DNA-Directed Alkylating Agents. 1. Structure-Activity Relationships for Acridine-Linked Aniline Mustards: Consequences of Varying the Reactivity of the Mustard

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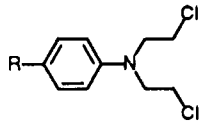
A series of DNA-targeted aniline mustards have been prepared, and their chemical reactivity and in vitro and in vivo cytotoxicity have been evaluated and compared with that of the corresponding simple aniline mustards. The alkylating groups were anchored to the DNA-intercalating 9-aminoacridine chromophore by an alkyl chain of fixed length attached at the mustard 4-position through a link group X, while the corresponding simple mustards possessed an electronically identical small group at this position. The link group was varied to provide a series of compounds of similar geometry but widely differing mustard reactivity. Variation in biological activity should then largely be a consequence of this varying reactivity. Rates of mustard hydrolysis in the two series related only to the electronic properties of the link group, with attachment of the intercalating chromophore having no effect. The cytotoxicities of the simple mustards correlated well with group electronic properties (with a 200–300-fold range in IC_{50}s). The corresponding DNA-targeted mustards were much more potent (up to 100-fold), but their IC_{50} values varied much less with linker group electronic properties. Most of the DNA-targeted mustards showed in vivo antitumor activity, being both more active and more dose-potent than either the corresponding untargeted mustards and chlorambucil. These results show that targeting alkylating agents to DNA by attachment to DNA-affinic units may be a useful strategy.

Bifunctional alkylating agents are an important class of anticancer drugs,[1] which express their cytotoxic and antitumor effects by cross-linking cellular DNA.[2] Although many potential alkylating moieties exist, the most common one employed in clinical drugs of this type is the *N,N*-bis(2-chloroethyl)aniline functionality. The simplest such compound, aniline mustard itself (**1**), has long been recognized for its experimental antitumor activity[3] and has also shown useful clinical results.[4] The most commonly used aniline mustards[5] are the substituted derivatives chlorambucil (**2**) and melphalan (**3**).



1: R = H
2: R = $(CH_2)_3COOH$
3: R = $CH_2CH(NH_2)COOH$

Although these compounds remain important as components in a variety of multidrug protocols,[1] they have a number of drawbacks which are common to all alkylating agents, due to their necessarily high chemical reactivity and the fact that, although they act by alkylating DNA, they have no particular affinity for it. Thus a high proportion of the drug is liable to be lost by hydrolysis, and by interactions with other cellular macromolecules, before reaching the DNA, leading to loss of potency. A significant amount of the drug may also reach the DNA with only one arm of the mustard intact, leading to monoalkylation events which are considered to be genotoxic rather than cytotoxic.[6,7] Finally, resistance to such reactive electrophiles is easily developed by an increase in the cellular level of low molecular weight thiols (particularly glutathione).[8,9]

Many of these drawbacks could in principle be ameliorated by attaching the mustard to a DNA-affinic carrier molecule, which would result in specifically targeting the mustard to the DNA. Such direction to the site of action would mean less chance of losing active drug by reaction with other cell components. Moreover, if a higher proportion of bifunctional alkylating agent can be delivered to the DNA and held there by the DNA-affinic carrier, a higher proportion of lethal cross-links with respect to more genotoxic monoalkylation events may result, leading to increased drug potency. Finally, the development of resistance to such compounds by elevated cellular thiol levels is likely to be less effective.

In spite of this logical rationale, relatively little work has gone into the development of DNA-targeted mustards, compared to the efforts expended in attaching them to a great variety of other carriers, including sugars, proteins, and steroids.[10] Creech et al. have studied a series of mono- and difunctional alkyl mustards linked to various heterocyclic chromophores, particularly acridines.[11] They showed these compounds (e.g. **4**) to be more potent than the corresponding simple mustards against ascitic tumors in vivo[12] and suggested that this was due to the high affinity of the chromophores for DNA, so that even the monofunctional mustards acted essentially as bifunctional agents.[13] A later analysis of the results[14] supported this

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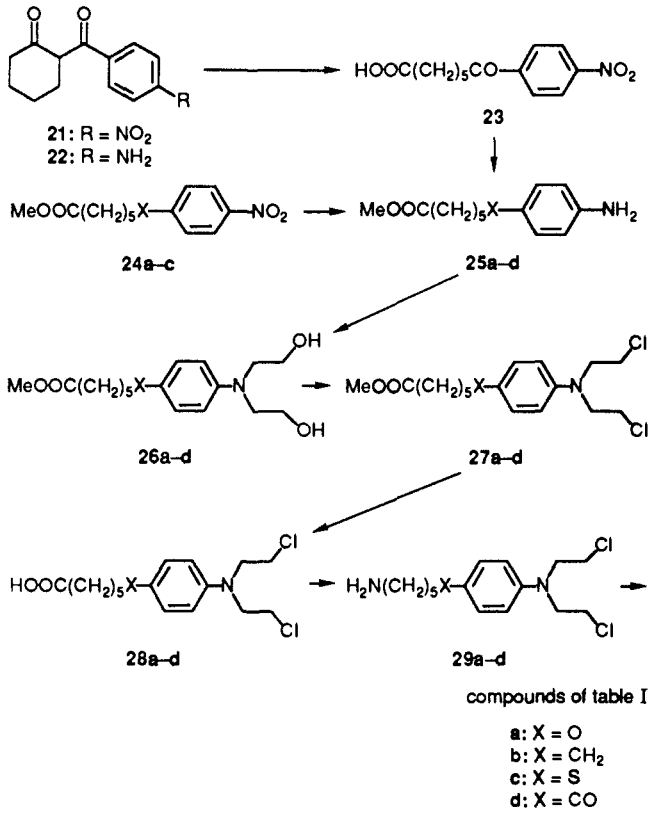
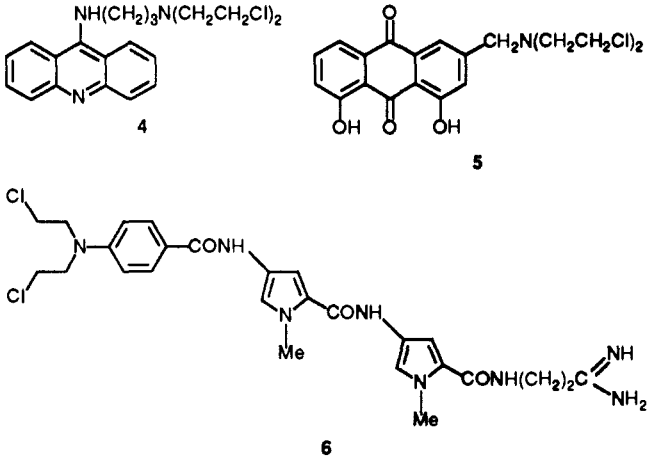
Scheme I

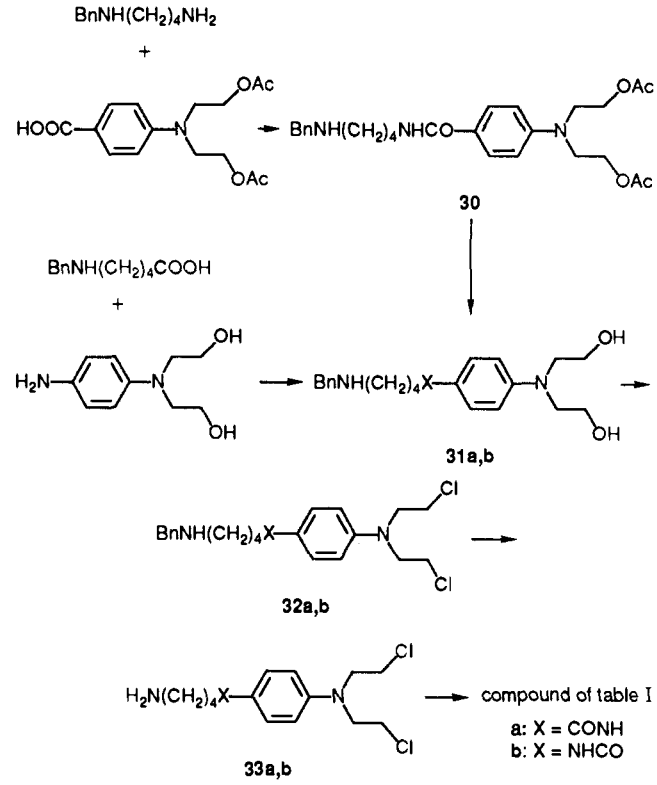


view, in that changes in the chromophore which would be expected to increase DNA binding resulted also in enhanced potency. More recently, a series of anthraquinone-linked alkyl mustards (e.g. **5**) were prepared as DNA-targeted alkylating agents,[15] and a benzoic acid mustard derivative of the DNA minor groove binder distamycin A (**6**) has also been shown[16] to have excellent antitumor activity.



In this paper we report on the synthesis and preliminary biological evaluation of two series of compounds: the parent 4-substituted aniline mustards (**7–13**) and a corresponding group of compounds (**14–20**) where the mustards are linked to the DNA-intercalating chromophore 9-aminoacridine by a chain of defined length and geometry. The 4-substituents X on the mustards (**7–13**) have been carefully chosen to span the largest practicable range of electronic parameter values (0.72 for CH_3SO_2 to −0.27 for CH_3O) compatible with the substituents also being able to serve as linker groups in the aniline series (**14–20**). Variations in biological activity within each series should then largely be a consequence of the differing reactivity of the mustard.

Scheme II



Chemistry

Syntheses of the substituted mustards **7–13** have been reported elsewhere.[3,17,18] The side chains (**29a–d**) for the acridine-linked mustards where X is O, CH_2, S, and CO were synthesized by the method shown in Scheme I. The O and CH_2 nitro esters (**24a,b**) were prepared by reported methods,[19,20] the S analogue (**24c**) from 4-nitrothiophenol and methyl 6-bromohexanoate, and the CO analogue (**24d**) from 2-(4-nitrobenzoyl)cyclohexanone (**21**) as shown. Reduction gave the 4-amino methyl esters **25**, which were treated with excess oxirane under mild acid catalysis to give the diols **26**. These were converted into the mustards **27** with $POCl_3$ or $SOCl_3$ except for the CO derivative **26d**. In this case the direct method was not suitable, and the mustard **27d** was instead prepared via the bismesylate. Acid hydrolysis of the methyl esters then gave the mustard acids **28**, which were cleanly converted by a modified Curtius reaction[21] into the desired alkylamines **29**. The

