Design, Synthesis, and Evaluation of New Noncross-Linking Pyrrolobenzodiazepine Dimers with Efficient DNA Binding Ability and Potent Antitumor Activity

Ahmed Kamal,*,† G. Ramesh,† N. Laxman,† P. Ramulu,† O. Srinivas,† K. Neelima,‡ Anand K. Kondapi,‡ V. B. Sreenu,§ and H. A. Nagarajaram§

Biotransformation Laboratory, Division of Organic Chemistry, Indian Institute of Chemical Technology, Hyderabad 500 007, India, Department of Biochemistry, School of Life Sciences, University of Hyderabad, Hyderabad 500 046, India, and Laboratory of Computational Biology and Bioinformatics, Centre for DNA Finger Printing and Diagnostics, Hyderabad 500 076, India

Received March 19, 2002

New sequence selective mixed imine-amide pyrrolobenzodiazepine (PBD) dimers have been developed that are comprised of DC-81 and dilactam of DC-81 subunits tethered to their C8 positions through alkanedioxy linkers (comprised of three to five and eight carbons). Thermal denaturation studies show that after 18 h of incubation with calf thymus DNA at a 5:1 DNA/ligand ratio, one of them (**5c**) increases the ΔT_m value by 17.0 °C. Therefore, these unsymmetrical molecules exhibit significant DNA minor groove binding affinity and **5c** linked through the pentanedioxy chain exhibits efficient DNA binding ability that compares with the cross-linking DSB-120 PBD dimer ($\Delta T_m = 15.4$ °C). Interestingly, this imine-amide PBD dimer has been linked with a five carbon chain linker unlike DSB-120, which has two DC-81 subunits with a three carbon chain linker, illustrating the effect of the noncross-linking aspect by introducing the noncovalent subunit. The binding affinity of the compounds has been measured by restriction endonuclease digestion assay based on inhibition of the restriction endonuclease *BamH*I. This study reveals the significance of noncovalent interactions in combination with covalent bonding aspects when two moieties of structural similarities are joined together. This allows the mixed imine-amide PBD dimer with a five carbon chain linker to achieve an isohelical fit within the DNA minor groove taking in to account both the covalent bonding and the noncovalent binding components. This has been supported by molecular modeling studies, which indicate that the PBD dimer with a five carbon chain linker gives rise to maximum stabilization of the complex with DNA at the minor groove as compared to the other PBD dimers with three, four, and eight carbon chain linkers. The energy of interaction in all of the complexes studied is correlated to the ΔT_m values. Furthermore, this dimer **5c** has significant cytotoxicity in a number of human cancer cell lines.

Introduction

There has been considerable interest in the design and development of DNA interactive ligands that are capable of binding to DNA in a sequence selective manner.[1−3] However, in spite of rational design of synthetic DNA intercalaters, few such compounds that are in current clinical use exhibit sequence selectivity. These compounds with the ability to target and then down regulate individual genes have potential use as drugs for therapy of genetic-based diseases including some cancers[4] and as research tools for using functional genomic studies. Therefore, the synthesis of small molecules, which exhibit DNA sequence selectivity, is of importance for the targeting of rapidly growing tumor cells.

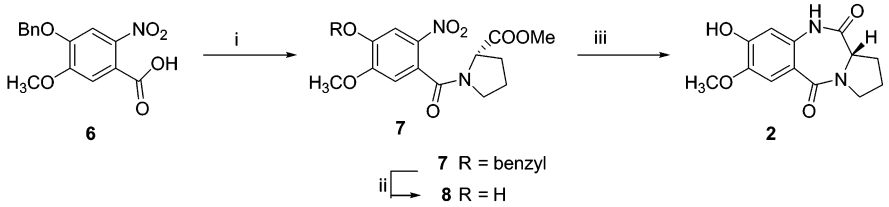
The pyrrolo[2,1-*c*][1,4]benzodiazepine (PBD) antitumor antibiotics are a well-known class of sequence selective DNA binding agents derived from *Streptomyces* species.[5] Their interactions with DNA are unique since they bind within the minor groove of DNA forming a covalent aminal bond between the C11 position of the central B-ring and the N2 amino group of a guanine base.[5,6] The cytotoxic and antitumor activity of PBDs are attributed to their ability to form covalent DNA adducts. Molecular modeling, solution NMR, fluorimetry, and DNA footprinting experiments have shown that these molecules have a preferred selectivity for Pu-G-Pu sequences[7,8] and can be oriented with their A-rings pointed either toward the 3′- or the -5′ end of the covalently bonded DNA strand as in the case of anthramycin and tomaymycin. The PBDs have been shown to inhibit endonuclease enzyme cleavage of DNA[9] and to block transcription by inhibiting DNA polymerase in a sequence specific manner,[10] processes which may be relevant for the biological activity.

The PBDs have also been used as a scaffold to attach ethylenediaminetetraacetic acid (EDTA),[11] epoxide,[12] polyamide,[13] and oligopyrrole[14] moieties leading to novel hybrids of PBD, which have exhibited sequence selective DNA-cleaving and cross-linking properties. Thurston and co-workers have synthesized homologous series of

* To whom correspondence should be addressed. Tel: +91-40-7193157. Fax: +91-40-7160512 or +91-40-7160757. E-mail: ahmedkamal@iict.ap.nic.in.
† Indian Institute of Chemical Technology.
‡ University of Hyderabad.
§ Centre for DNA Finger Printing and Diagnostics.

10.1021/jm020124h CCC: $22.00
Published on Web 09/17/2002

Scheme 1[a]



[a] Reagents and conditions: (i) $SOCl_2$, L-proline methylester hydrochloride, Et_3N, H_2O, 0 °C, 85%. (ii) $BF_3{\cdot}OEt_2-EtSH$, CH_2Cl_2, room temperature, 88%. (iii) Pd/C, H_2, 40 psi, 62%.

C8 diether-linked PBD dimers (DSB-120) that span approximately six base pairs of DNA and in which sequence selectivity has also increased (e.g., purine-GATC-pyrimidine).[15,16] DSB-120 exhibits potent in vitro cytotoxicity and enhances DNA binding affinity and sequence specificity as compared to the natural product DC-81. This improvement in biological activity has been attributed to the ability of these compounds to link to DNA irreversibly via guanine residues on opposite strands because of the presence of two active sites (i.e., two imine functionalities).[17] The in vivo studies of this compound were not encouraging, and the low therapeutic index observed was partly due to the reaction of this compound with cellular thiol-containing molecules before reacting at the tumor site.[18] Recently, another new cross-linking PBD dimer (SJG-136) having C2/C2′-exo unsaturation (that exhibits high DNA binding affinity) has been prepared by the same group.[19] This investigation has highlighted the effect of C2 unsaturation on the in vitro and in vivo cytotoxic activity. Interestingly, the comparison of this PBD dimer with its tetralactam analogue demonstrates that for maximum cytotoxicity an electrophilic imine or carbinolamine moiety is essential at the N10−C11 position of the PBD units. We have been interested in the structural modifications of the PBD ring system and development of new synthetic strategies.[20−29] It has been observed in the literature that no effort has been made to prepare and investigate PBD dimers with one imine functionality alone for exploring their cytotoxicity as noncross-linking agents. Therefore, it was of interest to synthesize and evaluate mixed dimers of PBD that contain an imino functionality in one of the PBD rings and an amido group in the other, which are linked at the C8 position by a suitable alkane spacer. Herein, we report the synthesis and biological evaluation of potent mixed imine-amide PBD dimers.

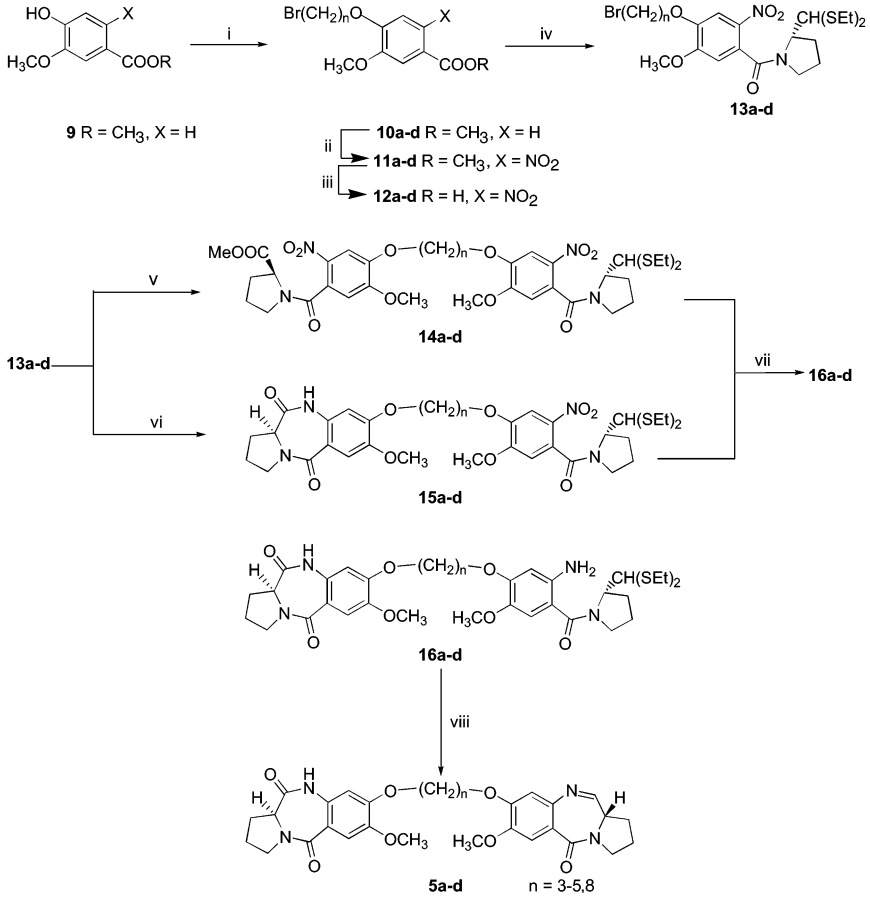
Results and Discussion

Chemistry. Synthesis of the imine-amide mixed dimers of PBD[30] has been carried out employing the commercially available vanillin. Oxidation of vanillin followed by benzylation and nitration by a literature method[31] provides the starting material **6**. L-Proline methyl ester has been coupled with **6** to give compound **7**, which upon debenzylation with $BF_3.OEt_2-EtSH$ gives compound **8**. Compound **2** has been prepared from compound **7** by hydrogenation over Pd/C at 40 psi (Scheme 1). The other precursor has been prepared from vanillic acid methyl ester **9** by its etherification with dibromoalkanes to afford **10**. The monoalkylation of **9** has been achieved by using 3 molar equiv of the dibromo alkanes. Nitration of **10** followed by ester hydrolysis and coupling with (2-*S*)-pyrrolidinecarbaxaldehyde diethyl thioacetal gives **13**. One key intermediate (**14**) has been prepared by linking **8** and **13** while the alternative key intermediate (**15**) has been prepared by linking **13** and **2**. Compounds **14** and **15** upon reduction with tin chloride provide **16**, which has a dilactam moiety at one end and the amino functionality on the other. Deprotection of the thioacetal group by using the method of Thurston and co-workers[16] affords the target molecules **5a**−**d** that contain both the imino and the amido functionalities (Scheme 2).

