DNA-Directed Alkylating Agents. 7. Synthesis, DNA Interaction, and Antitumor Activity of Bis(hydroxymethyl)- and Bis(carbamate)-Substituted Pyrrolizines and Imidazoles

Graham J. Atwell, Jun-Yao Fan, Karin Tan, and William A. Denny*

Auckland Cancer Society Research Centre, Faculty of Medicine and Health Science, The University of Auckland, Private Bag 92019, Auckland, New Zealand

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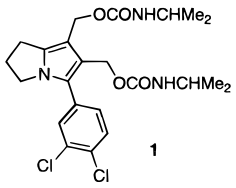
A series of bis(hydroxymethyl)-substituted imidazoles, thioimidazoles, and pyrrolizines and related bis(carbamates), linked to either 9-anilinoacridine (intercalating) or 4-(4-quinolinylamino)benzamide (minor groove binding) carriers, were synthesized and evaluated for sequence-specific DNA alkylation and cytotoxicity. The imidazole and thioimidazole analogues were prepared by initial synthesis of [(4-aminophenyl)alkyl]imidazole-, thioimidazole-, or pyrrolizine dicarboxylates, coupling of these with the desired carrier, and reduction to give the required bis(hydroxymethyl) alkylating moiety. The pyrrolizines were the most reactive alkylators, followed by the thioimidazoles, while the imidazoles were unreactive. The pyrrolizines and some of the thioimidazoles cross-linked DNA, as measured by agarose gel electrophoresis. Strand cleavage assays showed that none of the compounds reacted at purine N7 or N3 sites in the gpt region of the plasmid gpt2Eco, but the polymerase stop assay showed patterns of G-alkylation in C-rich regions. The corresponding thioimidazole bis(carbamates) were more selective than the bis(hydroxymethyl) pyrrolizines, with high-intensity bands at 5′-NC*C*N, 5′-NGC*N and 5′-NC*GN sequences in the PCR stopping assay (* indicates block sites). The data suggest that these targeted compounds, like the known thioimidazole bis(carbamate) carmethizole, alkylate exclusively at guanine residues via the 2-amino group, with little or no alkylation at N3 and N7 guanine or adenine sites. The cytotoxicities of the compounds correlated broadly with their reactivities, with the bis(hydroxymethyl)imidazoles being the least cytotoxic (IC_{50}s >1 μM; P388 leukemia) and with the intercalator-linked analogues being more cytotoxic than the corresponding minor-groove-targeted ones. This was true also for the more reactive thioimidazole bis(carbamates) (IC_{50}s 0.8 and 11 μM, respectively), but both were more active than the analogous "untargeted" carmethizole (IC_{50} 20 μM). The bis(hydroxymethyl)pyrrolizine analogues were the most cytotoxic, with IC_{50}s as low as 0.03 μM.

Alkylating agents remain an effective class of anticancer drugs, whose cytotoxic and therapeutic effects derive primarily from their ability to form DNA interstrand cross-links.[1] There is renewed interest in this general class of drugs, following recent demonstrations that both their sequence and regioselectivity of DNA alkylation can be altered by attaching them to a variety of DNA-affinic carriers and that this can result in a modified spectrum of biological action.[2−6]

While nitrogen mustards [*N*,*N*-bis(2-chloroethyl)-amines] have been the most widely used DNA interstrand cross-linking agents, other classes of compounds with similar activity are known. One class is the bis(hydroxymethyl)pyrrolizines and corresponding bis(carbamates). These "vinylogous carbinolamines" were developed initially from the pyrrolizine alkaloids and led to compounds such as IPP (**1**)[7] and the thioimidazole carmethizole (**3**), the latter of which has reached clinical evaluation.[8,9] Related bis-alcohols such as **2** are also DNA alkylators.[10] The mechanism of interaction of these compounds with DNA is considered to be via S_N1 reactions that result in electrophilic methides.[11] A recent study of **1** showed that it forms an interstrand cross-link with the short oligonucleotide 5′-ACGT at the 5′-CG site via the exocyclic amino groups of guanine residues in the minor groove.[12]



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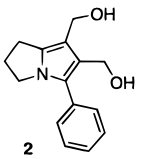

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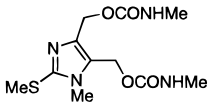

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As with the mustard alkylators, an important contemporary question is the degree to which the spectra of both DNA alkylation and biological activity of the bis(hydroxymethyl)pyrrolizines and analogues are altered by linking them to DNA-affinic carriers. A recent study[13] of the distamycin analogue **4** showed that it was about 1000-fold more potent than the corresponding "untargeted" alkylator **5**. In this paper we study the DNA alkylating ability and cytotoxicities of a series of these alkylating units of varying reactivity linked to either the DNA-intercalating carrier 9-anilinoacridine[14] (compounds **15**−**18**, **30**) or the DNA minor groove binding carrier 4-(4-quinolinylamino)benzamide[15] (compounds **22**−**25**, **32**, **37**).

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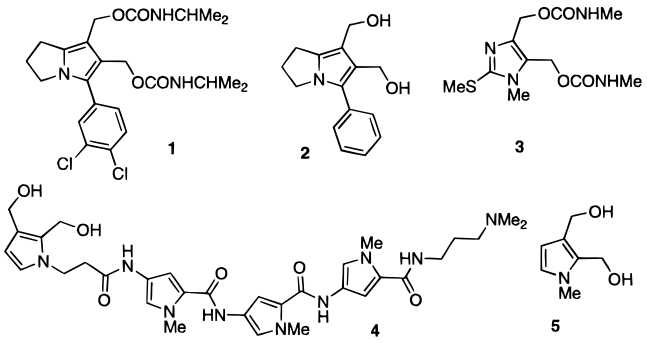


Chemistry

The target bis(*N*-methylcarbamates) **17**, **18**, **24**, and **25** were synthesized as shown in Schemes 2 or 3, via the key intermediates **11** or **12** (Scheme 1). Alkylation of imidazole **7** and the thioimidazole **8** with the alkyl bromide **6** furnished the diesters **9** and **10**. Subsequent nitro group reduction by Pt/C-catalyzed hydrogenation gave the amino diesters **11** and **12**. Lithium aluminum hydride reduction of diester **11** gave diol **13** (Scheme 2), which was coupled with 9-chloroacridine to provide the anilinoacridine **15**, and treatment of **15** with methyl isocyanate employing dibutyltin diacetate as catalyst[8] yielded the desired bis(carbamate) **17**. The amino diester **12** (Scheme 2) was coupled with 9-chloroacridine under acid-catalyzed conditions to give the anilinoacridine **13**, and subsequent hydride reduction yielded diol **16**. This was carbamoylated with methyl isocyanate to provide the desired product **18**.

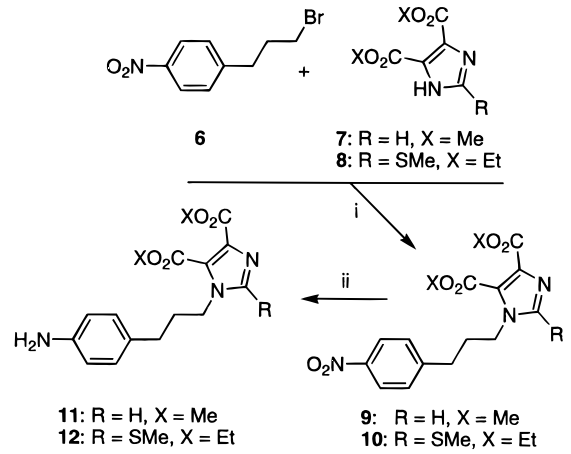
The zwitterionic acid **19** was reacted with amines **11** and **12**, employing BOP−Cl as the coupling reagent, to give the diesters **20** and **21**, respectively, in moderate yields (Scheme 3). Attempts to effect this reaction with a number of other coupling reagents were unsuccessful. Reduction of **20** and **21** with lithium aluminum hydride gave diols **22** and **23**, which were subsequently carbamoylated to provide the target compounds **24** and **25**.

Scheme 4 outlines the preparation of the bis(hydroxymethyl)pyrrolizines **30**, **32**, and **37**. The *N*-acylprolines **26** and **33**, which were obtained by reaction of (*S*)-proline with the appropriate acid chloride under Schotten−Baumann conditions,[10] were heated with acetic anhydride/dimethyl acetylenedicarboxylate to give the 1,3-dipolar cycloaddition products **27** and **34**, respectively.[10] Nitro group reduction by Pt/C-catalyzed hydrogenation yielded amines **28** and **35**. Compound **28** was coupled with 9-chloroacridine to furnish the anilinoacridine **29**, and subsequent reduction of this with lithium aluminum hydride yielded the desired diol **30**. The pyrrolizine diols **32** and **37** were obtained by hydride reduction of the corresponding diesters **31** and **36**, which in turn were prepared by BOP−Cl induced coupling of acid **19** with amines **28** and **35**, respectively.

Results and Discussion

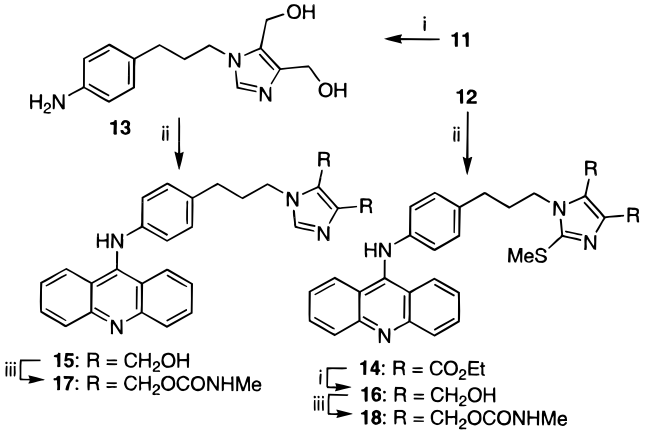
Mode of Noncovalent DNA Binding. The compounds studied are listed in Table 1. The ability of the acridine-linked compound **15** to intercalate was determined by electrophoresis studies with negatively supercoiled pBR322 (type I DNA) on 1% agarose gels containing varied concentrations of drug (Figure 1). These studies were carried out at 4 °C to minimize drug diffusion and alkylation processes. Compound **15** provided a concentration-dependent alteration in the degree of superhelicity of the DNA, analogous to the effect seen[16] with the classical DNA intercalator ethidium bromide (results not shown). At a drug concentration of 1 μM, the presence of the relaxed (type II) form of the DNA, migrating more slowly than the supercoiled

Scheme 1[a]



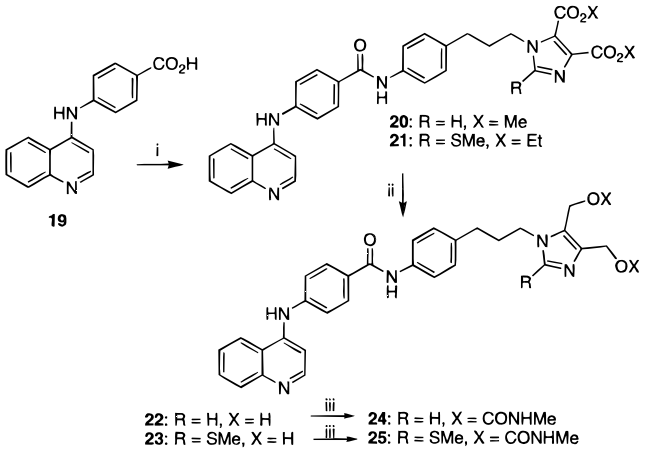
[a] (i) K_2CO_3/DMF/70−75 °C/2.5 h; (ii) Pt-C/H_2/MeOH/20 °C/2 h.

