Synthesis and Evaluation of DNA-Targeted Spatially Separated Bis(aniline mustards) as Potential Alkylating Agents with Enhanced DNA Cross-Linking Capability

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DNA-targeted separated bis-mustards were synthesized by attaching aniline mono-mustards at the 4- and 9-positions of the DNA-intercalating ligand 9-aminoacridine-4-carboxamide, with the intention of improving the low cross-link to monoadduct ratio found with most alkylating agents. The geometry of these compounds requires that, when the acridine binds to DNA by intercalation, one alkylating moiety is delivered to each DNA groove. Gel electrophoretic studies show that only one arm of these compounds (probably that attached to the 9-position) alkylates DNA, such alkylation occurring specifically in the major groove at the N7 of guanines. Cell-line studies confirm that the mode of cytotoxicity of these compounds (unlike that of untargeted aniline bis-mustards of comparable reactivity) is due to bulky DNA monoadduct formation. It is concluded that more information is required about the exact orientation of the initial monoadducts before ligands with specific DNA cross-linking ability can be designed.

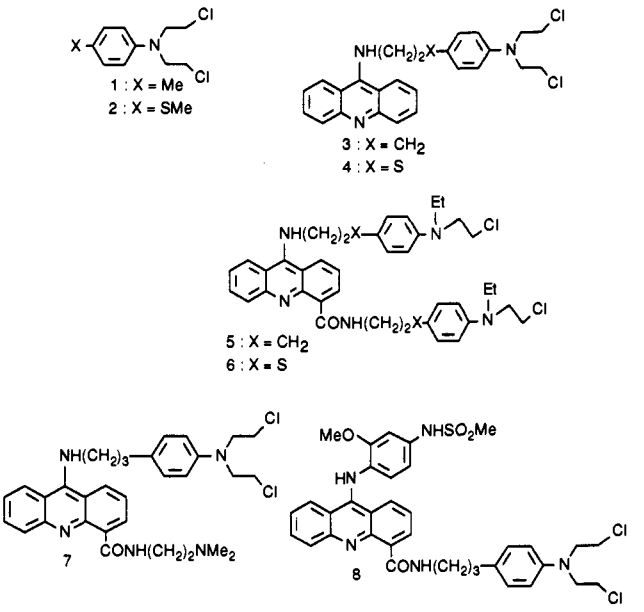
We and others[1,2] have recently discussed the concept[3,4] of targeting alkylating agents to DNA by attaching them to DNA-affinic carrier molecules, specifically DNA-intercalating ligands. The overall aims of this approach include increasing intrinsic drug potency,[1] altering the pattern of DNA lesions formed[5] (and their repair), and improving the low monoadduct (genotoxic lesions) to cross-link (cytotoxic lesions) ratio observed with nontargeted alkylating agents.[6]

We have recently shown[1] that the intrinsic cytotoxicities of simple aniline mustards (e.g. **1** and **2** Chart I) can be increased by up to 100-fold when they are attached to the DNA-affinic intercalating agent 9-aminoacridine.[1] The patterns of alkylation of DNA by the corresponding targeted compounds (e.g. **3** and **4**) are also altered in comparison with the parent mustards, particularly in terms of sequence selectivity of guanine alkylation.[7] The DNA-targeted mustards showed improved in vivo antitumor activity over both the parent mustards and the clinically used aniline mustard derivative chlorambucil.[1] However, these compounds do not appear to have greatly improved ratios of DNA cross-linking compared with monoadduct formation. Thus compound **3**, at a drug/base-pair ratio of 0.02, has been shown[7] to perform on average not more than one cross-link per 4362 base pairs in a linear pBR322 DNA fragment, whereas at the same input ratio 20 monoalkylation events occur on average. The ratio for the less reactive mustard **4** is even lower.[7]

The low level of cross-linking by bifunctional intercalating agents in general appears to result from the slow rate of the second alkylation event, which can take hours.[8,9] This exposes the second alkylating moiety to competitive hydrolysis, which can be accelerated in the microenvironment of the DNA. Recent work[10] on the cross-linking of DNA by mitomycin C has shown that the critical event determining the rate of the second alkylation event is favorable spatial orientation of the second alkylating moiety with respect to its nucleophilic DNA site. Thus, while mitomycin C forms the initial monoadducts at equal rates in both GpC and CpG sequences, cross-links are confined to CpG sequences, where the distance between the target sites (guanine 2-amino groups) in the free DNA is close to their separation in the cross-linked adduct.[10]

These results indicate that, to design DNA-targeted bifunctional alkylating agents with improved cross-linking properties, the carrier moiety must deliver the alkylating functions to the DNA in a specific orientation. This re-

Chart I



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(1) Gourdie, T. A.; Valu, K. K.; Gravatt, G. L.; Boritzki, T. J.; Baguley, B. C.; Wakelin, L. P. G.; Wilson, W. R.; Woodgate, P. D.; Denny, W. A. *J. Med. Chem.* **1990**, *33*, 1177.
(2) Koyama, M.; Kelly, T. R.; Wanatabe, K. A. *J. Med. Chem.* **1988**, *31*, 284.
(3) Kohn, K. W.; Hartley, J. A.; Mattes, W. B. *Nucleic Acids Res.* **1987**, *15*, 10531.
(4) Denny, W. A. *Anti-Cancer Drug Des.* **1989**, *4*, 241.
(5) Kohn, K. W.; Hartley, J. A.; Mattes, W. B. *Biochem. Pharmacol.* **1988**, *37*, 1799.
(6) Brendel, M.; Ruhland, A. *Mutat. Res.* **1984**, *13*, 51.
(7) Prakash, A. S.; Denny, W. A.; Gourdie, T. A.; Valu, K. K.; Woodgate, P. D.; Wakelin, L. P. G. *Biochemistry*, in press.
(8) Ross, W. E.; Ewing, R. A. G.; Kohn, K. W. *Cancer Res.* **1978**, *38*, 1502.
(9) Butour, J-L.; Johnson, N. P. *Biochemistry* **1986**, *25*, 4534.
(10) Teng, S. P.; Woodson, S. A.; Crothers, D. M. *Biochemistry* **1989**, *28*, 3901.

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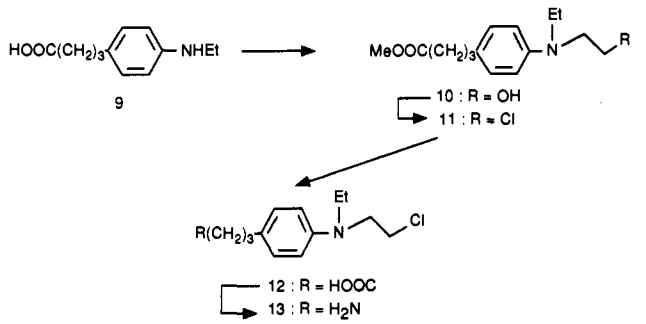
Scheme I



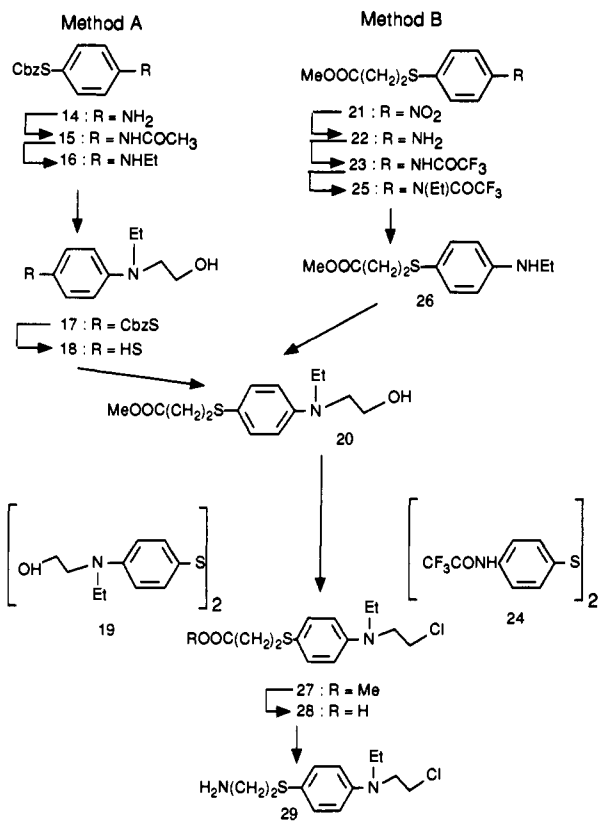
quires a suitable choice not only of the targeting ligand but also of the alkylating function. We have previously employed aniline mustards as alkylating functions in these classes of compounds because of their well-understood alkylation chemistry and because their reactivity can be modulated over a wide range by suitable choice of substituents in the aromatic ring.[1,11] However, the fixed geometry of the two alkylating moieties is a possible restriction, and in this study we explore the consequences of relaxing this criterion by employing two spatially separated aniline mono-mustards on a DNA-intercalating chromophore.

The compounds chosen for synthesis and evaluation were the acridine derivatives **5** and **6**. Studies with the corresponding "nonseparated" mustards **3** and **4** showed (particularly in the case of 4) that these compounds had a striking preference for alkylating guanines in 5′-GT sequences, probably in the N7 (major groove) site, due to the orientational preference of the chromophore and the rigidity of the short alkyl linker chain.[7] Previous work with related 4,9-disubstituted acridines has suggested that these compounds orient on DNA by maximal overlap of the acridine chromophore in the intercalation site, an arrangement which must place the two side chains at these positions in different DNA grooves.[12] This information suggests that compounds such as **5** and **6** will bind to DNA in a preferred orientation, "threading" through the DNA and placing the 9-side chain in the major groove where it can alkylate guanine N7 positions (as do the topologically related compounds **3** and 4).[7] In this orientation, the other side chain is then well-placed to alkylate the adenine N3 positions in the minor groove (the next most nucleophilic DNA site). The choice of mustard link groups (CH_2 and S) provide molecules of high and moderate alkylating reactivity, respectively.

Chemistry

The CH_2-substituted amine side chain **13** required for the synthesis of **5** is a homologue of the chlorambucil-related compounds previously prepared by Connors et al.[13] Our synthesis of **13** was based on use of a carboxylic acid as the amine synthon, employing a modified Curtius procedure[14] to expose the amine in the last step[1] (Scheme I). Reaction of methyl 4-[4-(ethylamino)phenyl]butanoate (**9**)[15] with oxirane gave the hydroxymethyl derivative **10**, which was converted to the chloride **11** in 92% yield via the mesylate.[16] The corresponding acid **12** was subjected to the Curtius rearrangement by using the method of Kaiser and Weinstock,[14] to give the desired amine **13** in 84% yield.