Thermal Denaturation Studies. The DNA binding affinity of the novel noncross-linking imine-amide PBD dimers (**5a**−**d**) was investigated by thermal denaturation using calf thymus (CT)-DNA.[9] The studies for these compounds (**5a**−**d**) were carried out at DNA/ligand molar ratios of 5:1. The increase in melting temperature (ΔT_m) for each compound was examined at 0 and 18 h incubation at 37 °C (Table 1). Data for DC-81 and DSB-120 are included in Table 1 for comparison. It is observed from the data that compound **5c** is an efficient stabilizing agent for double-stranded CT-DNA. This compound elevates the helix melting temperature of the CT-DNA by a remarkable 17 °C after incubation at 37 °C for 18 h. In the same experiment, the DC-81 dimer (DSB-120) gives a ΔT_m of 15.4 °C. This result illustrates the significant effect of introducing an amide functionality for one of the PBD rings with an increase in the size of the linker spacer from three carbons in DSB-120 to five carbons in **5c**. However, the naturally occurring DC-81 having one imine group exhibits a ΔT_m of 0.7 °C. It is interesting to note that as the linker chain increases from three to five carbons, there is an enhancement in the DNA binding affinity and with further increase of this chain from five to eight carbons the DNA stabilization is reduced as in the case of **5d**. As generally observed for the PBD dimers, compounds **5a**−**d** exert most of the effect upon the GC-rich or high-temperature regions of the DNA melting curves. In a manner similar to DSB-120, compound **5** provides about 75% of the stabilizing effect without prior incubation, suggesting the kinetic effect in the PBD reactivity profile.

These results clearly demonstrate that replacement of one of the electrophilic N10−C11 imine moieties present in DSB-120 with a lactam functionality and increasing from three to five the carbon linker length, although abolishing one of the covalent DNA interaction sites, presumably enhance the noncovalent component of interaction. Interestingly, a further increase in the length to an eight carbon linker dramatically reduces the DNA binding affinity.

Scheme 2[a]



[a] Reagents and conditions: (i) Br $(CH_2)_nBr$, K_2CO_3, CH_3COCH_3, reflux, 82−86%. (ii) $SnCl_4-HNO_3$, CH_2Cl_2, −25 °C, 88−91%. (iii) 1 M LiOH, THF, MeOH, H_2O (3:1:1), room temperature, 89−93%. (iv) $SOCl_2$, DMF, THF, H_2O, 2(*S*)-pyrrolidinecarbaldehyde diethyl thioacetal, Et_3N, 89−92%. (v) Compound **8**, K_2CO_3, CH_3COCH_3, reflux, 85−90%. (vi) Compound **2**, K_2CO_3, CH_3COCH_3, reflux, 72−76%. (vii) $SnCl_2$, $2H_2O$, MeOH, reflux, 80−85%. (viii) $HgCl_2$, $CaCO_3$, CH_3CN-H_2O (4:1), 55−61%.

Table 1. Thermal Denaturation Data for DC-81 (**1**), DSB-120 (**3**), and Compounds **5a**−**d** with Calf Thymus DNA

compd	[PBD]:[DNA] molar ratio[b]	induced ΔT_m (°C)[a] after incubation at 37 °C	
		0 h	18 h
5a	1:5	6.5	7.0
5b	1:5	5.0	8.5
5c[c]	1:5	14.0	17.0
5d	1:5	4.2	5.0
1	1:5	0.3	0.7
3	1:5	10.2	15.4

[a] For CT-DNA alone at pH 7.00 ± 0.01, ΔT_m = 66.5 °C ± 0.01 (mean value from 30 separate determinations); all ΔT_m values are ± 0.1−0.2 °C. [b] For a 1:5 molar ratio of [ligand]/[DNA], where CT-DNA concentration = 100 μM and ligand concentration = 20 μM in aqueous sodium phosphate buffer [10 mM sodium phosphate + 1 mM EDTA, pH 7.00 ± 0.01]. [c] The ΔT_m for **5c** at a [ligand]:[DNA] molar ratio of 1:5 increased to a value of 17.0 °C after 18 h of incubation.

Cytotoxicity. Compounds **5a**−**c** were evaluated in vitro against sixty human tumor cells derived from nine cancer types (leukemia, nonsmall-cell lung cancer, colon cancer, central nervous system (CNS) cancer, melanoma, ovarian cancer, renal cancer, prostate cancer, and breast cancer). For each compound, dose−response curves for each cell line were measured at a minimum of five concentrations at 10-fold dilutions. A protocol of 48 h of continuous drug exposure was used, and a sulforhodamine B (SRB) protein assay was used to estimate cell viability or growth. The concentration causing 50% cell growth inhibition (GI_{50}), total cell growth inhibition (TGI, 0% growth), and 50% cell death (LC_{50}, −50% growth) as compared with the control was calculated. The mean graph midpoint (MG MID) values of log TGI and log LC_{50} as well as log GI_{50} for **5a**−**c** are listed in Table 2. As demonstrated by mean graph pattern, compound **5c** exhibits an interesting profile of activity and selectivity for various cell lines. The MG MID of log TGI and log LC_{50} showed a pattern similar to the log GI_{50} MG MIDs.

Table 2. log GI_{50}, log TGI, and log LC_{50} MG MIDs of In Vitro Cytotoxicity Data for Compounds **5a**−**c** against Human Tumor Cell Lines[a]

compd	log GI_{50}	log TGI	log LC_{50}
5a	−4.88	−4.37	−4.08
5b	−5.14	−4.69	−4.30
5c	−6.92	−5.95	−4.56
5c[b]	−6.94	−5.65	−4.35

[a] GI_{50}, drug molar concentration causing 50% cell growth inhibition; TGI, drug concentration causing total cell growth inhibition (0% growth); LC_{50}, drug concentration causing 50% cell death (−50%); MG MID, mean graph midpoints, the average sensitivity of all cell lines toward the test agent. [b] Repeat testing in the primary screen values.

The comparison of the data in Table 3 reveals the importance of an alkane spacer. As the alkane spacer increased from three to five, the cytotoxic activity was moderately enhanced. The five carbon spacer of compound **5c** confers a suitable fit in the minor groove of

Table 3. Log LC_{50} (Concentration in mol/L Causing 50% Lethality) Values for Compounds **5a**−**c**[a]

cancer	5a	5b	5c	5c[b]
leukemia	−4.48	−4.62	−5.32	−4.00
nonsmall-cell lung	−4.00	−4.23	−4.10	−4.23
colon	−4.11	−4.24	−4.52	−4.46
CNS	−4.00	−4.34	−4.43	−4.44
melanoma	−4.08	−4.38	−5.48	−5.00
ovarian	−4.02	−4.23	−4.22	−4.06
renal	−4.04	−4.26	−4.35	−4.29
prostate	−4.00	−4.17	−4.00	−4.00
breast	−4.02	−4.27	−4.44	−4.42

[a] Each cancer type represents the average of 6−8 different cancer cell lines. [b] Repeat testing in the primary screen values.

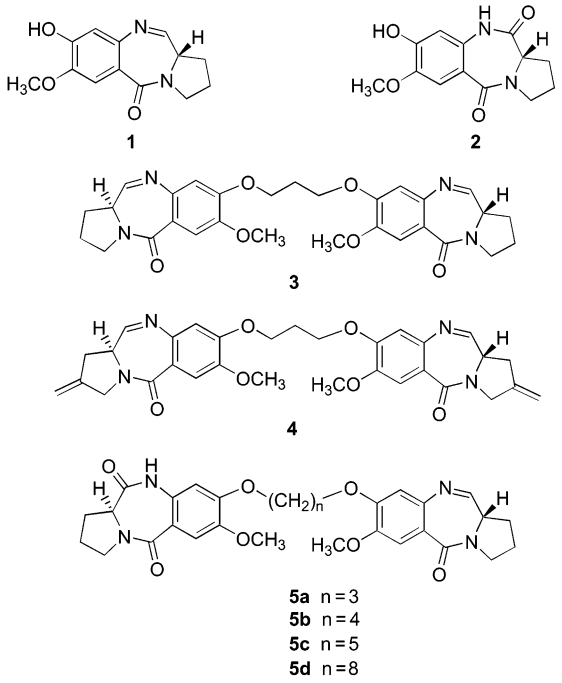


Figure 1. Structures of the PBD monomer DC-81 (**1**), PBD dilactam (**2**), and PBD dimers DSB-120 (**3**), SJG-136 (**4**), and **5**.

double helix DNA and shows slightly higher activity in this series of compounds **5a**−**c**. This observation was supported by molecular modeling and thermal denaturation studies. Furthermore, melanoma, leukemia, colon, and CNS are the more sensitive cancers to these compounds **5a**−**c**.

Inhibition of Restriction Endonuclease *BamH*I. The melting temperature study indicated that PBD possesses DNA binding affinity. To confirm their interaction with DNA restriction endonuclease, a protection assay was performed. The RED_{100} assay is based upon the ability of PBD to inhibit the cleavage activity of restriction endonuclease *BamH*I. The inhibition of *BamH*I cleavage activity by the ligand bound to the DNA suggests that PBD binding sites overlap with the *BamH*I cleavage site. The study was carried out to determine the stoichiometric ratio at which PBD inhibits the DNA linearization by *BamH*I. The results of this experiment for a representative compound **5a** as shown in Figure 2 suggest that the PBD inhibits *BamH*I digestion in a dose-dependent manner with a stoichiometric ratio and are illustrated in Table 4. It is observed that compound **5a** binds to DNA at a stoichiometric ratio of 2.05:1.