Scheme 2[a]



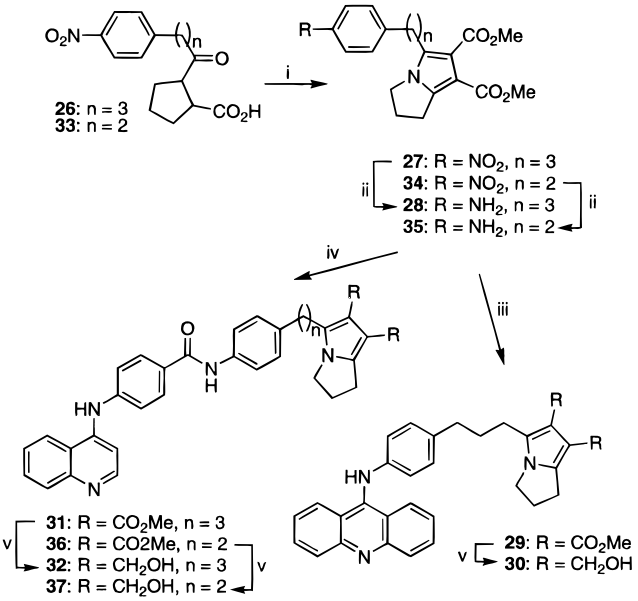
[a] (i) $LiAlH_4$/THF/10−20 °C/3 h; (ii) 9-chloroacridine/MeOH/20 °C/2−4 h; (iii) MeNCO/$Bu_2Sn(OAc)_2$/THF/20 °C/1.5−2.5 h.

Scheme 3[a]



[a] (i) Bis(2-oxo-3-oxazolidinyl)phosphinic chloride/DMF/20 °C/2 h, then **11** and **12**/*i*Pr_2EtN/20 °C/6 h; (ii) $LiAlH_4$/THF/10−20 °C/4 h; (iii) MeNCO/$Bu_2Sn(OAc)_2$/DMF/20 °C/2 h.

Scheme 4[a]



[a] (i) Dimethyl acetylenedicarboxylate/Ac_2O/130−140 °C/2 h; (ii) Pt-C/H_2/MeOH/THF/20 °C/4 h; (iii) 9-chloroacridine/MeOH/H^+/20−60 °C/30 min; (iv) **19**/bis(2-oxo-2-oxazolidinyl)phosphinic chloride/DMF/iPr$_2$EtN/20 °C/7.5 h; (v) $LiAlH_4$/THF/20 °C/3−19 h.

Table 1. Physicochemical and Biological Data for Analogues of Carmethizole and Related Agents

no.	alkylating unit	k_{obs}[a] ($s^{-1} \times 10^4$)	P388[b] IC_{50} (μM)
	Standard		
3	carmethizole	1.34	>20
	Intercalating Carriers		
15	bis(OH)imidazole	ND[c]	12
16	bis(OH)thioimidazole	v.slow	1.3
17	imidazole bis(carbamate)	ND	16
18	thioimidazole bis(carbamate)	1.61	0.8
30	bis(OH)pyrrolizine	1.87	1.3
	Minor Groove Carriers		
22	bis(OH)imidazole	ND	>20
23	bis(OH)thioimidazole	ND	>20
24	imidazole bis(carbamate)	ND	>20
25	thioimidazole bis(carbamate)	0.88	11
32	bis(OH)pyrrolizine	1.86	0.11
37	bis(OH)pyrrolizine	1.58	0.03

[a] Rate constants ($s^{-1} \times 10^4$) for loss of drug when incubated with calf thymus DNA in SHE buffer (pH 7.0) at 37 °C, as determined by HPLC (see text). [b] IC_{50}; concentration of drug (in μM) to reduce cell numbers to 50% of control values after a 72 h exposure. Values are the average of three independent determinations. [c] ND; not determined.

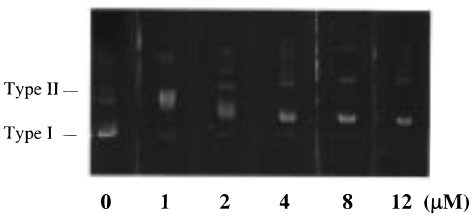


Figure 1. Unwinding of supercoiled plasmid pBR322 on 1% agarose gel containing various concentrations of compound **15**. Drug concentrations are labeled on the bottom of the gel.

form, indicates intercalation by the drug. As the drug concentration is further increased, positive superhelical turns are generated, resulting in an increase in mobility.

Kinetics of Alkylation (Covalent Binding). An estimate of the comparative reactivities of the compounds toward calf thymus DNA at 37 °C in pH 7.0 SHE buffer was determined by determining k_{obs} (the rate of loss of unbound drug) by HPLC. This kinetic rate constant is the sum of the rate of drug loss by both hydrolysis and by irreversible alkylation of the DNA. By this measure, under these conditions the bis(hydroxymethyl)imidazoles **15**, **16**, **22**, and **23** and the related 2-unsubstituted bis(carbamates) **17** and **24** were very stable, suggesting little DNA interaction. In contrast, carmethizole (**3**) and the related 2-thioimidazole bis(carbamates) **18** and **25** had half-lives of 86, 72, and 130 min, respectively, under these conditions, and these were broadly similar to that (128 min) reported previously for carmethizole at 25 °C in pH 7.4 phosphate buffer in the absence of DNA.[17] The most reactive compounds were the pyrrolizine analogues **30**, **32**, and **37**, with half-lives of 62, 62, and 73 min, respectively (see Table 1 for k_{obs} values).

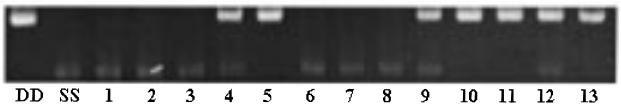


Figure 2. DNA interstrand cross-linking by the acridine-targeted and minor groove-targeted compounds at drug concentration of 0.25 mM. Linear pBR322 (*Eco*RI digested, 2 μg) was incubated with drugs at 37 °C for 30 min prior to denaturation in the mixture of SDS and CH_3HgOH at room temperature for 45 min and then was subjected to electrophoresis through 1% agarose. Control double-stranded DNA contains no drug and is nondenatured (DS) or denatured (SS). Lanes 1−13 represent compounds **15**−**18**, **30**, **22**−**25**, **32**, **37**, **3**, and **2**, respectively.

Efficiency of DNA Interstrand Cross-Linking. Previous studies[12,13] using short DNA oligomers have shown that both untargeted and targeted analogues (**1** and **4**, respectively) can cross-link double-stranded DNA. In the present work, the comparative interstrand cross-linking by the drugs was investigated by incubating them (0.25 mM) for 5, 30, or 60 min with pBR322 plasmid DNA linearized with *Eco*RI (2 μg), using methylmercury hydroxide and SDS as denaturing reagents.[18] 2-Mercaptoethanol was used to renature drug cross-linked DNA, which was separated from denatured single-strand DNA on an agarose gel. Maximal cross-linking was seen after 30 min incubation, and these comparative results are shown in Figure 2. No cross-linking was shown at any time point by the bis(hydroxymethyl)imidazoles **15** and **22** (lanes 1 and 6 in Figure 2), the bis(hydroxymethyl)thioimidazoles **16** and **23** (lanes 2 and 7), or the imidazole bis(carbamates) **17** and **24** (lanes 3 and 8). However, the more reactive thioimidazole bis(carbamates) **18** and **25** did cross-link at 0.25 mM (lanes 4 and 9), and the pyrrolizine analogues (**30, 32, 37**) were even more effective (lanes 5, 10, and 11). High cross-linking efficiencies were also observed for the "untargeted" alkylators **3** and **2** (lanes 12 and 13).

Sequence Selectivity of Drug Alkylation Determined by Polymerase Chain Reaction (PCR) Stop Assay. However, despite this observed cross-linking activity, none of the compounds showed alkylation patterns in the standard strand cleavage assays (heat ± piperazine)[19,20] (data not shown). The major products of the reaction of **1** and **4** with DNA have been

shown[12,13] (by isolation and structural identification) to be at the exocyclic 2-amino group of guanine, and it is known[21] that thermal strand cleavage methods do not readily cleave this type of adduct. Nevertheless, while these assays give no data on these adducts, the lack of other cleavage sites in the gels suggests that the present analogues do not react significantly at N7 and N3 purine sites.

To further examine the sequence specificity of DNA alkylation by these compounds, the PCR technique was used in conjunction with the gpt region of plasmid gpt2Eco (Figures 3 and 4). In this technique (polymerase stop assay),[22,23] the target DNA is reacted with drug, denatured, and reannealed with a complementary synthetic oligonucleotide primer which carries a single ^{32}P radioactive label at its 5′-terminus. This radioactive primer is then extended by copying the DNA strand in the nearby region of interest with Taq polymerase. DNA synthesis stops when the polymerase encounters any blocking lesion (inter- and intrastrand cross-links and monoalkylation events, including adducts at the guanine 2-amino group). This creates a truncated strand which still has the 5′-end of the original primer but a unique 3′-end corresponding to the blockage site and can be identified as usual by separation of the collection of extended primers on high-resolution DNA sequence gels followed by autoradiography. Only extended primers are observed upon autoradiography because the ^{32}P end label on the primer is the only source of radioactivity.[24]

Figures 3 and 4 show that the primer extension technique (primers 1 and 2 labeled by ^{32}P, respectively) can identify certain modified DNA sequences at single nucleotide resolution, and the patterns of alkylation by the more reactive drugs (**18, 25, 30, 32, 37**) are shown. The PCR blocking sites for the thioimidazole bis(carbamates) **18** and **25** (lanes 1 and 2 in both Figures 3 and 4) are quite different from the patterns caused by the pyrrolizine analogues **30, 32, 37** (lanes 4−6 in both Figures 3 and 4). The thioimidazole bis(carbamates) **18** and **25** clearly show higher DNA sequence recognition, with the PCR blocking sites mainly in C-rich regions (Figures 3 and 4). This suggests that the alkylation sites of these compounds are at guanines on the opposite strand of the DNA. The intercalator **18** (lane 1 in both Figures 3 and 4) is more reactive than the minor-groove-targeted **25** (lane 2 in both Figures 3 and 4), a result consistent with the previous drug stability studies.