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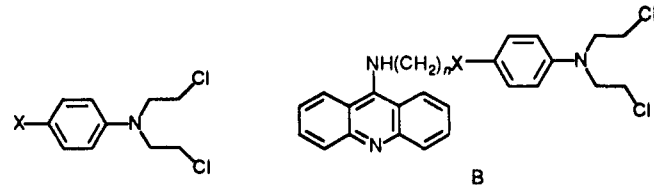
Table I. Physiochemical and Biological Properties of Aniline Mustards



					mustard reactivity		cytotoxicity				in vivo activity	
no.	formula	X	n	σ_p[a]	K_H,[b] $s^{-1} \times 10^4$	K_{NBP},[c] $s^{-1} \times 10^4$	P388, μM	AA8,[d] μM	UV4,[d] μM	HF[e]	P388 OD[f]	leukemia ILS[g]
2	chlorambucil						6.75	26 ± 3	0.6 ± 0.2	58 ± 19	225	37
4	quinacrine mustard							1.7 ± 0.3	0.14	11.5 ± 1.5		
7[h]	A	CH_3O		−0.27	40.9	99.0	0.63	2.9	0.34 ± 0.06	42	65	36
8[h]	A	CH_3		−0.17	4.71	111.0	0.41	3.2	0.17 ± 0.14	46	65	22
9[h]	A	CH_3S		0.0	1.24	9.97	1.47	5.3 ± 0.6	0.16 ± 0.03	33 ± 4	65	NA
10[i]	A	CH_3CONH		0.0	3.08	4.22	8.20	3.1 ± 0.5	0.10 ± 0.005	31 ± 65	65	NA
11[h]	A	$(CH_3)_2NCO$		0.36	0.29	0.98	19.2	28 ± 4	1.2 ± 0.3	52 ± 8	65	NA
12[i]	A	CH_3CO		0.50	0.56	0.24	>40	>40	5.5 ± 0.3	>8	45	NA
13[h]	A	CH_3SO_2		0.72	0.16	0.09	39	145	42 ± 9	3.5	100	NA
14	B	–O–	5	−0.27	10.0	11.5	0.04	0.21 ± 0.02	0.005	46 ± 26	20	28
15	B	$-CH_2-$	5	−0.17	3.39	15.7	0.13	0.53 ± 0.03	0.015	36 ± 15	13.3	22
16	B	–S–	5	0.0	0.59	1.69	0.36	0.71 ± 0.04	0.037	19 ± 6	45	23
17	B	–CONH–	4	0.0	0.29	0.25	3.2	0.90 ± 0.01	0.080	11.3 ± 0.8	45	58
18	B	–NHCO–	4	0.36	2.57	17.1	0.51	3.7 ± 0.2	0.673	5.5 ± 0.7	20	NA
19	B	–CO–	5	0.50	0.11	0.18	0.33	3.51 ± 0.05	0.593	5.9 ± 0.5	65	NA
20	B	$-SO_2-$	5	0.72	0.28	0.05	0.64	0.57 ± 0.02	0.57	1.0 ± 0.2	45	33

[a] Hammett electronic parameter; values from ref 37. [b] K_H: rate constant for hydrolysis of mustard in aqueous acetone; see the Experimental Section. [c] K_{NBP}: rate constant for alkylation of 4-(4-nitrobenzyl)pyridine; see the Experimental Section. [d] IC_{50} values determined against either P388, AA8, or UV4 cells, as described in the text, and the standard error of the mean. [e] HF: hypersensitivity factor = IC_{50} (AA8)/IC_{50} (UV4). Values are means of intraexperiment ratios and are therefore not identical with the ratio of the preceding two columns which include all data. [f] OD: optimal dose of drug in milligrams/kilograms, administered as a single dose in 0.1 or 0.2 mL of 30% v/v ethanol/water on day 1 after intraperitoneal inoculation of 10^6 P388 leukemia cells. [g] ILS: the percentage increase in lifespan of drug-treated tumor-bearing animals when treated at the optimal dose; values above 20% are considered statistically significant. [h] Reference 17. [i] Reference 18.

use of a methyl ester as a precursor group for the alkylamine needed in the last step is unusual in synthesis, but a number of other amine-protecting groups explored did not have the necessary stability to the acid-catalyzed reaction with oxirane, and conversion of the acids by the Curtius reaction proceeded in excellent yield (69–96%).

More conventional amine-protecting groups could be used in the synthesis of the carboxamido side chains (**33a,b**) (Scheme II), for here the preformed *N,N*-bis(2-hydroxyethyl)aniline synthons were reacted with the benzyloxycarbonyl-protected alkylamine components to give the intermediate diols **31a,b**. Finally, the SO_2 side chain (**36**) was prepared by perbenzoic acid oxidation of the methyl ester **27c** followed by the Curtius demasking (Scheme III).

The side chains (**29, 33, 36**) were coupled with 9-methoxyacridine[22] at 20 °C to give the desired compounds (**14–20**) in excellent yields.

Kinetics of Hydrolysis and Alkylation

Hydrolysis rates for the compounds were determined under the conditions employed previously by others,[3] namely 50% aqueous acetone at 66 °C. Aliquots were analyzed by HPLC using detection at 254 and 436 nm to determine the percentage of unreacted mustard remaining at each time point, and the first-order rate constants K_H were then calculated as described in the Experimental Section.

The rates of alkylation of 4-(4-nitrobenzyl)pyridine by the mustards were determined at 66 °C in 50% aqueous Me_2CO by the method of Bardos et al.,[23] using spectrophotometric quantitation.

Biological Evaluation

In vitro cytotoxicity was determined under aerobic conditions as described previously,[24] with use of the murine leukemia P388 and Chinese hamster ovary-derived cell lines AA8 and UV4. The UV4 subline of AA8[25,26] lacks the ability to perform the incision step of the excision repair pathway of DNA-damage repair. These cells are consequently from 2- to 10-fold more sensitive than AA8 to compounds which form bulky DNA monoadducts and from 8- to 200-fold more sensitive than AA8 to DNA interstrand cross-linking agents.[27] The ratio of the IC_{50} values of a drug in these two cell lines (hypersensitivity ratio) can be used to gain insight into the primary mechanism of cytotoxicity of DNA-reactive agents, with very large values suggesting cross-linking ability and lesser but significant ratios suggesting bulky monoadduct formation.

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

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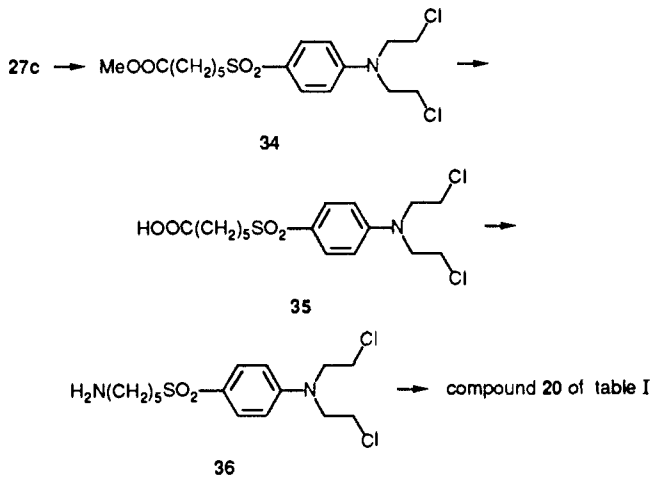
Scheme III



Results and Discussion

Table I gives physicochemical and biological data for the 4-substituted mustards **7–13** and the corresponding acridine-linked compounds **14–20**. Both sets of compounds showed a similar dependence of the rate constants for hydrolysis in aqueous acetone (K_H) on the electronic parameter σ, as shown by eqs 1 and 2 and the equations could be combined to give eq 3. For unknown reasons, compound **18** was much less stable than expected and was omitted from these equations and from eq 6.

compound **7–13**: $\log K_H\ (s^{-1}) = -2.02\ (\pm 0.41)\sigma - 3.49\ (\pm 0.57)$ (1)

$n = 7, r = -0.91, s = 0.37$

compounds **14–20**: $\log K_H\ (s^{-1}) = -1.48\ (\pm 0.59)\sigma - 3.71(\pm 0.88)$ (2)

$n = 6, r = -0.78, s = 0.51$

$\log K_H\ (s^{-1}) = -1.73\ (\pm 0.37)\sigma - 3.71\ (\pm 0.64)$ (3)

$n = 11, r = -0.82, s = 0.46$

The slopes of the equations are identical with that of eq 4, between the percentage hydrolysis at a fixed time and Hammett σ values, determined previously[28] under identical conditions[3] for a larger series of substituted aniline mustards. Equation 4 is derived from eq 22 of ref 28 by

$\log (\%\ \text{hydrolysis}) = -1.42\sigma + 0.51$ (4)

deleting the indicator variable I_o for ortho-substituted mustards and adjusting the constant term to allow for only chlorine mustards. Equations 1 and 2 indicate that, in general, attachment of the acridine chromophore has no effect on the rate of hydrolysis of the mustard, which is dependent only on the electronic effects of the substituent group. An exception is provided by compound **18**, which was not included in the derivation of eqs 2 and 3 and which undergoes hydrolysis 6 times faster than predicted by eq 2 and 5-fold faster than the corresponding compound **12**.

The rates at which the compounds alkylate 4-(4-nitrobenzyl)pyridine (NBP) were also determined by using published protocols,[23] and the rate constants (K_{NBP}) are given in Table I. These results are summarized by eq 5 and 6. In this case the slopes of the two equations are

compounds **7–13**: $\log K_{NBP}\ (s^{-1}) = -3.20\ (\pm 0.33)\sigma - 2.91\ (\pm 0.36)$ (5)

$n = 7, r = -0.97, s = 0.30$

compounds **14–20**: $\log K_{NBP}\ (s^{-1}) = -2.31\ (\pm 0.60)\sigma - 3.73\ (\pm 0.53)$ (6)

$n = 6, r = -0.89, s = 0.53$

significantly different, with the rate of alkylation of the parent mustards **7–13** being much more dependent on the electronic parameter of the substituent. The rates of alkylation by the most unreactive sulfone mustards (**13** and **20**) are very similar. However, the rates diverge as the mustard reactivity increases, so that for the most reactive (O-substituted) mustards, the parent compound (**7**) alkylates NBP about 10-fold more rapidly than the corresponding acridine-linked compound (**14**). We have no explanation for this.

The in vitro cytotoxicities of all the compounds were determined against P388 murine leukemia[29] and against the transformed Chinese hamster fibroblast lines AA8 and UV4, and are recorded in Table I. Cytotoxicities of the majority of the untargeted aniline mustards (**7–13**) in the Chinese hamster lines have been reported recently,[17] and data for chlorambucil (**2**), quinacrine mustard (**4**), the remaining two simple mustards (**10** and **12**), and the acridine-linked compounds (**14–20**) were determined under the same conditions, using log-phase adherent cultures of AA8 or UV4 cells exposed to drugs for 1 h and then incubated in the absence of drug for a further 95 h. Hypersensitivity factors (HF = IC_{50}(AA8)/IC_{50}(UV4)) were calculated and are also given in Table I.

For the untargeted mustards (**7–13**) there is a clear relationship in both cell lines between cytotoxic potency and substituent electronic parameter, as noted previously.[17] Against P388, the range in IC_{50} values was greater than 100-fold. In the wild-type AA8 cell line the range of IC_{50} values was approximately 50-fold (although many of the compounds were too insoluble for reliable data to be obtained), and against the UV4 cell line it was greater than 300-fold. The data for the P388 cell line (excluding compound **12** for which a toxic level could not be reached due to insolubility) could be summarized by eq 7 and a very similar relationship (eq 8) summarizes the UV4 data.[17] As expected, eq 8 has a similar slope to eq 4 of ref 17 ($\log IC_{50} = 2.50\sigma - 5.79$, $n = 13$, $r = 0.97$). In contrast, the IC_{50}

$\log IC_{50}(\text{molar}) = 2.01\ (\pm 0.38)\sigma - 5.66\ (\pm 0.73)$ (7)

$n = 6, r = 0.91, s = 0.31$

$\log IC_{50}(\text{molar}) = 2.30\ (\pm 0.52)\sigma - 6.42\ (\pm 0.69)$ (8)

$n = 7, r = 0.89, s = 0.46$

values of the corresponding acridine-linked mustards (**14–20**) were much lower and also varied much less with σ. The more reactive mustards (e.g. **14**, **15**) were 5–10-fold more potent than the analogous simple mustards, but the less reactive compounds (e.g. **19**, **20**) were over 100-fold more potent.