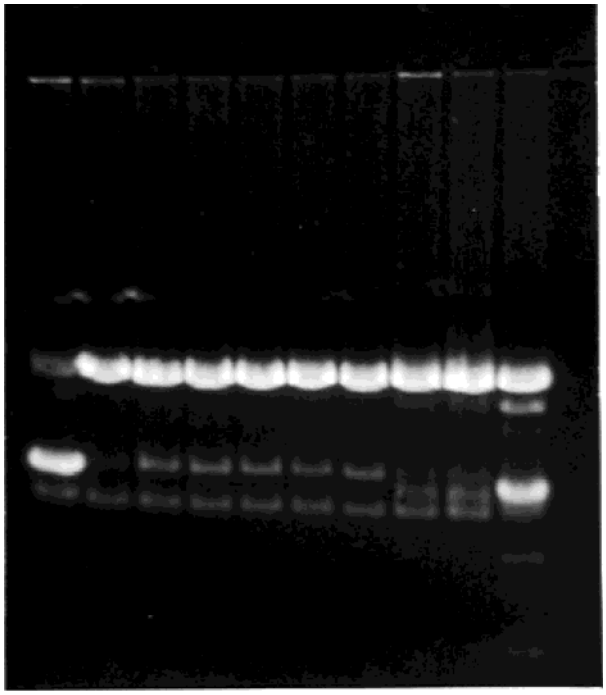


Figure 2. Lane 1, control plasmid DNA; lane 2, *BamH*I linearized DNA; lane 3−10, PBD−plasmid DNA complexes digested by *BamH*I with PBD (**5a**) concentrations of 1.8, 3.7, 5.5, 7.3, 9.2, 11.0, 12.8, and 14.6 μM, respectively.

Table 4. Stoichiometric Ratios of Drug to DNA Used for RED_{100} Assay

concn of drug (μM)	drug:DNA ratio	concn of drug (μM)	drug:DNA ratio
1.8	16.6:1	9.2	3.2:1
3.7	8.1:1	11.0	2.7:1
5.5	5.4:1	12.8	2.3:1
7.3	4.1:1	14.6	2.05:1

Because the drug was preincubated with PBD, the DNA−drug complexes were subjected to *BamH*I digestion, and the protection of cleavage of the G$^{\downarrow}$GATCC sequence by PBD suggests that these molecules selectively interact with G-rich sequences in DNA. The inability of PBD in protecting cleavage of AAT$^{\downarrow}$ATT by *Ssp*I suggests that these PBD have low affinity to AT-rich sequences. Therefore, PBD prefers G-rich sequences in DNA binding; this could be due to the affinity of PBDs in covalent interaction with the free amino group attached to the N2 of guanine in the DNA.[9]

Molecular Modeling Studies. Energetically favorable models of the DNA−PBD−dimer complexes for **5a**−**d** molecules were built using the systematic procedure as described in the methods section, involving molecular modeling and docking followed by detailed molecular dynamic simulations. The energy of interaction (E_{int}) between the DNA and the PBD−dimer molecule in a complex was calculated as a measure of stability of that complex. Table 5 gives the values of E_{int} obtained for the four complexes. The lowest value of E_{int} corresponds to the DNA−**5c** complex (Figure 3) indicating that the molecule **5c** forms energetically the most stable complex with DNA. It is interesting to note that the E_{int} progressively decreases indicating increase in stability as the number of alkane spacer units increases from three to five. However, further increase in the

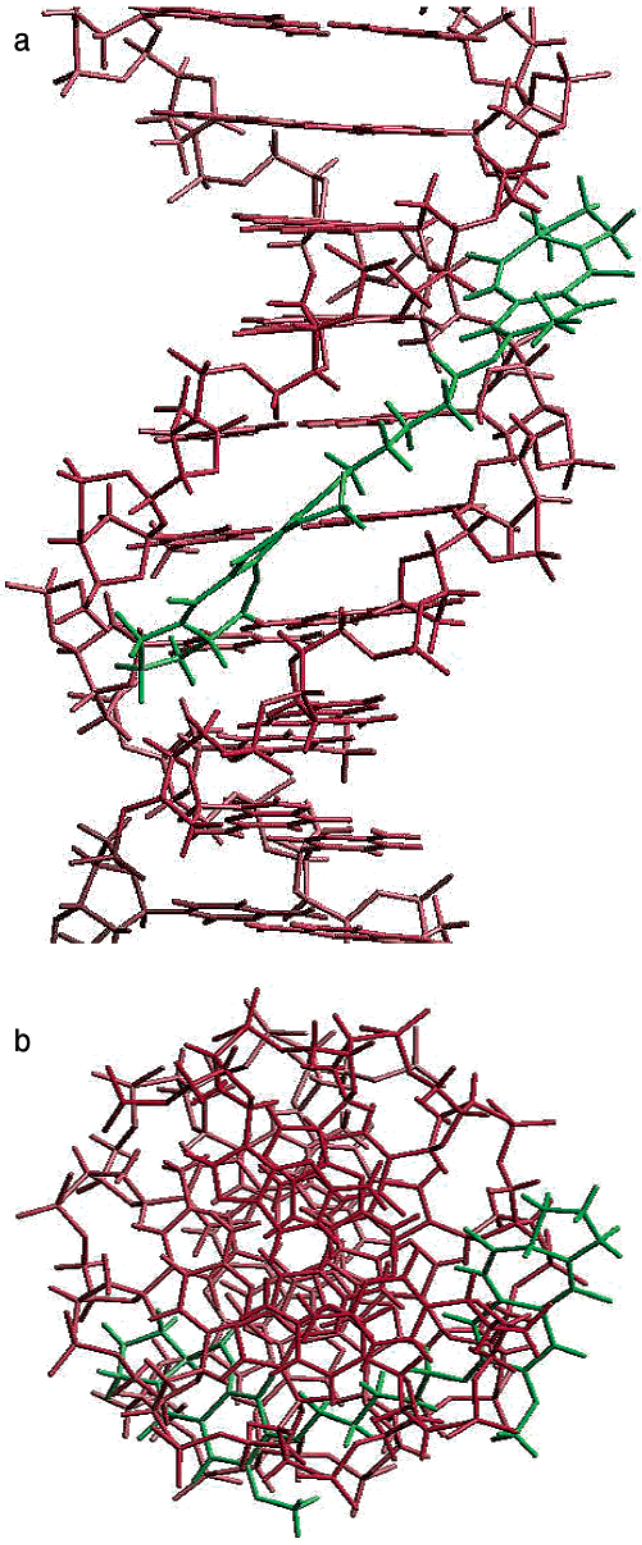


Figure 3. Projection diagram showing the DNA−**5c** complex. (a) Side-on view and (b) down the helix axis.

number of spacer units from five to eight does not lead to an increase in the E_{int}; in fact, E_{int} is lowered by about 2 kcal mol^{-1}. The E_{int} values are, in fact, correlated to the ΔT_m values measured from thermal denaturation experiments (Figure 4).

In general, a number of favorable van der Waals and Coulombic interactions are formed between the DNA and the PBD−dimer molecules and they together contribute to the stability in all of the complexes, in addition to the covalent linkage formed between the imine PBD subunit and the G8. Only in the cases of **5c**,**d**, the complexes are further stabilized by a hydrogen bond formed between the carbonyl oxygen of the C10−N11 amide functionality of the PBD subunit and the amino group of the 12th guanine nucleotide. It is worth noting that the amide PBD subunits in both the complexes of **5c**,**d** occupy the same site in the minor groove. The complex formed by **5c** is energetically more stable than the complex formed by **5d** because the chain of alkane spacer units in **5c** forms a better isohelical fit (and thus gives rise to more favorable nonbonded interactions) within the minor groove than the longer chain in **5d**.

Table 5. Values of Energy of Interactions Calculated for the DNA−PBD Dimer Complexes

complex	energy of interaction (E_{int}) in kcal mol^{-1}
DNA−**5a**	−123.4
DNA−**5b**	−129.2
DNA−**5c**	−139.7
DNA−**5d**	−137.5

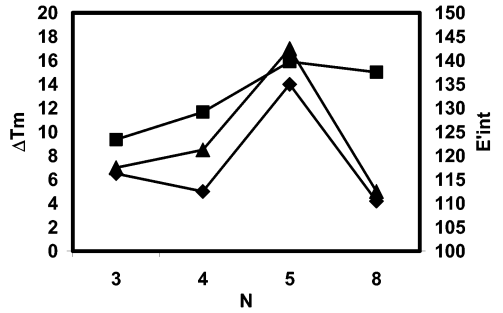


Figure 4. Plot of the calculated values of energy of interactions (E_{int} in kcal mol^{-1}, ■) and the melting temperatures ($\triangle T_m$ in °C, ◆ (0 h) and ▲ (18 h)) measured from thermal denaturation studies for the four DNA−PBD dimer complexes vs the number of spacer units.

Conclusions

Introduction of an amide functionality in one of the PBD rings and an increase of the dimer's linker length to a five carbon chain significantly enhance the DNA binding ability of the noncross-linking PBD dimer as evaluated by thermal denaturation studies. Modeling studies suggest that apart from the covalent linkage formed between the imine PBD subunit and the G8, there are a number of favorable van der Waals and Coulombic interactions that are formed between the DNA and the mixed imine-amide PBD dimer. Furthermore, the complex formed by compound **5c** (with a five carbon chain linker) is energetically more stable than the complexes formed by **5a**,**b**,**d** as it forms a better isohelical fit within the minor groove of DNA. The restriction endonuclease studies suggest that these molecules selectively interact with G sequences in DNA and have low affinity to AT-rich sequences. In addition, compound **5c** exhibits an interesting profile of in vitro cytotoxic activity and selectivity for various cell lines. This compound is being evaluated in the hollow fiber assay and a number of human tumor xenografts and is likely to be taken up for preclinical development.

Experimental Section

Synthetic Chemistry. Reaction progress was monitored by thin-layer chromatography (TLC) using GF_{254} silica gel with fluorescent indicator on glass plates. Visualization was achieved with UV light and iodine vapor unless otherwise stated. Chromatography was performed using Acme silica gel (100−200 mesh). The majority of reaction solvents were purified by distillation under nitrogen from the indicated drying agent and used fresh: dichloromethane (calcium hydride), tetrahydrofuran (THF) (sodium benzophenone ketyl), methanol (magnesium methoxide), acetonitrile (calcium hydride).

^{1}H NMR spectra were recorded on Varian Gemini 200 MHz spectrometer using tetramethylsilane (TMS) as an internal standard. Chemical shifts are reported in parts per million (ppm) downfield from TMS. Spin multiplicities are described as s (singlet), brs (broad singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Coupling constants are reported in Hertz (Hz). Low resolution mass spectra (LRMS) were recorded on VG-7070H Micromass mass spectrometer at 200 °C, 70 eV, with a trap current of 200 μA and 4 kV of acceleration voltage. High resolution mass spectra (HRMS) were recorded on VG Autospec N mass spectrometer at 200 °C, 70 eV, with a trap current of 200 μA and 7 kV of acceleration voltage.