Figure 5 summarizes the sites blocked by compound **18** (the gels are shown in Figures 3 and 4). The sequences 5′-NC*C*N, 5′-NGC*N, and 5′-NC*GN are found to be the most favored blocking sites (* indicate block sites). This suggests that drug alkylation may occur at guanines at the sequences 5′-N<u>GG</u>N, 5′-NC<u>G</u>N, and 5′-N<u>G</u>CN on the opposite strand of DNA (underlines represent alkylation sites). The untargeted thioimidazole bis(carbamate) carmethizole (**3**) shows a blocking pattern similar to those of **18** and **25**, although higher intensity bands are also observed at sequences 5′-TTAC*TGG and 5′-AGC*TAC*GAT at the top of the gel in Figure 3. In contrast, the PCR assay shows little sequence specificity in blocking sites for the more reactive pyrrolizine analogues **30**, **32**, **37** (lanes 4−6 in both Figures 3 and 4) and for the untargeted pyrrolizine

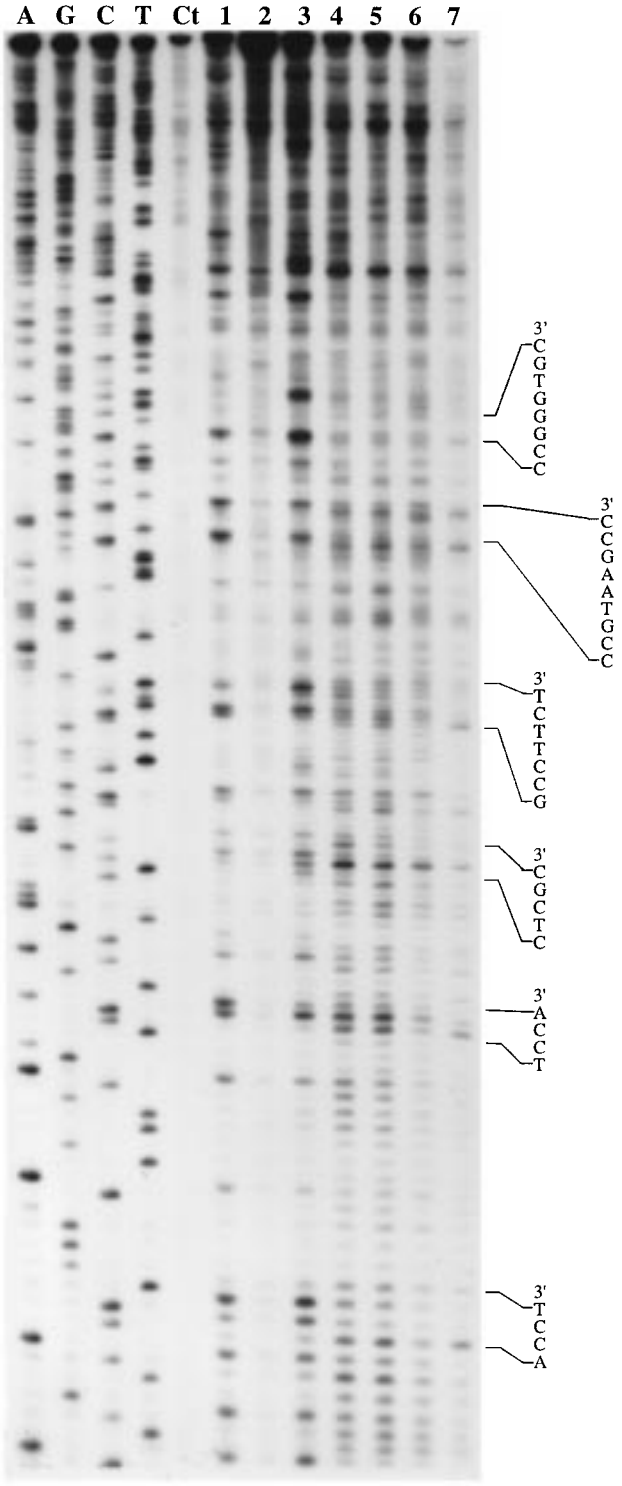


Figure 3. Primer 1 (labeled at 5′-end) extension blocked by the drug alkylation on the template DNA. Control tracks labeled "Ct" contained no drug. Tracks labeled A, G, C, and T represent the DNA sequence reactions upon the primer extension in the solution containing ddNTP mixtures and Taq polymerase. Lanes 1−7 represent compounds **18, 25, 3, 30, 32, 37, 2**.

2 (lane 7 in both Figures 3 and 4). For these compounds the blocked sites also occur one base next to the actual alkylation sites, probably due to the bulky nature of the pyrrolizine moiety.

Overall, Figures 2−5 provide strong evidence that these drugs (both targeted and untargeted) cross-link double-stranded DNA through alkylation at guanine

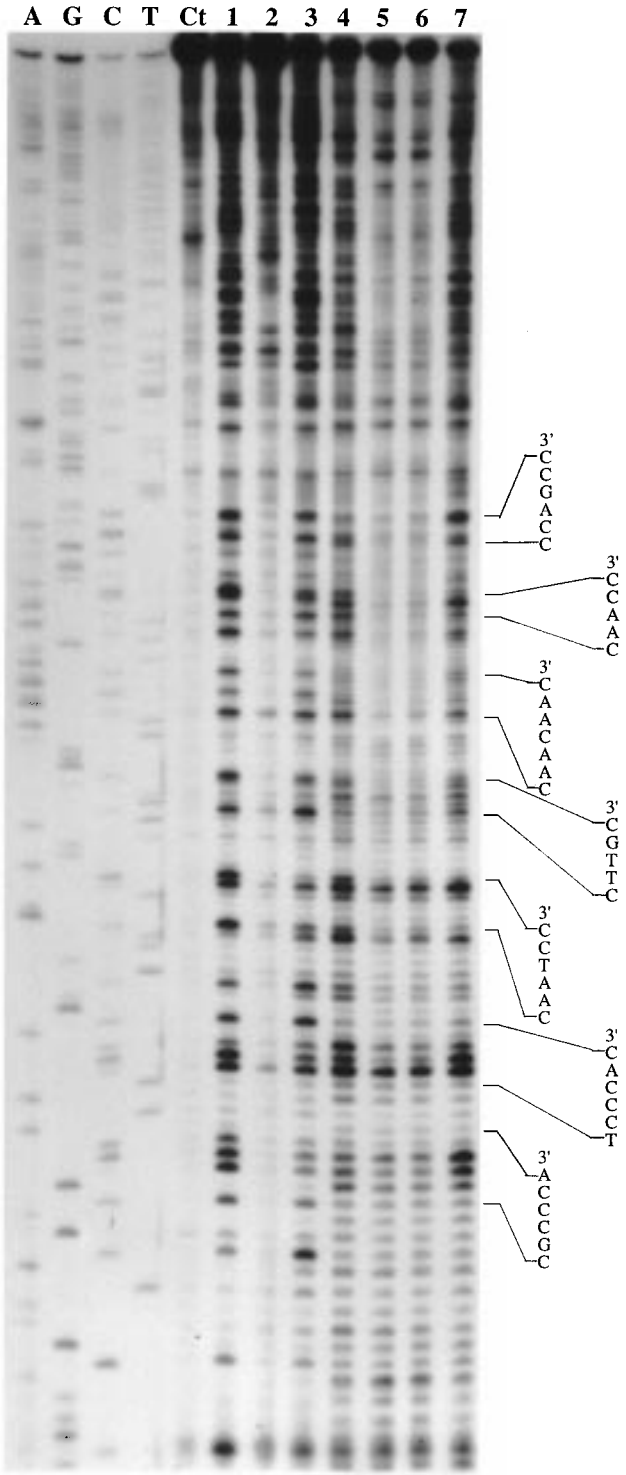


Figure 4. Primer 2 (labeled at 5′-end) extension blocked by the drug alkylation on the template DNA. Control tracks labeled "Ct" contained no drug. Tracks labeled A, G, C, and T represent the DNA sequence reactions upon the primer extension in the solution containing ddNTP mixtures and Taq polymerase. Lanes 1−7 represent compounds **18, 25, 3, 30, 32, 37, 2**.

residues (via the 2-amino group) and that there are few alkylations at N3 and N7 guanine and adenine sites. Although a recent study of the untargeted drug **1** showed that it formed an interstrand cross-link with the short oligonucleotide at sequence 5′-ACGT, we have found that the sequences 5′-NGGN, 5′-NCGN, and 5′-NGCN are the favored alkylation sites by the drugs.

-23 (nucleotide number)

*Primer 1**

5′-*GAT AAA CAG GCT GGG ACA CTT* CAC ATG AGC GAA AAA TAC ATC GTC ACC TGG GAC ATG TTG CAG ATC CAT GCA CGT AAA CTC GCA AGC CGA CTG ATG CCT TCT GAA CAA TGG AAA GGC ATT ATT GCC GTA AGC CGT GGC GGT CTG GTA CCG GGT GCG TTA CTG GCG CGT GAA CTG-3′ 150

480 (nucleotide number)

*Primer 2**

5′-*CAG TGC CAG GCG TTG AAA AGA* TTA GCG ACC GGA GAT TGG CGG GAC GAA TAC GAC GCC CAT ATC CCA CGG CTG TTC AAT CCA GGT ATC TTG CGG GAT ATC AAC AAC ATA GTC ATC AAC CAG CGG ACG ACC AGC CGG TTT TGC GAA GAT GGT GAC AAA GTG CGC TTT TGG ATA-3′ 310

Figure 5. Summary of the PCR blocking sites generated by compound **18** (from Figures 3 and 4).

These alkylation sites represent sites of drug monoalkylation and intrastrand and interstrand cross-linking. The PCR reaction (Figures 3 and 4) shows clear differences in alkylation patterns between the thioimidazole bis(carbamate) and the more reactive pyrrolizines, with the former alkylating with higher specificity.

Cytotoxicity. The compounds were evaluated for cytotoxicity against P388 murine leukemia cells in culture, and the results, as IC_{50} values, are shown in Table 1. The cytotoxicities correlated broadly with the reactivities of the alkylating units as judged by the drug stability studies. The least reactive bis(hydroxymethyl)-imidazoles **15**, **16**, **22**, and **23** and the related 2-unsubstituted bis(carbamates) **17** and **24** were the least active, with IC_{50}s $>1\ \mu$M. A comparison of pairs of intercalator-linked and minor groove binder linked pairs of compounds bearing indentical alkylating functions (**15** & **22**, **16** & **23**, **17** & **24**) showed in each case that the intercalator-linked compounds were the more active. Comparison of the more reactive thioimidazole bis-(carbamates) **3**, **18**, and **25** again showed the intercalator-linked analogue **18** to be the more potent (IC_{50} = 0.8 compared to 11 μM), but both **18** and **25** were more effective than the untargeted carmethizole **3** ($IC_{50} > 20\ \mu$M). As well as being the most reactive analogues, the pyrrolizines **30**, **32** and **37** were also the most cytotoxic, with IC_{50}s of 1.3, 0.11, and 0.03 μM, respectively (Table 1). In this series the minor-groove-targeted compounds were the more cytotoxic.

Conclusions

This study was designed to explore the degree to which the patterns of both DNA alkylation and biological activity of the bis(hydroxymethyl)pyrrolizines and analogues could be altered by attachment to various DNA-affinic carriers. The unwinding experiments show that some of the acridine-linked compounds (e.g., **15**) do intercalate prior to alkylation and had slightly faster rates of alkylation than the corresponding parent compounds, suggesting that initial intercalation by the acridine moiety somewhat enhances subsequent DNA alkylation. Overall, the bis(hydroxymethyl)imidazoles, bis(hydroxymethyl)thioimidazoles, and imidazole bis-(carbamates) are weak DNA alkylating agents, with the pyrrolizines being more effective. The cleavage and polymerase stop assays suggest that alkylation occurs

only at the 2-amino group of guanine residues, with the nature of the carrier group not significantly modulating the pattern. This is at variance with other work, which shows that the sites of alkylation of both nitrogen mustards[19,25] (N7 of guanine) and cyclopropylindolines[26] (N3 of adenine) can be altered by appropriate targeting, and suggests that this particular covalent reaction can only take place at exocyclic primary amines. The cytotoxicities of the compounds correlated broadly with their reactivities, and for compounds bearing identical alkylating units, the intercalator-linked analogues were in most cases the more potent. Both of the targeted thioimidazole bis(carbamates) were more cytotoxic than carmethizole, demonstrating the utility of DNA-targeting. The pyrrolizine analogues were the most reactive and the most cytotoxic, with the longer chain compound **37** having an IC_{50} of 0.03 μM.