All of the simple mustards with the exception of sulfone **13** showed high hypersensitivity factors (>40), suggesting that their primary mode of cytotoxicity is DNA cross-linking.[17,27] The value of 3.5 for sulfone **13** is still consistent with cross-linking, but less convincing. Hypersensitivity ratios for the DNA-targeted compounds (**14–20**) varied

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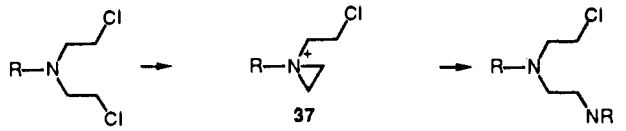
Scheme IV



aziridinium ion intermediate

more. For the most reactive mustards (**14**, **15**), the values were high enough to suggest that the primary cytotoxic effect is DNA cross-linking. However, the considerably lower HF values shown by the remainder of the compounds raise the possibility that another mode of DNA interaction (possibly bulky monoadduct formation) may be important.

In vivo antitumor activity was measured against P388 leukemia, taking care to employ a soluble drug formulation and giving only a single dose on day 1. This is a very stringent protocol, and under these conditions the clinical agent chlorambucil (**2**) gives an ILS of only 32% at a dose of 225 mg/kg (Table I). In contrast to the wide variation in cytotoxic potencies of the untargeted mustards (**7–13**), the optimal in vivo doses of these compounds varied by only 2-fold. The more reactive compounds were generally the more toxic, but there was no clear relationship. Only the two most reactive compounds (**7** and **8**) showed in vivo activity when this protocol was used. The acridine-linked mustards (**14–20**) were generally more dose-potent in vivo than the corresponding untargeted mustards, although only by 2–3-fold. Most of the compounds showed in vivo activity, with the best being the CONH-linked compound (**17**). This was clearly more active in vivo (ILS 58%) than either chlorambucil or the untargeted mustards.

Conclusions

This work was undertaken to determine the effects of DNA-targeting on a series of aniline mustards of identical alkylator chemistry but varying reactivity. Attachment of the DNA-targeting acridine chromophore to the simple mustards had no effect on the hydrolytic stability of the mustard, since the hydrolysis data for both sets of compounds **7–13** and **14–20** were well fit by a single equation in σ (eq 3). However, the rates of alkylation of NBP did appear to be affected by attachment of the chromophore, which reduced the alkylating ability of the more reactive mustards.

While the untargeted mustards **7–13** showed the expected correlation of in vitro cytotoxicity with mustard reactivity, this influence was almost entirely lost in the DNA-targeted compounds. Although the latter compounds were generally more potent, this differential became more marked as mustard reactivity declined. Thus, the reactive methylene-linked compounds **8** and **15** differed in potency by only 3-fold against P388, while the least reactive sulfone-linked compounds **13** and **20** differed by 60-fold.

There has been much debate about the mechanism of alkylation by aryl mustards,[10,23] but detailed kinetic studies of their reaction with DNA are not available. However, the general mechanism of alkylation by aryl nitrogen mustards with all but very powerful nucleophiles is considered to proceed through an aziridinium ion intermediate (**37**)[30,31] (Scheme IV). Recent kinetic studies using a related series of aryl nitrogen mustards have demonstrated that both hydrolysis and NBP alkylation reactions proceed via an S_N2 mechanism.[32] Therefore, alkylation reactions with soluble nucleophiles should exhibit bimolecular kinetics. These observations suggest that targeting an alkylating agent to DNA (and thus effectively raising the local concentration of the DNA nucleophile) should increase the rate of DNA alkylation.

In the present study, the in vitro cytotoxicities of the more reactive mustards were improved the least by DNA targeting, while those of the less reactive were improved the most. However, it must be remembered that in vitro cytotoxic potency is the resultant of a large number of events apart from the rate of formation of the DNA lesion. From a drug-design point of view, the important question is whether this strategy of DNA targeting can provide useful antitumor agents. The preliminary in vivo results reported here suggest that targeted aniline mustards (e.g. **17**) do show improved activity over the corresponding untargeted compounds, and that less reactive alkylating moieties can possibly be used.

Experimental Section

Elemental analyses were carried out in the Microchemical Laboratory, University of Otago, and are indicated by symbols of the elements where they were within ±0.4% of the calculated values. Melting points were determined on a Reichert-Kofler block and are uncorrected. High-resolution mass spectra were recorded on a VG-7070 spectrometer at nominal 3000 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.

Preparation of Compound 14 of Table I. Methyl 6-[4-[*N*,*N*-Bis(2-hydroxyethyl)amino]phenoxy]hexanoate (26a). Methyl 6-(4-nitrophenoxy)hexanoate[19] (**24a**) (5 g, 18.7 mmol) was hydrogenated over Pd/C in MeOH to give the crude amine **25a** (4.39 g, 100%): ^{1}H NMR (60 MHz) ($CDCl_3$) δ 6.63 (br s, 4 H, aromatics), 3.85 (t, J = 6 Hz, 2 H, OCH_2), 3.63 (s, 3 H, $COOMe$), 3.36 (br s, 2 H, NH), 2.35 (t, J = 6 Hz, 2 H, CH_2COOMe), 1.61 (m, 8 H, methylenes). The above amine (**25a**) (1.6 g, 6.75 mmol), AcOH (3 drops), and oxirane (0.9 g, 20.2 mmol) were dissolved in dry THF (20 mL), and the mixture was stirred at 110–120 °C for 48 h in a pressure vessel. The cooled mixture was concentrated under reduced pressure, and the residue was chromatographed on silica gel. Elution with EtOAc/petroleum ether (2:3) gave a brown solid which was recrystallized twice from benzene/petroleum ether to give **26a** as light brown plates (1.12 g, 51% yield): mp 59–61 °C; ^{1}H NMR ($CDCl_3$) δ 6.81 (d, J = 9.1 Hz, 2 H, H-2′,6′), 6.72 (d, J = 9.1 Hz, 2 H, H-3′,5′), 3.89 (t, J = 6.4 Hz, 2 H, H-6), 3.77 (t, J = 5.0 Hz, 4 H, CH_2OH), 3.67 (s, 3 H, COOMe), 3.44 (t, J = 5.0 Hz, 4 H, NCH_2), 2.34 (t, J= 7.6 Hz, 2 H, H-2), 1.77 (m, 2 H, H-5), 1.48 (m, 2 H, H-4); ^{13}C NMR δ 174.16 (q, C=O), 151.96 (q, C-1′), 142.60 (q, C-4′), 115.96 (CH, C-2′,6′), 115.69 (CH, C-3′,5′), 68.34 (CH_2, C-6), 60.68 (CH_2, CH_2OH), 55.97 (CH_2, NCH_2), 51.52 (CH_3, $COOCH_3$), 34.01 (CH_2, C-2), 29.08 (CH_2, C-5), 25.68 (CH_2, C-3), 24.71 (CH_2, C-4); mass spectrum m/z 325 ($M^{+\cdot}$), 294 (M − OMe) (100). Anal. ($C_{17}H_{27}NO_5$) C, H, N.

6-[4-[*N*,*N*-Bis(2-chloroethyl)amino]phenoxy]hexanoic Acid (28a). A solution of the above diol (**26a**) (1.14 g, 3.5 mmol) in benzene (15 mL) was treated with $POCl_3$ (0.65 mL, 7.0 mmol), and the mixture was heated under reflux for 1 h, cooled, and concentrated under reduced pressure to give the crude ester **27a**. This was dissolved in concentrated HCl (20 mL), and the mixture was warmed for 30 min and finally heated under reflux for an additional 10 min. The cooled mixture was concentrated under reduced pressure, the residue was diluted with ice–water, and the mixture was adjusted to neutral pH with concentrated NH_4OH. Extraction with EtOAc (3 × 100 mL) and workup of the organic layer gave the crude acid **28a** (1.07 g, 88% yield). Recrystallization from toluene/petroleum ether gave prisms: mp 94–96 °C; ^{1}H NMR ($CDCl_3$) δ 6.83 (d, J = 9.1 Hz, 2 H, H-2′,6′), 6.68 (d, J =

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9.1 Hz, 2 H, H-3′,5′), 3.90 (t, J = 6.4 Hz, 2 H, H-6), 3.64 (t, J = 6.5 Hz, 4 H, NCH_2), 3.59 (t, J = 6.6 Hz, 4 H, CH_2Cl), 2.40 (t, J = 7.5 Hz, 2 H, H-2), 1.78 (m, 2 H, H-5), 1.71 (m, 2 H, H-3), 1.52 (m, 2 H, H-4); ^{13}C NMR δ 179.31 (q, C=O), 151.92 (q, C-1′), 140.52 (q, C-4′), 115.95 (CH, C-2′,6′), 114.65 (CH, C-3′,5′), 68.25 (CH_2, C-6), 54.33 (CH_2, NCH_2), 40.77 (CH_2, CH_2Cl), 33.86 (CH_2, C-2), 27.03 (CH_2, C-5), 25.61 (CH_2, C-3), 24.43 (CH_2, C-4); mass spectrum, m/z 347/49/51 ($M^{\bullet+}$), 298 (M - CH_2Cl) (100). Anal. ($C_{16}H_{23}Cl_2NO_3$) C, H, N, Cl.

5-[4-[*N*,*N*-Bis(2-chloroethyl)amino]phenoxy]pentylamine (26a). Dry Et_3N (0.38 mL, 2.76 mmol) was added slowly to a solution of the above acid (**28a**) (0.8 g, 2.3 mmol) in Me_2CO (2 mL). A solution of ethyl chloroformate (0.26 mL, 2.76 mmol) in Me_2CO (2 mL) was added slowly, while the temperature was kept below 0 °C. After the mixture was stirred for a further 15 min at 0 °C, a solution of NaN_3 (0.295 g, 4.6 mmol) in water (2 mL) was added slowly, while the temperature was kept below 0 °C. After stirring for a further 30 min, the reaction mixture was diluted with ice–water and extracted with benzene (3 × 50 mL). The combined organic extracts were dried and filtered, heated under reflux for 1 h, and concentrated under reduced pressure. The residue was dissolved in 8 M HCl (20 mL) and heated under reflux for 10 min (or until CO_2 evolution ceased). The reaction mixture was concentrated under reduced pressure, the pH was adjusted to 12 with concentrated NH_4OH, and the mixture was then extracted with EtOAc (3 × 100 mL). Workup of the organic layer gave the crude amine **29a** (0.70 g, 96% yield): ^1H NMR (60 MHz) ($CDCl_3$) δ 6.73 (dd, J = 5, 12 Hz, 4 H, aromatics), 3.87 (t, J = 8 Hz, 2 H, OCH_2), 3.55 (br s, 8 H, NCH_2CH_2Cl), 2.68 (br s, 2 H, NH), 1.52 (br m, 8 H, methylenes).

Compound 14 of Table I. A mixture of the above crude amine **29a** (0.7 g, 2.2 mmol) and 9-methoxyacridine (0.49 g, 2.2 mmol) in dry MeOH (20 mL) was stirred for 24 h at 20 °C and then concentrated to dryness under reduced pressure. Chromatography of the residue on silica gel and elution with EtOAc gave the free base of **14** as a yellow oil (0.90 g, 83% yield). This was dissolved in EtOAc, and EtOAc saturated with dry HCl was added to precipitate the dihydrochloride salt, which was filtered and washed with EtOAc: mp ca. 50 °C dec; ^1H NMR (CD_3SOCD_3) δ 14.28 (s, 1 H, acridine N·*H*Cl), 10.08 (t, J = 5.5 Hz, 1 H, $NHCH_2$), 8.66 (br s, 2 H, acridine H-1″,8″), 8.03 (d, J = 8.5 Hz, 2 H, acridine H-3″,6″), 7.96 (t, J = 8.5 Hz, 2 H, acridine H-4″,5″), 7.52 (t, J = 7.4 Hz, 2 H, acridine H-2″,7″), 6.79 (m, 4 H, phenyl protons), 4.12 (q, J = 6.7 Hz, 2 H, H-1), 3.87 (t, J = 6.3 Hz, 2 H, H-5), 3.65 (br s, 8 H, NCH_2CH_2Cl), 1.98 (m, 2 H, H-2), 1.73 (m, 2 H, H-4), 1.53 (m, 2 H, H-3); ^{13}C NMR δ 157.19 (q, C-5a″,10a″), 134.79 (CH, C-1″,8″), 128.22 (CH, C-4″,5″), 123.22 (CH, C-3″,6″), 118.38 (CH, C-2″,7″), 115.56 (CH, C-2′,6′), 114.44 (CH, C-3′,5′), 67.48 (CH_2, C-5), 53.00 (CH_2, NCH_2), 48.48 (CH_2, C-1), 40.94 (CH_2, CH_2Cl), 28.44 (CH_2, C-4), 28.24 (CH_2, C-2), 22.80 (CH_2, C-3); mass spectrum, m/z 495/97/99 ($M^{\bullet+}$), 460 (M - Cl, 263, 207 (100). Anal. ($C_{28}H_{31}Cl_2N_3O$·2HCl·H_2O) C, H, N.