Methyl-(2*S*)-N-[4-benzyloxy-5-methoxy-2-nitrobenzoyl]-pyrrolidine-2-carboxylate (7). To a stirred suspension of compound **6** (303 mg, 1 mmol) and thionyl chloride (476 mg, 4.0 mmol) in dry benzene (15 mL) was added dimethylformamide (DMF) (4−5 drops), and the stirring was continued for 6 h. The benzene was evaporated in a vacuum, and the resultant oil was dissolved in dry THF (20 mL) and added dropwise over a period of 30 min to a stirred suspension of L-proline methylester hydrochloride (248 mg 1.5 mmol), triethylamine (303 mg, 3 mmol), and ice water (20 mL) cooled in an ice bath. After the addition was completed, the reaction mixture was brought to ambient temperature and stirred for an additional hour. The THF was evaporated in a vacuum, and the aqueous layer was washed with ethyl acetate (10 mL). The aqueous phase was then adjusted to pH 3 using 6 N HCl and extracted with ethyl acetate and washed with brine, dried over Na_2SO_4, and evaporated in a vacuum and was purified by column chromatography (30% EtOAc−hexane) to afford compound **7** as yellow oil (352 mg, 85%). ^{1}H NMR ($CDCl_3$): δ 1.80−2.10 (m, 3H), 2.12−2.35 (m, 1H), 3.10−3.18 (m, 1H),3.20−3.30 (m, 1H), 3.70 (s, 3H), 3.92 (s, 3H), 4.60−4.70 (m, 1H), 5.10 (s, 2H), 6.80 (s, 1H), 7.25−7.42 (m, 5H), 7.60 (s, 1H). MS (EI) *m*/*z* 414 $[M]^{+\cdot}$.

Methyl-(2*S*)-N-[4-hydroxy-5-methoxy-2-nitrobenzoyl]-pyrrolidine-2-carboxylate (8). To a stirred solution of EtSH (1.20 g, 19 mmol) and $BF_3{\cdot}OEt_2$ (1.419 g, 10 mmol) was added dropwise compound **7** (414 mg, 1 mmol) in dichloromethane (15 mL) at room temperature. Stirring was continued until the reaction was completed by TLC, and then, the solvent was evaporated in a vacuum. The residue was quenched with 5% $NaHCO_3$ solution (20 mL) and then extracted with dichloromethane (2 × 20 mL). The combined organic phases were washed with saturated brine (10 mL) and dried over Na_2SO_4, and the solvent was removed by vacuum and purified by column chromatography (70% EtOAc−hexane) to afford compound **8** as pale yellow oil (285 mg, 88%). ^{1}H NMR ($CDCl_3$): δ 1.87−2.50 (m, 4H), 3.15−3.40 (m, 2H), 3.80 (s, 3H), 4.0 (s, 3H), 4.55−4.70 (m, 1H), 6.80 (s, 1H), 7.68 (s, 1H). MS (EI) *m*/*z* 265 $[M\text{-}COOCH_3]^{+\cdot}$.

(11a*S*)-8-Hydroxy-7-methoxy-1,2,3,10,11,11a-hexahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11-dione (2). The compound **7** (414 mg, 1.0 mmol) was dissolved in methanol (10 mL), and 10% Pd−C (200 mg) was added. The mixture was hydrogenated at room temperature under atmospheric pressure for 10 h. The catalyst was removed by filtration through Celite, and then, the solvent was evaporated under vacuum and purified by column chromatography (80% EtOAc−hexane) to afford **2** as a pluffy solid (162 mg, 62%); mp 278−280 °C. ^{1}H NMR ($CDCl_3$): δ 1.90−2.10 (m, 3H), 2.75−2.85 (m,1H), 3.50−3.67 (m, 1H), 3.70−3.85 (m, 1H), 3.94 (s, 3H), 4.06 (m, 1H), 6.50 (s 1H), 7.45 (s,1H), 8.10 (br s, 1H). MS (EI) *m*/*z* 262 $[M]^{+\cdot}$.

Methyl-4-(3-bromopropoxy)-3-methoxybenzoate (10a). To a solution of vanillin methyl ester **9** (182 mg, 1 mmol) in acetone (30 mL) were added anhydrous K_2CO_3 (553 mg, 4 mmol) and 1,3-dibromo propane (605 mg, 3 mmol), and the mixture was refluxed in an oil bath for 48 h. The reaction was monitored by TLC using EtOAc−hexane (2:8), K_2CO_3 was removed by filtration, and the solvent was evaporated under the vacuum and was purified by column chromatography (10% EtOAc−hexane) to afford compound **10a** as white solid (260 mg, 86%); mp 117−118 °C. ^{1}H NMR ($CDCl_3$): δ 2.35−2.45 (m, 2H), 3. 64 (t, 2H, J = 6.3 Hz), 3.94 (s, 3H), 3.98 (s, 3H), 4.20 (t, 2H, J = 6 Hz), 6.88 (d, 1H, J = 8.2 Hz), 7.50 (s, 1H), 7.65 (d, 1H, J = 8.8 Hz). MS (EI) *m*/*z* 303 $[M]^{+\cdot}$.

Methyl-4-(4-bromobutoxy)-3-methoxybenzoate (10b). The compound **10b** was prepared according to the method described for the compound **10a** employing vanillin methyl ester **9** (182 mg, 1 mmol) and 1,4-dibromobutane (648 mg, 3 mmol), and the crude product was purified by column chromatography (10% EtOAc−hexane) to afford compound **10b** as white solid (260 mg, 82%); mp 122−124 °C. ^{1}H NMR ($CDCl_3$): δ 2.0−2.2 (m, 4H), 3.55 (t, 2H, J = 6.3 Hz), 3.94 (s, 3H), 3.97 (s, 3H), 4.15 (t, 2H, J = 6.1 Hz), 6.88 (d, 1H, J = 8.2 Hz), 7.55 (s, 1H), 7.65 (d, 1H, J = 8.8 Hz). MS (EI) *m*/*z* 317 $[M]^{+\cdot}$.

Methyl-4-(5-Bromopentyloxy)-3-methoxybenzoate (10c). The compound **10c** was prepared according to the method described for the compound **10a** employing vanillin methyl ester **9** (182 mg, 1 mmol) and 1,5-dibromopentane (689 mg, 3 mmol), and the crude product was purified by column chromatography (10% EtOAc−hexane) to afford compound **10c** as a white solid (275 mg, 83%); mp 125−127 °C. ^{1}H NMR ($CDCl_3$): δ 1.8−2.0 (m, 6H), 3.48 (t, 2H, J = 6.4 Hz), 3.93 (s, 3H), 3.96 (s, 3H), 4.15 (t, 2H, J = 5.9 Hz), 6.85 (d, 1H, J = 8.2 Hz), 7.55 (s, 1H), 7.65 (d, 1H, J = 8.8 Hz). MS (EI) *m*/*z* 331 $[M]^{+\cdot}$.

Methyl-4-(8-bromooctyloxy)-3-methoxybenzoate (10d). The compound **10d** was prepared according to the method described for the compound **10a** employing vanillin methyl ester **9** (182 mg, 1 mmol) and 1,8-dibromooctane (816 mg, 3 mmol), and the crude product was purified by column chromatography (10% EtOAc−hexane) to afford compound **10c** as an oil (275 mg, 83%). ^{1}H NMR ($CDCl_3$): δ 1.30−1.60 (m, 8H), 1.85−2.00 (m, 4H) 3.40 (t, 2H, J = 6.2 Hz), 3.93 (s, 3H), 3.96 (s, 3H), 4.15 (t, 2H, J = 6 Hz), 6.85 (d, 1H, J = 8.2 Hz), 7.55 (s, 1H), 7.65 (d, 1H, J = 8.8 Hz). MS (EI) *m*/*z* 373 $[M]^{+\cdot}$.

Methyl-4-(3-bromopropoxy)-5-methoxy-2-nitrobenzoate (11a). A freshly prepared mixture of stannic chloride (260 mg, 1.156 mmol) and fuming nitric acid (98 mg, 1.56 mmol) in dichloromethane was added dropwise over 5 min with stirring to a solution of **10a** (303 mg, 1 mmol) in dichloromethane (30 mL) at −25 °C (dry ice/carbon tetrachloride). The mixture was maintained at −25 °C for a further 5 min, quenched with water (20 mL), and then allowed to return to room temperature. The organic layer was separated, and the aqueous layer was extracted with dichloromethane (2 × 20 mL). The combined organic phase was dried (Na_2SO_4) and evaporated in a vacuum, and it was purified by column chromatography (20% EtOAc−hexane) to give **11a** as a yellow oily liquid (313 mg, 90%). ^{1}H NMR ($CDCl_3$): δ 2.30−2.48 (m, 2H), 3.55−3.65 (t, 2H, J = 6.2 Hz), 3.89 (s, 3H), 3.95 (s, 3H), 4.25 (t, 2H, J = 6.1 Hz), 7.05 (s, 1H), 7.45 (s, 1H). MS (EI) *m*/*z* 348 $[M]^{+\cdot}$.

Methyl-4-(4-bromobutoxy)-5-methoxy-2-nitrobenzoate (11b). Compound **10b** (317 mg, 1 mmol) was nitrated by the similar method described earlier for **11a** to get **11b** as a pale yellow oily liquid (218 mg, 88%). ^{1}H NMR ($CDCl_3$): δ 1.95−2.15 (m, 4H), 3.45 (t, 2H, J = 6.34 Hz), 3.85 (s, 3H), 3.95 (s, 3H), 4.10 (t, 2H, J = 6.12 Hz), 7.02 (s, 1H), 7.38 (s, 1H). MS (EI) *m*/*z* 362 $[M]^{+\cdot}$.

Methyl-4-(5-bromopentyloxy)-5-methoxy-2-nitrobenzoate (11c). Compound **10c** (331 mg, 1 mmol) was nitrated by the similar method described earlier for **11a** to get **11c** as a light yellow oily liquid (345 mg, 91%). ^{1}H NMR ($CDCl_3$): δ

1.75−2.0 (m, 6H), 3.40 (t, 2H, J = 6.31 Hz), 3.85 (s, 3H), 3.95 (s, 3H), 4.0−4.1 (t, 2H, J = 6 Hz), 7.0 (s,1H), 7.4 (s,1H). MS (EI) m/z 376 $[M]^{+\cdot}$.

Methyl-4-(8-bromooctyloxy)-5-methoxy-2-nitrobenzoate (11d). Compound **10d** (373 mg, 1 mmol) was nitrated by the similar method described earlier for **11a** to get **11d** as a light yellow oily liquid (376 mg, 90%). ^{1}H NMR ($CDCl_3$): δ 1.30−1.60 (m, 8H), 1.85−2.00 (m, 4H) 3.40 (t, 2H, J = 6.38 Hz), 3.93 (s, 3H), 3.96 (s, 3H), 4.15 (t, 2H, J = 6.21 Hz), 6.85 (s, 1H), 7.60 (s, 1H).