Experimental Section

Analyses were carried out in the Microchemical Laboratory, University of Otago, Dunedin, New Zealand. Melting points were determined on an Electrothermal 2300 digital melting point apparatus and are as read. NMR spectra were obtained on a Bruker AC-200 or AM-400 spectrometer and are referenced to Me_4Si. Mass spectra were determined on a VG 707 mass spectrometer at nominal 5000 resolution. Thin-layer chromatography was carried out on aluminum-backed silica gel plates (Merck 60 F_{254}). Column chromatography was carried out on silica gel, (Merck 230−400 mesh). Compounds for biological evaluation were of >99% purity, as determined by reverse-phase HPLC.

Dimethyl 1-[3-(4-Aminophenyl)propyl]imidazole-4,5-dicarboxylate (11) and Diethyl 2-(Methylthio)-1-[3-(4-aminophenyl)propyl]imidazole-4,5-dicarboxylate (12) (Scheme 1). A mixture of 3-(4-nitrophenyl)propyl bromide (**6**) (3.15 g, 12.9 mmol), dimethyl imidazole-4,5-dicarboxylate (**7**) (2.48 g, 13.5 mmol), powdered K_2CO_3 (2.68 g, 19.4 mmol), and DMF (30 mL) was stirred at 70 °C for 2.5 h. The hot mixture was filtered, the solids were washed with DMF, and the filtrate was concentrated under reduced pressure below 60 °C. Addition of water provided a solid which was chromatographed on silica gel. Elution with EtOAc/MeOH (20:1) gave dimethyl 1-[3-(4-nitrophenyl)propyl]imidazole-4,5-dicarboxylate (**9**) (3.95 g, 88%): mp (EtOAc/*i*-Pr_2O) 78−79 °C; ^{1}H NMR ($CDCl_3$) δ 8.16 (d, J = 8.6 Hz, 2 H, H-3′,5′), 7.54 (s, 1 H, H-2), 7.33 (d, J = 8.6 Hz, 2 H, H-2′,6′), 4.27 (t, J = 7.2 Hz, 2 H, NCH_2), 3.93 (s, 3 H, CH_3), 3.92 (s, 3 H, CH_3), 2.76 (t, J = 7.9 Hz, 2 H, $NCH_2CH_2CH_2$), 2.23−2.12 (m, 2 H, NCH_2CH_2). Anal. ($C_{16}H_{17}N_3O_6$) C, H, N.

Similar reaction of **6** and diethyl 2-(methylthio)imidazole-4,5-dicarboxylate[8] (**8**), followed by chromatography on silica gel, eluting with EtOAc, gave diethyl 2-(methylthio)-1-[3-(4-nitrophenyl)propyl]imidazole-4,5-dicarboxylate (**10**) (90% yield) as an oil: ^{1}H NMR ($CDCl_3$) δ 8.16 (d, J = 8.7 Hz, 2 H, H-3′,5′), 7.34 (d, J = 8.7 Hz, 2 H, H-2′,6′), 4.38 (q, J = 7.0 Hz, 2 H, CH_2CH_3), 4.32 (q, J = 7.0 Hz, 2 H, CH_2CH_3), 4.20 (t, J = 7.6 Hz, 2 H, NCH_2), 2.79 (t, J = 7.9 Hz, 2 H, $NCH_2CH_2CH_2$), 2.70 (s, 3 H, SCH_3), 2.16−2.06 (m, 2 H, $NHCH_2CH_2$), 1.38 (t, J = 7.1 Hz, 3 H, CH_2CH_3), 1.34 (t, J = 7.1 Hz, 3 H, CH_2CH_3). Anal. ($C_{19}H_{23}N_3O_6S\cdot0.5H_2O$) C, H, N, S.

A solution of **9** (4.00 g, 11.5 mmol) in MeOH (40 mL) was hydrogenated over Pt/C at 50 psi for 2 h. The crude product was chromatographed on silica gel, eluting with EtOAc/MeOH (19:1) to give **11** (3.47 g, 95%) as a waxy solid: mp 59−60 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 7.97 (s, 1 H, H-2), 6.82 (d, J = 8.2 Hz, 2 H, H-2′,6′), 6.49 (d, J = 8.3 Hz, 2 H, H-3′,5′), 4.86 (br s, 2 H, NH_2), 4.14 (t, J = 7.2 Hz, 2 H, NCH_2), 3.80 (s, 3 H, CH_3), 3.78 (s, 3 H, CH_3), 2.36 (t, J = 7.7 Hz, 2 H, $NCH_2CH_2CH_2$), 2.01−1.82 (m, 2 H, NCH_2CH_2). Anal. ($C_{16}H_{19}N_3O_4$) C, H, N.

Similar reduction of **10**, followed by removal of the catalyst and evaporation under reduced pressure (0.1 Torr) below 30 °C, gave the unstable **12** (99% yield) which was used immediately: ^{1}H NMR [$(CD_3)_2SO$] δ 6.84 (d, J = 8.3 Hz, 2 H, H-2′,6′), 6.49 (d, J = 8.3 Hz, 2 H, H-3′,5′), 4.88 (br s, 2 H, NH_2), 4.25 (q, J = 7.1 Hz, 2 H, CH_2CH_3), 4.24 (q, J = 7.1 Hz, 2 H, CH_2CH_3), 4.04 (t, J = 7.8 Hz, 2 H, NCH_2), 2.61 (s, 3 H, SCH_3), 2.42 (t, J = 7.6 Hz, 2 H, $NCH_2CH_2CH_2$), 1.91−1.81 (m, 2 H, NCH_2CH_2), 1.26 (t, J = 7.0 Hz, 3 H, CH_2CH_3), 1.24 (t, J = 7.1 Hz, 3 H, CH_2CH_3). HRMS (DEI) [M^+]: found, 391.1561; calcd for $C_{19}H_{25}N_3O_4S$, 391.1566.

1-[3-[4-(9-Acridinylamino)phenyl]propyl]-4,5-bis(*N*-methylcarbamoyloxymethyl)imidazole (17) (Scheme 2). A solution of **11** (900 mg, 2.84 mmol) in anhydrous THF (30 mL) was added dropwise over a 1 h period to a stirred suspension of $LiAlH_4$ (755 mg, 19.89 mmol) in anhydrous THF (60 mL) at 20 °C. The mixture was stirred for a further 2 h at 20 °C and then treated dropwise and sequentially with water (0.76 mL), 15% aqueous NaOH (0.76 mL), and water (2.3 mL). The mixture was warmed, filtered through a Celite pad, and the solids were washed with hot THF. The filtrate was concentrated to dryness under reduced pressure below 30 °C, and the residue was extracted with hot CH_2Cl_2, filtered, and then reevaporated. The residue was crystallized twice from EtOAc to give 1-[3-(4-aminophenyl)propyl]-4,5-bis(hydroxymethyl)-imidazole (**13**) (420 mg, 57%): mp 121−122 °C (hygroscopic); ^{1}H NMR [$(CD_3)_2SO$] δ 7.51 (s, 1 H, H-2), 6.84 (d, J = 8.3 Hz, 2 H, H-2′,6′), 6.48 (d, J = 8.3 Hz, 2 H, H-3′,5′), 4.86 (t, J = 5.3 Hz, 1 H, OH), 4.83 (s, 2 H, NH_2), 4.59 (t, J = 5.5 Hz, 1 H, OH), 4.44 (d, J = 5.1 Hz, 2 H, CH_2OH), 4.32 (d, J = 5.5 Hz, 2 H, CH_2OH), 3.92 (t, J = 7.3 Hz, 2 H, NCH_2), 2.40 (t, J = 7.8 Hz, 2 H, $NCH_2CH_2CH_2$), 2.00−1.90 (m, 2 H, NCH_2CH_2). Anal. ($C_{14}H_{19}N_3O_2\cdot0.5H_2O$) C, H, N.

9-Chloroacridine (0.32 g, 1.50 mmol) was added to a solution of **13** (0.44 g, 1.68 mmol) in anhydrous MeOH (25 mL), and the mixture was stirred at 20 °C for 4 h and then heated under reflux for 15 min. Addition of EtOAc to the cooled solution provided a semisolid which was dissolved in warm water. Filtration and basification with aqueous Na_2CO_3 yielded a crude product which was crystallized twice from MeOH/EtOAc to give 1-[3-[4-(9-acridinylamino)phenyl]propyl]-4,5-bis(hydroxymethyl)imidazole (**15**) (0.58 g, 88%): mp 201 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.83 (s), 9.17 (s), 8.30 (br s), 8.14 (d, J = 8.6 Hz), 8.08 (d, J = 8.7 Hz), 7.76 (t, J = 7.4 Hz), 7.55 (d, J = 9.3 Hz), 7.42 (t, J = 7.5 Hz), 7.28 (d, J = 8.0 Hz), 7.16 (d, J = 8.2 Hz), 7.05 (d, J = 4.2 Hz), 6.78 (d, J = 8.3 Hz), 6.68 (d, J = 8.2 Hz) [identifiable anilinoacridine protons, mixture of imino and amino forms], 7.56 (s, H-2), 4.92 (t, J = 5.2 Hz, 1 H, OH), 4.63 (t, J = 5.5 Hz, 1 H, OH), 4.48 (d, J = 5.1 Hz, 2 H, CH_2OH), 4.34 (d, J = 5.4 Hz, 2 H, CH_2OH), 3.99 (t, J = 7.3 Hz, 2 H, NCH_2), 2.59 (t, J = 7.6 Hz, 2 H, $NCH_2CH_2CH_2$), 2.15−2.02 (m, 2 H, NCH_2CH_2). HRMS (DEI) [M^+]: found, 438.2047; calcd for $C_{27}H_{26}N_4O_2$, 438.2056. Anal. ($C_{27}H_{26}N_4O_2$) C, H, N.

A suspension of **15** (0.48 mg, 1.09 mmol) in anhydrous THF (50 mL) was treated with methyl isocyanate (0.25 g, 4.39 mmol) followed by dibutyltin diacetate (1 drop) and stirred at 20 °C for 2.5 h. The resulting homogeneous solution was concentrated under reduced pressure below 30 °C, and the residue was triturated with EtOAc and cooled for a prolonged period to give **17** (0.55 g, 91%): mp MeOH/EtOAc) 198−199 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.83 (s), 9.16 (s), 8.13 (br s), 7.76 (t, J = 7.6 Hz), 7.69 (d, J = 8.9 Hz), 7.42 (t, J = 7.6 Hz), 7.28 (d, J = 8.1 Hz), 7.16 (d, J = 8.1 Hz), 6.77 (d, J = 8.2 Hz), 6.68 (d, J = 8.2 Hz) [identifiable anilinoacridine protons, mixture of imino and amino forms], 7.70 (s, H-2), 7.06 (q, J = 6.8 Hz, 1 H, $NHCH_3$), 6.94 (br d, J = 4.4 Hz, 1 H, $NHCH_3$), 5.10 (s, 2 H, CH_2O), 4.92 (s, 2 H, CH_2O), 3.99 (t, J = 7.3 Hz, 2 H, NCH_2), 2.64−2.48 (m, 8 H, 2 × CH_3, $NCH_2CH_2CH_2$), 2.10−1.99 (m, 2 H, NCH_2CH_2). HRMS (DAB) [M + H]$^+$: found, 553.2552; $C_{31}H_{33}N_6O_4$ requires 553.2563. Anal. ($C_{31}H_{32}N_6O_4$) C, H, N.

