pale gray powder (32%); mp 166−170 °C (dec); $[\alpha]_D$ +157° (*c* 0.151, THF); $^1H$ NMR (DMSO-$d_6$) $\delta$ 10.33 (s, 1 H), 10.20 (s, 1 H), 8.08 (d, $J$ = 8.2 Hz, 1 H), 7.99 (s, 1 H), 7.77 (d, $J$ = 8.3 Hz, 1 H), 7.48 (t, $J$ = 7.5 Hz, 1 H), 7.31 (t, $J$ = 7.6 Hz, 1 H), 7.23 (s, 1 H), 6.71 (s, 1 H), 4.37−4.30 (m, 1 H), 4.19−4.10 (m, 2 H), 4.08−3.93 (m, 4 H), 3.81−3.74 (m, 1 H), 3.77 (s, 3 H), 3.57−3.51 (m, 1 H), 3.48−3.41 (m, 1 H), 2.62−2.44 (m, 3 H), 1.98−1.65 (m, 7 H), 1.56−1.48 (m, 2 H). Anal. ($C_{32}H_{34}ClN_3O_6$·$H_2O$) C, H, N.

***tert*-Butyl 5-(Benzyloxy)-1-methyl-1,2-dihydro-3*H*-benzo[*e*]indole-3-carboxylate.** A solution of *tert*-butyl allyl[4-(benzyloxy)-1-bromo-2-naphthyl]carbamate (**37**)[50] (369 mg, 0.76 mmol), $Bu_3SnH$ (0.25 mL, 0.91 mmol), and AIBN (2,2′-azobisisobutyronitrile) (10 mg, 8%) in dry PhH (25 mL) was degassed by bubbling with $N_2$, then stirred at reflux under $N_2$ for 1 h. The solvent was evaporated and the residue diluted with petroleum ether and extracted with $CH_3CN$ (×4). The extracts were evaporated and the residue purified by column chromatography (1:20 EtOAc:petroleum ether) to give the title compound as a colorless oil (257 mg, 84%). A sample crystallized from petroleum ether, mp 89−90 °C; $^1H$ NMR ($CDCl_3$) $\delta$ 8.28 (d, $J$ = 8.4 Hz, 1 H), 7.87 (br s, 1 H), 7.68 (d, $J$ = 8.3 Hz, 1 H), 7.55 (br d, $J$ = 7.0 Hz, 2 H), 7.50−7.28 (m, 5 H), 5.26 (s, 2 H), 4.18 (dd, $J$ = 11.0, 9.4 Hz, 1 H), 3.84−3.67 (m, 2 H), 1.60 (s, 9 H), 1.38 (d, $J$ = 6.9 Hz, 3 H); $^{13}C$ NMR (two aromatic quaternary carbons not seen) $\delta$ 155.0, 152.8, 137.1, 130.2, 128.5, 127.9, 127.5, 127.0, 123.4, 122.8, 122.4, 96.6, 80.6, 70.2, 56.9, 32.9, 28.5, 21.5. Anal. ($C_{25}H_{27}NO_3$) C, H, N.

***tert*-Butyl 5-Hydroxy-1-methyl-1,2-dihydro-3*H*-benzo-[*e*]indole-3-carboxylate (38).** A mixture of the benzyl ether prepared above (94 mg, 0.24 mmol), aq $NH_4HCO_2$ (25%, 0.5 mL, 2.0 mmol), and Pd/C (10%, 25 mg) in THF (5 mL) was stirred under $N_2$ at 20 °C for 90 min. The catalyst was filtered off through Celite, and the filtrate was diluted with $H_2O$ and extracted with EtOAc (×2). The extracts were dried ($Na_2SO_4$) and evaporated, and the resulting oil was triturated with hot petroleum ether to give **38** as a white powder (67 mg, 93%); mp 175−177 °C; $^1H$ NMR ($CDCl_3$) $\delta$ 8.17 (d, $J$ = 8.1 Hz, 1 H), 7.79 (br s, 1 H), 7.67 (d, $J$ = 8.4 Hz, 1 H), 7.45 (t, $J$ = 7.5 Hz, 1 H), 7.31 (t, $J$ = 7.3 Hz, 1 H), 6.71 (br s, 1 H), 4.16 (dd, $J$ = 11.0, 9.3 Hz, 1 H), 3.80−3.67 (m, 2 H), 1.59 (s, 9 H), 1.38 (d, $J$ = 6.8 Hz, 3 H); $^{13}C$ NMR $\delta$ 153.1, 152.5, 139.6, 130.4, 126.9, 123.2, 122.6, 122.5, 121.4, 120.7, 99.3, 81.0, 57.0, 33.0, 28.5, 21.6. Anal. ($C_{18}H_{21}NO_3$) C, H, N.