Scheme II

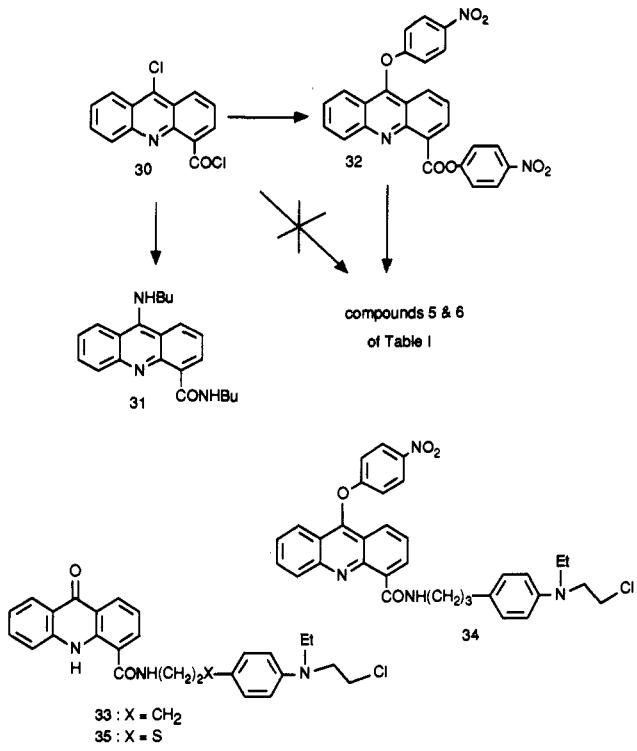


The initial route to the analogous sulfur side chain **29** began with *S*-(benzyloxycarbonyl)-4-aminobenzenethiol[17] (**14**) (Scheme II method a). Various attempts to N ethylate this material directly were not successful, so the corresponding *N*-acetate **15** was prepared and reduced with borane in THF in an inert atmosphere to give *S*-(benzyloxycarbonyl)-4-(ethylamino)benzenethiol (**16**) in 61% yield. This was then reacted with oxirane to give **17**. Removal of the benzyloxycarbonyl functionality from **17** proved to be difficult. Various methods were investigated, and the best appeared to be sodium hydroxide in ethanol at room temperature, which gave the free thiol **18** in 58% yield, although workup of the complex reaction mixture was tedious. This compound was relatively unstable, but was characterized by NMR and mass spectra. Also isolated from the reaction was the disulfide **19** (23%). The free thiol **18** was alkylated with methyl 3-bromopropanoate in refluxing methanol containing anhydrous potassium carbonate to give the desired methyl 3-[4-[(2-hydroxyethyl)ethylamino]phenyl]propanoate (**20**) (53%), together with the disulfide **19** (14%).

Due to the difficulty of isolating pure **18** from the deprotection reactions, and the ready formation of the disulfide **19** by aerial oxidation on further manipulations, an alternative approach to **20** was then considered (Scheme II method b). Reduction of methyl 3-[(4-nitrophenyl)thio]propanoate (**21**)[18] with stannous chloride in refluxing

(11) Palmer, B. D.; Wilson, W. R.; Pullen, S. M.; Denny, W. A. *J. Med. Chem.* **1990**, *33*, 112.
(12) Denny, W. A.; Wakelin, L. P. G. *J. Med. Chem.* **1990**, *33*, 2039.
(13) Connors, T. A.; Ross, W. C. J.; Wilson, J. G. *J. Chem. Soc.* **1960**, 2994.
(14) Kaiser, C.; Weinstock, J. *Org. Syn.* **1971**, *51*, 48.
(15) Hyde, K. A.; Edward, M. A.; Skinner, W. A.; Goodman, L.; Baker, B. R. *J. Org. Chem.* **1961**, *26*, 3551.
(16) Collington, E. W.; Meyers, A. I. *J. Org. Chem.* **1971**, *36*, 3044.
(17) Valu, K. K.; Gourdie, T. A.; Boritzki, T. J.; Gravatt, G. L.; Baguley, B. C.; Wilson, W. R.; Wakelin, L. P. G.; Woodgate, P. D.; Denny, W. A. *J. Med. Chem.*, in press.
(18) Fel'dman, I. K.; Mikhalova, V. N. *Zhur. Obshchei, Khim.* **1961**, *31*, 2115; *Chem. Abstr.* **1962**, *56*, 1377.

Scheme III



ethanol and reaction of the resulting amine **22** with trifluoroacetic anhydride in dichloromethane gave the *N*-trifluoroacetate **23** (85%), together with a small amount of the disulfide **24**. Refluxing **23** with 1.3 equiv of potassium hydroxide and excess iodoethane in acetone for 7 h (modified conditions of McEvoy and Allen[19]) gave the *N*-ethyl derivative **25** (70%), which on mild hydrolysis[20] gave an 89% yield of methyl 3-[[4-(ethylamino)phenyl]-thio]propanoate (**26**). Alkylation of this with oxirane in tetrahydrofuran and glacial acetic acid then gave **20** in 62% yield.

While both routes gave similar overall yields of **20** the latter (Scheme II method b) was the more reliable. This compound was then elaborated to the corresponding mustard **27** in 72% yield with use of methanesulfonyl chloride followed by lithium chloride. The presence of the mustard functionality was supported by the presence of a four proton multiplet at δ 3.61 in the ^{1}H NMR spectrum, and a shift in resonances in the ^{13}C NMR spectrum to δ 40.3 (CH_2Cl) and 52.2 (NCH_2). Hydrolysis of the methyl ester and treatment of the resulting acid **28** under the Curtius conditions mentioned above gave the desired amine **29** in 71% yield.

While, as mentioned above, the route chosen permits the attachment of different amine side chains at the 4 and 9 positions of acridine, the present work focused on attachment of the same side chain at both positions to give the target substituted 9-aminoacridine-4-carboxamide compounds **5** and **6**. Initial efforts employed 9-chloroacridine-4-carbonyl chloride[21] (**30**), as the functionalized acridine (Scheme III). The acid chloride was expected to react rapidly with the amine in the presence of a nonnucleophilic base to give the amide, with the slow step being nucleophilic displacement of the 9-chloro group (although displacement of this group with aliphatic amines under mild conditions has been achieved in good yields[22]).

In the event, while reaction of **30** with 2.2 equiv of butylamine (as a model amine) and triethylamine gave *N*,9-bis(butylamino)acridine-4-carboxamide (**31**) in 75% yield, the amine **29** under the same conditions gave none of the desired compound **6**, but only a mixture of the corresponding 9-chloroacridine and acridone compounds, due to the failure to react at the 9-position. Accordingly, alternative leaving groups were considered. Although 9-phenoxyacridines (prepared in situ) have been used widely for the preparation of 9-(alkylamino)acridines,[23] the concomitant formation of the relatively unreactive 4-phenoxy ester which would occur in the present case made the route unattractive. However, Atwell et al.[21] have successfully displaced the 4-nitrophenyl ester from 4-nitrophenyl 9-chloroacridine-4-carboxylate with aliphatic amines under mild conditions. Therefore, the doubly activated 4-nitrophenyl 9-(4-nitrophenoxy)acridine-4-carboxylate (**23**) was prepared by treating **30** with an excess of sodium 4-nitrophenolate in dry tetrahydrofuran at 0 °C under an inert atmosphere and then heating the mixture under reflux for 2.5 h. This compound was very labile, and the crude product was used directly.

Reaction of crude **32** with the amine **29** and triethylamine in dichloromethane at 0 °C, followed by heating under reflux for 5 h, gave a mixture from which was isolated the desired bis-mustard, *N*,9-bis[[2-[[4-[(2-chloroethyl)ethylamino]phenyl]thio]ethyl]amino]acridine-4-carboxamide (**6**), in 15% yield. The peaks at *m/z* 720/722/724 in the FAB mass spectrum of **6** corresponded to the expected molecular ion cluster of this compound ($C_{38}H_{43}Cl_2N_5OS_2$) plus a hydrogen from the glycerol matrix. The ^{1}H NMR spectrum of **6** confirmed the presence of both the 9-amino and 4-carboxamido side chains. A 6-proton multiplet at δ 1.15 and a 4-proton multiplet at 3.39 were attributed to the ethyl groups of both side chains. The NCH_2CH_2Cl methylene protons of the amido side chain corresponded to the 4-proton singlet at δ 3.57, and those of the amine side chain to the singlet at 3.59. The remaining methylene protons of the amide side chain appeared at δ 3.09 and 3.81, and the corresponding amine side chain protons appeared at 3.15 and 3.95. The ^{13}C NMR spectrum supported the ^{1}H NMR spectrum in confirming the presence of both side chains. Also isolated from the reaction was the acridone **35** (44%), which was identified from its ^{1}H NMR spectrum and chromatographic behavior.

Reaction of the corresponding amine **13** with **32** (using a slightly modified procedure, see Experimental Section) gave the methylene-linked analogue, *N*,9-bis[[3-[4-[(2-chloroethyl)ethylamino]phenyl]propyl]amino]acridine-4-carboxamide (**5**), also in 15% yield. The derived hydrochloride analyzed correctly for $C_{40}H_{47}Cl_2N_3O\cdot 3HCl$, indicating that both side chains and the acridine chromophore were protonated. The mass spectrum of **5** contained the molecular ion at *m/z* 683, corresponding to the molecular formula, $C_{40}H_{47}Cl_2N_3O$. The ^{1}H NMR spectrum supported the presence of both the 9-amino and 4-carboxamido side chains. A six proton triplet at δ 1.15 and a four proton multiplet at 3.38 indicated the presence of two *N*-ethyl groups. An eight proton singlet at δ 3.58 was attributed to the two NCH_2CH_2Cl groups. Two multiplets at δ 2.06 and 3.655 and a triplet at 2.69 were assigned to the remaining methylene protons of the amide side chain, while

(19) McEvoy, F. J.; Allen, G. R. *J. Med. Chem.* **1974**, *17*, 281.
(20) Newman, H. *J. Org. Chem.* **1965**, *30*, 1287.
(21) Atwell, G. J.; Cain, B. F.; Baguley, B. C.; Finlay, G. J.; Denny, W. A. *J. Med. Chem.* **1984**, *27*, 1481.
(22) Buchardt, O.; Ehrbar, U.; Larson, C.; Moller, J.; Nielsen, P. E.; Thomsen, T.; Watjen, F.; Hansen, J. B. *J. Org. Chem.* **1984**, *49*, 4123.
(23) Denny, W. A.; Atwell, G. J.; Baguley, B. C.; Wakelin, I. P. G. *J. Med. Chem.* **1985**, *28*, 1568.