1-[3-[4-(9-Acridinylamino)phenyl]propyl]-2-(methylthio)-4,5-bis(*N*-methylcarbamoyloxymethyl)imidazole (18) (Scheme 2). A mixture of powdered 9-chloroacridine (1.07 g, 5.01 mmol) and freshly prepared **12** (2.07 g, 5.29 mmol) in MeOH (25 mL) was stirred at 20 °C until homogeneous and then treated with 12 N HCl (0.05 mL). After the mixture was

stirred at 20 °C for a further 1 h, it was heated under reflux for 30 min and then concentrated to 5 mL and diluted with EtOAc. The resulting solid was crystallized from MeOH/aqueous $KHCO_3$, followed by purification by alumina chromatography (elution with MeOH). Appropriate fractions were pooled, triturated with *i*-Pr_2O, and crystallized from MeOH/*i*-Pr_2O to give diethyl 1-[3-[4-(9-acridinylamino)phenyl]propyl]-2-(methylthio)imidazole-4,5-dicarboxylate (**14**) (1.71 g, 60%): mp 143−145 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.83 (s), 9.18 (s), 8.31 (br s), 8.14 (d, J = 8.7 Hz), 8.08 (d, J = 8.6 Hz), 7.76 (t, J = 7.5 Hz), 7.42 (t, J = 7.5 Hz), 7.29 (d, J = 8.1 Hz), 7.16 (d, J = 8.1 Hz), 7.04 (d, J = 8.0 Hz), 6.78 (d, J = 7.9 Hz), 6.69 (d, J = 8.1 Hz) [identifiable anilinoacridine protons, mixture of imino and amino forms], 4.28 (q, J = 7.0 Hz, 2 H, CH_2CH_3), 4.26 (q, J = 7.0 Hz, 2 H, CH_2CH_3), 4.10 (t, J = 7.7 Hz, 2 H, NCH_2), 2.65−2.58 (m, 5 H, SCH_3, $NCH_2CH_2CH_2$), 2.04−1.90 (m, 2 H, NCH_2CH_2), 1.27 (t, J = 7.1 Hz, 6 H, 2 × CH_2CH_3). HRMS (EI) [M^+]: found, 568.2158. calcd for $C_{32}H_{32}N_4O_4S$, 568.2144. Anal. ($C_{32}H_{32}N_4O_4S$) C, H, N, S.

A solution of **14** (300 mg, 0.53 mmol) in anhydrous THF (25 mL) was added dropwise over a 1 h period to a stirred suspension of $LiAlH_4$ (200 mg, 5.27 mmol) in anhydrous THF (60 mL) at 5−10 °C. The mixture was stirred for a further 2 h at 10 °C, treated dropwise and sequentially with water (0.2 mL), 15% aqueous NaOH (0.2 mL), and water (0.6 mL). The mixture was filtered through a Celite pad, and the solids were washed with hot THF. The filtrate was concentrated under reduced pressure below 30 °C to 30 mL and then diluted with aqueous $KHCO_3$. The precipitated solid was extracted with hot EtOAc, impurities were removed by filtration, and the solution was then concentrated and diluted with *i*-Pr_2O to provide a crude product. Crystallization from THF-^{i}Pr_2O gave 1-[3-[4-(9-acridinylamino)phenyl]propyl]-2-(methylthio)-4,5-bis(2-hydroxymethyl)imidazole (**16**) (210 mg, 82%): mp 186−187 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.82 (s), 9.18 (s), 8.31 (br s), 8.14 (d, J = 8.6 Hz), 8.07 (d, J = 8.6 Hz), 7.75 (t, J = 7.6 Hz), 7.41 (t, J = 7.3 Hz), 7.28 (d, J = 7.7 Hz), 7.17 (d, J = 8.3 Hz), 7.06 (d, J = 8.4 Hz), 6.79 (d, J = 8.4 Hz), 6.69 (d, J = 8.3 Hz) [identifiable anilinoacridine protons, mixture of imino and amino forms], 4.94 (t, J = 5.3 Hz, 1 H, OH), 4.68 (t, J = 5.6 Hz, 1 H, OH), 4.46 (d, J = 5.3 Hz, 2 H, CH_2OH), 4.32 (d, J = 5.6 Hz, 2 H, CH_2OH), 3.93 (t, J = 7.8 Hz, 2 H, NCH_2), 2.64 (t, J = 7.4 Hz, 2 H, $NCH_2CH_2CH_2$), 2.57 (s, 3 H, SCH_3), 2.05−1.96 (m, 2 H, NCH_2CH_2). HRMS (FAB) [M + H]$^+$: Found, 485.1998; calcd for $C_{28}H_{29}N_4O_2S$, 485.2011. Anal. ($C_{28}H_{28}N_4O_2S$) C, H, N, Cl.

A mixture of **16** (402 mg, 0.83 mmol) and methyl isocyanate (284 mg, 4.98 mmol) in DMF (4 mL) and anhydrous THF (4 mL) was treated with dibutyltin diacetate (2 drops) and stirred at 20 °C for 1.5 h. The mixture was concentrated under reduced pressure below 30 °C to remove THF and then diluted with aqueous Na_2CO_3. The resulting solid was crystallized from MeOH/*i*-P_2O and then from MeOH to give **18** (446 mg, 90%): mp 159−162 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.82 (s), 9.17 (s), 8.31 (br s), 8.14 (d, J = 8.6 Hz), 8.08 (d, J = 8.7 Hz), 7.76 (t, J = 7.1 Hz), 7.41 (t, J = 7.5 Hz), 7.28 (d, J = 7.8 Hz), 7.18 (d, J = 8.2 Hz), 6.78 (d, J = 8.4 Hz), 6.69 (d, J = 8.2 Hz) [identifiable anilinoacridine protons, mixture of imino and amino forms], 7.11−7.00 (m, 1 H, N*H*CH_3), 6.96 (q, J = 4.5 Hz, 1 H, N*H*CH_3), 5.09 (s, 2 H, CH_2O), 4.90 (s, 2 H, CH_2O), 3.89 (t, J = 7.9 Hz, 2 H, NCH_2), 2.63 (t, J = 7.2 Hz, 2 H, $NCH_2CH_2CH_2$), 2.59−2.50 (m, 9 H, SCH_3, 2 × $NHCH_3$), 2.01−1.91 (m, 2 H, NCH_2CH_2). HRMS (FAB) [M + H]$^+$: found, 599.2427; calcd for $C_{32}H_{35}N_6O_4S$, 599.2441. Anal. ($C_{32}H_{34}N_6O_4S$) C, H, N, S.

1-[3-[4-[4-(4-Quinolinylamino)benzamido]phenyl]propyl]-4,5-bis(*N*-methylcarbamoyloxymethyl)imidazole (24) (Scheme 3). A suspension of 4-(4-quinolinylamino)-benzoic acid[27] (**19**) (0.80 g, 3.03 mmol) in DMF (12 mL) was treated with bis(2-oxo-3-oxazolidinyl)phosphinic chloride (97%, 0.95 g, 3.62 mmol) and stirred at 20 °C for 2 h. The mixture was cooled to −5 °C and treated with diisopropylethylamine (0.86 g, 6.65 mmol) immediately followed by **11** (1.01 g, 3.18 mmol). After stirring at 20 °C for a further 6 h, the mixture was diluted with aqueous $KHCO_3$, and the resulting solid was crystallized twice from MeOH/H_2O to give dimethyl 1-[3-[4-[4-(4-quinolinylamino)benzamido]phenyl]propyl]imidazole-4,5-dicarboxylate (**20**) (1.20 g, 70%): mp 206−207 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.11 (s, 1 H, NH), 9.24 (s, 1 H, NH), 8.58 (d, J = 4.9 Hz, 1 H, quinoline H-2), 8.38 (d, J = 8.5 Hz, 1 H, quinoline H-8), 8.02 (s, 1 H, H-2), 8.01 (d, J = 7.5 Hz, 2 H, PhH), 7.93 (d, J = 8.4 Hz, 1 H, quinoline H-5), 7.74 (t, J = 8.2 Hz, 1 H, quinoline H-7), 7.72 (d, J = 7.9 Hz, 2 H, PhH), 7.58 (t, J = 7.6 Hz, 1 H, quinoline H-6), 7.48 (d, J = 8.2 Hz, 2 H, PhH), 7.21 (d, J = 4.8 Hz, 1 H, quinoline H-3), 7.18 (d, J = 8.2 Hz, 2 H, PhH), 4.20 (t, J = 7.2 Hz, 2 H, NCH_2), 3.82 (s, 3 H, CH_3), 3.79 (s, 3 H, CH_3), 2.54 (t, J = 7.7 Hz, 2 H, $NCH_2CH_2CH_2$), 2.08−1.96 (m, 2 H, NCH_2CH_2). Anal. ($C_{32}H_{29}N_5O_5$) C, H, N.

A hot solution of **20** (1.36 g, 2.41 mmol) in anhydrous THF (300 mL) was cooled to 20 °C and added dropwise over a 1 h period to a stirred suspension of $LiAlH_4$ (1.10 g, 29.0 mmol) in anhydrous THF (130 mL) at 20 °C. The mixture was stirred for a further 2 h at 20 °C, then treated dropwise and sequentially with water (1.1 mL), 15% aqueous NaOH (1.1 mL), and water (3.3 mL). The mixture was diluted with MeOH (250 mL), warmed, and filtered through a Celite pad, and the solids were washed with MeOH/THF (1:1). The filtrate was concentrated under reduced pressure to 50 mL, and aqueous Na_2CO_3 was then added. The resulting solid was crystallized successively from MeOH/EtOAc and MeOH to give 1-[3-[4-[4-(4-quinolinylamino)benzamido]phenyl]propyl]-4,5-bis(hydroxymethyl)imidazole (**22**) (0.76 g, 63%): mp 149−153 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.11 (s, 1 H, NH), 9.24 (s, 1 H, NH), 8.59 (d, J = 5.2 Hz, 1 H, quinoline H-2), 8.39 (d, J = 7.9 Hz, 1 H, quinoline H-8), 8.01 (d, J = 8.7 Hz, 2 H, PhH), 7.94 (d, J = 8.0 Hz, 1 H, quinoline H-5), 7.74 (t, J = 7.5 Hz, 1 H, quinoline H-7), 7.72 (d, J = 8.5 Hz, 2 H, PhH), 7.58 (t, J = 7.5 Hz, 1 H, quinoline H-6), 7.56 (s, 1 H, H-2), 7.48 (d, J = 8.7 Hz, 2 H, PhH), 7.21 (d, J = 5.1 Hz, 1 H, quinoline H-3), 7.20 (d, J = 8.5 Hz, 2 H, PhH), 4.91 (t, J = 5.3 Hz, 1 H, OH), 4.62 (t, J = 5.5 Hz, 1 H, OH), 4.48 (d, J = 5.2 Hz, 2 H, CH_2OH), 4.34 (d, J = 5.4 Hz, 2 H, CH_2OH), 3.98 (t, J = 7.3 Hz, 2 H, NCH_2), 2.57 (t, J = 7.8 Hz, 2 H, $NCH_2CH_2CH_2$), 2.12−2.01 (m, 2 H, NCH_2CH_2). HRMS (FAB) [M + H]$^+$: found, 508.2343; calcd for $C_{30}H_{30}N_5O_3$, 508.2349. Anal. ($C_{30}H_{29}N_5O_3\cdot H_2O$) C, N. H: found, 6.4; requires 5.9%.