**(11a*S*)-8-{[6-(5-Hydroxy-1-methyl-1,2-dihydro-3*H*-benzo[*e*]indol-3-yl)-6-oxohexyl]oxy}-7-methoxy-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-5-one (39).** **38** was deprotected with HCl and coupled with the PBD acid **15e** using EDCI according to the general method described above, to give allyl (11a*S*)-11-hydroxy-8-{[6-(5-hydroxy-1-methyl-1,2-dihydro-3*H*-benzo[*e*]indol-3-yl)-6-oxohexyl]oxy}-7-methoxy-5-oxo-2,3,11,11a-tetrahydro-1*H*-pyrrolo[2,1-*c*][1,4]-benzodiazepine-10(5*H*)-carboxylate as a pale green oil (38%). The Aloc group was directly deprotected using $Pd(PPh_3)_4$, as described above, and the crude product was triturated with EtOAc to give **39** as a pale tan powder (70%); mp 170−176 °C; $^1H$ NMR ($CDCl_3$) (several signals split 1:1 due to diastereomers) $\delta$ 9.05, 9.01 (2×s, 1 H), 8.25 (d, $J$ = 8.4 Hz, 1 H), 7.68 (d, $J$ = 8.3 Hz, 1 H), 7.64 (d, $J$ = 4.3 Hz, 1 H), 7.51 (s, 1 H), 7.45 (t, $J$ = 7.3 Hz, 1 H), 7.32 (t, $J$ = 7.3 Hz, 1 H), 6.84, 6.83 (2×s, 1 H), 4.33−4.27 (m, 1 H), 4.19−4.07 (m, 2 H), 3.91 (s, 3 H), 3.86−3.77 (m, 3 H), 3.71−3.66 (m, 1 H), 3.61−3.54 (m, 1 H), 2.65−2.48 (m, 2 H), 2.34−2.25 (m, 2 H), 2.09−1.86 (m, 6 H), 1.68−1.58 (m, 2 H), 1.41, 1.40 (2×d, $J$ = 6.7 Hz, 3 H). Anal. ($C_{32}H_{35}N_3O_5$·EtOAc) C, H, N.

**In Vitro Cytotoxicity.** For the cell lines AA8, UV4, EMT6, and Skov3 in Table 1, inhibition of proliferation of log-phase monolayers was assessed in 96-well plates as described previously.[51] The drug exposure time was 4 h followed by sulforhodamine B staining 3 days later. The $IC_{50}$ was determined by interpolation as the drug concentration required to inhibit cell density to 50% of that of the controls on the same plate.

For the cell lines Colo205, HT29, WiDr, Skov3, and SiHa in Table 2, inhibition of [$^3H$]thymidine uptake by cell suspensions was assessed in 96-well plates as described previously.[52] Cells were exposed to drugs continuously for 5 days, with addition of [$^3H$]thymidine (0.25 $\mu$Ci/mL, 0.1 $\mu$M) and 5-fluoro-2′-deoxyuridine (0.1 $\mu$M) 4 h prior to culture termination. Pronase (2 mg/mL) in $Na_4EDTA$ (4 mM) (150 $\mu$L/well, 30−60 min treatment) was used to release any adherent cells from the plate prior to aspiration and filtration. The $IC_{50}$ was determined by interpolation as the drug concentration required to reduce [$^3H$]thymidine uptake to 50% of that of the controls on the same plate.

**Cross-Linking of Naked DNA.** A mixture containing pcDNA3 plasmid (Invitrogen, 5 $\mu$g, 5 $\mu$L), *Eco*RI restriction endonuclease (5 $\mu$L, 50 units), Boehringer buffer H (5 $\mu$L), and sterile $H_2O$ (17 $\mu$L) was incubated at 37 °C for 90 min to linearize the DNA. The DNA was labeled at the 3′ end by adding α-$^{32}$P-dATP (2 $\mu$L, 20 $\mu$Ci), Klenow polymerase (1 $\mu$L, 5 units), Boehringer buffer H (1 $\mu$L), and sterile $H_2O$ (8 $\mu$L) and incubating the mixture at 37 °C for 30 min. The labeled DNA was purified using a High Pure PCR purification kit (Boehringer) and resuspended in Tris-EDTA (TE, pH 7.5) buffer at 50 ng/$\mu$L ($OD_{260\ nm}$).

A mixture of the linearized labeled plasmid (1 $\mu$L, 50 ng) and test drug (1 $\mu$L, various concentrations in DMSO, to give the desired concentration in a final volume of 10 $\mu$L) in TE buffer (8 $\mu$L) was incubated at 37 °C for 18 h and then diluted with strand separation buffer (6 $\mu$L of DMSO, 2 $\mu$L of 0.5 M NaOH, and 2 $\mu$L of $H_2O$ containing 0.25% bromophenol blue, 0.25% xylene cyanol, and 30% glycerol). The mixture was allowed to stand at room temperature for 5−10 min and then loaded directly onto a 0.8% agarose gel. Control experiments showed that extending the alkali treatment time to 30 min gave no change in the amount of cross-linked DNA. Electro-

phoresis was performed at 75 V for 4 h. The gels were dried, and the bands were visualized and quantified by phosphorimagery, using Imagequant software. Results were expressed as percentage double-stranded DNA of the total present for each lane and averaged over 2−4 assays.

Control double-strand (ds) lanes were prepared in the absence of alkali treatment, and control single-strand (ss) lanes in the absence of drug. Control ds lanes were always free of ss DNA, but occasionally (3 of 16 gels) the ss lanes contained 2−4% ds DNA, indicating incomplete denaturation. This was not considered significant in calculating $C_{50}$. Each gel contained **3** as an internal standard, which gave good reproducibility on repeat assay.