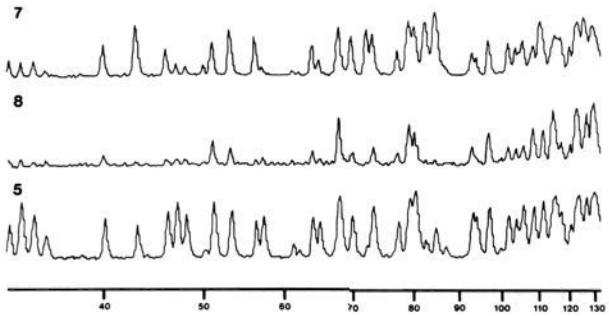


Figure 1. Densitometer scans obtained from the strand cleavage patterns of compounds **5** (bottom panel), **7** (top panel), and **8** (middle panel). Each scan is normalized to its most intense peak.

a multiplet at 2.17, a broad singlet at 3.95, and a triplet at 2.76 were attributed to the corresponding methylene protons in the amine side chain.

A mixture of the corresponding acridone **33** and the 9-(4-nitrophenoxy) compound **34** (75% by weight of the product mixture) was also obtained from this reaction. The presence of **34** was indicated by the complex aromatic region in the ^{1}H NMR spectrum and by the relatively large integral in this region. The mass spectrum of this mixture contained an ion at m/z 582, corresponding to the expected molecular ion for **34** and also an ion at 461 corresponding to the expected molecular ion for the acridone **33**. Extensive purification of the mixture gave a small amount (6%) of the pure acridone **33**.

DNA Alkylation

The interaction of compound **5** with DNA was studied in detail, with use of a 3′-end labeled 375 base-pair fragment of plasmid pBR322 DNA (between the EcoRI and BamH1 cleavage sites). Comparative studies were also carried out with compounds **7** and **8**, which were chosen[24] as the closest available analogues of **5** carrying CH_2-linked aniline mustard moieties of comparable reactivity at (respectively) the 9- and 4-acridine positions.

Densitometer scans of the strand cleavage patterns of DNA treated with drugs **5**, **7**, and **8** are shown in Figure 1. Compound **7** produces bands corresponding to every guanine in the fragment, but bands corresponding to adenines occur only for those in 5′-AC sequences (bands 43, 46, 56, 73, 83, and 85). In comparison compound **3**, which lacks the carboxamide side chain of **7**, has been shown to produce guanine bands only.[7] With compound **5**, strong bands for all guanines as well as for adenines occurring in runs of As were observed, while compound **8** (which carries the mustard attached to the acridine 4-position) shows bands for guanines only. The drug to base pair ratios required to produce similar levels of DNA alkylation for compounds **5**, **7**, and **8** were 0.40, 0.04, and 0.80, respectively.

Figure 2 shows the effect of Mg^{2+} on the alkylation patterns of **5** and **7**. For both compounds, the presence of Mg^{2+} dramatically reduces the intensities of adenine bands (bands 33–35, 46–48, 56, 57, 61, 62, 65, and 85) but affects the guanine bands only moderately when compared with the patterns obtained in the presence of Na^+ of equal ionic strength (0.04). In contrast, increasing the ionic strength from 0.01 to 0.04 by the addition of Na^+ reduces the overall alkylation by **5**, but does not affect the band pattern at all (see lanes 5, 5′, and 5″). In our earlier work[7] we had shown that Mg^{2+} inhibits alkylation of adenine at N7 sites in the major groove but not at N3 sites in the

(24) Syntheses of compounds **7** and **8** will be reported shortly.

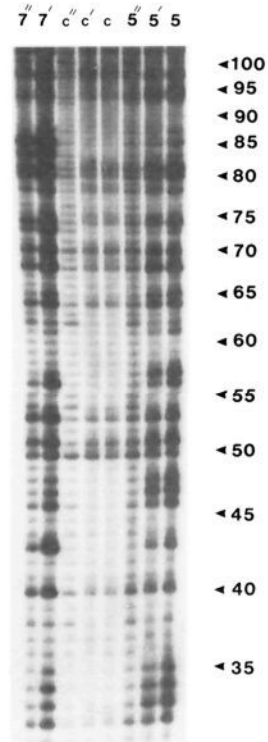


Figure 2. Autoradiograph showing the effects of Mg^{2+} on the alkylation pattern of **5** and **7**. Lanes c and 5 correspond to DNA control and DNA treated with compound **5** in buffer only (ionic strength 0.01). Lanes c′, 5′, and 7′correspond to control DNA sample and drug-treated samples in the presence of 30 mM Na^+, and lanes c″, 5″, and 7″ correspond to control DNA sample and drug-treated DNA samples in the presence of 10 mM Mg^{2+} (ionic strength 0.04).

minor groove. The above results with Mg^{2+} imply that the adenine alkylation by both compounds **5** and **7** occurs in the major groove.

Kinetic studies of the dissociation of DNA complexes of 9-aminoacridine-4-carboxamides related to **7** have suggested that the 4-substituted carboxamide chain resides in the minor groove,[25] with the compounds binding much more tightly to DNA than do the corresponding 9-aminoacridines. It is also known that ligand binding in the minor groove leads to opening up of the major groove,[26] and examples of minor groove binding ligands modifying the reactivity of alkylating agents in the major groove have been reported.[27,28] Thus it is not surprising that **7** shows enhanced major groove reactivity at N7 of adenines in 5′-AC sequences, in comparison with compound **3** which lacks this minor groove binding side chain.

The lack of alkylation in the minor groove by compound **5** (implied by the Mg^{2+} results) suggests that the monomustard group on the 4-substituted carboxamide side chain (which is likely to reside in the minor groove[25]) is not contributing to the observed adenine alkylation. The preference of **5** for alkylating runs of As is similar to that of the simple mustards **1** and **2**, which also have been

(25) Wakelin, L. P. G.; Atwell, G. J.; Rewcastle, G. W.; Denny, W. A. *J. Med. Chem.* **1987**, *30*, 855.
(26) Kopka, M. L.; Yoon, C.; Goodsell, D.; Pjura, P.; Dickerson, R. E. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *182*, 1376.
(27) Rajalakshmi, S.; Rao, P. M.; Sarma, D. S. R. *Teratog., Carcinog., and Mutagen.* **1980**, *1*, 97.
(28) Wilmanska, D.; Malagocka, E.; Szimigiero, L.; Gniazdowski, M. *Biochim. Biophys. Acta* **1983**, *782*, 285.

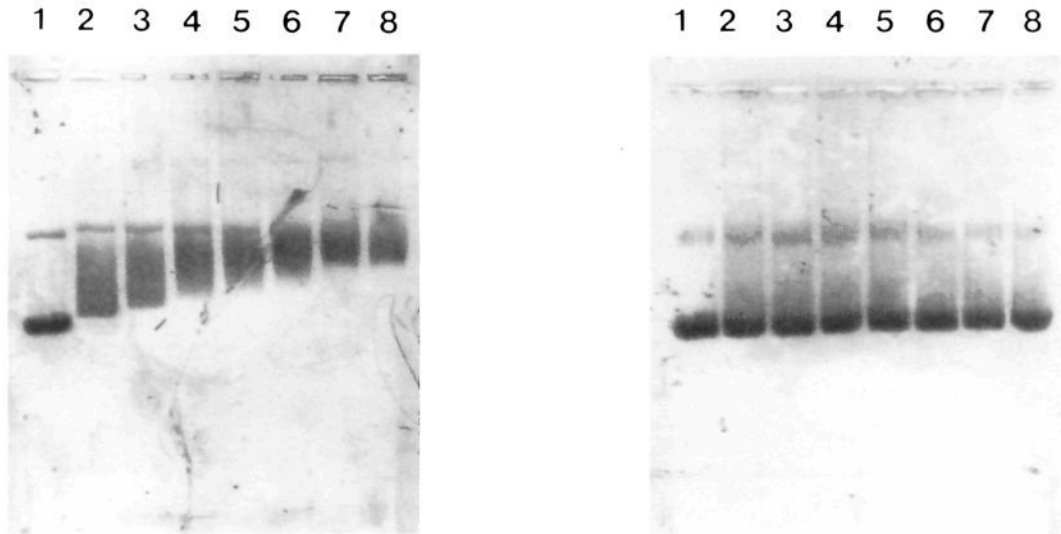


Figure 3. Results of unwinding of closed circular superhelical plasmid pBR322 in the presence of compounds **5** (right) and **7** (left), at drug to base pair ratios 0.00, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35 and 0.40 (lanes numbered 1–8, respectively).

shown to alkylate at the N7 of adenines in the major groove.[7] Further evidence that the 4-substituted side chain is not involved in the minor groove alkylation comes from studies with compound **8**, which carries a full mustard moiety on its 4-substituted carboxamide side chain, yet shows no reactivity toward adenines whatsoever and produces only weak alkylation at guanines at a high drug to base-pair ratio (0.8).

Helix unwinding assays (Figure 3) show that **7** removes the superhelical turns from covalently closed circular pBR322 DNA quite efficiently, but the equivalence point is not reached even at a drug/base-pair ratio of 0.4. In comparison, compound **3** was shown[7] to induce supercoiling in the reverse direction, above a ratio of 0.2. This suggests that, while **7** binds strongly to DNA, the presence of the minor groove binding carboxamide side chain prevents the DNA from rewinding. Compound **5** shows no effects on supercoiling, presumably due to its low reactivity. At a drug/base-pair ratio of 0.40 only about 10 to 15 sites on the DNA are alkylated, whereas similar levels of alkylation occurs with compound **7** at a drug/base-pair ratio of 0.04.