Preparation of Compound 15 of Table I. Methyl 7-[4-[*N*,*N*-Bis(2-hydroxyethyl)amino]phenyl]heptanoate (26b). Esterification of 7-(4-nitrophenyl)heptanoic acid[20] with $MeOH/H_2SO_4$ gave methyl 7-(4-nitrophenyl)heptanoate (**24b**), which crystallized from toluene/petroleum ether: mp 50–51 °C; ^1H NMR (60 MHz) ($CDCl_3$) δ 8.12 (d, J = 8.0 Hz, 2 H, H-3′,5′), 7.28 (d, J = 8.0 Hz, 2 H, H-2′,6′), 3.65 (br s, 3 H, COOMe), 2.67 (t, J = 7.0 Hz, 2 H, $PHCH_2$), 2.28 (t, J = 7.0 Hz, 2 H, CH_2COOMe), 1.49 (m, 8 H, methylenes). Reduction ($Pd/C/H_2$) in MeOH gave the amine **25b**, which was used directly. A mixture of the amine (1.17 g, 7.23 mmol), oxirane (2 mL), and AcOH (2 drops) in dry THF (25 mL) was stirred at 80–90 °C in a pressure vessel for 48 h. The reaction mixture was then concentrated under reduced pressure, and the residue was chromatographed on silica gel. Elution with EtOAc/petroleum ether (2:3) gave **26b** (2.0 g, 86% yield) as an oil: ^1H NMR ($CDCl_3$) δ 7.02 (d, J = 8.7 Hz, 2 H, H-2′,6′), 6.61 (d, J = 8.7 Hz, 2 H, H-3′,5′), 3.81 (t, J = 5.0 Hz, 4 H, CH_2OH), 3.63 (s, 3 H, COOMe), 3.51 (t, J = 4.8 Hz, 4 H, NCH_2), 2.48 (t, J = 7.9 Hz, 2 H, $PhCH_2$), 2.30 (t, J = 7.7 Hz, 2 H, CH_2COOMe, 1.33 and 1.58 (m, 8 H, methylenes); ^{13}C NMR δ 174.40 (q, C=O), 145.92 (q, C-1′), 131.22 (q, C-4′), 129.15 (CH, C-2′,6′), 112.77 (CH, C-3′,5′), 60.82 (CH_2, CH_2OH), 55.54 (CH_2, NCH_2), 51.49 (CH_3, $COOCH_3$), 34.69 (CH_2, C-7), 34.08 (CH_2, C-2), 31.58 (CH_2, C-6), 29.01 (CH_2, C-3), 28.89 (CH_2, C-5), 24.89 (CH_2, C-4); mass spectrum, m/e 323 ($M^{\bullet+}$), 292 (M - OMe) (100). Anal. ($C_{18}H_{29}NO_4$·0.5 H_2O) C, H, N.

7-[4-[*N*,*N*-Bis(2-chloroethyl)amino]phenyl]heptanoic Acid (28b). Treatment of **26b** with $POCl_3$ in benzene followed by acid hydrolysis as detailed above gave a brown oil, which was crystallized from toluene and then from benzene/petroleum ether to tive **28b** as a colorless solid (49% yield): mp 80–83 °C; ^1H NMR ($CDCl_3$) δ 7.06 (d, J = 8.7 Hz, 2 H, H-2′,6′), 6.62 (d, J = 8.7 Hz, 2 H, H-3′,5′), 3.69 (t, J = 6.1 Hz, 4 H, NCH_2), 3.62 (t, J = 6.1 Hz, 4 H, CH_2Cl), 2.51 (t, J = 7.9 Hz, 2 H, $PhCH_2$), 2.34 (t, J = 7.5 Hz, 2 H, CH_2COOH), 1.35 and 1.60, 8 H, m, methylenes); ^{13}C NMR δ 179.53 (q, C=O), 144.07 (q, C-1′), 131.93 (q, C-4′), 129.57 (CH, C-2′,6′), 112.10 (CH, C-3′,5′), 53.64 (CH_2, NCH_2), 40.55 (CH_2, CH_2Cl), 34.66 (CH_2, C-7), 33.92 (CH_2, C-2), 31.49 (CH_2, C-6), 28.91 (CH_2, C-3), 28.84 (CH_2, C-5), 24.61 (CH_2, C-4); mass spectrum, m/z 345/47/49 ($M^{\bullet+}$), 296 (M - CH_2Cl) (100). Anal. ($C_{17}H_{25}Cl_2NO_2$) C, H, N, Cl.

Compound 15 of Table I. The above acid (**28b**) was subjected to the modified Curtius reaction described above, to give the crude amine **29b** in 94% yield. This was coupled with 9-methoxyacridine as described above to give an 88% yield of the free base of compound **15** as a yellow oil. The dihydrochloride salt, prepared as described above, was a bright yellow solid: mp ca. 50 °C dec; ^1H NMR (CD_3SOCD_3) δ 10.07 (t, J = 5.7 Hz, 1 H, NH), 8.68 (br s, 2 H, acridine H-1″,8″), 8.03 (d, J = 8.6 Hz, 2 H, acridine H-3″,6″), 7.96 (t, J = 8.5 Hz, 2 H, acridine H-4″,5″), 7.52 (t, J = 7.4 Hz, 2 H, acridine H-2″,7″), 6.98 (d, J = 8.7 Hz, 2 H, H-2′,6′), 6.64 (d, J = 8.7 Hz, 2 H, H-3′,5′), 4.08 (q, J = 7.12 Hz, 2 H, H-1), 3.67 (br s, 8 H, NCH_2CH_2Cl) 2.41 (t, J = 7.6 Hz, 2 H, H-6), 1.90 (m, 2 H, H-5), 1.49 (m, 2 H, H-3), 1.31 (m, 2 H, H-4); ^{13}C NMR δ 157.13 (q, C-5a″,10a″), 144.07 (a, C-1′), 134.69 (CH, C-1″,8″), 130.39 (q, C-4′), 129.10 (CH, C-2′,6′), 123.15 (CH, C-3″,6″), 118.37 (CH, C-2″,7″), 111.83 (CH, C-3′,5′), 52.16 (CH_2, NCH_2), 48.53 (CH_2, C-1), 39.40 (CH_2, CH_2Cl), 33.82 (CH_2, C-6), 30.99 (CH_2, C-2), 28.66 (CH_2, C-5), 28.07 (CH_2, C-3), 25.93 (CH_2, C-4); mass spectrum, m/z 493/95/97 ($M^{\bullet+}$), 458 (M - Cl), 263, 207. Anal. ($C_{29}H_{33}Cl_2N_3$·2HCl·0.5H_2O) C, H, N.

Preparation of Compound 16 of Table I. Methyl 6-[(4-Nitrophenyl)thio]hexanoate (24c). A mixture of sodium 4-nitrothiophenolate (17.7 g, 0.1 mol) and methyl 6-bromohexanoate (21.1 g, 0.1 mol) in MeOH (400 mL) was heated under reflux for 1.5 h, and the solvent was then removed under reduced pressure. The residue was partitioned between CH_2Cl_2 and water, and the organic phase was washed four times with 2 M NaOH and once with brine, and the solvent was evaporated to give a solid. This was recrystallized from aqueous MeOH to give **24c** (28.1 g, 99% yield) as yellow needles: mp 48.5–49.5 °C; ^1H NMR ($CDCl_3$) δ 8.12 (d, J = 9.08 Hz, 2 H, H-2′,6′), 7.31 (d, J = 9.08 Hz, 2 H, H-3′,5′), 3.66 (s, 3 H, COOMe), 3.03 (t, J = 7.30 Hz, 2 H, SCH_2), 2.34 (t, J = 7.32 Hz, 2 H, CH_2COOMe), 1.75 (m, 2 H, H-5), 1.67 (m, 2 H, H-3), 1.51 (m, 2 H, H-4); ^{13}C NMR δ 173.89 (q, C=O), 147.84 (q, C-1′), 144.90 (q, C-4′), 126.06 (CH, C-2′,6′), 123.96 (CH, C-3′,5′), 51.59 (CH_3, OMe), 33.77 (CH_2, SCH_2), 31.69 (CH_2, CH_2COMe), 28.28 (CH_2, C-5), 28.14 (CH_2, C-3), 24.37 (CH_2, C-4); mass spectrum, m/z 283 ($M^{\bullet+}$), 266 (M - OH), 253. Anal. ($C_{13}H_{17}NO_4S$) C, H, N, S.

Methyl 6-[[4-[*N*,*N*-Bis(2-hydroxyethyl)amino]phenyl]-thio]hexanoate (26c). The above nitro ester (**24c**) was hydrogenated over Pd/C in MeOH for 17 h to give the crude amine **25c** in 85% yield. A solution of this amine (1.50 g, 5.9 mmol) in CH_2Cl_2 (15 mL) and 2 drops of AcOH was cooled to 0 °C and oxirane (0.2 g, 5 mmol) was added slowly. The solution was kept at 20 °C for 3 days, with successive additions of oxirane (0.2 mL) at 24 and 48 h. The reaction mixture was then diluted with CH_2Cl_2, washed with saturated $NaHCO_3$ (2×) and brine, and concentrated. The residue was chromatographed on silica gel with EtOAc as eluant, giving the diol **26c** (0.53 g, 27% yield) as an oil: ^1H NMR ($CDCl_3$) δ 7.28 (d, J = 8.61 Hz, 2 H, H-2′,6′), 6.59 (d, J = 8.61 Hz, 2 H, H-3′,5′), 3.75 (t, J = 6.54 Hz, 4 H, CH_2OH), 3.61 (s, 3 H, COOMe), 3.50 (t, J = 6.54 Hz, 4 H, NCH_2), 2.74 (t, J = 6.90 Hz, 2 H, H-6), 2.36 (t, J = 6.70 Hz, 2 H, CH_2COOMe), 1.58 (m, 4 H, H-3,5), 1.38 (m, 2 H, H-4); ^{13}C NMR δ 174.27 (q, C=O) 147.13 (q, C-1′), 134.01 (CH, C-2′,6′), 121.32 (q, C-4′), 112.82 (CH, C-3′,5′), 60.54 (CH_2, CH_2OH), 55.31 (CH_2, NCH_2), 51.55 (CH_3, OMe), 36.28 (CH_2, SCH_2), 33.93 (CH_2, CH_2CO_2Me) (C-2), 29.04 (CH_2, C-5), 28.06 (CH_2, C-3), 24.47 (CH_2, C-4); mass spectrum,

m/z 341 ($M^{\bullet+}$), 310 (M - OMe), 181, 136, 87, 69, 45.