The data points were fitted to a Logistic 3 parameter sigmoidal curve using Sigmaplot software. The curve is defined by the equation $y = a/[1 + (x/x_0)^b]$ where a is the maximum $y$ value (constrained to 100 in this case), $b$ is a measure of the slope, and $x_0$ is the midpoint (equal to $C_{50}$). In all cases the data converged to this fitted curve and was tolerated. The individual data points, fitted curves, and curve parameters are included in the Supporting Information.

**Cross-Linking of Cellular DNA (Comet Assay).** This was performed as previously described,[72] except that Skov3 cell suspensions were used ($10^5$ cells/mL) under aerobic conditions with a drug exposure time of 1 h. The cross-linking index (CI) was calculated from the median tail moment (TM) for each treatment as follows:

$$\mathrm{CI} = \frac{\mathrm{TM(radiation\ only)} - \mathrm{TM(drug + radiation)}}{\mathrm{TM(radiation\ only)} - \mathrm{TM(control)}}$$

and the cross-linking index of 50% ($CI_{50}$) was derived by interpolation from Figure 5.

**Thermal Cleavage Assay.** This was performed as previously described,[31c] except that the 5′-$^{32}$P-end labeled DNA was a 512 bp fragment of the *lac UV5* promoter. To generate this fragment the TC1 primer (5′-ctggcacgacaggtttcc-3′) was end-labeled using T4 PNK and PCR was performed using this, unlabeled TC3 primer (5′-tgggctgcaggtcgacgctct-3′), and the plasmid pCC1.[73] Alkylation of the labeled DNA and thermal cleavage followed the method described,[31c] with sequencing lanes generated using the *fmol* DNA sequencing system (Promega) according to manufacturer's instructions, with primer TC1 and plasmid pCC1.

**Toxicity and Antitumor Activity in Mice.** All compounds were formulated in 100% DMSO and administered as single doses at 0.001 mL/g of body weight. At this dose level no toxicity was observed in vehicle only treated controls via ip or iv routes. The toxicity in nontumor-bearing female C3H/HeN mice was determined using dose increments of 1.33-fold on a fixed scale with a 60 day observation time. The MTD was defined as the highest dose causing no deaths in a group of 6 mice and ≤15% mean body weight loss on day 4.

For the growth delay assay, WiDr cells ($10^7$ cells/mouse in 100 $\mu$L) were injected subcutaneously into the flank of CD-1 nude female mice. Mice were randomized to treatment groups (5−11 mice/group) when mean tumor diameter reached 8 mm and received a single dose either ip or iv at the predetermined MTD (day 0). Tumor size was measured periodically thereafter using calipers and mice were culled once the mean tumor diameter reached 17 mm. The statistical significance of drug effects was evaluated by ANOVA followed by Dunnett's test to determine $p$ values for individual groups.

**Acknowledgment.** We would like to thank Prof. David E. Thurston for the gift of some 3-({(11a*S*)-10-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-11-hydroxy-7-methoxy-5-oxo-2,3,5,10,11,11a-hexahydro-1*H*-pyrrolo[2,1-*c*]-[1,4]benzodiazepin-8-yl}oxy)propanoic acid (the FMOC-protected analogue of **15c**). We would also like to thank Dr Swarna A. Gamage for the synthesis of **6**, Karin Tan for the chiral HPLC resolution of this material, Drs. Michael A. Gieseg and Charley de Bock for preliminary work on the cross-linking of plasmid DNA, Dr. Jared B. Milbank for assistance in the statistical analysis of the cross-linking results, and Hui Hui Phua for assistance with the comet assay. We acknowledge the National Cancer Institute's Developmental Therapeutics Program for performing the in vitro 60 cell line screen and COMPARE analysis. This work was carried out with the support of the Auckland Division of the Cancer Society of New Zealand and the Health Research Council of New Zealand.

**Supporting Information Available:** Synthesis of **3**, **16**, **30**, **31**, **32*R***, **32*S***; chiral HPLC of **16**; optical rotation measurements for **3**; cross-linking of linearized plasmid DNA by **3** and each CBI−PBD compound; relationship between cross-linking ability and cytotoxicity in UV4, EMT6, and Skov3 cell lines; cross-linking by **3** and **27c*R*** after 2 and 18 h; COMPARE analysis results; in vivo toxicity data; growth delay curves for WiDr xenografts in nude mice treated with iv **27e*S***. This material is available free of charge via the Internet at http://pubs.acs.org.