Biological Activity

The in vitro cytotoxicities of the compounds were determined as described previously[29] against the murine leukemia P388 and Chinese hamster ovary derived AA8 and UV4 cell lines and are given in Table I. The UV4 subline of AA8[30,31] lacks the ability to perform the incision step of the excision repair pathway of DNA damage repair, and these cells are consequently from 2–10-fold more sensitive than AA8 to compounds, which form bulky DNA monoadducts, and from 8–200-fold more sensitive than AA8 to DNA interstrand cross-linking agents.[32] The ratio of the IC_{50} values of a drug in these two cell lines (hypersensitivity factor, HF) can thus provide information about the primary mechanism of cytotoxicity of DNA-reactive agents. Large HFs (>20) are indicative of DNA cross-linking ability, while lesser but significant ratios (3–15) are suggestive of bulky monoadduct formation. The DNA-targeted separated mustards **5** and **6** are more potent cytotoxins than the corresponding untargeted mustards (**1** and **2**, respectively), with the compound bearing the more reactive methylene-linked alkylating moiety showing the greater cytotoxicity in each case. The DNA-targeted full mustard **7** was also very cytotoxic, but **8** was much less so, in agreement with the gel results, which suggest it is a less efficient alkylator. As shown previously,[1] both of the untargeted mustards **1** and **2** have HFs exceeding 30, indicative of DNA cross-linking, and the full mustards **7** and **8** also appeared to cross-link. However, the separated mustards **5** and **6** have HFs of 5–7, suggesting that while their cytotoxic effects are due to DNA alkylation they result from monoadducts and not cross-links.

The more active methylene-linked mustard **5** was evaluated against P388 leukemia in vivo,[33] but showed only

Table I. Biological Activity of Mustard Derivatives

	in vitro cytotoxicity				
	IC_{50}[a] μM			P388 in vivo	
compd	P388	AA8	HF[b]	OD[c]	ILS[d]
1[e]	0.63	2.9	42	65	36
2[e]	1.47	5.3	33	65	NA[f]
5	0.29	1.5	5.4	45	25
6	0.55	9.4	7.4	ND[g]	ND[g]
7	0.06	0.21	19	13.3	NA
8	0.18	>4000	>50	65	26
chlorambucil[e]	6.8	26	58	225	37

[a] IC_{50} values determined against either P388 or AA8 cells, as described in ref 22 and outlined in the text, and the standard error of the mean. [b] HF = hypersensitivity factor = IC_{50} (AA8)/IC_{50} (UV4), as outlined in the text. [c] OD = optimal dose of drug in milligrams/kilogram, administered as a single dose in 0.1 or 0.2 mL at 30% v/v ethanol/water on day 1 after intraperitoneal inoculation of 10^6 P388 leukemia cells. [d] ILS = the percentage increase in lifespan of drug-related tumor-bearing animals when heated at the optimal dose (determined by spanning a range of doses from inactive to toxic at 1.5-fold intervals). Values above 20% are considered statistically significant. [e] Data taken from ref 1. [f] No activity seen at all dose levels, including toxic ones. [g] Not tested.

(29) Wilson, W. R.; Thompson, L. H.; Anderson, R. F.; Denny, W. A. *J. Med. Chem.* **1989**, *32*, 31.
(30) Thompson, L. H.; Rubin, J. S.; Cleaver, J. E.; Whitmore, G. F.; Brookman, K. *Somatic Cell Genet.* **1980**, *6*, 391.
(31) Westerfeld, A.; Hoeijmakers, J. H. H.; van Duin, M.; de Witt, J.; Odijk, H.; Pastink, A.; Wood, R. D.; Bootsma, D. *Nature* **1984**, *310*, 425.
(32) Hoy, C. A., Thompson, L. H., Mooney, C. L., Salazar, E. P. *Cancer Res.* **1985**, *45*, 1737.
(33) Baguley, B. C.; Wilson, W. R. *Eur. J. Cancer Clin. Oncol.* **1987**, *23*, 607.

low activity (Table I), which is not unexpected in view of its lack of DNA cross-linking ability.

Conclusions

Both the electrophoresis studies of DNA alkylation and the cell line studies indicate clearly that the bis-mustards **5** and **6** only monoalkylate DNA. The principal reaction is at N7 of guanines in the major groove. While it is less clear which of the mustards reacts, the weight of evidence (i.e. comparison of **5** and **8**) favors alkylation by the 9-linked side chain. These results reinforce the conclusions of Crothers et al.[10] that the critical event in completing a cross-linking event is correct positioning of the second alkylator with respect to a nucleophilic DNA site. While the full mustard moiety has obvious structural limitations, it does have the correct geometry to bridge between guanine N7 sites. Although the 4,9-disubstituted acridine carrier molecule in these studies was carefully selected as having a well-understood DNA orientation,[12] it is clear that more information is required about the exact orientation of the initial monoadducts before ligands with specific DNA cross-linking ability can be designed.

Experimental Section

Chemistry. Elemental analyses were carried out by the Microchemical Laboratory, University of Otago, and are indicated by the symbols of the elements where they are within ±0.4% of theoretical values. Melting points were determined on an Electrothermal apparatus and are uncorrected. High-resolution mass spectra were recorded on a VG-70 spectrometer at nominal 5000 resolution. NMR spectra were recorded on Bruker WP-60 or AM-400 spectrometers (400 MHz unless noted otherwise) and are reported as chemical shifts in ppm downfield from Me_4Si.

A. CH_2 Side Chain. Methyl 4-[4-[Ethyl(2-hydroxyethyl)amino]phenyl]butanoate (10). Oxirane (1.0 mL, 20.1 mmol) was added to a solution of methyl 4-[4-(ethylamino)phenyl]butanoate[15] (**9**) (6.40 g, 0.29 mmol) in THF (50 mL) and glacial AcOH (50 mL). The mixture was stirred for 4 days with a further addition of oxirane (1.0 mL, 20.1 mmol) being made on the second day, then diluted with water, neutralized with solid $NaHCO_3$, and extracted with EtOAc. The organic layer was worked up to give a residue which was chromatographed on silica gel. Elution with EtOAc/hexanes (1:9) gave recovered amine **9** (0.67 g, 10%), and elution with EtOAc/hexanes (1:1) gave methyl 4-[4-[ethyl(2-hydroxyethyl)amino]phenyl]butanoate (**10**) (6.90 g, 90%) as a colorless oil: ^1H NMR ($CDCl_3$; 60 MHz) δ 1.10 (t, J = 6.9 Hz, 3 H, Me), (1.90, m, 2 H, $CH_2CH_2CH_2CO$), 2.35 (m, 4 H, $CH_2CH_2CH_2CO$), 3.41 (m, 4 H, NCH_2CH_3, NCH_2CH_2OH), 3.63 (s, 3 H, OMe), 3.72 (m, 2 H, CH_2OH), 6.66 (d, J = 8.9 Hz, 2 H, H-3), 7.16 (d, J = 8.9 Hz, 2 H, H-2).

4-[4-[(2-Chloroethyl)ethylamino]phenyl]butanoic Acid (12). To an ice-cold solution of **10** (4.20 g, 15.8 mmol) in dry CH_2Cl_2 (150 mL) was added Et_3N (3.31 mL, 23.8 mmol) followed by methanesulfonyl chloride (1.85 mL, 24.0 mmol). The solution was stirred for 30 min at 0 °C, then diluted with CH_2Cl_2, washed with aqueous $NaHCO_3$, and worked up as usual. The crude mesylate (4.34 g, 80%) was dissolved in dry DMF (20 mL), LiCl (1.34 g, 31.6 mmol) was added, and the mixture was heated to 120 °C for 5 min. The solvent was removed under reduced pressure, and the residue was chromatographed on silica gel. Elution with EtOAc/hexanes (1:19) gave methyl 4-[4-[(2-chloroethyl)ethylamino]phenyl]butanoate (**11**) (3.35 g, 71%) as a colorless oil. This was hydrolyzed by heating under reflux in concentrated HCl for 45 min, cooling, neutralizing with concentrated aqueous ammonia, and extracting with EtOAc. Usual workup gave 4-[4-[(2-chloroethyl)ethylamino]phenyl]butanoic acid (**12**) (2.92 g, 92%) as a white solid, mp (benzene/hexanes) 61–62 °C (lit.[15] mp 61–62 °C).

3-[4-[(2-Chloroethyl)ethylamino]phenyl]propanamine (13). According to the procedure of Kaiser and Weinstock,[14] a solution of 4-[4-[(2-chloroethyl)ethylamino]phenyl]butanoic acid (**12**) (2.92 g, 10.9 mmol) in dry Me_2CO (60 mL) at 0 °C was treated successively with Et_3N (1.35 g, 9.78 mmol) and a solution of ethyl chloroformate (0.93 mL, 9.78 mmol) in dry Me_2CO (15 mL). The mixture was stirred at 0 °C for 10 min, and a solution of NaN_3 (0.90 g, 16.7 mmol) in water (15 mL) was added. The mixture was stirred for a further 30 min, poured into ice water, and extracted with toluene (2 × 200 mL). The combined extracts were dried (Na_2SO_4) and then heated under reflux for 1 h. Solvent was removed under reduced pressure, and the residue was dissolved in 2 N HCl (20 mL) and heated under reflux for 10 min. The pH of the cooled mixture was adjusted to 12 with concentrated ammonia, and it was extracted twice with EtOAc. Workup of the organic phase gave 3-[4-[(2-chloroethyl)ethylamino]phenyl]propanamine (**13**) (2.18 g, 84%) as a yellow-brown oil: ^1H NMR ($CDCl_3$; 60 MHz) δ 1.11 (t, J = 7.1 Hz, 3 H, Me), 1.80 (m, 2 H, $NH_2CH_2CH_2$), 2.52 (m, 4 H, $NH_2CH_2CH_2CH_2$), 3.29 (m, 2 H, NCH_2CH_3), 3.56 (s, 4 H, NCH_2CH_2Cl), 6.52 (d, J = 8.3 Hz, 2 H, H-3), 7.00 (d, J = 8.4 Hz, 2 H, H-2). The compound was not characterized further, and was used directly.