4-(3-Bromopropoxy)-5-methoxy-2-nitrobenzoic acid (12a). An amount of 2 N lithium hydroxide monohydrate (1.22 mL) was added to a solution of **11a** (348 mg, 1 mmol) in THF−H_2O−MeOH (4:1:1), and the mixture was stirred at room temperature for 12 h. After most of the THF and methanol was evaporated, the aqueous phase was acidified with 12 N HCl to pH 7 and reextracted with EtOAc to give a **12a** as a white solid (297 mg, 89%); mp 118−120 °C. ^{1}H NMR ($CDCl_3$): δ 2.28−2.48 (m, 2H), 3.6 (t, 2H, J = 6.4 Hz), 3.98 (s, 3H), 4.25 (t, 2H, J = 6.2 Hz), 7.20 (s, 1H), 7.40 (s, 1H).

4-(4-Bromobutoxy)-5-methoxy-2-nitrobenzoic Acid (12b). The compound (**12b**) was prepared by the same method described earlier for **12a** employing **11b** (362 mg, 1 mmol), which gave **12b** as a white solid (324 mg, 93%); mp 176−178 °C. ^{1}H NMR ($CDCl_3$): δ 2.0−2.15 (m, 4H), 3.50 (t, 2H, J = 6.35 Hz), 3.96 (s, 3H), 4.15 (t, 2H, J = 6.11 Hz), 7.18 (s, 1H), 7.38 (s, 1H).

4-(5-Bromopentyloxy)-5-methoxy-2-nitrobenzoic Acid (12c). The compound (**12c**) was prepared by the same method described earlier for **12a** employing **11c** (376 mg, 1 mmol), which gave **12c** as a white solid (330 mg, 91%); mp 166−168 °C. ^{1}H NMR ($CDCl_3$): δ 1.9−2.1 (m, 6H), 3.45 (t, 2H, J = 6.3 Hz), 3.95 (s, 3H), 4.1−4.15 (t, 2H, J = 6.2 Hz), 7.18 (s, 1H), 7.38 (s, 1H).

4-(8-Bromooctyloxy)-5-methoxy-2-nitrobenzoic Acid (12d). The compound (**12d**) was prepared by the same method described earlier for **12a** employing **11d** (418 mg, 1 mmol), which gave **12d** as an oil (355 mg, 88%). ^{1}H NMR ($CDCl_3$): δ 1.30−1.60 (m, 8H), 1.85−2.00 (m, 4H) 3.40 (t, 2H, J = 6.32 Hz), 3.96 (s, 3H), 4.15 (t, 2H, J = 6 Hz), 6.85 (s, 1H), 7.60 (s, 1H). MS (EI) m/z 404 $[M]^{+\cdot}$.

(2*S*)-N-[4-(3-Bromopropoxy)-5-methoxy-2-nitrobenzoyl]-pyrrolidine-2-carboxaldehyde Diethyl Thioacetal (13a). DMF was added to a stirred suspension of compound **12a** (334 mg, 1 mmol) and thionyl chloride (476 mg, 4 mmol) in dry benzene (15 mL), and the stirring was continued for 6 h. The benzene was evaporated in vacuo, and the resultant oil was dissolved in dry THF (20 mL) and added dropwise over a period of 30 min to a stirred suspension of (2*S*)-pyrrolidine-2-carboxaldehyde diethyl thioacetal (205 mg, 1 mmol), triethylamine (303 mg, 3 mmol), and ice water (20 mL) cooled in an ice bath. After the addition was completed, the reaction mixture was brought to ambient temperature and stirred for an additional hour. The THF was evaporated in a vacuum, and the aqueous layer was washed with ethyl acetate (10 mL). The aqueous phase was then adjusted to pH 3 using 6 N HCl and extracted with ethyl acetate and washed with brine, dried over Na_2SO_4, and evaporated in a vacuum, and the crude product was purified by column chromatography (30%EtoAc−hexane) to afford **13a** as a oily liquid (464 mg, 89%). NMR ($CDCl_3$): δ 1.20−1.40 (m, 6H), 1.70−2.50 (m, 6H), 2.60−2.90 (m, 4H), 3.20−3.35 (m, 2H), 3.65 (t, 2H, J = 6.22 Hz), 3.95 (s, 3H), 4.25(t, 2H, J = 5.98 Hz), 4.63−4.75 (m, 1H), 4.85 (d, 1H, J = 4.3 Hz), 6.80 (s, 1H), 7.70 (s, 1H). MS (FAB) 521 $[M]^{+\cdot}$.

(2*S*)-N-[4-(4-Bromobutoxy)-5-methoxy-2-nitrobenzoyl]-pyrrolidine-2-carboxaldehyde Diethyl Thioacetal (13b). The compound **13b** was prepared according to the method described for the compound **13a** by employing **12b** (348 mg, 1 mmol) and (2*S*)-pyrrolidine-2-carboxaldehyde diethyl thioacetal, which gave **13b** as a oily liquid (476 mg, 89%). ^{1}H NMR ($CDCl_3$): δ 1.21−1.45 (m, 6H), 1.70−2.40 (m, 8H), 2.60−2.90 (m, 4H), 3.15−3.30 (m, 2H), 3.50 (t, 2H, J = 6.25 Hz), 3.95 (s, 3H), 4.10 (t, 2H, J = 6 Hz), 4.60−4.71 (m, 1H), 4.85 (d, 1H, J = 4.3 Hz), 6.80 (s, 1H), 7.65 (s, 1H). MS (FAB) 535 $[M]^{+\cdot}$.

(2*S*)-N-[4-(5-Bromopentyloxy)-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehyde Diethyl Thioacetal (13c). The compound **13c** was prepared according to the method described for the compound **13a** by employing **12c** (362 mg, 1 mmol) and (2*S*)-pyrrolidine-2-carboxaldehyde diethyl thioacetal, which gave **13c** as a oily liquid (505 mg, 92%). ^{1}H NMR ($CDCl_3$): δ 1.21−1.40 (m, 8H), 1.60−2.40 (m, 8H), 2.65−2.85 (m, 4H), 3.15−3.30 (m, 2H), 3.45 (t, 2H, J = 6.34 Hz), 3.95 (s, 3H), 4.10 (t, 2H, J = 6 Hz), 4.60−4.71 (m, 1H), 4.85 (d, 1H, J = 4.33 Hz), 6.80 (s, 1H), 7.65 (s, 1H). MS (FAB) 549 $[M]^{+\cdot}$.

(2*S*)-N-[4-(5-Bromooctyloxy)-5-methoxy-2-nitrobenzoyl]-pyrrolidine-2-carboxaldehyde Diethyl Thioacetal (13d). The compound **13d** was prepared according to the method described for the compound **13a** by employing **12d** (404 mg, 1 mmol) and (2*S*)-pyrrolidine-2-carboxaldehyde diethyl thioacetal, which gave **13d** as an oily liquid (532 mg, 90%). ^{1}H NMR ($CDCl_3$): δ 1.21−1.60 (m, 14H), 1.80−2.20 (m, 8H), 2.65−2.85 (m, 4H), 3.15−3.30 (m, 2H), 3.45 (t, 2H, J = 6.2 Hz), 3.95 (s, 3H), 4.10 (t, 2H, J = 6.1 Hz), 4.60−4.71 (m, 1H), 4.85 (d, 1H, J = 4.3 Hz), 6.80 (s, 1H), 7.70 (s, 1H).

(2*S*)-N-{4-[Methyl-(2*S*)-N-(5-methoxy-2-nitrobenzoyl)-pyrrolidine-carboxylate-4-yloxy]propoxy-5-methoxy-2-nitrobenzoyl}pyrrolidine-2-carboxaldehyde Diethyl Thioacetal (14a). To a solution of **13a** (521 mg, 1 mmol) in dry acetone (30 mL) was added anhydrous K_2CO_3 (552 mg, 4 mmol) and **8** (324 mg, 1 mmol). The reaction mixture was refluxed in an oil bath for 48 h. The reaction was monitored by TLC using EtOAc−hexane (6:4) as a solvent system. K_2CO_3 was removed by filtration, and the solvent was removed under vacuum. The crude product was purified by column chromatography (60%EtoAc−hexane) to afford a yellow oil (688 mg, 90%). ^{1}H NMR ($CDCl_3$): δ 1.20−1.40 (m, 6H), 1.50−2.40 (m, 10H), 2.60−2.90 (m, 4H), 3.10−3.40 (m, 4H), 3.80 (s 3H), 3.90 (s, 3H), 3.98 (s, 3H), 4.18−4.38 (m, 4H), 4.60−4.75 (m, 2H), 4.85 (d, 1H, J = 4.4 Hz), 6.70 (s, 1H), 6.80 (s, 1H), 7.6 (s, 1H), 7.70 (s, 1H). MS (FAB) 765 $[M + H]^{+\cdot}$.

(2*S*)-N-{4-[Methyl-(2*S*)-N-(5-methoxy-2-nitrobenzoyl)-pyrrolidine-carboxylate-4-yloxy]butoxy-5-methoxy-2-nitrobenzoyl}pyrrolidine-2-carboxaldehyde Diethyl Thioacetal (14b). The compound **14b** was prepared according to the method described for **14a** by employing **8** and **13b** (535 mg, 1 mmol) to afford **14b** as a yellow oil (662 mg, 85%). ^{1}H NMR ($CDCl_3$): δ 1.20−1.40 (m, 6H), 1.50−2.40 (m, 12H), 2.60−2.90 (m, 4H), 3.10−3.40 (m, 4H), 3.80 (s 3H), 3.88 (s, 3H), 3.98 (s, 3H), 4.10−4.20 (m, 4H), 4.60−4.80 (m, 2H), 4.85 (d, 1H, J = 4.3 Hz), 6.70 (s, 1H), 6.80 (s, 1H), 7.6 (s, 1H), 7.70 (s, 1H). MS (FAB) 779 $[M + H]^{+\cdot}$.

(2*S*)-N-{4-[Methyl-(2*S*)-N-(5-methoxy-2-nitrobenzoyl)-pyrrolidine-carboxylate-4-yloxy]pentyloxy-5-methoxy-2-nitrobenzoyl}pyrrolidine-2-carboxaldehyde Diethyl Thioacetal (14c). The compound **14c** was prepared according to the method described for **14a** by employing **8** and **13c** (549 mg, 1 mmol) to afford **14c** as a yellow oil (697 mg, 88%). ^{1}H NMR ($CDCl_3$): δ 1.20−1.40 (m, 8H), 1.50−2.40 (m, 12H), 2.60−2.90 (m, 4H), 3.10−3.40 (m, 4H), 3.80 (s 3H), 3.88 (s, 3H), 3.98 (s, 3H), 4.10−4.20 (m, 4H), 4.60−4.80 (m, 2H), 4.85 (d, 1H, J = 4.2 Hz), 6.70 (s, 1H), 6.80 (s, 1H), 7.6 (s, 1H), 7.70 (s, 1H). MS (FAB) 794 $[M + H]^{+\cdot}$.