A mixture of **22** (140 mg, 0.28 mmol), methyl isocyanate (96 mg, 1.68 mmol), anhydrous DMF (1 mL), and anhydrous THF (2 mL) was treated with dibutyltin diacetate (1 drop) and stirred at 20 °C for 2 h. The mixture was diluted with aqueous Na_2CO_3, and the resulting solid was dried and dissolved in MeOH at 20 °C. The solution was clarified by filtration, diluted with EtOAc, and then concentrated under reduced pressure below 30 °C. The product which separated was crystallized from MeOH/EtOAc to give **24** (143 mg, 83%): mp 198−199 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.11 (s, 1 H, NH), 9.26 (s, 1 H, NH), 8.58 (d, J = 5.2 Hz, 1 H, quinoline H-2), 8.39 (d, J = 8.2 Hz, 1 H, quinoline H-8), 8.00 (d, J = 8.6 Hz, 2 H, PhH), 7.94 (d, J = 8.3 Hz, 1 H, quinoline H-5), 7.77−7.67 (m, 4 H, H-2, quinoline H-7, PhH), 7.58 (t, J = 7.5 Hz, 1 H, quinoline H-6), 7.48 (d, J = 8.5 Hz, 2 H, PhH), 7.24−7.15 (m, 3 H, quinoline H-3, PhH), 7.06 (q, J = 4.6 Hz, 1 H, N*H*CH_3), 6.94 (q, J = 4.5 Hz, 1 H, N*H*CH_3), 5.10 (s, 2 H, CH_2O), 4.92 (s, 2 H, CH_2O), 3.97 (t, J = 7.3 Hz, 2 H, NCH_2), 2.62−2.50 (m, 8 H, 2 × CH_3, $NCH_2CH_2CH_2$), 2.10−1.96 (m, 2 H, NCH_2CH_2). HRMS (FAB) [M + H]$^+$: found, 622.2780; calcd for $C_{34}H_{36}N_7O_5$, 622.2778. Anal. ($C_{34}H_{35}N_7O_5\cdot 2H_2O$). C, N. H: found, 5.5; requires 6.0%.

2-(Methylthio)-1-[3-[4-[4-(4-quinolinylamino)benzamido]phenyl]propyl]-4,5-bis(*N*-methylcarbamoyloxymethyl)imidazole (25) (Scheme 3). A suspension of **19** (1.60 g, 6.05 mmol) in DMF (25 mL) was treated with bis(2-oxo-3-oxazolidinyl)phosphinic chloride (97%, 1.91 g, 7.26 mmol) and stirred at 20 °C for 2 h. The mixture was cooled to −5 °C and treated sequentially with *N*,*N*-diisopropylethylamine (1.73 g, 13.38 mmol) and freshly prepared **12** (2.49 g, 6.36 mmol). After stirring at 20 °C for a further 6 h, the mixture was diluted with aqueous $KHCO_3$ to provide a semisolid. This was dried, triturated with EtOAc, and crystallized successively from

MeOH/EtOAc/*i*-Pr_2O and then EtOAc to give diethyl 2-(methylthio)-1-[3-[4-[4-(4-quinolinylamino)benzamido]-phenyl]-propyl]imidazole-4,5-dicarboxylate (**21**) (2.38 g, 62%): mp 148−150 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.11 (s, 1 H, NH), 9.24 (s, 1 H, NH), 8.59 (d, J = 5.2 Hz, 1 H, quinoline H-2), 8.38 (d, J = 7.9 Hz, 1 H, quinoline H-8), 8.01 (d, J = 8.7 Hz, 2 H, PhH), 7.94 (d, J = 8.1 Hz, 1 H, quinoline H-5), 7.74 (t, J = 7.6 Hz, 1 H, quinoline H-7), 7.72 (d, J = 8.5 Hz, 2 H, PhH), 7.58 (t, J = 7.7 Hz, 1 H, quinoline H-6), 7.48 (d, J = 8.7 Hz, 2 H, PhH), 7.24−7.15 (m, 3 H, quinoline H-3, PhH), 4.27 (q, J = 6.9 Hz, 2 H, CH_2CH_3), 4.25 (q, J = 6.9 Hz, 2 H, CH_2CH_3, 4.10 (t, J = 7.6 Hz, 2 H, NCH_2), 2.63 (s, 3 H, SCH_3), 2.60 (t, J = 7.6 Hz, 2 H, $NCH_2CH_2CH_2$), 2.03−1.90 (m, 2 H, NCH_2CH_2), 1.27 (t, J = 7.1 Hz, 3 H, CH_2CH_3), 1.26 (t, J = 7.1 Hz, 3 H, CH_2CH_3). Anal. ($C_{35}H_{35}N_5O_5S$) C, H, N, S.

A solution of **21** (1.15 g, 1.80 mmol) in anhydrous THF (125 mL) was added dropwise over a 1 h period to a stirred suspension of $LiAlH_4$ (0.68 g, 17.92 mmol) in anhydrous THF (70 mL) at 10 °C. After the mixture was stirred at 10 °C for a further 3 h, it was treated dropwise and sequentially with water (0.7 mL), 15% aqueous NaOH (0.7 mL), and water (2.1 mL). This mixture was diluted with MeOH (200 mL), heated, and filtered through a Celite pad, and the solids were washed with MeOH/THF (1:1). The filtrate was concentrated under reduced pressure below 30 °C to 50 mL and then diluted with aqueous Na_2CO_3 to precipitate the crude product. Two crystallizations from MeOH/EtOAc gave 2-(methylthio)-1-[3-[4-[4-(4-quinolinylamino)benzamido]phenyl]propyl]-4,5-bis(2-hydroxymethyl)imidazole (**23**) (0.76 g, 76%): mp 196−197 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.11 (s, 1 H, NH), 9.24 (s, 1 H, NH), 8.58 (d, J = 5.2 Hz, 1 H, quinoline H-2), 8.38 (d, J = 8.0 Hz, 1 H, quinoline H-8), 8.01 (d, J = 8.7 Hz, 2 H, PhH), 7.94 (d, J = 8.2 Hz, 1 H, quinoline H-5), 7.74 (t, J = 7.6 Hz, 1 H, quinoline H-7), 7.72 (d, J = 8.5 Hz, 2 H, PhH), 7.58 (t, J = 7.3 Hz, 1 H, quinoline H-6), 7.48 (d, J = 8.6 Hz, 2 H, PhH), 7.24−7.18 (m, 3 H, quinoline H-3, PhH), 4.94 (t, J = 5.3 Hz, 1 H, OH), 4.68 (t, J = 5.5 Hz, 1 H, OH), 4.45 (d, J = 5.1 Hz, 2 H, CH_2OH), 4.32 (d, J = 5.3 Hz, 2 H, CH_2OH), 3.92 (t, J = 7.7 Hz, 2 H, NCH_2), 2.62 (t, J = 7.6 Hz, 2 H, $NCH_2CH_2CH_2$), 2.52 (s, 3 H, SCH_3), 2.05−1.93 (m, 2 H, NCH_2CH_2). HRMS (FAB) $[M + H]^+$: found, 554.2216; calcd for $C_{31}H_{32}N_5O_3S$, 554.2226. Anal. ($C_{31}H_{31}N_5O_3S$) C, H, N, S.

A mixture of **23** (360 mg. 0.65 mmol) in DMF (4 mL) and THF (4 mL) was treated with methyl isocyanate (222 mg, 3.90 mmol), followed by dibutyltin diacetate (2 drops), and stirred at 20 °C for 2 h. The mixture was concentrated under reduced pressure below 30 °C to remove THF and then diluted with aqueous Na_2CO_3. The resulting solid was dissolved in warm MeOH and clarified by filtration, and then EtOAc was added. The precipitated solid was crystallized from MeOH/EtOAc to give **25** (370 mg, 85%): mp 181−182 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.11 (s, 1 H, NH), 9.24 (s, 1 H, NH), 8.59 (d, J = 5.2 Hz, 1 H, quinoline H-2), 8.38 (d, J = 7.9 Hz, 1 H, quinoline H-8), 8.01 (d, J = 8.7 Hz, 2 H, PhH), 7.93 (d, J = 7.9 Hz, 1 H, quinoline H-5), 7.74 (t, J = 7.6 Hz, 1 H, quinoline H-7), 7.72 (d, J = 8.5 Hz, 2 H, PhH), 7.58 (t, J = 7.6 Hz, 1 H, quinoline H-6), 7.48 (d, J = 8.7 Hz, 2 H, PhH), 7.24−7.17 (m, 3 H, quinoline H-3, PhH), 7.08 (q, J = 4.6 Hz, 1 H, $NHCH_3$), 6.97 (q, J = 4.6 Hz, 1 H, $NHCH_3$), 5.08 (s, 2 H, CH_2O), 4.90 (s, 2 H, CH_2O), 3.87 (t, J = 7.8 Hz, 2 H, NCH_2), 2.61 (t, J = 7.3 Hz, 2 H, $NCH_2CH_2CH_2$), 2.59 (d, J = 4.5 Hz, 3 H, $NHCH_3$), 2.55 (d, J = 4.7 Hz, 3 H, $NHCH_3$), 2.54 (s, 3 H, SCH_3), 2.03−1.88 (m, 2 H, NCH_2CH_2). HRMS (FAB) $[M + H]^+$: found, 668.2679. calcd for $C_{35}H_{38}N_7O_5S$, 668.2655. Anal. ($C_{35}H_{37}N_7O_5S$) C, H, N, S.

2,3-Dihydro-5-[3-[4-(9-acridinylamino)phenyl]propyl]-6,7-bis(hydroxymethyl)-1*H*-pyrrolizine (30) (Scheme 4). A solution of 4-(4-nitrophenyl)butyryl chloride (7.62 g, 33 mmol) in Et_2O (40 mL) was added dropwise to a vigorously stirred solution of (S)-proline (4.62 g, 40 mmol) and NaOH (2.91 g, 73 mmol) in H_2O (28 mL) at 5 °C. The mixture was stirred at 5 °C for 1 h, then at 20 °C for 1 h, followed by concentration to remove Et_2O. Acidification with 12 N HCl gave a precipitated oil which was chromatographed on silica gel, eluting with EtOAc, to give crude (*S*)-1-[4-(4-nitrophenyl)-butyryl]pyrrolidine-2-carboxylic acid (**26**) (8.03 g, 78%) as a gum: ^{1}H NMR ($CDCl_3$) δ 8.14 (d, J = 8.7 Hz, 2 H, H-3′,5′), 7.8 (br s, 1 H, CO_2H), 7.36 (d, J = 8.7 Hz, 2 H, H-2′,6′), 4.61−4.54 (m, 1 H, $CHCO_2H$), 3.59−3.49 (m, 1 H, CH), 3.48−3.39 (m, 1 H, CH), 2.81 (t, J = 7.7 Hz, 2 H, CH_2), 2.45−2.30 (m, 3 H, CH_2, CH), 2.13−1.97 (m, 5 H, 2 × CH_2, CH). HRMS (DEI) $[M^+]$: found, 306.1217; calcd for $C_{15}H_{18}N_2O_5$, 306.1216.

A mixture of **26** (7.00 g, 23 mmol), dimethyl acetylenedicarboxylate (5.7 mL, 46 mmol), and Ac_2O (57 mL) was stirred and heated at 130−140 °C until CO_2 evolution had ceased (ca. 2 h). The mixture was concentrated under reduced pressure, and the residue was shaken with water to give an oil which was chromatographed on silica gel. Elution with EtOAc gave a solid that was crystallized twice from EtOAc/petroleum ether (decolorizing charcoal) to give dimethyl 2,3-dihydro-5-[3-(4-nitrophenyl)propyl]-1*H*-pyrrolizine-6,7-dicarboxylate (**27**) (6.48 g, 73%): mp 94 °C; ^{1}H NMR ($CDCl_3$) δ 8.14 (d, J = 8.8 Hz, 2 H, H-3′,5′), 7.32 (d, J = 8.8 Hz, 2 H, H-2′,6′), 3.83 (t, J = 7.1 Hz, 2 H, NCH_2), 3.80 (s, 3 H, CH_3), 3.78 (s, 3 H, CH_3), 3.03 (t, J = 7.5 Hz, 2 H, CH_2), 2.86−2.72 (m, 4 H, 2 × CH_2), 2.58−2.40 (m, 2 H, NCH_2CH_2), 2.03−1.84 (m, 2 H, $PhCH_2CH_2$). Anal. ($C_{20}H_{22}N_2O_6$) C, H, N.