B. S Side Chain. *S*-(Benzyloxycarbonyl)-4-acetamidobenzenethiol (15). Concentrated aqueous HCl (6.62 mL, 72.6 mmol) was added to a suspension of *S*-(benzyloxycarbonyl)-4-aminobenzenethiol[17] (**14**) (18.8 g, 72.6 mmol) in water (350 mL) and THF (150 mL). Acetic anhydride (7.60 mL, 80.3 mmol) and sodium acetate (6.00 g, 73.1 mmol) were added sequentially, and the resulting mixture was shaken vigorously for 10 min, cooled on ice, and filtered. The resulting solid was dissolved in EtOAc and washed with water and brine, and the organic phase was concentrated under reduced pressure to give *S*-(benzyloxycarbonyl)-4-acetamidobenzenethiol (**15**) (19.4 g, 89%): mp (MeOH) 138 °C; IR(KBr) λ_{max} 3235, 1725 (OCO), 1676 (NHCO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.12 (s, 3 H, Me), 5.26 (s, 2 H, CH_2), 7.36 (m, phenyl-H), 7.43 (d, J = 8.7 Hz, 2 H, H-3), 7.52 (d, J = 8.7 Hz, 2 H, H-2), 7.64 (br s, 1 H, NH); ^{13}C NMR ($CDCl_3$) δ 24.6 (CH_3), 69.5 (CH_2), 120.1 (C-3), 121.9 (C-1), 128.65, 128.5, (phenyl-C), 134.9 (C-ipso), 135.8 (C-2) 139.5, (C-4), 168.5 (NHCO), 170.5, OCO; mass spectrum, m/z 301 (M, 7), 257 (M - CO_2), 167 ($HSC_6H_4NHCOMe$, 100), 124 (167 - MeCO), 91 ($PhCH_2$), 80, 65, 51, 43 (MeCO). Anal. ($C_{16}H_{15}NO_3S$) C, H, N.

***S*-(Benzyloxycarbonyl)-4-(ethylamino)benzenethiol (16).** A solution of S-(benzyloxycarbonyl)-4-acetamidobenzenethiol (**15**) (17.5 g, 58.1 mmol) in THF (90 mL) was treated slowly with a 1.0 M solution of borane in THF (100 mL, 0.10 mol) under N_2, and the mixture was heated under reflux for 20 h. The cooled reaction mixture was quenched with water (10 mL), the solvent was removed under reduced pressure, and the residue was partitioned between EtOAc and brine. The organic phase yielded *S*-(benzyloxycarbonyl)-4-(ethylamino)benzenethiol (**16**) (10.2 g, 61%): mp (PhH/hexane) 108 °C; IR(KBr) λ_{max} 1710 (CO), 1595, 1120 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.30 (t, J = 7.1 Hz, 3 H, Me), 5.28 (s, 2 H, CH_2), 3.22 (m, J = 7.2 Hz, 2 H, $NHCH_2$), 5.28 (s, 2 H, CH_2O), 6.62 (d, J = 8.8 Hz, 2 H, H-3), 7.34 (d, J = 8.8 Hz, 2 H, H-2), 7.40, m, 5 H, phenyl-H); ^{13}C NMR ($CDCl_3$) δ 14.7 (Me), 38.1 ($NHCH_2$), 69.1 (CH_2O), 112.8 (C-1), 113.0 (C-3), 128.6 (phenyl-C), 135.3 (C-ipso), 136.6 (C-2), 149.6 (C-4), 171.4 (CO); mass spectrum, m/z 287 (M, 9), 243 (M - CO_2), 152 (243 - $PhCH_2$, 100), 137 (152 - Me), 124. 91 ($PhCH_2$), 80, 65. Anal. ($C_{16}H_{17}NO_2S$) C, H, N.

***S*-(Benzyloxycarbonyl)-4-[ethyl(2-hydroxyethyl)amino]benzenethiol (17).** Oxirane (2.00 mL, 40.0 mmol) was added to a suspension of **16** (10.2 g, 35.5 mmol) in THF (50 mL) and glacial AcOH (50 mL). The reaction was stirred for 4 days at room temperature with a further addition of oxirane (1.00 mL, 20 mmol) being made after 2 days. The mixture was then diluted with water, neutralized with solid $NaHCO_3$ and extracted twice with EtOAc. The combined organic phase was washed with aqueous $NaHCO_3$ and worked up to give a solid which was chromatographed on silica gel. Elution with EtOAc/hexanes (1:19) removed a trace of unreacted amine (0.59 g, 6%), while elution with EtOAc/hexanes (1:9) gave *S*-(benzyloxycarbonyl)-4-[ethyl(2-hydroxyethyl)amino]benzenethiol (**17**) as a colorless oil (11.9 g, 96%): IR ($CHCl_3$) λ_{max} 3508, 1709 (CO), 1599, 1503 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.16 (t, J = 6.8 Hz, 3 H, Me), 3.42 (m, J = 7.1 Hz, 2 H, NCH_2CH_3), 3.46 (t, J = 6.1 Hz, 2 H, NCH_2CH_2OH), 3.77 (t, J = 5.9 Hz, 2 H, CH_2OH), 5.24 (s, 2 H, $PhCH_2$), 6.71 (d, J = 9.1 Hz, 2 H, H-3), 7.32 (d, J = 9.1 Hz, 2 H, H-2), 7.35 (m, 5 H, phenyl-H); ^{13}C NMR ($CDCl_3$) δ 11.8 (Me), 45.5 (NCH_2CH_3), 52.2 (NCH_2CH_2OH), 60.05 (CH_2OH), 69.05 ($PhCH_2$), 111.9 (C-1), 112.5 (C-3), 128.5, 128.4 (phenyl-C), 135.2 (C-ipso), 136.6 (C-2), 149.2 (C-4), 171.4 (CO); mass spectrum, m/z 331 (M, 11), 300 (M

- CH_2OH), 287(M - CO_2), 256 (287 - CH_2OH), 196 (287 - $PhCH_2$, 100), 165 (196 - CH_2OH), 150,136, 109; HRMS (M^+) calcd for $C_{18}H_{21}NO_3S$ 331.1242, found 331.1246.

4-[Ethyl(2-hydroxyethyl)amino]benzenethiol (18). A suspension of 17 (11.9 g, 36.0 mmol) in EtOH (300 mL) containing NaOH (7.20 g, 18.0 mmol) was stirred for 20 h at room temperature. The ethanol was removed under reduced pressure, and the residue was partitioned between EtOAc and brine. The organic phase was worked up to give an oil which was chromatographed on silica gel. Elution with EtOAc/hexanes (1:9) gave an unidentified yellow oil (1.85 g).

Continued elution with EtOAc/hexanes (1:3) gave 4-[ethyl(2-hydroxyethyl)amino]benzenethiol (18) as a yellow oil (4.50 g, 64%): ^1H NMR ($CDCl_3$; 60 MHz) δ 1.11 (t, J = 7.1 Hz, 3 H, Me), 2.24 (s, 1 H, OH), 3.34 (m, 4 H, NCH_2CH_2OH), 3.77 (m, 2 H, NCH_2CH_3), 6.51 (d, J = 8.8 Hz, 2 H, H-3), 7.22 (d, J = 8.4 Hz, 2 H, H-2); mass spectrum, m/z 197 (M, 20), 166 (M - CH_2OH, 100), 138 (166 - CH_2=CH_2), 108, 79, 65; HRMS (M^+) calcd for $C_{10}H_{15}NO_3$ 197.0874, found 197.0886.

Elution with neat EtOAc gave the disulfide 19 as a yellow-green oil (3.80 g, 27%): ^1H NMR ($CDCl_3$) δ 1.11 (t, J = 6.60 Hz, 3 H, Me), 1.25 (br s, 1 H, OH), 3.40 (m, 2 H, NCH_2CH_3), 3.47 (t, J = 5.9 Hz, 2 H, NCH_2OH), 3.79 (t, J = 5.94 Hz, 2 H), 6.66 (d, J = 9.9 Hz, 2 H, H-3), 7.33 (d, J = 9.0 Hz, 2 H, H-2); mass spectrum, m/z 392 (M, 15), 374 (M - H_2O), 196 ($SC_6H_4N(CH_2CH_3)CH_2CH_2OH$), 166 (196 - CHOH, 100).

Methyl 3-[[4-[Ethyl(2-hydroxyethyl)amino]phenyl]thio]propanoate (20). A mixture of the 2-hydroxyethyl derivative 18 (2.50 g, 12.7 mmol), methyl 3-bromopropanoate (2.33 g, 14.0 mmol), and dry K_2CO_3 (1.93 g, 14.0 mmol) was heated under reflux for 0.5 h in dry MeOH (100 mL). The MeOH was removed under reduced pressure, and the residue was partitioned between EtOAc and water. The organic phase was worked up to give a solid which was chromatographed on silica gel. Elution with EtOAc/hexanes (3:2) gave methyl 3-[[4-[ethyl(2-hydroxyethyl)amino]phenyl]thio]propanoate (20) (1.92 g, 53%) as a colorless oil: IR ($CHCl_3$) λ_{max} 1731 (CO), 1600, 1505 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.15 (t, J = 7.1 Hz, 3 H, Me), 2.57 (t, J = 7.6 Hz, 2 H, $COCH_2$), 2.98 (t, J = 7.6 Hz, 2 H, $COCH_2$), 2.98 (t, J = 7.6 Hz, 2 H, SCH_2), 3.41 (m, J = 7.1 Hz, 2 H, NCH_2CH_3), 3.47 (t, J = 5.9 Hz, 2 H, NCH_2CH_2OH), 3.67 (s, 3 H, OMe), 3.79 (t, J = 5.9 Hz, 2 H, CH_2OH), 6.69 (d, J = 9.0 Hz, 2 H, H-3), 7.31 (d, J = 9.0 Hz, 2 H, H-2); ^{13}C NMR ($CDCl_3$) δ 11.8 (Me), 31.7 ($COCH_2$), 34.5 (SCH_2), 45.6 (NCH_2CH_3), 51.7 (OMe), 52.4 (NCH_2CH_2OH), 60.1 (CH_2OH), 112.9 (C-3), 119.3 (C-1), 135.0 (C-2), 148.0 (C-4), 172.6 (CO); mass spectrum, m/z 283 (M, 23.5), 266 (M - OH), 252 (M - OMe, 100), 223 (252 - CH_2CH_3), 196 (M - $CH_3OCOCH_2CH_2$), 165 (196 - CH_2OH), 150 (165 - Me), 136 ($SC_6H_4NCH_2$); HRMS (M^+) calcd for $C_{14}H_{21}NO_3S$ 283.1242, found 283.1249.

Elution with EtOAc gave a 14% yield of bis[4-[ethyl(2-hydroxyethyl)amino]phenyl] disulfide (19).