6-[[4-[*N*,*N*-Bis(2-chloroethyl)amino]phenyl]thio]hexanoic Acid (28c). A solution of the above diol (**26c**) (1.60 g, 4.69 mmol) in 1,2-dichloroethane (7 mL) and DMF (1 drop) was treated with $SOCl_2$ (0.75 mL, 10 mmol). The mixture was heated under reflux for 25 min, the volatiles were then removed under reduced pressure, and the residue was partitioned between EtOAc and water. Workup of the organic layer gave the crude mustard **27c** as a brown oil (0.97 g, 55% yield): ^{1}H NMR ($CDCl_3$) δ 7.33 (d, J = 8.60 Hz, 2 H, H-2′,6′), 6.63 (d, J = 8.72 Hz, 2 H, H-3′,5′), 3.74 (t, J = 6.69 Hz, 4 H, NCH_2), 3.66 (s, 3 H, OCH_3), 3.61 (t, J = 6.92 Hz, 4 H, CH_2Cl), 2.76 (t, J = 7.26 Hz, 2 H, H-6), 2.30 (t, J = 7.40 Hz, 2 H, H-2), 1.60 (m, 4 H, H-3,5), 1.42 (m, 2 H, H-4). A solution of this crude mustard (0.82 g, 2.17 mmol) in concentrated HCl was heated to 90 °C for 1 h. The solution was cooled to 20 °C and the pH was adjusted to 7 with ammonia, and the mixture was extracted with EtOAc (3×). The combined organic layers were worked up to give an oil which was filtered through a column of charcoal and silica gel in EtOAc to give the acid **28c** as a solid (0.72 g, 92% yield). Crystallization from CH_2Cl_2/hexane gave white crystals: mp 93–94 °C; ^{1}H NMR ($CDCl_3$) δ 7.33 (d, J = 8.89 Hz, 2 H, H-2′,6′), 6.63 (d, J = 8.93 Hz, 2 H, H-3′,5′), 3.74 (t, J = 1.12 Hz, 4 H, NCH_2), 3.62 (t, J = 1.30 Hz, 4 H, CH_2Cl), 2.77 (t, J = 7.16 Hz, 2 H, SCH_2), 2.34 (t, J = 7.37 Hz, 2 H, CH_2COOH), 1.61 (m, 4 H, H-3,5), 1.42 (m, 2 H, H-4); ^{13}C NMR δ 174.09 (q, C=O), 145.33 (q, C-1′), 134.09 (CH, C-2′,6′), 122.80 (q, C-4′), 112.42 (CH, C-3′,5′), 53.45 (CH_2, NCH_2), 51.52 (CH_3, OMe), 40.31 (CH_2, CH_2Cl), 36.08 (CH_2, SCH_2), 33.92 (CH_2, CH_2CO_2Me) (C-2), 29.04 (CH_2, C-5), 28.12 (CH_2, C-3), 24.50 (CH_2, C-4); mass spectrum, m/z 363 ($M^{\bullet+}$), 314 (M - CH_2Cl), 251, 199, 136, 69. Anal. ($C_{16}H_{23}Cl_2NO_2S$) C, H, N, Cl.

Compound 16 of Table I. The acid **28c** was subjected to the modified Curtius reaction described above to give a 69% yield of the crude amine **29c** as a brown oil. This was coupled directly with 9-methoxyacridine to give a 60% yield of the free base of compound **16** as a yellow oil. The dihydrochloride salt crystallized from MeOH/EtOAc as a yellow solid: mp 85–86 °C; ^{1}H NMR (CD_3SOCD_3) δ 14.05 (s, 1 H, acridine N·HCl), 8.61 (br s, 2 H, acridine H-1″,8″), 7.97 (m, 4 H, acridine H-2″,4″,5″,7″), 7.54 (t, J = 4.15 Hz, 2 H, H-3″,6″), 7.20 (d, J = 8.74 Hz, 2 H, H-2′,6′), 6.68 (d, J = 8.74 Hz, 2 H, H-3′,5′), 4.02 (t, J = 6.84 Hz, 2 H, H-5), 3.85 (br s, 4 H, NCH_2), 3.71 (br s, 4 H, CH_2Cl), 2.76 (t, J = 6.54 Hz, 2 H, H-1), 1.89 (t, J = 7.49 Hz, 2 H, H-4), 1.51 (m, 4 H, H-2,3); ^{13}C NMR δ 157.28 (q, C-5a″,10a″), 145.50 (q, C-1′), 134.81 (C-2′,6′,C-1″,8″), 133.23 (q, C-9), 133.13 (CH, C-3″,6″), 120.85 (CH, C-4″,5″), 118.39 (CH, C-2″,7″,C-4′), 112.36 (CH, C-3′,5′), 51.84 (CH_2, NCH_2), 48.52 (CH_2, SCH_2, C-5), 40.89 (CH_2, CH_2Cl); mass spectrum, m/z 515.1597 (12), 513.1616 (72), 511.1628 (100), 476 (M - Cl), 460, 440, 424. Anal. ($C_{28}H_{31}Cl_2N_3S$·2HCl) C, H, N.

Preparation of Compound 19 of Table I. 6-(4-Aminobenzoyl)hexanoic Acid (23). 2-(4-Nitrobenzoyl)cyclohexanone (**21**) was hydrogenated over Pd/C in MeOH to give the amine **22** in 75% yield. Crystallization from CH_2Cl_2 gave needles: mp 175–177 °C; ^{1}H NMR (CD_3SOCD_3) δ 7.62 (d, J = 8.7 Hz, 2 H, H-2′,6′), 6.55 (d, J = 8.7 Hz, 2 H, H-3′,5′), 6.07 (br s, 2 H, NH_2), 4.48 (t, J = 7 Hz, 1 H, H-2), 2.08 (m, 8 H, H-3,4,5,6); ^{13}C NMR δ 209.16 (q, PhC=O), 194.74 (q, C=O, C-1), 153.62 (q, C-1′), 130.69 (CH, C-2′,6′), 124.41 (q, C-4′), 112.30 (CH, C-3′,5′), 56.62 (CH, C-2), 41.72 (CH_2, C-6), 29.65 (CH_2, C-3), 26.82 (CH_2, C-5), 22.97 (CH_2, C-4); mass spectrum, m/z 217 ($M^{\bullet+}$), 120 (100). Anal. ($C_{13}H_{15}NO_2$) C, H, N. This amine (2.0 g, 9.2 mmol) was suspended in water (50 mL) containing KOH (1.56 g) and heated under reflux until homogeneous (ca. 15 min) and for a further 1 h. The cooled mixture was acidified with dilute HCl and extracted with EtOAc (3 × 50 mL). The combined organic layers were worked up, and the residue was chromatographed on silica gel. EtOAc/petroleum ether (1:2) eluted the desired acid (**23**) (1.32 g, 61% yield). Crystallization from benzene gave crystals: mp 136–138 °C; ^{1}H NMR (CD_3OD) δ 7.75 (d, J = 8.7 Hz, 2 H, H-2′,6′), 6.63 (d, J = 8.7 Hz, 2 H, H-3′,5′), 2.86 (t, J = 7.4 Hz, 2 H, $COCH_2$), 2.29 (t, J = 7.4 Hz, 2 H, H-2), 1.64 (m, 4 H, H-3.5), 1.39 (m, 2 H, H-4); ^{13}C NMR δ 201.36 (q, C=O, C-7), 177.60 (q, C=O, C-1), 155.21 (q, C-1′), 131.90 (CH, C-2′,6′), 126.67 (q, C-4′), 114.25 (CH, C-3′,5′), 38.46 (CH_2, C-6), 34.82 (CH_2, C-2), 30.00 (CH_2, C-5), 26.01 (CH_2, C-3), 25.96 (CH_2, C-4); mass spectrum, m/z 235 ($M^{\bullet+}$), 135. Anal. ($C_{13}H_{17}NO_3$) C, H, N.

Methyl 6-[4-[*N*,*N*-Bis(2-hydroxyethyl)amino]benzoyl]hexanoate (26d). Esterification of the above amino acid (**23**) with $MeOH/H_2SO_4$ gave an 87% yield of the methyl ester (**25d**): mp (toluene/CH_2Cl_2) 93–95 °C; ^{1}H NMR ($CDCl_3$) δ 7.80 (d, J = 8.5 Hz, 2 H, H-2′,6′), 6.65 (d, J = 8.5 Hz, 2 H, H-3′,5′), 4.18 (br s, 2 H, NH_2), 3.66 (s, 3 H, COOMe), 2.87 (t, J = 7.5 Hz, 2 H, $COCH_2$), 2.33 (t, J = 7.5 Hz, 2 H, CH_2COOH), 1.68 and 1.40 (m, 8 H, methylenes); mass spectrum, m/z 249 ($M^{\bullet+}$), 135. Anal. ($C_{14}H_{19}NO_3$) C, H, N. A suspension of the above ester (4.4 g, 17.7 mmol) in AcOH/THF (1:1) (200 mL) was treated with oxirane (3.2 g, 73 mmol), and the mixture was stirred at 20 °C for 168 h, with five periodic additions of 1-mL portions of oxirane. At the end of this time the solution was concentrated under reduced pressure and poured into ice–water (300 mL). The mixture was extracted with EtOAc (4 × 100 mL), and the combined organic layers were washed with ice-cold saturated $NaHCO_3$ and brine and concentrated to give an oil, which was chromatographed on silica gel. Elution with EtOAc/petroleum ether (1:1) gave the diol **26d** (2.25 g, 38% yield), which crystallized from benzene: mp 64–66 °C; ^{1}H NMR ($CDCl_3$) δ 7.80 (d, J = 9.2 Hz, 2 H, H-2′,6′), 6.65 (d, J = 9.2 Hz, 2 H, H-3′,5′), 3.89 (t, J = 5.0 Hz, 4 H, CH_2OH), 3.72 (s, 2 H, OH), 3.66 (s, 3 H, COOMe), 3.66 (t, J = 5.0 Hz, 4 H, NCH_2), 2.85 (t, J = 7.6 Hz, 2 H, $COCH_2$), 2.32 (t, J = 7.6 Hz, 2 H, CH_2COOH), 1.68 (m, 4 H, H-3,5), 1.39 (m, 2 H, H-4); ^{13}C NMR δ 198.77 (q, C=O, C-7), 174.28 (q, C=O, C-1), 151.45 (q, C-1′), 130.37 (CH, C-2′,6′), 125.52 (q, C-4′), 111.24 (CH, C-3′,5′), 60.47 (CH_2, CH_2OH), 55.07 (CH_2, NCH_2), 51.52 (CH_3, $COOCH_3$), 37.61 (CH_2, C-6), 33.92 (CH_2, C-2), 28.91 (CH_2, C-5), 24.78 (CH_2, C-3), 24.50 (CH_2, C-4); mass spectrum, m/z 337 ($M^{\bullet+}$), 306 (M - OMe) (100). Anal. ($C_{18}H_{27}NO_5$) C, H, N.