A solution of **27** (3.50 g, 9.1 mmol) in 1:1 MeOH/THF (40 mL) was hydrogenated over Pt/C at 60 psi for 2 h. The crude product was chromatographed on silica gel, eluting with CH_2Cl_2/EtOAc (3:2), to give dimethyl 2,3-dihydro-5-[3-(4-aminophenyl)propyl]-1*H*-pyrrolizine-6,7-dicarboxylate (**28**) (3.07 g, 95%): mp (EtOAc/petroleum ether) 121 °C; ^{1}H NMR ($CDCl_3$) δ 6.95 (d, J = 8.4 Hz, 2 H, H-2′,6′), 6.62 (d, J = 8.4 Hz, 2 H, H-3′,5′), 3.79 (s, 3 H, CH_3), 3.77 (s, 3 H, CH_3), 3.77 (t, J = 7.2 Hz, 2 H, NCH_2; partially obscured), 3.56 (br s, 2 H, NH_2), 3.01 (t, J = 7.5 Hz, 2 H, CH_2), 2.73 (t, J = 7.8 Hz, 2 H, CH_2), 2.54 (t, J = 7.6 Hz, 2 H, CH_2), 2.54−2.36 (m, 2 H, NCH_2CH_2), 1.91−1.72 (m, 2 H, $PhCH_2CH_2$). Anal. ($C_{20}H_{24}N_2O_4$) C, H, N.

A solution of **28** (0.40 g, 1.12 mmol) in MeOH (25 mL) was treated in one portion with powdered 9-chloroacridine (0.22 g, 1.03 mmol). The mixture was stirred at 20 °C until homogeneous, 12 N HCl (half drop) was added, and the solution was heated under reflux for 15 min. The solution was diluted with EtOAc, concentrated, and cooled. The resulting orange crystals were treated with MeOH/aqueous $KHCO_3$ to provide a solid which was crystallized from EtOAc/CH_2Cl_2/*i*-Pr_2O to give dimethyl 2,3-dihydro-5-[3-[4-(9-acridinylamino)-phenyl]propyl]-1*H*-pyrrolizine-6,7-dicarboxylate (**29**) (0.48 g, 87%): mp 73−77 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.82 (s), 9.15 (s), 8.30 (br s), 8.13 (d, J = 8.6 Hz), 8.07 (d, J = 8.6 Hz), 7.75 (t, J = 7.5 Hz), 7.41 (t, J = 7.5 Hz), 7.28 (d, J = 8.1 Hz), 7.14 (d, J = 8.2 Hz), 7.03 (d, J = 8.1 Hz), 6.77 (d, J = 8.1 Hz), 6.67 (d, J = 8.2 Hz) [identifiable anilinoacridine protons, mixture of imino and amino forms], 3.92 (t, J = 7.1 Hz, 2 H, NCH_2), 3.66 (s, 3 H, CH_3), 3.65 (s, 3 H, CH_3), 2.91 (t, J = 7.4 Hz, 2 H, CH_2), 2.70 (t, J = 7.7 Hz, 2 H, CH_2), 2.59 (t, J = 7.4 Hz, 2 H, CH_2), 2.49−2.36 (m, 2 H, NCH_2CH_2), 1.88−1.74 (m, $PhCH_2CH_2$). HRMS (EI) $[M^+]$: found, 533.2293; calcd for $C_{33}H_{31}N_3O_4$, 533.2314. Anal. ($C_{33}H_{31}N_3O_4$) C, H, N.

A solution of **29** (0.60 g, 1.12 mmol) in anhydrous THF (80 mL) was added dropwise over a 1 h period to a stirred suspension of $LiAlH_4$ (0.45 g, 1.19 mmol) in anhydrous THF (80 mL) at 20 °C. The mixture was stirred for a further 2 h at 20 °C and then treated dropwise and sequentially with water (0.45 mL), 15% aqueous NaOH (0.45 mL), and water (1.4 mL). The mixture was warmed and filtered through a Celite pad, and the solids were washed with hot THF. The filtrate was concentrated to dryness under reduced pressure below 30 °C. The residue was dissolved in hot anhydrous THF, diluted with *i*-Pr_2O to precipitate impurities (which were removed by filtration), and then further diluted with *i*-Pr_2O to provide the crude product. Crystallization from THF/EtOAc/*i*-Pr_2O gave **30** (0.38 g, 71%): mp 265−270 °C (hygroscopic); ^{1}H NMR [$(CD_3)_2SO$] δ 10.81 (s), 9.16 (s), 8.31 (br s), 8.14 (d, J = 8.7 Hz), 8.07 (d, J = 8.7 Hz), 7.75 (t, J = 7.4 Hz), 7.41 (t, J = 7.5 Hz), 7.28 (d, J = 8.0 Hz), 7.15 (d, J = 8.2 Hz), 7.04 (d, J = 8.3 Hz), 6.78 (d, J = 8.2 Hz), 6.67 (d, J = 8.2 Hz) [identifiable

anilinoacridine protons, mixture of imino and amino forms], 4.43 (t, $J = 5.1$ Hz, 1 H, OH), 4.33−4.23 (1 H, OH; obscured), 4.30 (d, $J = 4.9$ Hz, 2 H, CH_2OH), 4.27 (d, $J = 4.7$ Hz, 2 H, CH_2OH), 3.76 (t, $J = 6.9$ Hz, 2 H, NCH$_2$), 2.71 (t, $J = 7.2$ Hz, 2 H, CH$_2$), 2.60 (t, $J = 7.4$ Hz, 2 H, CH$_2$), 2.53 (t, $J = 7.4$ Hz, 2 H, CH$_2$), 2.41−2.30 (m, 2 H, NCH$_2$CH_2), 1.83−1.68 (m, 2 H, PhCH$_2$CH_2). HRMS (FAB) [M + H]$^+$: found, 478.2486; calcd for $C_{31}H_{32}N_3O_2$, 478.2495. Anal. ($C_{31}H_{31}N_3O_2$) H, N. C: found, 77.4; requires 77.9%.

2,3-Dihydro-5-[3-[4-[4-(4-quinolinylamino)benzamido]-phenyl]propyl]-6,7-bis(hydroxymethyl)-1*H*-pyrrolizine (32) (Scheme 4). A suspension of **19** (1.11 g, 4.20 mmol) in DMF (15 mL) was treated with bis(2-oxo-3-oxazolidinyl)-phosphinic chloride (1.32 g of 97%, 5.03 mmol). The mixture was stirred at 20 °C for 1.5 h, then cooled to −5 °C, and treated with diisopropylethylamine (1.19 g, 9.24 mmol) immediately followed by amine **28** (1.57 g, 4.41 mmol). After being stirred at 20 °C for a further 6 h, the mixture was diluted with aqueous KHCO$_3$. Trituration of the resulting solid with cold MeOH provided a crude product which was crystallized from DMF/MeOH/H$_2$O to give dimethyl 2,3-dihydro-5-[3-[4-[4-(4-quinolinylamino)benzamido]phenyl]propyl]-1*H*-pyrrolizine-6,7-dicarboxylate (**31**) (1.79 g, 71%): mp 257 °C; ^{1}H NMR [(CD$_3$)$_2$SO] δ 10.09 (s, 1 H, NH), 9.24 (s, 1 H, NH), 8.58 (d, $J = 5.2$ Hz, 1 H, quinoline H-2), 8.38 (d, $J = 7.9$ Hz, 1 H, quinoline H-8), 8.01 (d, $J = 8.7$ Hz, 2 H, PhH), 7.94 (d, $J = 8.1$ Hz, 1 H, quinoline H-5), 7.74 (td, $J = 7.1$, 1.2 Hz, 1 H, quinoline H-7), 7.70 (d, $J = 8.5$ Hz, 2 H, PhH), 7.58 (td, $J = 7.6$, 1.1 Hz, 1 H, quinoline H-6), 7.48 (d, $J = 8.7$ Hz, 2 H, PhH), 7.21 (d, $J = 5.2$ Hz, 1 H, quinoline H-3), 7.18 (d, $J = 8.6$ Hz, 2 H, PhH), 3.91 (t, $J = 7.2$ Hz, 2 H, NCH$_2$), 3.65 (s, 6 H, 2 × CH$_3$), 2.91 (t, $J = 7.5$ Hz, 2 H, CH$_2$), 2.69 (t, $J = 7.7$ Hz, 2 H, CH$_2$), 2.57 (t, $J = 7.6$ Hz, 2 H, CH$_2$), 2.48−2.36 (m, 2 H, NCH$_2$CH_2), 1.87−1.73 (m, $J = 7.6$ Hz, 2 H, PhCH$_2$CH_2). Anal. ($C_{36}H_{34}N_4O_5$) C, H, N.

Powdered diester **31** (750 mg, 1.24 mmol) was added portionwise over a 1.5 h period to a stirred suspension of LiAlH$_4$ (470 mg, 12.38 mmol) in THF (210 mL) at 20 °C under N$_2$. Stirring was continued for 18 h at 20 °C and then the mixture was treated dropwise and sequentially with water (0.5 mL), 15% aqueous NaOH (0.5 mL), and water (1.5 mL). The warmed mixture was filtered and the solids were washed with hot THF. The filtrate was concentrated under reduced pressure below 30 °C to 15 mL and diluted with EtOAc to provide the crude product. Repeated crystallization from dry THF/*i*-Pr$_2$O gave **32** (428 mg, 63%): mp 225−230 °C (hygroscopic); ^{1}H NMR [(CD$_3$)$_2$SO] δ 10.09 (s, 1 H, NH), 9.24 (s, 1 H, NH), 8.58 (d, $J = 5.2$ Hz, 1 H, quinoline H-2), 8.38 (d, $J = 8.0$ Hz, 1 H, quinoline H-8), 8.01 (d, $J = 8.7$ Hz, 2 H, PhH), 7.93 (d, $J = 7.8$ Hz, 1 H, quinoline H-5), 7.74 (t, $J = 7.7$ Hz, 1 H, quinoline H-7), 7.70 (d, $J = 8.5$ Hz, 2 H, PhH), 7.58 (t, $J = 7.7$ Hz, 1 H, quinoline H-6), 7.48 (d, $J = 8.7$ Hz, 2 H, PhH), 7.23−7.15 (m, 3 H, H-3, quinoline H-3, PhH), 4.42 (t, $J = 5.2$ Hz, 1 H, OH), 4.30 (d, $J = 5.3$ Hz, 3 H, CH$_2$OH; OH obscured), 4.26 (d, $J = 5.0$ Hz, 2 H, CH_2OH), 3.76 (t, $J = 6.9$ Hz, 2 H, NCH$_2$), 2.70 (t, $J = 7.3$ Hz, 2 H, CH$_2$), 2.58 (t, $J = 7.6$ Hz, 2 H, CH$_2$), 2.52 (t, $J = 7.6$ Hz, 2 H, CH$_2$), 2.40−2.30 (m, 2 H, NCH$_2$CH_2), 1.82−1.70 (m, 2 H, PhCH$_2$CH_2). HRMS (FAB) [M + H]$^+$: found, 547.2735; calcd for $C_{34}H_{35}N_4O_3$, 547.2709. Anal. ($C_{34}H_{34}N_4O_3$·0.5H$_2$O) C, H, N.