(2*S*)-N-{4-[Methyl-(2*S*)-N-(5-methoxy-2-nitrobenzoyl)-pyrrolidine-carboxylate-4-yloxy]octyloxy-5-methoxy-2-nitrobenzoyl}pyrrolidine-2-carboxaldehyde Diethyl Thioacetal (14d). The compound **14d** was prepared according to the method described for **14a** by employing **8** and **13d** (591 mg, 1 mmol) to afford **14d** as a yellow oil (697 mg, 88%). ^{1}H NMR ($CDCl_3$): δ 1.20−1.60 (m, 14H), 1.80−2.40 (m, 12H), 2.60−2.90 (m, 4H), 3.10−3.40 (m, 4H), 3.80 (s 3H), 3.88 (s, 3H), 3.98 (s, 3H), 4.10−4.20 (m, 4H), 4.60−4.80 (m, 2H), 4.85 (d, 1H, J = 4.38 Hz), 6.70 (s, 1H), 6.80 (s, 1H), 7.6 (s, 1H), 7.70 (s, 1H). MS (FAB) 835 $[M]^{+\cdot}$.

(2*S*)-N-{4-[3-(7-Methoxy-(11a*S*)-1,2,3,10,11,11a-hexahydro-5*H*-pyrrolo[2,1-*c*]-[1,4]benzodiazepine-5,11-dione-8-

yloxy)propoxy]-5-methoxy-2-nitrobenzoyl}pyrrolidine-2-carboxaldehyde Diethyl Thioacetal (15a). To a solution of **13a** (521 mg, 1 mmol) in dry acetone (30 mL) was added anhydrous K_2CO_3 (552 mg, 4 mmol) and **2** (262 mg, 1 mmol). The reaction mixture was refluxed in an oil bath for 48 h. The reaction was monitored by TLC using EtOAc−hexane (9:1) as a solvent system. K_2CO_3 was removed by filtration, and the solvent was removed under vacuum. The crude product was purified by column chromatography (90%EtoAc−hexane) to afford a yellow oil (688 mg, 90%). ^{1}H NMR ($CDCl_3$): δ 1.20−1.65 (m, 6H), 1.70−2.60 (m, 9H), 2.65−2.98 (m, 5H), 3.20−3.40 (m, 2H), 3.50−3.75 (m, 1H), 3.75−3.90 (m, 1H) 3.95 (s 3H), 4.0 (s, 3H), 4.06 (m, 1H), 4.20−4.45 (m, 4H), 4.65−4.85 (m, 1H), 4.90 (d, 1H, J = 4.28 Hz), 6.55 (s, 1H), 6.85 (s, 1H), 7.48 (s, 1H), 7.75 (s, 1H), 7.90 (br s, NH exchangeable). MS (FAB) 703 $[M + H]^{+\cdot}$.

(2*S*)-N-{4-[4-(7-Methoxy-(11a*S*)-1,2,3,10,11,11a-hexahydro-5*H*-pyrrolo[2,1-*c*]-[1,4]benzodiazepine-5,11-dione-8-yloxy)butoxy]-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehyde Diethyl Thioacetal (15b). The compound **15b** was prepared according to the method described for **15a** by employing **2** and **13b** (535 mg, 1 mmol) to afford **15b** as a yellow oil (662 mg, 85%). ^{1}H NMR ($CDCl_3$): δ 1.20−1.60 (m, 6H), 1.60−2.20 (m, 11H), 2.50−3.0 (m, 5H), 3.15−3.40 (m, 2H), 3.50−3.80 (m, 2H), 3.85 (s 3H), 3.95 (s, 3H), 4.0−4.30 (m, 5H), 4.60−4.75 (m, 1H), 4.85 (d, 1H, J = 4.4 Hz), 6.40 (s, 1H), 6.78 (s, 1H), 7.40 (s, 1H), 7.65 (s, 1H), 8.20−8.40 (br s, NH exchangeable). MS (FAB) 717 $[M + H]^{+\cdot}$.

(2*S*)-N-{4-[5-(7-Methoxy-(11a*S*)-1,2,3,10,11,11a-hexahydro-5*H*-pyrrolo[2,1-*c*]-[1,4]benzodiazepine-5,11-dione-8-yloxy)pentyloxy]-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehyde Diethyl Thioacetal (15c). The compound **15c** was prepared according to the method described for **15a** by employing **2** and **13c** (549 mg, 1 mmol) to afford **15c** as a yellow oil (697 mg, 88%). ^{1}H NMR ($CDCl_3$): δ 1.20−1.50 (m, 6H), 1.50−2.50 (m, 13H), 2.70−2.90 (m, 5H), 3.20−3.40 (m, 2H), 3.50−3.90 (m, 2H), 3.95 (s 3H), 4.0 (s, 3H), 4.0−4.25 (m, 5H), 4.65−4.80 (m, 1H), 4.90 (d, 1H, J = 4.38 Hz), 6.45 (s, 1H), 6.85 (s, 1H), 7.48 (s, 1H), 7.70 (s, 1H), 8.40 (br s, NH exchangeable). MS (FAB) 731 $[M + H]^{+\cdot}$.

((2*S*)-N-{4-[8-(7-Methoxy-(11a*S*)-1,2,3,10,11,11a-hexahydro-5*H*-pyrrolo[2,1-*c*]-[1,4]benzodiazepine-5,11-dione-8-yloxy)octyloxy]-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehyde Diethyl Thioacetal (15d). The compound **15d** was prepared according to the method described for **15a** by employing **2** and **13d** (591 mg, 1 mmol) to afford **15d** as a yellow oil (687 mg, 89%). ^{1}H NMR ($CDCl_3$): δ 1.20−1.60 (m, 12H), 1.80−2.20 (m, 13H), 2.70−2.90 (m, 5H), 3.20−3.40 (m, 2H), 3.50−3.90 (m, 2H), 3.95 (s 3H), 4.0 (s, 3H), 4.0−4.25 (m, 5H), 4.65−4.80 (m, 1H), 4.80 (d, 1H, J = 4.32 Hz), 6.45 (s, 1H), 6.75 (s, 1H), 7.40 (s, 1H), 7.60 (s, 1H), 8.80 (br s, NH exchangeable).

(2*S*)-N-{4-[3-(7-Methoxy-(11a*S*)-1,2,3,10,11,11a-hexahydro-5*H*-pyrrolo[2,1-*c*]-[1,4]benzodiazepine-5,11-dione-8-yloxy)propoxy]-5-methoxy-2-aminobenzoyl}pyrrolidine-2-carboxaldehyde Diethyl Thioacetal (16a). The compounds **14a**/**15a** (764 mg/702 mg, 1 mmol) were dissolved in methanol (20 mL) and added $SnCl_2 \cdot 2H_2O$ (2.256 g, 10 mmol/1.125 g, 5 mmol) was refluxed for 1.5 h or until the TLC indicated that the reaction was complete. The methanol was evaporated under vacuum, and the aqueous layer was then carefully adjusted to pH 8 with 10% $NaHCO_3$ solution and then extracted with ethyl acetate (2 × 30 mL). The combined organic phase was dried over Na_2SO_4 and evaporated under vacuum to afford the amino diethyl thioacetal, **16a**, as a yellow oil, which because of potential stability problems,[32] was briefly characterized by ^{1}H NMR and then used directly in the next step (537 mg, 80%/571 mg, 85%). ^{1}H NMR ($CDCl_3$): δ 1.20−1.40 (m, 6H), 1.50−2.40 (m, 9H), 2.50−2.85 (m, 5H), 3.45−3.70 (m, 4H), 3.75 (s, 3H), 3.90 (s, 3H), 4.0−4.20 (m, 5H), 4.55−4.75 (m, 2H), 6.20 (s, 1H), 6.50 (s, 1H), 6.85 (s, 1H), 7.40 (s, 1H), 8.50 (br s, NH exchangeable).

(2*S*)-N-{4-[4-(7-Methoxy-(11a*S*)-1,2,3,10,11,11a-hexahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11-dione-8-yloxy)butoxy]-5-methoxy-2-aminobenzoyl}pyrrolidine-2-carboxaldehyde Diethyl Thioacetal (16b). The compound **16b** was prepared according to the method described for the compound **16a** employing the compound **14b/15b** (778 mg/716 mg, 1 mmol) to afford the amino diethyl thioacetal **16b** as a yellow liqiud (549 mg, 80%/562 mg, 82%). ^{1}H NMR ($CDCl_3$): δ 1.20−1.40 (m, 6H), 1.50−2.40 (m, 11H), 2.50−2.85 (m, 5H), 3.45−3.70 (m, 4H), 3.75 (s, 3H), 3.90 (s, 3H), 4.0−4.20 (m, 5H), 4.55−4.75 (m, 2H), 6.20 (s, 1H), 6.50 (s, 1H), 6.85 (s, 1H), 7.40 (s, 1H), 8.45 (br s, NH exchangeable).

(2*S*)-N-{4-[5-(7-Methoxy-(11a*S*)-1,2,3,10,11,11a-hexahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11-dione-8-yloxy)pentyloxy]-5-methoxy-2-aminobenzoyl}pyrrolidine-2-carboxaldehyde Diethyl Thioacetal (16c). The compound **16c** was prepared according to the method described for the compound **16a** employing the compound **14c/15c** (792 mg/730 mg, 1 mmol) to afford the amino diethyl thioacetal **16c** as a yellow liquid (574 mg, 80%/588 mg, 84%). ^{1}H NMR ($CDCl_3$): δ 1.20−1.40 (m, 8H), 1.50−2.40 (m, 11H), 2.50−2.85 (m, 5H), 3.45−3.70 (m, 4H), 3.75 (s, 3H), 3.90 (s, 3H), 4.0−4.20 (m, 5H), 4.55−4.75 (m, 2H), 6.20 (s, 1H), 6.50 (s, 1H), 6.85 (s, 1H), 7.40 (s, 1H), 8.52 (br s, NH exchangeable).

(2*S*)-N-{4-[8-(7-Methoxy-(11a*S*)-1,2,3,10,11,11a-hexahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11-dione-8-yloxy)octyloxy]-5-methoxy-2-aminobenzoyl]pyrrolidine-2-carboxaldehyde Diethyl Thioacetal (16d). The compound **16d** was prepared according to the method described for the compound **16a** employing the compound **14d/15d** (834 mg/772 mg, 1 mmol) to afford the amino diethyl thioacetal **16d** as a yellow liquid (593 mg, 80%/623 mg, 84%). ^{1}H NMR ($CDCl_3$): δ 1.20−1.60 (m, 14H), 1.80−2.30 (m, 11H), 2.50−2.85 (m, 5H), 3.45−3.70 (m, 4H), 3.75 (s, 3H), 3.90 (s, 3H), 4.0−4.20 (m, 5H), 4.55−4.75 (m, 2H), 6.20 (s, 1H), 6.50 (s, 1H), 6.85 (s, 1H), 7.40 (s, 1H), 8.40 (br s, NH exchangeable).