6-[4-[*N*,*N*-Bis(2-chloroethyl)amino]benzoyl]hexanoic Acid (28d). A solution of the diol **26d** (0.2 g, 0.6 mmol) and Et_3N (0.25 mL, 1.8 mmol) in dry CH_2Cl_2 (5 mL) was treated dropwise at 0 °C with MsCl (0.12 mL, 1.5 mmol). The mixture was stirred for a further 3 h at 20 °C and partitioned between water and additional CH_2Cl_2. The organic layer was washed with water, ice-cold aqueous $NaHCO_3$, and water, separated, dried, and evaporated under reduced pressure to give the crude dimesylate. This was dissolved in DMF (5 mL), excess NaCl was added, and the mixture was heated under reflux for 5 min. Excess DMF was removed by evaporation at low pressure, and the residue was partitioned between EtOAc and water. Workup of the organic layer gave crude **27d** as a brown oil, which was heated in concentrated HCl (10 mL) at 80 °C for 1 h. Extraction of the cooled mixture with EtOAc gave the mustard **28d** (0.17 g, 90% yield, which crystallized from benzene: mp 91–93 °C; ^{1}H NMR ($CDCl_3$) δ 7.90 (d, J = 9.1 Hz, 2 H, H-2′,6′), 6.68 (d, J = 9.1 Hz, 2 H, H-3′,5′), 3.82 (t, J = 6.9 Hz, 4 H, NCH_2), 3.67 (t, J = 6.9 Hz, 4 H, CH_2Cl), 2.89 (t, J = 7.6 Hz, 2 H, H-6), 2.38 (t, J = 6.7 Hz, 2 H, H-2), 1.73 (m, 4 H, H-3,5), 1.44 (m, 2 H, H-4); ^{13}C NMR δ 198.43 (q, C=O, C-7), 179.24 (q, C=O, C-1), 149.72 (q, C-1′), 130.70 (CH, C-2′,6′), 126.62 (q, C-4′), 110.85 (CH, C-3′,5′), 53.26 (CH_2, NCH_2), 40.11 (CH_2, CH_2Cl), 37.56 (CH_2, C-6), 33.80 (CH_2, C-2), 28.82 (CH_2, C-5), 24.54 (CH_2, C-3), 24.33 (CH_2, C-4); mass spectrum, m/z 347/49/51 ($M^{\bullet+}$), 298 (M - CH_2Cl) (100). Anal. ($C_{17}H_{23}Cl_2NO_3$) C, H, N, Cl.

Compound 19 of Table I. The acid mustard **24d** was subjected to the modified Curtius reaction described above to give the crude amine mustard **25d** in 94% yield; ^{1}H NMR ($CDCl_3$) (80 MHz) δ 7.89 (d, J = 10 Hz, 2 H, H-2′,6′), 6.66 (d, J = 10 Hz, 2 H, H-3′,5′), 3.72 (m, 8 H, NCH_2CH_2Cl), 2.85 (br s, 2 H, NH_2), 2.75 (m, 2 H, H-5), 1.48 (m, 8 H, H-1,2,3,4); ^{13}C NMR δ 198.54 (q, C=O, C-7), 174.22 (q, C=O, C-1), 151.01 (q, C-1′), 130.51 (CH, C-2′,6′), 127.58 (q, C-4′), 113.76 (CH, C-3′,5′), 51.50 (CH_3, $COOCH_3$), 37.66 (CH_2, C-6), 33.93 (CH_2, C-2), 28.91 (CH_2, C-5), 24.80 (CH_2, C-3), 24.32 (CH_2, C-4). This was coupled directly with 9-methoxyacridine as described above to give a 74% yield of the free base of **19** as an oil. The dihydrochloride salt was prepared as described above and crystallized from MeOH/EtOAc as a yellow solid: mp 185–189 °C; ^{1}H NMR (CD_3SOCD_3) δ 14.00 (s, 1 H, acridine N·HCl), 8.60 (br s, 2 H, acridine H-1″,8″), 7.97 (d, J = 3.9 Hz, 4 H, acridine H-3″,6″,4″,5″), 7.79 (d, J = 9.0 Hz, 2 H, H-2′,6′), 7.57 (m, 2 H, acridine H-2″,7″), 6.80 (d, = 9.1 Hz, 2 H, H-3′,5′), 4.10 (q, J = 7.0 Hz, 2 H, H-1), 3.82 (t, J = 5.7 Hz, 4 H, NCH_2), 3.77 (t, J = 5.5 Hz, 2 H, CH_2Cl), 2.88 (t, J = 7.1 Hz, 2 H, H-5), 1.95 (m, 2 H, H-2), 1.65 (m, 2 H, H-4), 1.44 (m, 2 H, H-3); ^{13}C NMR δ 197.31

(q, C=O), 157.26 (q, C-5a″,10a″), 150.08 (q, C-1′), 134.77 (CH, C-1″,8″), 130.07 (CH, C-2′,6′), 125.3 (q, C-4′), 123.13 (CH, C-3″,6″), 118.36 (CH, C-2″,7″), 110.87 (CH, C-3′,5′), 51.55 (CH_2, NCH_2), 48.54 (CH_2, C-1), 40.83 (CH_2, CH_2Cl), 36.75 (CH_2, C-5), 28.59 (CH_2, C-2), 25.80 (CH_2, C-4), 23.70 (CH_2, C-3); mass spectrum, m/z 507 ($M^{\bullet+}$), 472 (M - Cl), 263, 207 (100). Anal. ($C_{29}H_{31}Cl_2N_3O$·HCl) C, H, N, Cl.

Preparation of Compound 17 of Table I. *N*,*N*-Bis(2-hydroxyethyl)-4-[5-[*N*-(benzyloxycarbonyl)amino]pentanamido]aniline (31a). A mixture of 5-[*N*-(benzyloxycarbonyl)-amino]pentanoic acid[33] (0.55 g, 3.5 mmol) and 1,1′-carbonyldiimidazole (0.55 g, 3.5 mmol) in dry DMF (5 mL) was heated at 40 °C for 30 min and then cooled. To this solution was added a solution of *N*,*N*-bis(2-hydroxyethyl)-4-aminoaniline (0.69 g, 3.5 mmol; freshly prepared by hydrogenation of *N*,*N*-bis(2-hydroxyethyl)-4-nitroaniline in dry DMF (5 mL)), and the mixture was stirred at 20 °C for 10 h. Solvent was then removed under reduced pressure, and the residue was partitioned between EtOAc and dilute aqueous Na_2CO_3. The aqueous layer was further extracted with EtOAc (2 × 50 mL), and the combined organic extracts were worked up to give a purple solid which was chromatographed on silica gel. Elution with EtOAc/MeOH (100:1) gave **31a** (0.35 g, 78% yield), which was crystallized from EtOAc/hexane as tan crystals: mp 90–93 °C; ^{1}H NMR ($CDCl_3$) δ 6.15 (m, 5 H, phenyl protons), 6.12 (d, J = 9.1 Hz, H-2′,6′), 5.51 (d, J = 9.1 Hz, 2 H, H-3′,5′), 3.87 (s, 2 H, OCH_2), 2.51 (t, J = 6.0 Hz, 4 H, CH_2OH), 2.31 (t, J = 6.0 Hz, 4 H, NCH_2) 1.96 (t, J = 6.8 Hz, 2 H, H-5), 1.14 (t, J = 7.5 Hz, 2 H, H-2), 0.50 (m, 2 H, H-4), 0.37 (m, 2 H, H-3); ^{13}C NMR 171.23 (q, C=O, C-1) 157.01 (q, C=O, C-6), 143.21 (q, C-1′), 136.82 (q, C-1″), 129.02 (q, C-4′), 128.59 (CH, C-3″,5″), 128.32 (CH, C-4″), 128.13 (CH, C-2″,6″), 123.04 (CH, C-3′,5′), 113.12 (CH, C-2′,6′), 66.87 (CH_2, $CbzCH_2$), 60.51 (CH_2, CH_2OH), 53.87 (CH_2, NCH_2), 40.54 (CH_2, C-5), 36.82 (CH_2, C-2), 29.88 (CH_2, C-4), 22.81 (CH_2, C-3); mass spectrum, m/z 429 ($M^{\bullet+}$), 321, 290, 191, 108, 107. Anal. ($C_{23}H_{31}N_3O_5$) C, H, N.

***N*,*N*-Bis(2-chloroethyl)-4-[5-[*N*-(benzyloxycarbonyl)-amino]pentanamido]aniline (32a).** A stirred suspension of the diol **31a** (1.5 g, 3.9 mmol) in dry 1,2-dichloroethane (10 mL) and $SOCl_2$ (0.35 g, 4.7 mmol) was heated under reflux for 30 min. Volatiles were removed under reduced pressure, and the residue was chromatographed on silica gel. Elution with EtOAc/hexane (10:1) gave the mustard **32a** (0.45 g, 25% yield), which was recrystallized from benzene: mp 120–123 °C; ^{1}H NMR ($CDCl_3$) δ 7.57 (br s, 1 H, NH), 7.34 (m, 7 H, phenyl protons and H-2′,6′), 6.62 (d, J = 9.0 Hz, 2 H, H-3′,5′), 5.10 (s, 2 H, OCH_2), 4.97 (br s, 1 H, NH), 3.69 (t, J = 6.4 Hz, 4 H, CH_2OH), 3.60 (t, J = 6.4 Hz, 4 H, NCH_2), 3.22 (m, 2 H, H-5), 2.35 (t, J = 7.4 Hz, 2 H, H-2), 1.74 (m, 2 H, H-4), 1.58 (m, 2 H, H-3); ^{13}C NMR δ 171.02 (q, C=O, C-1), 156.76 (q, C=O, C-6), 143.03 (q, C-1′), 136.52 (q, C-1″), 128.78 (q, C-4′), 128.54 (CH, C-3″,5″), 128.13 (CH, C-4″), 128.02 (CH, C-2″,6″), 122.27 (CH, C-3′,5′), 112.52 (CH, C-2′,6′), 66.68 (CH_2, $CbzCH_2$), 53.68 (CH_2, NCH_2), 40.51 (CH_2, CH_2Cl), 40.09 (CH_2, C-5), 36.52 (CH_2, C-2), 29.38 (CH_2, C-4), 22.51 (CH_2, C-3); mass spectrum, m/z 465 ($M^{\bullet+}$), 416, 357, 308 (100). Anal. ($C_{23}H_{29}Cl_2N_3O_3$) C, H, N, Cl.

Compound 17 of Table I. A suspension of the mustard **32a** (0.38 g, 0.82 mmol) in anhydrous HBr/AcOH (45% w/v) (5 mL) was stirred vigorously at 20 °C for 4 h. Dilution with Et_2O gave a precipitate which was collected by decantation and dissolved in dilute ammonia. This mixture was extracted with EtOAc (3 × 50 mL) and the organic layer was worked up to give the crude amine **33a** as an oil (0.26 g, 96% yield). This was coupled directly with 9-methoxyacridine as described above, and the product was chromatographed on silica gel. Elution with EtOAc/MeOH (10:1) gave the free base of compound **17** as a yellow solid (0.32 g, 75% yield): ^{1}H NMR ($CDCl_3$) δ 8.55 (br s, 1 H, NH), 8.19 (d, J = 7.6 Hz, 2 H, acridine H-1″,8″), 7.97 (d, J = 8.6 Hz, 2 H, acridine H-3″,6″), 7.55 (t, J = 7.6 Hz, 2 H, acridine H-4″,5″), 7.49 (d, J = 9.0 Hz, 2 H, H-2′,6′), 7.23 (m, 2 H, acridine H-2″,7″), 6.59 (d, J = 9.0 Hz, 2 H, H-3′,5′), 3.87 (t, J = 5.6 Hz, 2 H, $NHCH_2$), 3.65 (t, J = 6.3 Hz, 4 H, NCH_2), 3.57 (t, J = 6.3 Hz, 4 H, CH_2Cl), 2.49 (t, J = 6.3 Hz, 2 H, H-2), 1.88 (m, 4 H, H-3,4); ^{13}C NMR δ 171.32 (q, C=O), 153.64 (q, C-5a″,10a″), 145.60 (q, C-8a″,9a″), 142.98 (q, C-1′), 131.56 (CH, C-1″,8″), 129.02 (q, C-4′), 125.21 (CH, C-3″,6″), 123.87 (CH, C-4″,5″), 123.03 (CH, C-2″,7″), 122.29 (CH, C-3′,5′), 114.89 (q, C-9), 112.47 (CH, C-2′,6′), 53.64 (CH_2, NCH_2), 49.44 (CH_2, C-1), 40.56 (CH_2, CH_2Cl), 36.05 (CH_2, C-4), 30.08 (CH_2, C-2), 22.40 (CH_2, C-3). The free base was dissolved in the minimum amount of MeOH and a solution of excess dry HCl in EtOAc was added to precipitate the dihydrochloride salt as a light yellow solid, mp 155–157 °C. Anal. ($C_{28}H_{30}Cl_2N_4O$·HCl·H_2O) C, H, N, Cl.