Methyl 3-[(4-Aminophenyl)thio]propanoate (22). A suspension of methyl 3-[(4-nitrophenyl)thio]propanoate (21)[18] (23.3 g, 0.10 mmol) and $SnCl_2 \cdot 2H_2O$ (109 g, 0.48 mol) in EtOAc (500 mL) was heated under reflux for 4 h under N_2. The cooled mixture was basified with ammonia and filtered, and the precipitate was washed repeatedly with EtOAc. The combined filtrates were washed with water and worked up to give methyl 3-[(4-aminophenyl)thio]propanoate (22) as an orange oil (17.1 g, 84%): ^1H NMR ($CDCl_3$; 60 MHz) δ 2.53 (t, J = 4.3 Hz, 2 H, CH_2CO), 2.96 (t, J = 5.8 Hz, 2 H, SCH_2), 3.62 (s, 5 H, OMe, NH_2), 6.55 (d, J = 8.8 Hz, 2 H, H-3,5), 7.22 (d, J = 9.1 Hz, 2 H, H-2,6). The compound was used immediately without further purification.

Methyl 3-[[4-(Trifluoroacetamido)phenyl]thio]propanoate (23). Trifluoroacetic anhydride (8.80 mL, 62.3 mmol) was added dropwise to an ice-cold solution of 22 (11.5 g, 54.5 mmol) in dry CH_2Cl_2 (200 mL) resulting in a blue-black mixture. After stirring for a further 10 min at 0 °C and 20 min at room temperature, the mixture was diluted with ice/water and extracted with CH_2Cl_2. The organic phase was washed with aqueous $NaHCO_3$ and worked up to give methyl 3-[[4-(trifluoroacetamido)phenyl]thio]propanoate (23) (11.3 g, 67%): mp (PhH/hexanes) 111 °C; IR (KBr) λ_{max} 3355, 1730 (OCO), 1710 (CF_3CO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.63 (t, J = 7.3 Hz, 2 H, CH_2CO), 3.16 (t, J = 7.3 Hz, 2 H, CH_2CH_2CO), 3.69 (s, 3 H, MeO), 7.38 (d, J = 8.7 Hz, 2 H, H-3), 5.53 (d, J = 6.8 Hz, 2 H, H-2); ^{13}C NMR ($CDCl_3$) δ 29.3 (CH_2CO), 34.1 (CH_2CH_2CO), 51.9 (OMe), 121.1 (H-3), 131.1 (H-2), 133.2 (C-1), 133.8 (C-4), 172.15 (CO). Anal. ($C_{12}H_{12}F_3NO_3S$) C, H, N.

Chromatography of the mother liquors from the above crystallization on silica gel and elution with EtOAc/hexanes (1:9) gave an additional 2.95 g (18%) of 23, followed by bis[4-[(trifluoroacetyl)amino]phenyl] disulfide (24) as a white solid (1.02 g, 4%): ^1H NMR ($CDCl_3/CD_3COCD_3$; 60 MHz) δ 2.56 (br s, 2 H, NH), 7.39–7.78 (m, 8 H, aromatics); mass spectrum, m/z 440 (M, $(CF_3CONHC_6H_4S)_2$, 90%), 408 (M - S), 252 (M - $CF_3CONHC_6H_4$), 220 ($CF_3CONHC_6H_4S$, 100%), 69 (CF_3).

Methyl 3-[[4-(*N*-Ethyltrifluoroacetamido)phenyl]thio]propanoate (25). To an ice-cold solution of 23 (93 mg, 0.30 mmol) in Me_2CO (5 mL) was added sequentially powdered KOH (22 mg, 0.39 mmol) and EtI (0.032 mL, 0.40 mmol). The mixture was then heated under reflux for 7 h, with a further addition of EtI (0.032 mL, 0.40 mmol) after 2 h. Solvent was removed from the cooled mixture under reduced pressure, and the residue was chromatographed on silica gel. Elution with ethyl acetate/hexanes (1:9) gave methyl 3-[[4-(*N*-ethyltrifluoroacetamido)phenyl]thio]propanoate (25) (0.071 g, 70%) as a colorless oil: IR(neat) λ_{max} 2550, 1730 (OCO), 1690 (CF_3CO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.18 (t, J = 7.1 Hz, 3 H, Me), 2.68 (t, J = 7.4 Hz, 2 H, $COCH_2$), 3.23 (t, J = 7.4 Hz, 2 H, SCH_2), 3.71 (s, 3 H, OMe), 3.78 (m, J = 7.2 Hz, 2 H, NCH_2), 7.14 (d, J = 8.4 Hz, 2 H, H-3), 7.37 (d, J = 8.4 Hz, 2 H, H-2); ^{13}C NMR ($CDCl_3$) δ 12.2 Me; 28.3, $COCH_2$; 33.9, SCH_2; 46.9, NCH_2; 52.0, OMe; 129.0, C-3; 129.4, C-2; 136.7, C-1; 137.45, CF_3; 148.4, C-4; 171.9, CF_3CO; 172.5, OCO; mass spectrum, m/z 335 (M, 100), 304 (M - OMe), 275 (304 - CH_2CH_3), 248, 223, 154, 150, 124; HRMS (M^+) calcd for $C_{14}H_{16}F_3NO_3S$ 335.0803, found 335.0806.

Methyl 3-[[4-(Ethylamino)phenyl]thio]propanoate (26). A solution of 25 (1.80 g, 5.37 mmol) in 90% aqueous MeOH (50 mL) containing K_2CO_3 (1.48 g, 10.7 mmol) was stirred for 20 h at room temperature, and the solvent was removed under reduced pressure. The residue was partitioned between EtOAc and water, and the organic phase was worked up as usual to give methyl 3-[[4-(ethylamino)phenyl]thio]propanoate (26) (1.14 g, 89%) as an oil: IR(neat) λ_{max} 3400, 2960, 1730 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.25 (t, J = 7.1 Hz, 3 H, Me), 2.55 (t, J = 7.5 Hz, 2 H, $COCH_2$), 2.97 (t, J = 8.2 Hz, 2 H, SCH_2), 3.14 (m, J = 7.1 Hz, 2 H, NCH_2), 3.66 (s, 3 H, OMe), 6.63 (d, J = 8.6 Hz, 2 H, H-3), 7.28 (d, J = 8.6 Hz, 2 H, H-2); ^{13}C NMR ($CDCl_3$) δ 14.8 (Me), 31.8 ($COCH_2$), 34.5 (SCH_2), 38.3 (NCH_2), 51.7 (OMe), 113.1 (C-3), 119.8 (C-1), 135.2 (C-2), 148.4 (C-4), 172.5 (CO); mass spectrum, m/z 239 (M), 224 (M - Me), 208 (M - OMe), 166, 152 (M - $CH_3OCOCH_2CH_2$, 100); HRMS (M^+) calcd for $C_{12}H_{17}NO_2S$ 239.0980, found 239.0985.

Methyl 3-[[4-[Ethyl(2-hydroxyethyl)amino]phenyl]thio]propanoate (20). A solution of 26 (3.93 g, 16.4 mmol) in THF (50 mL) and glacial AcOH (50 mL) was treated with oxirane and worked up as previously. The resulting product was chromatographed on silica gel. Elution with EtOAc/hexanes (1:19) removed unreacted amine (0.32 g, 8%), while elution with EtOAc/hexanes (1:3) gave methyl 3-[[4-[ethyl(2-hydroxyethyl(2-hydroxyethyl)amino]phenyl]thio]propanoate (20) (2.87 g, 62%).

Methyl 3-[[4-[(2-Chloroethyl)ethylamino]phenyl]thio]propanoate (27). A solution of 20 (2.85 g, 10.1 mmol) in dry CH_2Cl_2 (150 mL) was treated as detailed above for compound 10 to give methyl 3-[[4-[(2-chloroethyl)-N-ethylamino]phenyl]thio]propanoate (27) (2.17 g, 72%) as a colorless oil: IR ($CHCl_3$) λ_{max} 1736 (CO), 1600 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.18 (t, J = 7.2 Hz, 3 H, Me), 2.58 (t, J = 6.7 Hz, 2 H, $COCH_2$), 2.99 (t, J = 6.5 Hz, 2 H, SCH_2), 3.42 (m, J = 7.1 Hz, 2 H, NCH_2CH_3), 3.61 (m, 4 H, NCH_2CH_2Cl), 3.67 (s, 3 H, OMe), 6.62 (d, J = 9.0 Hz, 2 H, H-3), 7.31 (d, J = 9.0 Hz, 2 H, H-2; ^{13}C NMR ($CDCl_3$) δ 12.3 (Me), 31.7 ($COCH_2$), 34.5 (SCH_2), 40.3 (CH_2Cl), 45.4 (NCH_2CH_3), 51.7 (OMe), 52.2 (NCH_2CH_2Cl), 112.2 (C-3), 119.45 (C-1), 135.2 C-2), 146.8 (C-4), 172.4 (CO); mass spectrum, m/z 301 (M, 28), 303 (M + 2, 11), 286 (M - Me), 252 (M - CH_2Cl, 100), 214, 165, 136. HRMS (M^+) calcd for $C_{14}H_{20}ClNO_2S$ 301.0903, found 301.0912.

3-[[4-[(2-Chloroethyl)ethylamino]phenyl]thio]propanoic Acid (28). A solution of the above ester 27 (2.43 g, 8.07 mmol) in concentrated HCl (100 mL) was heated under reflux for 1 h, cooled, neutralized with concentrated NH_4OH, and extracted with EtOAc (2 × 150 mL). The organic phase was treated with charcoal and worked up to give 3-[[4-[(2-chloroethyl)ethylamino]-

phenyl]thio]propanoic acid (**28**) (2.20 g, 95%): mp (PhH/hexanes) 78.5–79 °C; IR ($CHCl_3$) λ_{max} 2960, 1695 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.18 (t, J = 7.1 Hz, 3 H, Me), 2.62 (t, J = 7.4 Hz, 2 H, $COCH_2$), 2.97 (t, J = 7.3 Hz, 2 H, SCH_2), 3.43 (m, J = 7.1 Hz, 2 H, NCH_2CH_3), 3.61 (m, 4 H, NCH_2CH_2Cl), 6.62 (d, J = 9.0 Hz, 2 H, H-3), 7.34 (d, J = 9.0 Hz, 2 H, H-2); ^{13}C NMR ($CDCl_3$) δ 12.4 (Me), 31.4 ($COCH_2$), 34.3 (SCH_2), 40.3 (CH_2Cl), 45.45 (NCH_2CH_3), 52.2 (NCH_2CH_2Cl), 112.2 (C-3), 119.2 (C-1), 135.4 (C-2), 146.9 (C-4), 177.6 (CO); mass spectrum, m/z 287 (M, 25), 289 (M + 2, 9.5), 238 (M – CH_2Cl, 100), 209 (238 – CH_2CH_3), 165, 136, 73, 57, 43. Anal. ($C_{13}H_{18}ClNO_2S$) C, H, N.