## References

(1) Rajski, S. R.; Williams, R. M. DNA Cross-Linking Agents as Antitumor Drugs. *Chem. Rev.* **1998**, *98*, 8, 2723−2795.
(2) Woynarowski, J. M.; Napier, C.; Trevino, A. V.; Arnett, B. Region-Specific DNA Damage by AT-Specific DNA-Reactive Drugs is Predicted by Drug Binding Specificity. *Biochemistry* **2000**, *39*, 9917−9927.
(3) Woynarowski, J. M.; Trevino, A. V.; Rodriguez, K. A.; Hardies, S. C.; Benham, C. J. AT-rich Islands in Genomic DNA as a Novel Target for AT-Specific DNA-Reactive Antitumor Drugs. *J. Biol. Chem.* **2001**, *276*, 40555−40566.
(4) Bando, T.; Iida, H.; Saito, I.; Sugiyama, H. Sequence-Specific DNA Interstrand Cross-Linking by Imidazole-Pyrrole CPI Conjugate. *J. Am. Chem. Soc.* **2001**, *123*, 5158−5159.
(5) Thurston, D. E. Nucleic Acid Targeting: Therapeutic Strategies for the 21st Century. *Br. J. Cancer* **1999**, *80*, 65−85.
(6) Boger, D. L.; Johnson, D. S. CC-1065 and the Duocarmycins: Understanding Their Biological Function Through Mechanistic Studies. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1438−1474.
(7) Thurston, D. E.; Bose, D. S. Synthesis of DNA-Interactive Pyrrolo[2,1-*c*][1,4]benzodiazepines. *Chem. Rev.* **1994**, *94*, 433−465.
(8) Fukuda, Y.; Seto, S.; Furuta, H.; Ebisu, H.; Oomori, Y.; Terashima, S. Cyclopropapyrroloindole (CPI) Bisalkylators. *Drugs Future* **1998**, *23*, 1317−1330.
(9) *Seco*-CPI is the ring-opened form of the CPI (1,2,8,8a-tetrahydro-7-methylcyclopropa[*c*]pyrrolo[3,2-*e*]indol-4-one) alkylation subunit. In general the *seco* forms of cyclopropaindolones readily ring close under culture conditions and have the same biological properties as the cyclo parents.[6]
(10) Mitchell, M. A.; Kelly, R. C.; Wicnienski, N. A.; Hatzenbuhler, N. T.; Williams, M. G.; Petzold, G. L.; Slightom, J. L.; Siemieniak, D. R. Synthesis and DNA Cross-Linking by a Rigid CPI Dimer, *J. Am. Chem. Soc.* **1991**, *113*, 8994−8995.
(11) Cross-linking occurs between the adenine marked with an asterisk and that opposite the underlined thymine.
(12) Sun, D.; Hurley, L. H. Analysis of the Monoalylation and Cross-Linking Sequence Specificity of Bizelesin, a Bifunctional Alkylation Agent Related to (+)-CC-1065. *J. Am. Chem. Soc.* **1993**, *115*, 5925−5933.
(13) Carter, C. A.; Waud, W. R.; Li, L. H.; DeKoning, T. F.; McGovren, J. P.; Plowman, J. Preclinical Antitumor Activity of Bizelesin in Mice. *Clin. Cancer Res.* **1996**, *2*, 1143−1149.
(14) Pitot, H. C.; Reid, J. M.; Sloan, J. A.; Ames, M. M.; Adjei, A. A.; Rubin, J.; Bagniewski, P. G.; Atherton, P.; Rayson, D.; Goldberg, R. M.; Erlichman, C. A Phase I Study of Bizelesin (NSC 615291) in Patients with Advanced Solid Tumors. *Clin. Cancer Res.* **2002**, *8*, 712−717.
(15) Volpe, D. A.; Tomaszewski, J. E.; Parchment, R. E.; Garg, A.; Flora, K. P.; Murphy, M. J.; Grieshaber, C. K. Myelotoxic Effects of the Bifunctional Alkylating Agent Bizelesin on Human, Canine and Murine Myeloid Progenitor Cells. *Cancer Chemother. Pharmacol.* **1996**, *39*, 143−149.
(16) (a) Markovic, S. N.; Suman, V. J.; Vukov, A. M.; Fitch, T. R.; Hillman, D. W.; Adjei, A. A.; Alberts, S. R.; Kaur, J. S.; Braich, T. A.; Leitch, J. M.; Creagan, E. T. Phase II Trial of KW2189 in Patients With Advanced Malignant Melanoma. *Am. J. Clin. Oncol.* **2002**, *25*, 308−312. (b) Pavlidis, N.; Aamdal, S.; Awada, A.; Calvert, H.; Fumoleau, P.; Sorio, R.; Punt, C.; Verweij, J.; van Oosterom, A.; Morant, R.; Wanders, J.; Hanauske, A−R.

Carzelesin Pase II Study in Advanced Breast, Ovarian, Colorectal, Gastric, Head and Neck Cancer, Non-Hodgkin's Lymphoma and Malignant Melanoma: a Study of the EORTC Early Clinical Studies Group (ECSG). *Cancer Chemother. Pharmacol.* **2000**, *46*, 167−171. (c) Cristofanilli, M.; Bryan, W. J.; Miller, L. L.; Chang, A. Y-C.; Gradishar, W. J.; Kufe, D. W.; Hortobagyi, G. N. Phase II Study of Adozelesin in Untreated Metastatic Breast Cancer. *Anti-Cancer Drugs* **1998**, *9*, 779−782.

(17) Mitchell, M. A.; Johnson, P. D.; Williams, M. G.; Aristoff, P. A. Interstrand DNA Cross-Linking with Dimers of the Spirocyclopropyl Alkylating Moiety of CC-1065. *J. Am. Chem. Soc.* **1989**, *111*, 6428−6429.