Preparation of Compound 18 of Table I. 4-[*N*,*N*-Bis(2-hydroxyethyl)amino]benzoic Acid. A solution of methyl 4-[*N*,*N*-bis(2-hydroxyethyl)amino]benzoate[34] (3.61 g, 15 mmol) in MeOH (50 mL) and 10% aqueous NaOH (25 mL) was heated under reflux for 2 h, cooled, and diluted with water. Acidification and extraction into EtOAc gave the acid diol (2.6 g, 79% yield): mp (EtOAc) 178.5–179.5 °C; ^{1}H NMR (CD_3COCD_3) δ 7.82 (d, J = 9.06 Hz, 2 H, H-2′,6′), 6.77 (d, J = 9.07 Hz, 2 H, H-3′,5′), 3.77 (t, J = 5.82 Hz, 4 H, NCH_2), 3.65 (t, J = 5.66 Hz, 4 H, CH_2OH), 2.09 (br s, 1 H, OH); ^{13}C NMR δ 167.85 (q, COOH), 152.73 (q, C-1′), 132.20 (CH, C-2′,6′), 117.63 (q, C-4′), 111.64 (CH, C-3′,5′), 59.97 (CH_2, CH_2OH), 54.89 (CH_2, NCH_2); mass spectrum, m/z 225 ($M^{\bullet+}$), 194 (M - CH_2OH), 150. Anal. ($C_{11}H_{15}NO_4$) C, H, N.

4-[*N*,*N*-Bis(2-acetoxyethyl)amino]benzoic Acid. A mixture of the above acid diol (1.56 g, 6.93 mmol) and Ac_2O (3.93 mL, 41.6 mmol) in pyridine (20 mL) was stirred at 20 °C for 20 h. The mixture was poured onto ice, and the precipitate was collected, washed well with water, and dried to give the diacetate (1.75 g, 85% yield). Crystallization from CH_2Cl_2/hexane gave crystals: mp 129.5 °C; ^{1}H NMR ($CDCl_3$) δ 7.91 (d, J = 5.69 Hz, 2 H, H-2′,6′), 6.74 (d, J = 6.09 Hz, 2 H, H-3′,5′), 4.26 (t, J = 12.18 Hz, 4 H, NCH_2), 3.69 (t, J = 11.90 Hz, 4 H, CH_2OH), 2.045 (s, 3 H, OCOMe); ^{13}C NMR δ 170.89 (q, $OCOCH_3$), 167.15 (q, COOH), 150.772 (q, C-1′), 131.55 (CH, C-2′,6′), 118.07 (q, C-4′), 110.79 (CH, C-3′,5′), 61.08 (CH_2, CH_2OCCH_3), 49.47 (CH_2, NCH_2), 20.85 (CH_3, CCH_3); mass spectrum, m/z 309 ($M^{\bullet+}$), 236, 176, 150. Anal. ($C_{15}H_{19}NO_6$) C, H, N.

***N*-[4-[*N*-(Benzyloxycarbonyl)amino]butyl]-4-[*N*,*N*-bis(2-acetoxyethyl)amino]benzamide (30).** *N*-(Benzyloxycarbonyl)butane-1,4-diamine[33] (2.94 g, 5.75 mmol) and 4-[*N*,*N*-bis(2-acetoxyethyl)amino]benzoic acid (1.75 g, 5.89 mmol) were coupled in DMF with use of 1,1′-carbonyldiimidazole as described above to give **30** in 89% yield. The product was crystallized from EtOAc/hexane as needles: mp 100–101 °C; ^{1}H NMR ($CDCl_3$) δ 7.68 (d, J = 8.92 Hz, 2 H, H-2′,6′), 7.32 (m, 5 H, phenyl protons), 6.72 (d, J = 9.00 Hz, 2 H, H-3′,5′), 5.25 (s, 1 H, NH), 5.08 (s, 2 H, CH_2O), 4.23 (t, J = 6.28 Hz, 4 H, CH_2OAc), 3.86 (br s, 1 H, NH), 3.65 (t, J = 6.10 Hz, 4 H, NCH_2), 3.41 (t, 2 H, H-5), 3.19 (t, J = 6.20 Hz, 2 H, H-2), 2.09 (s, 6 H, OCOMe), 1.60 (m, 4 H, H-3,4); ^{13}C NMR δ 170.94 (q, $OCOCH_3$), 167.37 (q, NHCO), 156.63 (q, $COCH_2Ph$), 149.64 (q, Ph), 136.50 (q, C-1′), 128.14 (CH, phenyl and C-2″,6″), 122.24 (q, C-4′), 111.03 (CH, C-3′,5′), 66.74 (CH_2, CH_2O), 61.08 (CH_2, CH_2OAC), 49.51 (CH_2, NCH_2), 40.63 (CH_2, C-5), 39.47 (CH_2, C-2), 27.45 (CH_2, C-4), 26.88 (CH_2, C-3), 20.87 (CH_3OCMe); mass spectrum, m/z 513 ($M^{\bullet+}$), 440, 435, 345, 332, 108, 107. Anal. ($C_{27}H_{35}N_3O_7$) C, H, N.

***N*-[4-[*N*-(Benzyloxycarbonyl)amino]butyl]-4-[*N*,*N*-bis(2-hydroxyethyl)amino]benzamide (31b).** Hydrolysis of the above diacetate in methanolic NaOH was complete in a few minutes, giving an essentially quantitative yield of the diol **31b**, which crystallized from EtOAc/hexane as needles: mp 80–81 °C; ^{1}H NMR ($CDCl_3$) δ 7.58 (d, J = 8.88 Hz, 2 H, H-2′,6′), 7.32 (m, 5 H, phenyl protons), 7.09 (s, 1 H, NH), 5.02 (s, 2 H, CH_2O), 6.62 (d, J = 8.88 Hz, 2 H, H-3′,5′), 3.72 (m, 4 H, CH_2OH), 3.52 (m, 4 H, NCH_2), 3.30 (s, 2 H, H-5), 3.14 (m, 4 H, H-2), 1.50 (br s, 6 H, H-3,4); ^{13}C NMR δ 167.96 (q, NHC), 156.83 (q, $COCH_2Ph$), 149.63 (q, Ph C), 136.59 (q, C-1′), 128.49 (CH, phenyl CH, C-2′,6′), 122.28 (q, C-4′), 111.27 (CH, C-3′,5′), 66.66 (CH_2, CH_2O), 60.23 (CH_2, CH_2OH), 59.54 (CH_2, NCH_2), 40.61 (CH_2, C-5), 39.55 (CH_2, C-2), 27.29 (CH_2, C-4), 26.75 (CH_2, C-3). Anal. ($C_{23}H_{31}N_3O_5$·0.5H_2O) C, H, N.

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N-[4-[N-(Benzyloxycarbonyl)amino]butyl]-4-[N,N-bis(2-chloroethyl)amino]benzamide (32b). A solution of the diol **31b** (1.60 g, 3.47 mmol) in THF/CH_2Cl_2 (1:4, 20 mL) was cooled to 0 °C, and Et_3N (1.21 mL, 8.68 mmol) was added, followed by methanesulfonyl chloride (0.67 mL, 8.68 mmol). The mixture was stirred at 0 °C for 10 min, then diluted with CH_2Cl_2, and washed twice with cold aqueous $NaHCO_3$ and brine, dried, and evaporated. The resulting crude dimesylate and LiCl (0.7 g, 16.5 mmol) were dissolved in DMF (30 mL), and the mixture was slowly heated to 110 °C and kept there for 5 min. Solvent was removed under reduced pressure, and the residue was partitioned between CH_2Cl_2 and water. Workup of the organic layer and recrystallization of the product from EtOAc gave the mustard **32b** as a white solid (1.65 g, 92%): mp 120–120 °C; ^1H NMR ($CDCl_3$) δ 7.70 (d, J = 8.78 Hz, 2 H, H-2′,6′), 7.35 (m, 5 H, aromatic protons), 6.62 (d, J = 8.98 Hz, 2 H, H-3′,5′), 6.33 (br s, 1 H, NH), 5.09 (s, 2 H, CH_2O), 3.77 (t, J = 7.18 Hz, 4 H, $ArNCH_2$), 3.64 (t, J = 6.81 Hz, 4 H, CH_2Cl), 3.44 (q, J = 6.15 Hz, 2 H, H-5), 3.23 (q, J = 6.46 Hz, 2 H, H-2), 1.60 (m, 4 H, H-3,4); ^{13}C NMR δ 167.05 (q, NHCO), 156.69 (q, $COCH_2Ph$), 148.48 (q, phenyl C), 136.59 (q, C-1′), 128.93, 128.51, 128.09, 128.06 (CH, phenyl CH, C-2″,6″), 123.14 (q, C-4′), 111.04 (CH, C-3′,5′), 66.62 (CH_2, OCH_2Ph), 53.27 (CH_2, NCH_2), 40.63 (CH_2, C-5), 40.17 (CH_2, CH_2Cl), 39.47 (CH_2, C-2), 27.75 (CH_2, C-4), 26.82 (CH_2, C-3); mass spectrum, m/z 465/467/469 ($M^{\bullet+}$), 4.16 (M - CH_2Cl), 357, 318 (100). Anal. ($C_{23}H_{29}Cl_2N_3O_3$) C, H, N, Cl.

Compound 18 of Table I. Hydrolysis of **32b** with anhydrous HBr/AcOH as described above gave the crude amine mustard **33b** in 72% yield. This compound (0.65 g, 2.50 mmol) was coupled directly with 9-methoxyacridine (0.62 g, 2.75 mmol) as described above, and the product was chromatographed on silica gel. EtOAc/MeOH (9:1) eluted the free base of compound 18, which was crystallized from MeOH/EtOAc/HCl to give the dihydrochloride salt (0.72 g, 50%) as bright yellow hygroscopic crystals: mp 127–130 °C; ^1H NMR (CD_3COCD_3) δ 8.41 (d, J = 8.37 Hz, 2 H, acridine H-1,8), 7.91 (m, 4 H, acridine H-4,5 and H-2′,6′), 7.66 (t, J = 7.09 Hz, 2 H, acridine, H-2,7), 7.33 (t, J = 7.29 Hz, 2 H, acridine H-3,6), 6.84 (d, J = 8.88 Hz, 2 H, H-3′,5′), 4.05 (t, J = 6.79 Hz, 2 H, H-2), 3.87 (t, J = 6.47 Hz, 4 H, $ArNCH_2$), 3.78 (t, J = 6.31 Hz, 4 H, CH_2Cl), 3.49 (t, J = 6.15 Hz, 2 H, H-5), 1.98 (t, J = 6.17 Hz, 2 H, H-3), 1.80 (t, J = 6.38 Hz, 2 H, H-4); ^{13}C NMR δ 167.35 (q, CONH), 152.90 (q, C-1′), 149.53 (q, C-5a″,10a″), 148.69 (q, C-8a″,9a″), 130.61 (CH, C-1″,8″,3″,6″), 129.79 (CH, C-2′,6′), 125.10 (CH, C-4″,5″), 122.84 (CH, C-2″,7″), 117.47 (q, C-4′), 111.77 (CH, C-3′,5′), 53.38 (CH_2, NCH_2), 51.07 (CH_2, C-5), 41.36 (CH_2, CH_2Cl), 39.82 (CH_2, C-2), 29.80 (CH_2, C-4), 20.78 (CH_2, C-3); mass spectrum, m/z 551 ($M^{\bullet+}$ (508) + C_3H_7), 509 (M + H), 473 (M - Cl), 455, 447, 424, 411, 265, 244 (100). Anal. ($C_{28}H_{30}Cl_2N_4O\cdot 2HCl\cdot 4H_2O$) C, H, N.