3-[[4-[(2-Chloroethyl)ethylamino]phenyl]thio]propanamine (29). According to the procedure of Kaiser and Weinstock,[14] 3-[[4-[(2-chloroethyl)ethylamino]phenyl]thio]propanoic acid (**28**) (0.24 g, 0.83 mmol) gave 3-[[4-[(2-chloroethyl)ethylamino]phenyl]thio]propanamine (**29**) (0.21 g, 71%) as a brown oil: ^1H NMR ($CDCl_3$, 60 MHz) δ 1.58 (t, J = 6.4 Hz, 3 H, Me), 2.61 (s, 2 H, NH_2), 3.22 (m, 4 H, NCH_2CH_2S), 3.88 (m, 2 H, NCH_2CH_3), 4.02 (s, 4 H, NCH_2CH_2Cl), 7.03 (d, J = 7.4 Hz, 2 H, H-3), 7.73 (d, J = 8.5 Hz, 2 H, H-2); mass spectrum, m/z 258 (M, 54), 260 (M + 2, 20), 240, 209 (M – CH_2Cl, 100), 180 (209 – CH_2CH_3), 166 (180 – CH_2), 150 (166 – NH_2), 136, 106, 91, 77, 65, 44. This compound was not characterized further, but used directly.

***N*,9-Bis[[3-[4-[(2-chloroethyl)ethylamino]phenyl]propyl]amino]acridine-4-carboxamide (5).** Freshly prepared 9-chloroacridine-4-carbonyl chloride[21] (**30**) (1.25 g, 4.54 mmol) was dissolved in THF (100 mL), and the solution was treated with 4-nitrophenol (1.39 g, 9.99 mmol) at 0 °C under N_2. Et_3N (1.39 mL, 9.99 mmol) was added, and the mixture was stirred at 0 °C for 10 min and then brought to room temperature. The suspension was finally heated under reflux for 15 min and then cooled to 0 °C, when the amine **13** (2.18 g, 9.08 mmol) was added, followed by more Et_3N (0.69 mL, 5.00 mmol). The suspension was stirred at 0 °C for a further 10 min, brought to room temperature, and then heated under reflux for 8 h. Solvents were removed from the mixture under reduced pressure, and the residue was partitioned between EtOAc and water. Usual workup of the organic phase gave a residue which was chromatographed on silica gel. Elution with EtOAc/hexanes (1:19) gave initially a mixture of **33** and **34** as a red oil (1.80 g): ^1H NMR ($CDCl_3$; 60 MHz) δ 1.13 (t, J = 7.1 Hz, 3 H, Me), 2.02 (t, J = 5.1 Hz, 2 H, $NHCH_2CH_2$), 2.65 (t, J = 5.7 Hz, 2 H, $NHCH_2CH_2CH_2$), 3.28 (m, 8 H, NCH_2CH_2Cl, $CONHCH_2$, NCH_2CH_3), 6.67 (d, J = 8.4 Hz, 2 H, H-3′), 6.74–7.95 (m, 12 H, aromatics), 8.95 (m, 1 H, aromatic); mass spectrum, m/z 582 (M of **34**, 14), 584 (M + 2 of **34**, 6), 533 (582 – CH_2Cl), 479, 461 (M of **33**, 1), 425 (461 – HCl), 412 (461 – CH_2CH_2Cl), 373, 316, 272, 174, 118, 91, 65.

Further elution with the same solvent mixture then gave pure *N*-[3-[4-[(2-chloroethyl)ethylamino]phenyl]propyl]acridone-4-carboxamide (**33**) as a yellow solid (0.12 g, 6%): mp 139–141 °C; ^1H NMR ($CDCl_3$; 60 MHz) δ 1.18 (t, J = 7.0 Hz, 3 H, Me), 2.00 (t, J = 5.8 Hz, 2 H, $NHCH_2CH_2$), 2.61 (t, J = 5.8 Hz, 2 H, $NHCH_2CH_2CH_2$), 3.28 and 3.56 (m, s, 8 H, NCH_2CH_3, $NHCH_2CH_2$, NCH_2CH_2Cl), 6.64 (d, J = 8.9 Hz, 2 H, H-31), 7.06 and 7.58 and 8.49 (m, 9 H, aromatics); mass spectrum, m/z 461 (M, 1), 446 (M – Me), 425 (M – HCl), 412 (M – CH_2Cl), 398 (M – CH_2CH_2Cl), 355 (398 – NCH_2CH_3), 252, 222 (M – $NHCH_2CH_2CH_2C_6H_4N(CH_2CH_2Cl)CH_2CH_3$, 100), 174, 118, 91.

Finally, elution with EtOAc gave *N*,9-bis[[3-[4-[(2-chloroethyl)ethylamino]phenyl]propyl]amino]acridine-4-carboxamide (**5**) as a yellow oil (0.48 g, 15%). Crystallization from EtOAc saturated with gaseous HCl gave the yellow, hygroscopic trihydrochloride salt: mp 128–130 °C; IR (neat) λ_{max} 3350, 2550, 1710 (CO), 1615, 1550 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.15 (t, J = 7.0 Hz, 6 H, Me), 2.06 (m, 2 H, $CONHCH_2CH_2$), 2.17 (m, 2 H, $NHCH_2CH_2$), 2.69 (t, J = 7.1 Hz, 2 H, $CONHCH_2CH_2CH_2$), 2.76 (t, J = 7.1 Hz, 2 H, $NHCH_2CH_2CH_2$), 3.38 (m, 4 H, NCH_2CH_3), 3.58 (s, 8 H, NCH_2CH_2Cl), 3.66 (m, 2 H, $CONHCH_2$), 3.95 (br s, 2 H, $NHCH_2$), 6.61 (2d, J = 8.9 Hz, 4 H, H-3′), 7.03 (d, J = 8.64 Hz, 2 H, H-2′ (amide side chain)), 7.13 (d, J = 8.6 Hz, 2 H, H-2′ (amine side chain)), 7.30 (m, 2 H, H-2,6), 7.53 (m, 1 H, H-7), 7.70 (m, 2 H, H-3,5), 7.98 (br s, 2 H, H-1,8), 8.74 (br s, 1 H, CONH); ^{13}C NMR ($CDCl_3$) δ 12.5, 12.55 (Me), 23.0 ($CONHCH_2CH_2$), 23.7 ($NHCH_2CH_2$), 28.9 ($CONHCH_2CH_2CH_2$), 30.3 ($NHCH_2CH_2CH_2$), 32.6 ($CONHCH_2$), 38.7 ($NHCH_2$), 40.6 (CH_2Cl), 45.45 (NCH_2CH_3), 52.4, 52.5 (NCH_2), 112.1, 112.2 (C-31), 123.2 (C-8a), 128.4 (C-9a), 128.8 (C-7), 129.5 (C-21), 129.8 (C-10a), 130.9 (C-6), 132.4 (C-11), 134.6 (C-1, 8), 145.2 (C-41), 145.5 (C-9), 167.8 (CO); mass spectrum, m/z 683 (M, 18), 685 (M + 2, 8), 647 (M – HCl), 620 (M – CH_2CH_2Cl), 546, 498, 425, 217 (100), 118, 91. Anal. ($C_{40}H_{47}Cl_2N_5O \cdot 3HCl$) C, H, N.

***N*,9-Bis[[2-[[4-[(2-chloroethyl)ethylamino]phenyl]thio]ethyl]amino]acridine-4-carboxamide (6).** Freshly prepared **30** (81 mg, 0.29 mmol) was dissolved in THF (5 mL), and the solution was treated with sodium 4-nitrophenolate (95 mg, 0.59 mmol) at 0 °C under N_2. The mixture was stirred at this temperature for 15 min, allowed to come to room temperature, and finally heated under reflux for 3 h. The mixture was cooled and filtered through Celite, and the filtrate was concentrated to dryness under reduced pressure. The residue of crude 4-nitrophenyl 9-(4-nitrophenoxy)acridine-4-carboxylate (**32**) was suspended in dry CH_2Cl_2 (2 mL) under N_2 and treated slowly with a solution of the amine (**18**) (152 mg, 0.59 mmol) in dry CH_2Cl_2 (2 mL). Et_3N (0.45 mL, 3.24 mmol) was added at 0 °C, and the mixture was then brought to room temperature, and finally heated under reflux for 5 h. The reaction was diluted with CH_2Cl_2, the organic layer was worked up as usual, and the residue was chromatographed on silica gel. Initial elution with EtOAc/hexanes (1:19) gave foreruns, while later eluates gave *N*-[2-[[4-[(2-chloroethyl)ethylamino]phenyl]thio]ethyl]acridone-4-carboxamide (**35**) as a yellow solid (62 mg, 44%): ^1H NMR ($CDCl_3$) δ 1.12 (t, J = 7.0 Hz, 3 H, Me), 3.09 (t, J = 6.3 Hz, 2 H, SCH_2), 3.35 (m, J = 5.6 Hz, 2 H, NCH_2CH_3), 3.54 (s, 4 H, NCH_2CH_2Cl), 3.71 (m, J = 5.7 Hz, 2 H, $CONHCH_2$), 6.56 (d, J = 8.7 Hz, H-3′), 7.10 (t, J = 7.7 Hz, H-6), 7.27 (t, J = 7.6 Hz, 2 H, H-7), 7.37 (t, J = 8.4 Hz, 2 H, H-3,2′), 7.67 (t, J = 8.4 Hz, 1 H, H-2), 7.76 (d, J = 7.6 Hz, 1 H, H-5), 8.41 (d, J = 8.0 Hz, 1 H, H-8), 8.56 (d, J = 8.0 Hz, 1 H, H-1), 12.24 (s, 1 H, NHCO); ^{13}C NMR ($CDCl_3$) δ 12.3 (Me), 35.8 (CH_2S), 39.7 ($CONHCH_2$), 40.2 (CH_2Cl), 45.4 (NCH_2CH_3), 52.2 (NCH_2CH_2Cl), 112.3 (H-31), 117.5 (C-8a), 117.8 (C-6), 119.6 (C-2), 121.1 (C-9a), 122.1 (C-7), 122.4 (C-11), 126.1 (C-21), 126.8 (C-5), 131.7 (C-3), 134.0 (C-8), 134.7 (C-1), 140.2 (C-5a), 140.9 (C-10a), 168.3 (CONH), 178.1 (C-9).