(18) (a) Park, H-J.; Kelly, R. C.; Hurley, L. H. The Chemical Evolution of DNA-DNA Interstrand Cross-Linkers That Recognize Mixed AT and GC Sequences. *J. Am. Chem. Soc.* **1996**, *118*, 10041−10051. (b) Fukuda, Y.; Seto, S.; Furuta, H.; Ebisu, H.; Oomori, Y.; Terashima, S. Novel Seco Cyclopropa[*c*]pyrrolo[3,2-*e*]indole Bisalkylators Bearing a 3,3′-Arylenebisacryloyl Group as a Linker. *J. Med. Chem.* **2001**, *44*, 1396−1406. (c) Boger, D. L.; Searcey, M.; Tse, W. C.; Jin, Q. Bifunctional Alkylating Agents Derived from Duocarmycin SA: Potent Antitumor Activity with Altered Sequence Selectivity. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 495−498.

(19) Jia, G.; Lown, J. W. Design, Synthesis and Cytotoxicity Evaluation of 1-Chloromethyl-5-hydroxy-1,2-dihydro-3*H*-benz[*e*]indole (*seco*-CBI) Dimers. *Bioorg. Med. Chem.* **2000**, *8*, 1607−1617.

(20) Boger, D. L.; Ishizaki, T.; Kitos, P. A.; Suntornwat, O. Synthesis of *N*-(*tert*-Butyloxycarbonyl)-CBI, CBI, CBI−$CDPI_1$, and CBI−$CDPI_2$: Enhanced Functional Analogues of CC-1065 Incorporating the 1,2,9,9a-Tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one (CBI) Left-Hand Subunit. *J. Org. Chem.* **1990**, *55*, 5823−5832.

(21) Bose, D. S.; Thompson, A. S.; Ching, J.; Hartley, J. A.; Berardini, M. D.; Jenkins, T. C.; Neidle, S.; Hurley, L. H.; Thurston, D. E. Rational Design of a Highly Efficient Irreversible DNA Interstrand Cross-Linking Agent Based on the Pyrrolobenzodiazepine Ring System. *J. Am. Chem. Soc.* **1992**, *114*, 4939−4941.

(22) Bose, D. S.; Thompson, A. S.; Smellie, M.; Berardini, M. D.; Hartley, J. A.; Jenkins, T. C.; Neidle, S.; Thurston, D. E. Effect of Linker Length on DNA-Binding Affinity, Cross-Linking Efficiency and Cytotoxicity of C8−Linked Pyrrolobenzodiazepine Dimers. *J. Chem. Soc., Chem. Commun.* **1992**, 1518−1520.

(23) Jenkins, T. C.; Hurley, L. H.; Neidle, S.; Thurston, D. E. Structure of a Covalent Minor Groove Adduct with a Pyrrolobenzodiazepine Dimer: Evidence for Sequence-Specific Interstrand Cross-Linking. *J. Med. Chem.* **1994**, *37*, 4529−4537.

(24) Walton, M. I.; Goddard, P.; Kelland, L. R.; Thurston, D. E.; Harrap, K. R. Preclinical Pharmacology and Antitumor Activity of the Novel Sequence-Selective DNA Minor-Groove Cross-Linking Agent DSB-120. *Cancer Chemother. Pharmacol.* **1996**, *38*, 431−438.

(25) Gregson, S. J.; Howard, P. W.; Hartley, J. A.; Brooks, N. A.; Adams, L. J.; Jenkins, T. C.; Kelland, L. R.; Thurston, D. E. Design, Synthesis, and Evaluation of a Novel Pyrrolobenzodiazepine DNA-Interactive Agent with Highly Efficient Cross-Linking Ability and Potent Cytotoxicity. *J. Med. Chem.* **2001**, *44*, 737−748.

(26) Hartley, J. A.; Spanswick, V. J.; Pedley, B.; Hollingshead, M. G.; Alley, M. C.; Schultz, R.; Gregson, S. J.; Howard, P. H.; Thurston, D. E. In Vitro Antitumor Activity and In Vivo DNA Interstrand Cross-linking by the Novel Pyrrolobenzodiazepine Dimer SJG-136 (NSC 694501). *Proceedings of the American Association for Cancer Research*; 93rd Annual Meeting, San Francisco, April 6−10, 2002, Vol. 43, Abstract 2432 (http://www.aacr.org).

(27) Zhou, Q.; Duan, W.; Simmons, D.; Shayo, Y.; Raymond, M. A.; Dorr, R. T.; Hurley, L. H. Design and Synthesis of a Novel DNA-DNA Interstrand Adenine-Guanine Cross-Linking Agent. *J. Am. Chem. Soc.* **2001**, *123*, 4865−4866.

(28) Hurley, L. H.; Warpehoski, M. A.; Lee, C−S.; McGovren, J. P.; Scahill, T. A.; Kelly, R. C.; Mitchell, M. A.; Wicnienski, N. A.; Gebhard, I.; Johnson, P. D.; Bradford, V. S. Sequence Specificity of DNA Alkylation by the Unnatural Enantiomer of CC-1065 and Its Synthetic Ananlogues. *J. Am. Chem. Soc.* **1990**, *112*, 4633−4649.