7-Methoxy-8-{3-[7-methoxy-(11a*S*)-1,2,3,10,11,11a-hexahydro-5*H*-PBD-5,11-dione-8-yloxy]propoxy}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-5-one (5a). A solution of **16a** (672 mg, 1 mmol), $HgCl_2$ (613 mg, 2.26 mmol), and $CaCO_3$ (246 mg, 2.46 mmol) in MeCN−water (4:1) was stirred slowly at room temperature until TLC indicated complete loss of starting material. The reaction mixture was diluted with EtOAc (30 mL) and filtered through a Celite bed. The clear yellow organic supernatant was extracted with saturated 5% $NaHCO_3$ (20 mL) and brine (20 mL), and the combined organic phase was dried (Na_2SO_4). The organic layer was evaporated in a vacuum and purified by column chromatography (90% CH_2Cl_2−MeOH) to give compound **5a** as pale yellow oil (318 mg, 58%). This material was repeatedly evaporated from $CHCl_3$ in vacuo to generate the imine form. ^{1}H NMR (DMSO-d_6 + $CDCl_3$): δ 1.80−2.18 (m, 7H), 2.20−2.85 (m, 3H), 3.45−3.85 (m, 5H), 3.85−4.0 (m, 7H), 4.08−4.35 (m, 4H), 6.65 (s, 1H), 6.80 (s, 1H); 7.30 (s, 1H), 7.50 (s, 1H), 7.65 (d, 1H, J = 4.8 Hz), 9.95 (br s, NH exchangeable). MS (FAB) 549 $[M + H]^{+\cdot}$. HRMS $[M + H]^{+\cdot}$ calcd for $C_{29}H_{33}N_4O_7$ m/z 549.234925; obsd (FAB) m/z 549.235223; $[\alpha]_D^{25}$ +202.6° (c = 0.5, $CHCl_3$); reverse phase HPLC (C_4 stationary phase, 75% MeOH/H_2O mobile phase, 254 nm) R_t = 4.85 min, % peak area = 98.54%. Calcd for $C_{29}H_{32}N_4O_7$: C, 63.49; H, 5.87; N, 10.21.

7-Methoxy-8-{4-[7-methoxy-(11a*S*)-1,2,3,10,11,11a-hexahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11-dione-8-yloxy]butoxy}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-5-one (5b). The compound **5b** was prepared according to the method described for the compound **5a** employing **16b** (686 mg, 1 mmol) to afford **5b** as a pale yellow oil (344 mg, 61%). ^{1}H NMR (DMSO-d_6 + $CDCl_3$): δ 1.80−2.18 (m, 9H), 2.20−2.80 (m, 3H), 3.45−3.85 (m, 5H), 3.85 (s, 3H), 3.90 (s, 3H), 3.98−4.20 (m, 5H), 6.55 (s, 1H), 6.75 (s, 1H); 7.30 (s, 1H), 7.41 (s, 1H), 7.60 (d, 1H, J = 5 Hz), 9.85 (br s, NH exchangeable). MS (FAB) 563 $[M + H]^{+\cdot}$. HRMS $[M + H]^{+\cdot}$ calcd for $C_{30}H_{35}N_4O_7$ m/z 563.250575; obsd (FAB) m/z 563.251967; $[\alpha]_D^{25}$ +294.33° (c = 0.5, $CHCl_3$); reverse phase HPLC (C_4 stationary phase, 75% MeOH/H_2O

mobile phase, 254 nm) R_t = 4.99 min, % peak area = 98.8%. Calcd for $C_{30}H_{34}N_4O_7$: C, 64.04; H, 6.09; N, 9.96.

7-Methoxy-8-{5-[7-methoxy-(11a*S*)-1,2,3,10,11,11a-hexahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11-dione-8-yloxy]pentyloxy}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-5-one (5c). The compound **5c** was prepared according to the method described for the compound **5a** employing **16c** (700 mg, 1 mmol) to afford **5c** as a pale yellow oil (317 mg, 55%). ^{1}H NMR (DMSO-d_6 + $CDCl_3$): δ 1.50−2.18 (m, 11H), 2.20−2.80 (m, 3H), 3.45−3.85 (m, 5H), 3.85 (s, 3H), 3.90 (s, 3H), 3.98−4.20 (m, 5H), 6.58 (s, 1H), 6.75 (s, 1H); 7.25 (s, 1H), 7.38 (s, 1H), 7.65 (d, 1H, J = 5.1 Hz), 9.85 (br s, NH exchangeable). MS (FAB) 577 [M + H]$^{+\cdot}$. HRMS [M + H]$^{+\cdot}$ calcd for $C_{31}H_{37}N_4O_7$ m/z 577.266225, obsd (FAB) m/z 577.264803; $[\alpha]_D^{25}$ +303.00° (c = 0.5, $CHCl_3$); reverse phase HPLC (C_4 stationary phase, 75% MeOH/H_2O mobile phase, 254 nm) R_t = 5.17 min, % peak area = 99%. Calcd for $C_{31}H_{36}N_4O_7$: C, 64.57; H, 6.29; N, 9.71.

7-Methoxy-8-{8-[7-methoxy-(11a*S*)-1,2,3,10,11,11a-hexahydro-5*H*-pyrrolo[2,1-*c*]-[1,4]benzodiazepine-5,11-dione-8-yloxy]octyloxy}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-5-one (5d). The compound **5d** was prepared according to the method described for the compound **5a** employing **16d** (742 mg, 1 mmol) to afford **5d** as a pale yellow oil (346 mg, 56%). ^{1}H NMR (DMSO-d_6 + $CDCl_3$): δ 1.20−2.40 (m, 17H), 2.60−2.80 (m, 3H), 3.45−3.85 (m, 5H), 3.85 (s, 3H), 3.90 (s, 3H), 3.98−4.20 (m, 5H), 6.65 (s, 1H), 6.80 (s, 1H); 7.38 (s, 1H), 7.45 (s, 1H), 7.60 (d, 1H, J = 5 Hz), 10.05 (br s, NH exchangeable). MS (FAB) 619 [M + H]$^{+\cdot}$; $[\alpha]_D^{25}$ +435.20° (c = 0.25, $CHCl_3$); reverse phase HPLC (C_4 stationary phase, 75% MeOH/H_2O mobile phase, 254 nm) R_t = 5.78 min, % peak area = 96.07%. Calcd for $C_{34}H_{42}N_4O_7$: C, 66.00; H, 6.84; N, 9.06.

Thermal Denaturation Studies. Compounds were subjected to thermal denaturation studies with duplex form CT-DNA using an adaptation of a reported procedure.[33] Working solutions in aqueous buffer (10 mM NaH_2PO_4/Na_2HPO_4, 1 mM Na_2EDTA, pH 7.00 + 0.01) containing CT-DNA (100 μM in phosphate) and the PBD (20 μM) were prepared by addition of concentrated PBD solutions in MeOH to obtain a fixed [PBD]/[DNA] molar ratio of 1:5. The DNA−PBD solutions were incubated at 37 °C for 0 and 18 h prior to analysis. Samples were monitored at 260 nm using a Hitachi 150-20 spectrophotometer fitted with KPC-6 thermoprogramer and SPR-7 temperature controller, and heating was applied at 1 °C min^{-1} in the 40−90 °C range. DNA helix → coil transition temperatures (T_m) were obtained from the maxima in the d(A_{260})/dT derivative plots. Results are given as the mean ± standard deviation from three determinations and are corrected for the effects of MeOH cosolvent using a linear correction term.[34] Drug-induced alterations in DNA melting behavior are given by ΔT_m = T_m(DNA + PBD) − T_m(DNA alone), where the T_m value for the PBD-free CT-DNA is 66.50 ± 0.01. The fixed [PBD]/[DNA] ratio used did not result in binding saturation of the host DNA duplex for any compound examined.

RED$_{100}$ Restriction Endonuclease Digestion Assay. PBD−DNA complexes were made by preincubating plasmid DNA (0.5 μg) in concentration ranges of 1.8−14.6 μM of PBD in restriction buffer (150 mM NaCl, 10 mM tris HCl, 1 mM dithithreitol, 100 μg BSA (bovine serum albumin)) in a reaction volume of 49 μL for 1 h at 37 °C. The PBD−DNA complexes were added with 20 units of *BamH*I (1 μL) to initiate the restriction digestion and incubated at 37 °C. The reaction was stopped by addition of loading buffer (60% sucrose, 0.25% bromophenol blue, 0.5% xylene cyanol, 10 mM Tris HCl). The DNA was separated by 1.0% agarose gel electrophoresis in Tris-acetate EDTA buffer (TAE-40 mM Tris base, pH 8.0, 18 mM acetic acid, 1 mM EDTA) at 100 V for 2 h. The gels were stained with ethidium bromide and photographed.[9]

Molecular Modeling Studies. All of the calculations described below were performed using INSIGHT-II suite of software (MSI, Inc.) running on a Silicon Graphics OCTANE system.

(a) Modeling of DNA Duplex and PBD Dimer Structures. The 15-mer sequence GGGGCGAGAGAGGGG with central AGA triplet representing the preferred binding site Pu-G-Pu for the PBD molecule was chosen for modeling the B-DNA duplex structure. The model was constructed using the BIOPOLYMER module. Models for the PBD dimers **5a**−**d** were built using the BUILDER module. First, the PBD molecules were "sketched" in two dimensions (2D) and then converted into three dimensional (3D) entities using the 2D to 3D conversion tool in the BUILDER module. During modeling, it was assumed that both of the PBD units are having (*S*)-stereochemistry at the chiral C11 atom.

(b) Docking Studies. Docking of a PBD dimer into the minor groove of the DNA duplex was carried out manually, such that the N10−C11 imine functionality and the exocyclic C2-amino group of G8 are nearly at a bonding distance from each other so that a covalent bond can be formed. After the covalent bond was created, the PBD dimer in the minor groove was manually oriented in such a way that the PBD with amide functionality is oriented toward the 3′ end of a covalently linked DNA strand. Some of the dihedral angles about the C−C bonds of the spacer units were manually adjusted such that the entire PBD dimer has an isohelical fit within the minor groove of the DNA duplex. The potentials and the charges were fixed using the CVFF force field. The complex so formed was subjected to energy minimization using a conjugate gradient technique until full convergence (energy gradient ≤ 0.001) was reached. During energy minimization and MD simulations, constraints were applied to fix the DNA duplex structure.