Preparation of Compound 20 of Table I. 6-[[4-[N,N-Bis(2-chloroethyl)amino]phenyl]sulfonyl]hexanoic Acid (35). A solution of the sulfide **27c** (3.0 g, 7.96 mmol) in dry CH_2Cl_2 (80 mL) was treated with 3-chloroperbenzoic acid (3.43 g, 20 mmol) in portions, and the mixture was stirred at 20 °C for 8 h. It was then diluted with CH_2Cl_2 and washed with $NaHCO_3$, water, and brine and concentrated to dryness. The residue was filtered through silica gel in EtOAc to give methyl 6-[[4-[N,N-(bis(2-chloroethyl)amino]phenyl]sulfonyl]hexanoate (**34**) (2.74 g, 84% yield) as a pale yellow oil: ^1H NMR ($CDCl_3$) δ 7.73 (d, J = 8.98 Hz, 2 H, H-2′,6′), 6.75 (d, J = 9.08 Hz, 2 H, H-3′,5′), 3.84 (t, J = 6.93 Hz, 4 H, NCH_2), 3.68 (t, J = 6.75 Hz, 4 H, CH_2Cl), 3.66 (s, 3 H, OCH_3), 3.05 (t, J = 8.08 Hz, 2 H, CH_2SO_2), 2.28 (t, J = 7.33 Hz, 2 H, H-2), 1.72 (m, 2 H, H-5), 1.59 (m, 2 H, H-3), 1.41 (m, 2 H, H-4); ^{13}C NMR δ 173.81 (q, C=O), 150.13 (q, C-1′), 130.17 (CH, C-2′,6′), 126.41 (q, C-4′), 111.31 (CH, C-3′,5′), 56.46 (CH_2, CH_2SO_2), 53.26 (CH_2, NCH_2), 51.59 (CH_3, OMe), 39.95 (CH_2, CH_2Cl), 33.58 (CH_2, CCH_2), 27.76 (CH_2, C-5), 24.33 (CH_2, C-3), 22.70 (CH_2, C-4); mass spectrum, m/z 409.881 ($M^{\bullet+}$), 394 (M - CH_3), 374 (M - Cl), 360, 347, 266, 69, 41.

Hydrolysis in concentrated HCl for 80 min at 90 °C gave the acid **35** in 93% yield: ^1H NMR ($CDCl_3$) δ 7.73 (d, J = 9.07 Hz, 2 H, H-2′,6′), 6.75 (d, J = 9.07 Hz, 2 H, H-3′,5′), 3.83 (t, J = 6.96 Hz, 4 H, NCH_2), 3.68 (t, J = 6.65 Hz, 4 H, CH_2Cl), 3.05 (t, J = 7.95, 2 H, CH_2SO_2), 2.34 (t, J = 7.28, 2 H, H-2), 1.73 (m, 2 H, H-5), 1.62 (m, 2 H, H-3), 1.43 (m, 2 H, H-4); ^{13}C NMR δ 178.94 (q, C=O), 150.13 (q, C-1′), 130.14 (CH, C-2′,6′), 126.30 (q, C-4′), 111.29 (CH, C-3′,5′), 56.37 (CH_2, CH_2SO_2), 53.21 (CH_2, NCH_2), 39.92 (CH_2, CH_2Cl), 33.45 (CH_2, CCH_2), 27.61 (CH_2, C-5), 24.01 (CH_2, C-3), 22.62 (CH_2, C-4); mass spectrum, m/z 395.07249 ($M^{\bullet+}$), 360 (M - Cl); 346 (M - CH_2Cl), 310, 297 (3446 - CH_2Cl).

Compound 20 of Table I. The acid **35** was subjected to the modified Curtius reaction as described above to give a 69% yield of the crude amine **36** as a brown oil. This was coupled directly with 9-methoxyacridine as described above, and the product was chromatographed on silica gel. Elution with EtOAc/MeOH (10:1) gave the free base of compound **20** of Table I in 60% yield. Crystallization from MeOH/EtOAc/HCl gave the hydrochloride salt as a yellow solid: mp 85–86 °C; ^1H NMR (CD_3OD) δ 8.48 (d, J = 8.67 Hz, 2 H, acridine-H-1″,8″), 7.96 (t, J = 7.58 Hz, 2 H, acridine H-4″,5″), 7.81 (d, J = 8.49 Hz, 2 H, acridine H-2″,7″), 7.67 (d, J = 9.01 Hz, 2 H, H-2′,6′), 7.55 (d, J = 7.65 Hz, 2 H, H-3′,5′), 6.89 (d, J = 9.07 Hz, 2 H, H-3′,5′), 4.11 (m, 2 H, H-5), 3.86 (t, J = 6.84 Hz, 4 H, NCH_2), 3.71 (t, J = 6.52 Hz, 4 H, CH_2Cl), 3.16 (t, J = 7.61 Hz, 2 H, H-1), 1.97 (m, 2 H, H-4), 1.75 (m, 2 H, H-2), 1.57 (m, 2 H, H-3); ^{13}C NMR δ 159.71 (q, C-5a″,10a″), 152.28 (q, C-1′), 136.47 (CH, C-1″,8″), 133.15 (q, C-9″), 131.16 (CH, C-2′,6′), 131.02 (CH, C-3″,6″), 126.73 (q, C-8a,9a), 124.97 (CH, C-4″,5″), 119.62 (CH, C-2″,7″), 113.71 (q, C-4′), 112.73 (CH, C-3′,5′), 61.56 (CH_2, CH_2SO_2), 56.88 (CH_2, $NHCH_2$), 53.91 (CH_2, NCH_2), 41.31 (CH_2, CH_2Cl), 30.05 (CH_2, C-5), 26.37 (CH_2, C-3), 23.76 (CH_2, C-4); mass spectrum, m/z 543.14987 ($M^{\bullet+}$), 508 (M - Cl), 472 (M - 2Cl), 432, 263, 207, 179. Anal. ($C_{28}H_{31}Cl_2N_3O_2S\cdot HCl\cdot H_2O$) C, H, N, Cl.

Hydrolysis of Mustards. Drugs were dissolved in DMSO to make 2 mM solutions, and 50 μL of this solution was added to 950 μL of 50% w/w acetone/water in 1.5-mL capped microcentrifuge tubes at 66 °C. At appropriate times, 50-μL aliquots were analyzed by HPLC, using a 0.4 × 30 cm μ-Bondapak C-18 column eluted at 1 mL/min with a mobile phase of 70% MeOH, 10% CH_3CN, and 20% 1 M aqueous ammonium acetate, containing 1 mM triethylamine and 1 mM heptanesulfonic acid. Absorbance was monitored at 254 and 436 nm, and peaks corresponding to the parent mustard, and half-mustard, and the diol were integrated. The percentage of parent compound remaining at each time point was determined and used to calculate a pseudo-first-order rate constant for the hydrolysis reaction according to the formula

$$K_H = \ln((A - x_1)/(A - x_2))/(t_2 - t_1)$$

where A = initial concentration and $A - x$ is the concentration at time t.

NBP Alkylation Assays. These were determined by a modification of the standard NBP colorimetric assay.[23] Water (950 μL) and 1 mL of a 50 mM solution of NPB in Me_2CO were mixed in 13 × 100 mm glass tubes and then placed on ice. A 2 mM solution of the appropriate drug (50 μL in DMSO) was added, and the tubes were tightly capped, mixed vigorously, and placed in a 66 °C waterbath for various times. The pH was monitored and remained at 6.5 ± 0.2 during the experiment. Reactions were terminated by placing the tubes on ice for 1 min, followed by addition of EtOAc (1 mL) and 0.3 M NaOH (1 mL). Samples were then placed in a 20 °C waterbath for 5 min, mixed vigorously, and centrifuged at 500g for 3 min to separate the phases. The blue product in the EtOAc (upper) layer was quantified spectrophotometrically at 500 nm, and the K' values were calculated by the formula

$$K' = (A_2 - A_1)/(T_2 - T_1)$$

where A = absorbance at 550 nm and T = incubation time. Since the K' values are determined from initial rates in the presence of excess NBP, they are proportional to (but not the same as) the pseudo-first-order rate constants for NBP alkylation.[23]

Cytotoxicity Assays. 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.[17,29] IC_{50} values were determined with 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, using eight control cultures on each microplate. For P388 cultures, drug was present throughout the growth period (72 h), and final cell densities were determined by using a minor modification of the MTT method of Mossman.[35]

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.[36]

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Development of a Novel Series of (2-Quinolinylmethoxy)phenyl-Containing Compounds as High-Affinity Leukotriene Receptor Antagonists. 1. Initial Structure–Activity Relationships

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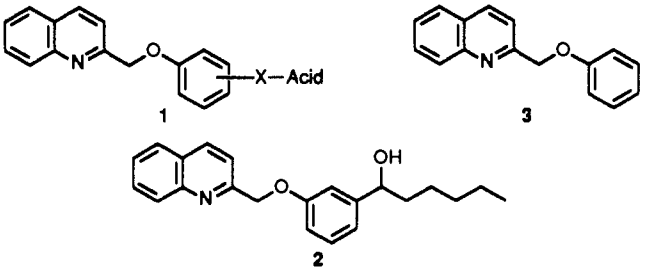
This series of reports describes the development of orally active, highly potent, specific antagonists of the peptidoleukotrienes containing a (2-quinolinylmethoxy)phenyl moiety. Described in this first report are the structure–activity relationships that led to more than a 20-fold improvement of the potency and selectivity of the initial chemical lead (RG 5901). From this series of compounds, RG 7152 (**16**) was identified and selected for further evaluation in the clinic as an antiasthmatic agent. Compound **16** competitively inhibits [^{3}H]LTD_4 binding to membranes from guinea pig lung (K_i = 38 ± 6 nM) and the spasmogenic activity of LTC_4, LTD_4, and LTE_4 in parenchymal lung strips from guinea pigs. Unlike the original lead (RG 5901), compound **16** does not inhibit 5-lipoxygenase from guinea pig PMNs. Following oral administration to guinea pigs, **16** blocks LTD_4-induced dermal permeability (ED_{50} = 6.9 mg/kg), LTD_4-induced bronchoconstriction (ED_{50} = 1.1 mg/kg), antigen-induced bronchoconstriction (ED_{50} = 2.5 mg/kg), and anaphylactic-induced mortality (ED_{50} = 16 mg/kg). These studies on structure–activity relationships indicate that there is a requirement for an acidic function and the presence of the (2-quinolinylmethoxy)phenyl moiety in a specific geometric arrangement.

Several lines of evidence have suggested that the sulfidopeptide leukotrienes (LTC_4, LTD_4, and LTE_4) play a pathophysiological role in hypersensitivity diseases.[1] Several laboratories[2-7] have been searching for potent and selective antagonists of these leukotrienes for the potential therapeutic use in treating diseases such as asthma. Some of the earlier reported leukotriene antagonists were evaluated in clinical studies, but the results have been disappointing.[8] However, it is highly possible that the antagonists under evaluation in the clinic have lacked sufficient potency and/or the appropriate pharmacokinetic profile to determine if this pharmaceutical approach has therapeutic utility. In this series of papers, we describe the development of a chemical series containing a (2-quinolinylmethoxy)phenyl moiety into high-affinity, orally active leukotriene receptor antagonists.

Initially, we concentrated on structure–activity studies of a series of compounds represented by generic structure

1. The development of this chemical series evolved from



the observations that RG 5901 (**2**) is not only a competitive

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