(29) Boger, D. L.; Yun, W. CBI-TMI: Synthesis and Evaluation of a Key Analogue of the Duocarmycins. Validation of a Direct Relationship between Chemical Solvolytic Stability and Cytotoxic Potency and Confirmation of the Structural Features Responsible for the Distinguishing Behavior of Enantiomeric Pairs of Agents. *J. Am. Chem. Soc.* **1994**, *116*, 7996−8006.

(30) Fukuyama, T.; Liu, G.; Linton, S. D.; Lin, S−C.; Nishino, H. Total Synthesis of (+)-Porothramycin B. *Tetrahedron Lett.* **1993**, *34*, 2577−2580.

(31) (a) Boger, D. L.; McKie, J. A. An Efficient Synthesis of 1,2,9,9a-Tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one (CBI): An Enhanced and Simplified Analogue of the CC-1065 and Duocarmycin Alkylation Subunits. *J. Org. Chem.* **1995**, *60*, 1271−1275. (b) Boger, D. L.; Boyce, C. W.; Garbaccio, R. M.; Searcey, M. Synthesis of CC-1065 and Duocarmycin Analogues via Intramolecular Aryl Radical Cyclization of a Tethered Vinyl Chloride. *Tetrahedron Lett.* **1998**, *39*, 2227−2230. (c) Gieseg, M. A.; Matejovic J.; Denny, W. A. Comparison of the Patterns of DNA Alkylation by Phenol and Amino *seco*-CBI-TMI Compounds: Use of a PCR Method for the Facile Preparation of Single End-Labeled Double-Stranded DNA. *Anti-Cancer Drug Design* **1999**, *14*, 77−84.

(32) Petrusek, R. L.; Anderson, G. L.; Garner, T. F.; Fannin, Q. L.; Kaplan, D. J.; Zimmer, S. G.; Hurley, L. H. Pyrrolo[1,4]-benzodiazepine Antibiotics. Proposed Structures and Characteristics of the In Vitro Deoxyribonucleic Acid Adducts of Anthramycin, Tomaymycin, Sibiromycin, and Neothramycins A and B. *Biochemistry* **1981**, *20*, 1111−1119.

(33) Althuis, T. H.; Hess, H−J. Synthesis and Identification of the Major Metabolites of Prazosin Formed in Dog and Rat. *J. Med. Chem.* **1977**, *20*, 146−149.

(34) (a) Sagnou, M. J.; Howard, P. H.; Gregson, S. J.; Eno-Amooquaye, E.; Burke, P. J.; Thurston, D. E. Design and Synthesis of Novel Pyrrolobenzodiazepine (PBD) Prodrugs for ADEPT and GDEPT. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2083−2086. (b) Thurston, D. E.; Howard, P. W. Preparation of Pyrrolobenzodiazepines (PBDs) as Antitumor Antibiotics. PCT Int. Appl. WO 0012507, 2000.

(35) Wright, I. G.; Ashbrook, C. W.; Goodson, T.; Kaiser, G. V.; Van Heyningen, E. M. Chemistry of Cephalosporin Antibiotics. 23. 2-Methyl- and 2-methylenecephalosporins. *J. Med. Chem.* **1971**, *14*, 420−426.

(36) Just, G.; Grozinger, K. A Selective, Mild Cleavage of Trichloroethyl Esters, Carbamates, and Carbonates to Carboxylic Acids, Amines, and Phenols using Zinc/Tetrahydrofuran/pH4.2−7.2 Buffer. *Synthesis* **1976**, 457−459.

(37) Jou, G.; Gonzalez, I.; Albericio F.; Lloyd-Williams, P.; Giralt, E. Total Synthesis of Dehydrodidemnin B. Use of Uronium and Phosphonium Salt Coupling Reagents in Peptide Synthesis in Solution. *J. Org. Chem.* **1997**, *62*, 354−366.

(38) (a) Baraldi, P. G.; Balboni, G.; Cacciari, B.; Guiotto, A.; Manfredini, S.; Romagnoli, R.; Spalluto, G.; Thurston, D. E.; Howard, P. H.; Bianchi, N.; Rutigliano, C.; Mischiati, C.; Gambari, R. Synthesis, In Vitro Antiproliferative Activity, and DNA-Binding Properties of Hybrid Molecules Containing Pyrrolo[2,1-*c*][1,4]-benzodiazepine and Minor-Groove-Binding Oligopyrrole Carriers. *J. Med. Chem.* **1999**, *42*, 5131−5141. (b) Thurston, D. E.; Bose, D. S.; Howard, P. H.; Jenkins, T. C.; Leoni, A.; Baraldi, P. G.; Guiotto, A.; Cacciari, B.; Kelland, L. R.; Foloppe, M−P.; Rault, S. Effect of A-Ring Modifications on the DNA-Binding Behavior and Cytotoxicity of Pyrrolo[2,1-*c*][1,4]benzodiazepines. *J. Med. Chem.* **1999**, *42*, 1951−1964.

(39) Dalcanale, E.; Montanari, F. Selective Oxidation of Aldehydes to Carboxylic Acids with Sodium Chlorite-Hydrogen Peroxide. *J. Org. Chem.* **1986**, *51*, 567−569.