2,3-Dihydro-5-[2-[4-[4-(4-quinolinylamino)benzamido]-phenyl]ethyl]-6,7-bis(hydroxymethyl)-1*H*-pyrrolizine (37). Reaction of 3-(4-nitrophenyl)propionyl chloride with (*S*)-proline as described for **26** gave crude (S)-1-[3-(-4-nitrophenyl)propionyl]pyrrolidine-2-carboxylic acid (**33**) (80%) as a gum: ^{1}H NMR (CDCl$_3$) δ 8.1 (br s, 1 H, CO$_2$H), 8.13 (d, $J = 8.8$ Hz, 2 H, H-3′,5′), 7.40 (d, $J = 8.8$ Hz, 2 H, H-2′,6′), 4.63−4.50 (m, 1 H, CHCO$_2$H), 3.70−3.35 (m, 2 H, CH$_2$), 3.20−3.02 (m, 2 H, CH$_2$), 2.80−2.65 (m, 2 H, CH$_2$), 2.33−1.85 (m, 4 H, 2 × CH$_2$). HRMS (EI) [M$^+$]: found, 292.1053; calcd for $C_{14}H_{16}N_2O_5$, 292.1059.

Reaction of **33** with dimethyl acetylenedicarboxylate by the method described for **27** gave dimethyl 2,3-dihydro-5-[2-(4-nitrophenyl)ethyl]-1*H*-pyrrolizine-6,7-dicarboxylate (**34**) (73% yield): mp (EtOAc) 104−105 °C; ^{1}H NMR (CDCl$_3$) δ 8.12 (d, $J = 8.7$ Hz, 2 H, H-3′,5′), 7.26 (d, $J = 8.7$ Hz, 2 H, H-2′,6′), 3.82 (s, 3 H, CH$_3$), 3.79 (s, 3 H, CH$_3$), 3.52 (t, $J = 7.2$ Hz, 2 H, NCH$_2$), 3.10−2.92 (m, 6 H, 3 × CH$_2$), 2.46−2.27 (m, 2 H, NCH$_2$CH_2). Anal. ($C_{19}H_{20}N_2O_6$) C, H, N.

Hydrogenation of **34** in MeOH/THF (1:1) over Pt/C at 60 psi gave dimethyl 2,3-dihydro-5-[2-(4-aminophenyl)ethyl]-1*H*-pyrrolizine-6,7-dicarboxylate (**35**) (92% yield): mp (EtOAc/petroleum ether) 153−154 °C; ^{1}H NMR (CDCl$_3$) δ 6.82 (d, $J = 7.9$ Hz, 2 H, H-2′,6′), 6.56 (d, $J = 7.9$ Hz, 2 H, H-3′,5′), 3.83 (s, 3 H, CH$_3$), 3.78 (s, 3 H, CH$_3$), 3.59 (br s, 2 H, NH$_2$), 3.35 (t, $J = 7.2$ Hz, 2 H, NCH$_2$), 2.98−2.87 (m, 4 H, 2 × CH$_2$), 2.76 (t, $J = 7.2$ Hz, 2 H, CH$_2$), 2.33−2.23 (m, 2 H, NCH$_2$CH_2). Anal. ($C_{19}H_{22}N_2O_4$) C, H, N.

Reaction of **35** with **19** by the method described for **31** gave a crude product that was triturated with MeOH/EtOAc and crystallized from THF/MeOH/H$_2$O to give dimethyl 2,3-dihydro-5-[2-[4-[4-(4-quinolinylamino)benzamido]phenyl]ethyl]-1*H*-pyrrolizine-6,7-dicarboxylate (**36**) (75% yield): mp 208−209 °C; ^{1}H NMR [(CD$_3$)$_2$SO] δ 10.12 (s, 1 H, NH), 9.26 (s, 1 H, NH), 8.59 (d, $J = 5.2$ Hz, 1 H, quinoline H-2), 8.39 (d, $J = 8.4$ Hz, 1 H, quinoline H-8), 8.02 (d, $J = 8.6$ Hz, 2 H, PhH), 7.94 (d, $J = 8.4$ Hz, 1 H, quinoline H-5), 7.74 (t, $J = 7.6$ Hz, 1 H, quinoline H-7), 7.69 (d, $J = 8.3$ Hz, 2 H, PhH), 7.59 (t, $J = 7.6$ Hz, 1 H, quinoline H-6), 7.49 (d, $J = 8.6$ Hz, 2 H, PhH), 7.22 (d, $J = 5.2$ Hz, 1 H, quinoline H-3), 7.10 (d, $J = 8.3$ Hz, 2 H, PhH), 3.74 (t, $J = 7.1$ Hz, 2 H, NCH$_2$), 3.69 (s, 3 H, CH$_3$), 3.67 (s, 3 H, CH$_3$), 2.94 (t, $J = 7.5$ Hz, 2 H, CH$_2$), 2.89 (t, $J = 7.4$ Hz, 2 H, CH$_2$), 2.78 (t, $J = 7.5$ Hz, 2 H, CH$_2$), 2.39−2.28 (m, 2 H, NCH$_2$CH_2). Anal. ($C_{35}H_{32}N_4O_5$) C, H, N.

Reduction of diester **36** using the method described for **32**, followed by crystallization of the crude product from MeOH/EtOAc (twice) then THF/EtOAc, gave **37** (61% yield): mp 222−227 °C; ^{1}H NMR [(CD$_3$)$_2$SO] δ 10.09 (s, 1 H, NH), 9.24 (s, 1 H, NH), 8.59 (d, $J = 5.2$ Hz, 1 H, quinoline H-2), 8.38 (d, $J = 8.2$ Hz, 1 H, quinoline H-8), 8.01 (d, $J = 8.7$ Hz, 2 H, PhH), 7.94 (d, $J = 8.3$ Hz, 1 H, quinoline H-5), 7.74 (t, $J = 7.6$ Hz, 1 H, quinoline H-7), 7.69 (d, $J = 8.5$ Hz, 2 H, PhH), 7.58 (t, $J = 7.3$ Hz, 1 H, quinoline H-6), 7.48 (d, $J = 8.6$ Hz, 2 H, PhH), 7.21 (d, $J = 5.2$ Hz, 1 H, quinoline H-3), 7.16 (d, $J = 8.5$ Hz, 2 H, PhH), 4.40 (t, $J = 4.6$ Hz, 1 H, OH), 4.33−4.25 (1 H, OH, obscured), 4.30 (d, $J = 4.8$ Hz, 2 H, CH_2OH), 4.27 (d, $J = 4.6$ Hz, 2 H, CH_2OH), 3.67 (t, $J = 6.9$ Hz, 2 H, NCH$_2$), 2.80−2.65 (m, 6 H, 3 × CH$_2$), 2.37−2.25 (m, 2 H, NCH$_2$CH_2). Anal. ($C_{33}H_{32}N_4O_3$·H$_2$O) C, H, N.

Reactivity of Compounds with Calf Thymus DNA. Stock solutions (10 mM) of the compounds in DMSO were prepared freshly. Calf thymus DNA was dissolved in SHE buffer (2 mM Hepes/9 mM NaCl/10 μM EDTA, pH 7.0), and the solution was adjusted to an accurately determined concentration of between 7.5 and 8.5 μM (in base pairs). Solutions of drug (100 μL) and DNA (900 μL) were mixed and immediately placed at 37 °C. At various times (up to 8 h), 45 μL aliquots of the mixture were removed and mixed with 20 μL of 3 N aqueous NaOAc solution and 65 μL of 1-butanol. The mixture was vortexed vigorously and then centrifuged briefly. The top butanol layer contained the unreacted drug, including drug that may have bound noncovalently with DNA. An aliquot (25 μL) of the 1-butanol layer was injected directly into the HPLC, using a 300 × 3.9 mm Bondclone C-18 column and isocratic elution on a Phillips PU-4100 instrument with Unicam software. The mobile phase was a mixture of A, acetonitrile/water (80:20), and B, 0.05 M sodium formate buffer (pH 4.5). The ratio of these two solvents varied (for different compounds) from 70% to 50% of the organic phase. Detection was at 254 nm using a Phillips PU 4120 diode array detector. The data were fitted to the equation $\ln(A)_t = -k_{obs}t + \ln(A)_o$, where $(A)_t$ is the integrated peak area of the unreacted drug at time t and k_{obs} is kinetic rate constant observed for the sum of the rate of drug loss by both by hydrolysis and by alkylation of the DNA. A plot of $\ln(A)_t$ versus time yielded straight-line plots of slope $-k_{obs}$.

DNA Labeling. The primers 1 and 2 (10 pmol; complementary to the gpt2Eco template DNA) were 5′-labeled using [γ-^{32}P]-ATP and T4 polynucleotide kinase. The sample was

then heated at 80 °C for 3 min to inactive the kinase. No further purification is required for the labeled primer. The sequences for primers 1 and 2 are shown in Figure 5.

Cross-Linking Assay. A linear pBR322 plasmid DNA (4.36 kb digested at *Eco*RI, 2 μg) was incubated with drug at concentration of 0.25 mM in TE buffer (10 mM Tris/1 mM EDTA, pH 7.4) at 37 °C for 30 min. The reaction adducts were denatured by adding sodium dodecyl sulfate (SDS) and methylmercury hydroxide at room temperature for 1 h (final concentrations of 0.1% SDS and 25 mM methylmercury hydroxide). The denatured samples were mixed with 0.1 vol of 2-mercaptoethanol and loading buffer, and electrophoresis was carried out on a 1% agarose gel in TAE buffer containing 10% DMSO at 50 V for 2 h. The gel was stained for 30 min in TAE buffer containing 0.5 μg/mL ethidium bromide and photographed using a Polaroid MP 4 Land camera.

Unwinding Assay. Drugs were dissolved in melted 1% agarose gel solution, and six mini-gels containing various drug concentrations (0−12 μM) were made at the same size. The pBR322 DNA (1 μg) was mixed with loading buffer and added to the mini-gels, then electrophoresed on 1% agarose gel in TAE buffer at 50 V for 2.5 h in a cool room (4 °C). The gel was stained for 30 min in TAE buffer containing 0.5 μg/mL ethidium bromide and photographed using a Polaroid MP 4 Land camera.

PCR Reaction (Polymerase Stop Assay). The template was the gpt region (503 bp) of plasmid gpt2Eco amplified using primers 1 and 2. A typical reaction containing 50 ng DNA and 1 μL of 5 mM drug stock in DMSO was carried out in SHE buffer, pH 7.4, for 3 h at 37 °C (total volume 20 μL). Following the reactions, excess drugs were moved by DNA precipitating in ethanol twice. To the dried alkylated DNA was added 5′-end labeled primers 1 and 2 (0.7 μL, 1 pmol/μL), 3 units of Taq DNA polymerase, and 1.25 μL of 10× reaction buffer. The samples were mixed well and chilled on ice before adding 4 μL of 600 mM dNTP solution and one drop of oil. The tubes were returned to ice before placing in the PCR machine preheated to 94 °C. The PCR reaction was performed as follows: cycles 1, 2.5 min at 94 °C; cycles 2−20, 30 s at 94 °C, 30 s at 62 °C, 30 s at 72 °C. The extension products were denatured at 90 °C for 2 min in sequencing gel loading buffer before sequencing gel electrophoresis on a 8% denaturing polyacrylamide gel containing 8.3 M urea. The gels were dried and autoradiographed at −70 °C with an intensifying screen.

Cytotoxicity Assays. These were carried out as previously described.[28] IC_{50} values were determined with log-phase cultures in 96-well microtiter plates, with drug present throughout the growth period (72 h). The IC_{50} values are the average of at least two consistent and independent determinations, calculated as the nominal drug concentration needed to reduce the cell density to 50% of control values. There were eight control wells on each plate.

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