Elution with EtOAc gave *N*,9-bis[[2-[[4-[(2-chloroethyl)ethylamino]phenyl]thio]ethyl]amino]acridine-4-carboxamide (**6**) (31 mg, 15%) as a yellow oil: ^1H NMR ($CDCl_3$) δ 1.15 (m, 6 H, Me), 3.09 (m, 2 H, $CONHCH_2CH_2S$), 3.15 (t, J = 6.4 Hz, 2 H, $NHCH_2CH_2S$), 3.385 (m, J = 7.1 Hz, 4 H, NCH_2CH_3), 3.57 (s, 4 H, NCH_2CH_2Cl (amide side chain)), 3.59 (m, 4 H, NCH_2CH_2Cl (amine side chain)), 3.81 (m, 2 H, $CONHCH_2$), 3.95 (br s, 2 H, $NHCH_2$), 6.58 (d, J = 9.0 Hz, 4 H, H-3′), 7.325 (d, J = 8.8 Hz, 2 H, H-2′ (amide side chain)), 7.40 (t, J = 7.7 Hz, 2 H, H-2,6), 7.46 (d, J = 8.9 Hz, 2 H, H-2′ (amine side chain)), 7.73 (t, J = 7.3 Hz, 1 H, H-7), 8.13 (d, J = 8.4 Hz, 1 H, H-5), 8.26 (br s, 2 H, H-3,8), 8.82 (br s, 1 H, H-1), 12.7 (br s, 1 H, NHCO); ^{13}C NMR ($CDCl_3$) δ 12.4 (Me), 36.5 ($CONHCH_2CH_2S$), 38.8 ($NHCH_2CH_2S$), 40.3 (CH_2Cl), ($CONHCH_2$) 45.4 (NCH_2CH_3), 48.3 ($NHCH_2$), 52.3 (NCH_2CH_2Cl), 112.3, 112.4 (C-3′), 119.6 (C-11) 122.25 (C-7), 123.7 (C-2′), 135.2, 135.5 (C-21), 146.7 (C-9) 147.2 (C-4); FAB mass spectrum, m/z 720 (M + H, 100), 722 (M + 2, 78), 724 (M + 4, 19), 684 (720 – HCl), 658, 539, 505.

***N*,9-Bis(butylamino)acridine-4-carboxamide (31).** Et_3N (0.25 mL, 1.82 mmol) was added slowly to an ice-cold mixture of **30** (0.20 g, 0.73 mmol) and butylamine (0.15 mL, 1.59 mmol) in dry THF (25 mL), and the mixture was then stirred at room temperature for 20 h. The solvent was removed under reduced pressure, and the residue was partitioned between EtOAc and water and worked up as usual to give N9-bis(butylamino)-acridine-4-carboxamide (**31**) (0.19 g, 75%) as a bright yellow solid: mp (MeOH) 116 °C; IR (KBr) λ_{max} 3400 (NH), 3000, 1740 (CO), 1645, 1600, 1550 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.96 (t, J = 6.7 Hz, 3 H, Me (amide side chain)), 1.05 (t, J = 7.4 Hz, 3 H, Me (amine side chain)), 1.46 (m, 2 H, $CONHCH_2CH_2CH_2$), 1.61 (m, 2 H, $NHCH_2CH_2CH_2$), 1.80 (m, 4 H, $NHCH_2CH_2$), 3.67 (t, J = 6.9 Hz, 2 H, $CONHCH_2$), 3.84 (t, J = 7.1 Hz, $NHCH_2$), 7.41 (m, 2 H, H-2,6), 7.72 (t, J = 7.4 Hz, 1 H, H-7), 7.96 (d, J = 8.5 Hz, 1 H, H-3), 8.10 (d, J = 8.4 Hz, H-5), 8.21 (d, J = 8.6 Hz, 1 H, H-8), 8.87 (d, J = 6.4 Hz, 1 H, H-1); ^{13}C NMR ($CDCl_3$) δ 13.8, 13.9 (Me), 20.05 ($CONHCH_2CH_2CH_2$), 20.6 ($NHCH_2CH_2CH_2$), 31.7 ($CONHCH_2CH_2$), 33.8 ($NHCH_2CH_2$), 39.5 ($CONHCH_2$), 50.9 ($NHCH_2$), 115.6 (C-8a) 116.4 (C-9a), 122.1 (C-2), 122.5 (C-7), 123.4 (C-3),

126.4 (C-5), 129.3 (C-3), 130.7 (C-1), 134.7 (C-8), 147.3, 147.8 (C-5a, 10a), 152.7 (C-9), 166.4 (NHCO); mass spectrum, m/z 349 (M), 306 (M - $CH_2CH_2CH_3$) 277, 250 (100). Anal. ($C_{22}H_{27}N_3O$) C, H, N.

Preparation and Alkylation of Labeled DNA Fragment. The plasmid pBR322 DNA was obtained from Boehringer-Mannheim. The restriction enzymes, EcoRI and Bam HI, and the Klenow fragment of DNA polymerase I were all purchased from Pharmacia. EcoRI digested pBR322 was 3'-end labeled by using Klenow fragment in the presence of ^{32}P-dATP and cold mixture of dNTPs. The fragment was then further digested with BamHI and the 375 base-pair labeled fragment was isolated on a 4% nondenaturing polyacrylamide gel. The partial sequence is given in ref 7.

Appropriate conditions for DNA alkylation were established as previously described.[7] Labeled DNA (ca. 30 000 counts) was incubated with the compounds **5**, **7**, or **8** in the presence of 1 μg of sonicated calf thymus DNA for 30 minutes at 37 °C. The drug to base-pair ratio was adjusted against the calf thymus DNA. For Mg^{2+} experiments, the reactions were carried out in the presence of either 30 mM NaCl or 10 mM $MgCl_2$ (final ionic strength = 0.04).

The modified DNA was then precipitated with EtOH, dissolved in 100 μL of 0.01 SHE buffer (pH = 7.3), and heated at 90 °C for 10 min. This leads to depurination at alkylated purine sites.[7] The DNA was then again precipitated with EtOH, dissolved in 100 μL of freshly prepared 1 M piperidine and heated at 90 °C for 10 min which leads to strand breaks at all depurinated sites.[7] The sample was then lyophilized overnight, washed twice with 10 μL of distilled water, and lyophilized briefly. The pellet was dissolved in a sequencing dye (80% deionized formamide, 1% xylene cyanol, and 1% bromophenol blue) and denatured at 90 °C for 2 min before loading on a 0.4 mm thick, 8% polyacrylamide sequencing gel containing 8 M urea. The gel was run on a Sequi-Gen kit from Biorad for 2 h in the TBE buffer at a constant 2500 V. It was then transferred to 3-mm Whatman filter paper, covered with plastic wrap and dried in a Biorad gel drier model 1125B for 30 min at 80 °C. The gel was then placed in intimate contact with X-AR5 Kodak film and exposed for 10–15 h before developing. The densitometer scans were performed on a Zeineh soft laser scanning densitometer.

Helix Unwinding Assay. The covalently closed circular pBR322 plasmid (1 μg) was incubated with various concentrations of drug in 10 μl of 0.01 SHE for 30 min at 37 °C. The drug to base-pair ratios used are 0.0, 0.1, 0.15, 0.20, 0.25, 0.30, 0.35, and 0.40. At the end of the incubation 2 μL of 1% bromophenol blue dye was added, and the solutions were directly loaded on to a nondenaturing 1-cm thick 1% agarose minigel (noncovalently bound drug molecules will quickly run out of the gel and hence they will not play a part in the unwinding of the superhelix). The gel was run for 90 min at 80 V. It was then stained with ethidium bromide and the pattern observed under a UV light was photographed.

Determination of in Vitro Cytotoxicity and in Vivo Antileukemic Activity. Cell lines were maintained in exponential growth phase by subculturing in RPMI 1640 (P388) or Alpha MEM (AA8, UV4) containing 10% fetal calf serum as previously described.[11,33] IC_{50} values were determined by using log phase cultures in 96-well microculture plates and are calculated as the nominal drug concentration required to reduce the cell density to 50% of control values, with eight control cultures used on each microplate. For P388 cultures, drug was present throughout the growth period (72 h) and final cell densities were determined with use of a minor modification of the MTT method of Mossman.[34] For AA8 and UV4 cultures, drug exposure was terminated after 18 h by washing three times with fresh medium. Cultures were grown for a further 72 h before determining cell density by staining with methylene blue.[35]

The in vivo antileukemic activities of the compounds were determined in mice bearing the P388 lymphocytic leukemia cell line,[36] by using a single-dose protocol (see Experimental Section).

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(34) Mossman, T. *J. Immunol. Methods* **1983**, *65*, 55.
(35) Finlay, G. J.; Baguley, B. C.; Wilson, W. R. *Anal. Biochem.* **1986**, *22*, 655.
(36) Geran, R. I.; Greenburg, N. H.; MacDonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep.* **1972**, *3*, 9.

Dopamine Autoreceptor Agonists as Potential Antipsychotics. 2. (Aminoalkoxy)-4*H*-1-benzopyran-4-ones

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The synthesis and pharmacological properties of a novel type of [(arylpiperazinyl)alkoxy]-4*H*-1-benzopyran-4-ones with dopaminergic activity are described. The nature of the arylpiperazine (AP) moiety determines the dopamine (DA) agonist/antagonist character of this series of compounds; when the aryl portion of the AP is unsubstituted the compounds appear to be DA autoreceptor agonists while substituted aryl groups seem to impart DA antagonist activity. A heterocyclic piperazine, 7-[3-[4-(2-pyridinyl)-1-piperazinyl]propoxy]-4*H*-1-benzopyran-4-one (**31**, PD 119819) has been identified as an extremely selective DA autoreceptor agonist in tests that include [^{3}H]haloperiodol binding, inhibition of spontaneous locomotor activity, inhibition of brain DA synthesis, inhibition of brain DA neuronal firing, stereotypy assessment, and reversal of 6-hydroxydopamine (6-OHDA) induced akinesia in rats. In addition, **31** possesses good oral activity in the Sidman avoidance test in squirrel monkeys, a predictor of clinical antipsychotic efficacy. In another primate model, **31** has been found to lack the liability for extrapyramidal side effects observed with currently available antipsychotic drugs.

During recent years several research groups, including our own, have focused on the development of dopamine (DA) autoreceptor agonists, compounds with selectivity for central nervous system (CNS) presynaptic DA receptors, as potential agents for the treatment of schizophrenia.[1] This approach is based on evidence that DA auto-

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