(40) Seltzman, H. H.; Berrang, B. D. Nickel Boride Reduction of Aryl Nitro Compounds. *Tetrahedron Lett.* **1993**, *34*, 3083−3086.

(41) Kunz, H.; Unverzagt, C. Protective Group Dependent Stability of Intersaccharide Bond. Synthesis of Fucosylchitobiose-Glucopeptides. *Angew. Chem.* **1988**, *100*, 1763−1765.

(42) Thurston, D. E.; Murty, V. S.; Langley, D. R.; Jones, G. B. *O*-Debenzylation of a Pyrrolo[2,1-*c*][1,4]benzodiazepine in the Presence of a Carbinolamine Functionality: Synthesis of DC-81. *Synthesis* **1990**, 81−84.

(43) Molina, P.; Diaz, I.; Tarraga, A. Synthesis of Pyrrolo[2,1-*c*][1,4]-benzodiazepines via an Intramolecular Aza-Wittig Reaction. Synthesis of the Antibiotic DC-81. *Tetrahedron* **1995**, *51*, 5617−5630.

(44) Bose, D. S.; Jones, G. B.; Thurston, D. E. New Approaches to Pyrrolo[2,1-*c*][1,4]benzodiazepines: Synthesis, DNA-Binding and Cytotoxicity of DC-81. *Tetrahedron* **1992**, *48*, 751−758.

(45) These $[\alpha_D]$ values in turn are considerably higher than that measured for the natural product itself, $[\alpha_D] = +135°$, but this has been attributed to likely epimerization during the isolation steps.[42]

(46) The recorded value in 2% EtOH/$CHCl_3$ was +567°, but this does not necessarily represent the equilibrium situation. For further discussion see Supporting Information.

(47) Thurston, D. E.; Bose, D. S.; Thompson, A. S.; Howard, P. H.; Leoni, A.; Croker, S. J.; Jenkins, T. C.; Neidle, S.; Hartley, J. A.; Hurley, L. H. Synthesis of Sequence-Selective C8−Linked Pyrrolo[2,1-*c*][1,4]benzodiazepine DNA Interstrand Cross-Linking Agents. *J. Org. Chem.* **1996**, *61*, 8141−8147.

(48) Jones, G. B.; Davey, C. L.; Jenkins, T. C.; Kamal, A.; Kneale, G. G.; Neidle, S.; Webster, G. D.; Thurston, D. E. The Non-Covalent Interaction of Pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11-diones with DNA. *Anti-Cancer Drug Design* **1990**, *5*, 249−264.

(49) **13e** was found to have almost completely hydrolyzed on storage, so following Aloc deprotection, an extra EDCI-mediated dehydration step was used to reform the N10/C11 amide bond.