(c) Molecular Dynamics Simulations. The following protocol was used to carry out the molecular dynamics studies: heating phase (equilibration) = 0−10 ps and length of MD simulation after the heating phase = 100 ps.

During simulations, all of the intermittent structures of the complexes formed at every successive 5 ps were saved and later they were subjected to energy minimization. The minima obtained for each complex were screened to identify the lowest energy minimum, and that was taken as a representative of energetically favorable complex for further studies. Energy of interaction between the DNA and the PBD dimer molecule in a complex was calculated as follows:

$$E_{int} = E_{complex} - (E_{DNA} + E_{PBD})$$

where E_{int} = energy of interaction of a complex, $E_{complex}$ = total energy of the complex, and E_{DNA} and E_{PBD} are the individual total energies of the DNA and the PBD dimer molecules calculated after they are separated from each other.

Acknowledgment. We thank the National Cancer Institute, Maryland, for in vitro anticancer assay in human cell lines. G.R., N.L., P.R., and O.S. are thankful to CSIR, New Delhi, for the award of research fellowships.

References

(1) Kamal, A.; Laxman, N.; Ramesh, G.; Neelima, K.; Kondapi, A. K. Synthesis of Novel Non-Cross-Linking Pyrrolobenzodiazepines with Remarkable DNA Binding Affinity and Potent Antitumour Activity. *Chem. Commun.* **2001**, 437−438.

(2) White, S.; Szewczyk, J. W.; Turner, J. M.; Baird, E. E.; Dervan, P. B. Recognition of the Four Watson−Crick Base Pairs in the DNA Minor-Groove by Synthetic Ligands. *Nature* **1998**, *391*, 468−471.

(3) Thurston, D. E. Nucleic Acid Targeting: Therapeutic Strategies for the 21st Century. *Br. J. Cancer* **1999**, *80*, 65−85.

(4) Neidle, S.; Thurston, D. E. *New Targets for Cancer Chemotherapy*; Kerr, D. J., Workmann, P., Eds.; CRC Press: London, U.K., 1994; p 159.

(5) Thurston, D. E. Advances in the Study of Pyrrolo[2,1-*c*][1, 4]-benzodiazepine (PBD) Antitumour Antibiotics. *Molecular Aspects of Anticancer Drug−DNA Interactions*; The Macmillan Press Ltd.; London, U.K., 1993; pp 54−88.

(6) Petrusek, R. L.; Uhlenhopp, E. L.; Duteau, N.; Hurley, L. H. Reaction of Anthramycin with DNA. *J. Biol. Chem.* **1982**, *257*, 6207−6216.

(7) Hurley, L. H.; Reck, T.; Thurston, D. E.; Langley, D. R.; Holden, K. G.; Hertzberg, R. P.; Hoover, J. R. E.; Gallagher, G., Jr.; Faucette, L. F.; Mong, S. M.; Johnson, R. K. Pyrrolo[1,4]-benzodiazepine Antitumour Antibiotics: Relationship of DNA Alkylation and Sequence Specificity to the Biological Activity of Natural and Synthetic Compounds. *Chem. Res. Toxicol*. **1988**, *1*, 258−268.

(8) Boyd, F. L.; Stewart, D.; Remers, W. A.; Barkley, M. D.; Hurley, L. H. Characterization a Unique Tomaymycin d(CICGAAT-TCICG)$_2$ Adduct Containing Two Drug Molecules per Duplex by NMR Fluorescence, and Molecular Modeling Studies. *Biochemistry* **1990**, *29*, 2387−2403.

(9) Puvvada, M. S.; Hartley, J. A.; Jenkins, T. C.; Thurston, D. E. A Quantitative Assay to Measure Relative DNA-Binding Affinity of Pyrrolo[2,1-*c*][1,4]benzodiazepine (PBD) Antitumour Antibiotics Based on the Inhibition of Restriction-Endonuclease *Bam*HI. *Nucleic Acids Res.* **1993**, *21*, 3671−3675.

(10) Puvvada, M. S.; Forrow, S. A.; Hartley, J. A.; Stephenson, P.; Gibson, I.; Jenkins, T. C.; Thurston, D. E. Inhibition of Bacteriophage T7 RNA Polymerase In Vitro Transcription by DNA-Binding Pyrrolo[2,1-*c*][1,4]benzodiazepines. *Biochemistry* **1997**, *36*, 2478−2484.

(11) Thurston, D. E.; Morris, S. J.; Hartley, J. A. Synthesis of a Novel GC−Specific Covalent-Binding DNA Affinity-Cleavage Agent Based on Pyrrolobenzodiazepines (PBDs). *Chem. Commun.* **1996**, 563−565.

(12) Wilson, S. C.; Howard, P. W.; Forrow, S. M.; Hartley, J. A.; Adams, L. J.; Jenkins, T. C.; Kelland, L. R.; Thurston, D. E. Design, Synthesis and Evaluation of a Novel Sequence-Selective Epoxide-Containing DNA Cross-Linking Agent Based on the Pyrrolo[2,1-*c*][1,4]benzodiazepine System. *J. Med. Chem*. **1999**, *42*, 4028−4041.

(13) Reddy, B. S. P.; Damayanthi, Y.; Reddy, B. S. N.; Lown, W. J. Design, Synthesis and In Vitro Cytotoxicity Studies of Novel Pyrrolo[2,1-*c*][1,4]benzodiazepine (PBD)-Polyamide Conjugates and 2,2′-PBD dimers. *Anti-Cancer Drug Des.* **2000**, *15*, 225−238.

(14) Baraldi, P. G.; Balboni, G.; Cacciari, B.; Guiotto, A.; Manfredini, S.; Romagnoli, R.; Spalluto, G.; Thurston, D. E.; Howard, P. W.; Bianchi, N.; Rutigiiano, C.; Mischiati, C.; Gambari, R. Synthesis, In Vitro Antiproliferative Activity, and DNA-Binding Properties of Hybrid Molecules Containig Pyrrolo[2,1-*c*][1,4]benzodiazepine and Minor-Groove-Binding Oligopyrrole Carriers. *J. Med. Chem*. **1999**, *42*, 5131−5141.

(15) 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.

(16) Thurston, D. E.; Bose, D. S.; Thompson, A. S.; Howard, P. W.; 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.

(17) 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 2nd-Arm Cross-Link Reactions. *Anal. Biochem*. **1991**, *193*, 131−134.

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

(19) 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 Pyrrolo- benzodiazepine DNA-Interactive Agent with Highly Efficient Cross-Linking Ability and Potent Cytotoxicity. *J. Med. Chem*. **2001**, *44*, 737−748.

(20) Kamal, A.; Howard, P. W.; Reddy, B. S. N.; Reddy, B. S. P.; Thurston, D. E. Synthesis of Pyrrolo[2,1-*c*][1,4]benzodiazepine Antibiotics: Oxidation of Cyclic Secondary Amine with TPAP. *Tetrahedron* **1997**, *53*, 3223−3230.

(21) Kamal, A.; Damayanthi, Y.; Reddy, B. S. N.; Laxminarayana, B.; Reddy, B. S. P. Novel Biocatalytic Reduction of Aryl Azides: Chemoenzymatic Synthesis of Pyrrolo- [2,1-*c*][1,4]benzodiazepine Antibiotics. *Chem. Commun*. **1997**, 1015−1016.

(22) Kamal, A.; Rao, M. V.; Reddy, B. S. N. The Newer Synthetic Strategies For DNA- Binding Pyrrolo benzodiazepine Antibiotics. *Khim. Geterosil. Soedin. Chem*. (*Chem. Heterocycl. Compd. Engl. Trasl*.) **1998**, *12*, 1588−1604.

(23) Kamal, A.; Laxman, E.; Laxman, N.; Rao, N. V. Synthesis of Pyrrolo[2,1-*c*]-[1,4]benzodiazepines via Reductive Cyclization of *w*-Azido Carbonyl Compounds by TMSI.; An Efficient Preparation of Antibiotic DC-81 and its Dimers. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2311−2313.

(24) Kamal, A.; Laxman, E.; Arifuddin, M. An Efficient Reduction of Azides to Amines: Synthesis of DNA Interactive Pyrrolo[2,1-*c*]-[1,4]benzodiazepines, *Tetrahedron Lett*. **2000**, *41*, 7743−7746.

(25) Kamal, A.; Laxman, E.; Reddy, P. S. M. M. Reductive Cyclization of *w*-Azido/Nitrocarbonyl Compounds by Samarium Iodide: A Facile Preparation of DNA-Binding PBD and its Dimers. *Tedrahedron Lett*. **2000**, *41*, 8631−8634.

(26) Kamal, A.; Laxman, E.; Reddy, P. S. M. M. A Mild and Efficient Dethioacetalisation Employing $FeCl_3 \cdot 6H_2O$: Synthesis of DNA-Linking PBD Ring and its Dimers. *Synlett* **2000**, *10*, 1476−1478.

(27) Kamal, A.; Reddy, G. S. K.; Reddy, K. L. Efficient Reduction of Nitro/Azido Groups on Solid Support Employing Indium: Synthesis of Pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11-diones. *Tedrahedron Lett*. **2001**, *42*, 6969−6971.

(28) Kamal, A.; Reddy, G. S. K.; Reddy, K. L.; Raghavan, S. Efficient Solid-phase Synthesis of DNA-interactive Pyrrolo[2,1-*c*][1,4]-benzodiazepine antitumour Antibiotics. *Tedrahedron Lett*. **2002**, *43*, 2103−2106.

(29) Kamal, A.; Rao, M. V.; Laxman, N.; Ramesh, G.; Reddy, G. S. K. Recent Developments in the Design, Synthesis and Structure−Activity Relationship Studies of Pyrrolo[2,1-c][1,4]benzodiazepines as DNA-Interactive Antitumour Antibiotics. *Curr. Med. Chem.*—*Anti-Cancer Agents* **2002**, *2*, 215−254.

(30) Kamal, A.; Laxman, N.; Ramesh, G.; Ramulu, P.; Srinivas, O. Process for the Preparation of Antitumour Agents. U.S. Patent No. 6,362,331 B1, Mar 26, 2002.

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

(32) Langley, D. R.; Thurston, D. E. A Versatile and Efficient Synthesis of Carbinolamine-Containing Pyrrolo[1,4]benzodiazepines via the Cyclization of N-(2-Aminobenzoyl)pyrrolidine-2-carboxaldehyde Diethyl Thioacetals: Total Synthesis of Prothracarcin. *J. Org. Chem*. **1987**, *52*, 91−97.

(33) 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 Des*. **1990**, *5*, 249−264.

(34) McConnaughie, A. W.; Jenkins, T. C. Novel Acridine-Triazenes as Prototype Combilexins: Synthesis, DNA-Binding, and Biological activity. *J. Med. Chem*. **1995**, *38*, 3488−3501.

JM020124H