(50) Boger, D. L.; Mesini, P. Design, Synthesis and Evaluation of CC-1065 and Duocarmycin Analogues Incorporating the 2,3,10,10a-Tetrahydro-1*H*-cyclopropa[*d*]benzo[*f*]quinol-5-one (CBQ) Alkylation Subunit: Identification and Structural Origin of Subtle Stereoelectronic Features That Govern Reactivity and Regioselectivity. *J. Am. Chem. Soc.* **1994**, *116*, 11335−11348.
(51) Wilson, W. R.; Denny, W. A.; Pullen, S. M.; Thompson, K. M.; Li, A. E.; Patterson, L. H.; Lee, H. H. Tertiary Amine N−Oxides as Bioreductive Drugs: DACA N−Oxide, Nitracrine N−Oxide and AQ4N. *Br. J. Cancer* **1996**, *74* (Suppl. XXVII), S43−S47.
(52) Marshall, E. S.; Finlay, G. J.; Matthews, J. H. L.; Shaw, J. H. F.; Nixon, J.; Baguley, B. C. Microculture-Based Chemosensitivity Testing: A Feasibility Study Comparing Freshly Explanted Human Melanoma Cells With Human Melanoma Cell Lines. *J. Natl. Cancer Inst.* **1992**, *84*, 340−345.
(53) Hoeijmakers, J. H. J.; Bootsma, D. Molecular Genetics of Eukaryotic DNA Excision Repair. *Cancer Cells* **1990**, *2*, 311−320.
(54) Hoy, C. A.; Salazar, E. P.; Thompson, L. H. Rapid Detection of DNA-Damaging Agents Using Repair-Deficient CHO Cells. *Mutat. Res.* **1984**, *130*, 321−332.
(55) Boger, D. L.; Garbaccio, R. M. Shape-Dependent Catalysis: Insights into the Source of Catalysis for the CC-1065 and Duocarmycin DNA Alkylation Reaction. *Acc. Chem. Res.* **1999**, *32*, 1043−1052.
(56) (a) Monks, A.; Scudiero, D. A.; Johnson, G. S.; Paull, K. D.; Sausville, E. A. The NCI Anti-Cancer Drug Screen: A Smart Screen to Identify Effectors of Novel Targets. *Anti-Cancer Drug Design* **1997**, *12*, 533−541. (b) Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A.; Gray-Goodrich, M.; Campbell, H.; Mayo, J.; Boyd, M. Feasibility of a High-Flux Anticancer Drug Screen Using a Diverse Panel of Cultured Human Tumor Cell Lines. *J. Natl. Cancer Inst.* **1991**, *83*, 757−766.
(57) Paull, K. D.; Shoemaker, R. H.; Hodes, L.; Monks, A.; Scudiero, D. A.; Rubinstein, L.; Plowman, J.; Boyd, M. R. Display and Analysis of Patterns of Differential Activity of Drugs Against Human Tumor Cell Lines: Development of Mean Graph and COMPARE Algorithm. *J. Natl. Cancer Inst.* **1989**, *81*, 1088−1092.
(58) Hartley, J. A.; Berardini, M. D.; Souhami, R. L. An Agarose Gel Method for the Determination of DNA Interstrand Cross-linking Applicable to the Measurement of the Rate of Total and "Second-Arm" Cross-link Reactions. *Anal. Biochem.* **1991**, *193*, 131−134.
(59) Lee, C−S.; Gibson, N. W. DNA Damage and Differential Cytotoxicity Produced in Human Carcinoma Cells by CC-1065 Analogues, U-73,975 and U-77,779. *Cancer Res.* **1991**, *51*, 6586−6591.
(60) Reynolds, V. L.; Molineux, I. J.; Kaplan, D. J.; Swenson, D. H.; Hurley, L. H. Reaction of the Antitumor Antibiotic CC-1065 with DNA. Location of the Site of Thermally Induced Strand Breakage and Analysis of DNA Sequence Specificity. *Biochemistry* **1985**, *24*, 6228−6237.
(61) (a) Warpehoski, M. A.; Harper, D. E.; Mitchell, M. A.; Monroe, T. J.Reversibility of the Covalent Reaction of CC-1065 and Analogues with DNA. *Biochemistry* **1992**, *31*, 2502−2508. (b) Boger, D. L.; Yun, W. Reversibility of the Duocarmycin A and SA DNA Alkylation Reaction. *J. Am. Chem. Soc.* **1993**, *115*, 9872−9873.
(62) See Supporting Information for graphs of the individual data points and fitted curves for each of the compounds.
(63) A $C_{50}$ value for **3** has been reported (0.055 $\mu$M).[22] Although similar to the value found here the results are not directly comparable since a different DNA sequence (pBR322 plasmid, 4400 bp) and exposure time (2 h) were used.
(64) Fairbairn, D. W.; Olive, P. L.; O'Neill, K. L. The Comet Assay: a Comprehensive Review. *Mutat. Res.* **1995**, *339*, 37−59.
(65) Boger, D. L.; Munk, S. A.; Zarrinmayeh, H.; Ishizaki, T.; Haught, J.; Bina, M. An Alternative and Convenient Strategy for Generation of Substantial Quantities of Singly 5′-$^{32}$P-End-Labeled Double-Stranded DNA for Binding Studies: Development of a Protocol for Examination of Functional Features of (+)-CC-1065 and the Duocarmycins that Contribute to their Sequence-Selective DNA Alkylation Properties. *Tetrahedron* **1991**, *47*, 2661−2682.
(66) Smellie, M.; Kelland, L. R.; Thurston, D. E.; Souhami, R. L.; Hartley, J. A. Cellular Pharmacology of Novel C8-Linked Anthramycin-Based Sequence-Selective DNA Minor Groove Cross-Linking Agents. *Br. J. Cancer* **1994**, *70*, 48−53.
(67) Paull, K. D.; Hamel, E.; Malspeis, L. Prediction of Biochemical Mechanism of Action from the In Vitro Antitumor Screen of the National Cancer Institute. In *Cancer Chemotherapeutic Agents*, Foye, W. O., Ed.; American Chemical Society: Washington, DC, 1995; pp 9−45.
(68) http://dtp.nci.nih.gov/docs/cancer/searches/cancer_open_compounds.html, bizelesin (NSC 615291) at log(high concentration) −5.00, $LC_{50}$ comparison.
(69) Seuring, B.; Seebach, D. Syntheses and Determinations of the Absolute Configurations of Norpyrenophorin, Pyrenophorin, and Vermiculine. *Justus Liebigs Ann. Chem.* **1978**, *12*, 2044−2073.
(70) Trost, B. M.; Verhoeven, T. R. Cyclization Catalyzed by Palladium(0). Initial Studies and Macrolide Formation. *J. Am. Chem. Soc.* **1980**, *102*, 4743−4763.
(71) Alexander, J.; Renyer, M. L.; Veerapanane, H. A Convenient Method for the Conversion of Halides to Alcohols. *Synth. Commun.* **1995**, *25*, 3875−3881.
(72) Tercel, M.; Lee, A. E.; Hogg, A.; Anderson, R. F.; Lee, H. H.; Siim, B. G.; Denny, W. A.; Wilson, W. R. Hypoxia-Selective Antitumor Agents. 16. Nitroarylmethyl Quaternary Salts as Bioreductive Prodrugs of the Alkylating Agent Mechlorethamine. *J. Med. Chem.* **2001**, *44*, 3511−3522.
(73) White, R. J.; Phillips, D. R. Transcriptional Analysis of Multisite Drug−DNA Dissociation Kinetics: Delayed Termination of Transcription by Actinomycin D. *Biochemistry* **1988**, *27*, 9122−9132.

JM020526P